Initial IND Application Submission

October 28, 2016

IND Application Title:	**Autologous Wharton's Jelly for Augmented Repair of Cleft Palate**
Biological Product:	**Autologous Human Wharton's Jelly**
IND Number:	**Not Yet Assigned**
Serial Number:	**0000**
Sponsor-Investigator:	**Charles S. Cox, Jr., M.D.** **UTHealth McGovern Medical School at Houston** **Phone: 713.500.7300 Fax: 713.500.0714** **charles.s.cox@uth.tmc.edu**

UTHealth™
The University of Texas
Health Science Center at Houston

Medical School
Charles S. Cox, Jr., M.D.
The George & Cynthia Mitchell Distinguished Chair in Neurosurgery
Director, Children's Program in Regenerative Medicine
Professor, Department of Pediatric Surgery
6431 Fannin Street, Suite 5.246
Houston, Texas 77030
Office: 713.500.7300
Fax: 713.500.0714
charles.s.cox@uth.tmc.edu

October 19th, 2016

Patrick Riggins, PhD.
FDA Center for Biologics and Evaluation and Research
Office of Cellular, Tissues and Gene Therapies
Document Control Center, WO71-G-112
10903 New Hampshire Ave.
Silver Springs, MD 20993-0002

RE: Investigational New Drug Application, Serial # 0000

Protocol Title: Autologous Wharton's Jelly for Augmented Repair of Cleft Palate
UTHealth IRB# HSC-MS-16-0738

Dear Dr. Riggins:

Enclosed please find 3 copies of an investigational new drug application to evaluate the safety and feasibility of using autologous Wharton's Jelly in the repair of cleft palate. The clinical trial will not be initiated prior to thirty days after receipt of this IND by your Center.

Please do not hesitate to contact me if there are any questions or concerns regarding this submission. Additionally, you may contact my clinical trial program manager, Steven Kosmach, at 713.500.7329, steven.kosmach@uth.tmc.edu.

Sincerely,

Charles S. Cox, Jr., M.D.

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SERIAL # 0000

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(Title 21, Code of Federal Regulations (CFR) Part 312)

Form Approved: OMB No. 0910-0014
Expiration Date: February 28, 2019
See PRA Statement on page 3.

NOTE: No drug/biologic may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40)

1. Name of Sponsor
Charles S. Cox, Jr., M.D.

2. Date of Submission *(mm/dd/yyyy)*
10/19/2016

3. Sponsor Address

Address 1 *(Street address, P.O. box, company name c/o)*
UTHealth McGovern Medical School, 6431 Fannin Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*
Department of Pediatric Surgery, MSB 5.246

City: Houston
State/Province/Region: Texas
Country: USA
ZIP or Postal Code: 77030

4. Telephone Number *(Include country code if applicable and area code)*
713.500.7300

5. Name(s) of Drug *(Include all available names: Trade, Generic, Chemical, or Code)*
Human Native Wharton's Jelly

Continuation Page for #5

6. IND Number *(If previously assigned)*

7. (Proposed) Indication for Use
Augmented Repair of Cleft Palate

Is this indication for a rare disease (prevalence <200,000 in U.S.)? ☐ Yes ☑ No

Does this product have an FDA Orphan Designation for this indication? ☐ Yes ☑ No

If yes, provide the Orphan Designation number for this indication:

Continuation Page for #7

8. Phase(s) of Clinical Investigation to be conducted
☑ Phase 1 ☐ Phase 2 ☐ Phase 3 ☐ Other *(Specify)*: _____

9. List numbers of all Investigational New Drug Applications (21 CFR Part 312), New Drug Applications (21 CFR Part 314) , Drug Master Files (21 CFR Part 314.420) , and Biologics License Applications (21 CFR Part 601) referred to in this application.

10. IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 0000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 0001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.

Serial Number: 0000

11. This submission contains the following *(Select all that apply)*

☑ Initial Investigational New Drug Application (IND)
☐ Request For Reactivation Or Reinstatement
☐ Development Safety Update Report (DSUR)
☐ Response to Clinical Hold
☐ Annual Report
☐ Other *(Specify)*: _____
☐ Response To FDA Request For Information
☐ General Correspondence

Protocol Amendment(s)
☐ New Protocol
☐ Change in Protocol
☐ New Investigator
☐ PMR/PMC Protocol

Information Amendment(s)
☐ Chemistry/Microbiology
☐ Pharmacology/Toxicology
☐ Clinical ☐ Statistics
☐ Clinical Pharmacology

Request for
☐ Meeting
☐ Proprietary Name Review
☐ Special Protocol Assessment
☐ Formal Dispute Resolution

IND Safety Report(s)
☐ Initial Written Report
☐ Follow-up to a Written Report

12. Select the following only if applicable. *(Justification statement must be submitted with application for any items selected below. Refer to the cited CFR section for further information.)*

Expanded Access Use, 21 CFR 312.300

☐ Emergency Research Exception From Informed Consent Requirements, 21 CFR 312.23 (f)
☐ Charge Request, 21 CFR 312.8
☐ Individual Patient, Non-Emergency 21 CFR 312.310
☐ Individual Patient, Emergency 21 CFR 312.310(d)
☐ Intermediate Size Patient Population, 21 CFR 312.315
☐ Treatment IND or Protocol, 21 CFR 312.320

For FDA Use Only

CBER/DCC Receipt Stamp	DDR Receipt Stamp	Division Assignment
		IND Number Assigned

PSC Publishing Services (301) 443-6740 EF

13. Contents of Application – This application contains the following items *(Select all that apply)*

- [x] 1. Form FDA 1571 *(21 CFR 312.23(a)(1))*
- [x] 2. Table of Contents *(21 CFR 312.23(a)(2))*
- [x] 3. Introductory statement *(21 CFR 312.23(a)(3))*
- [x] 4. General Investigational plan *(21 CFR 312.23(a)(3))*
- [x] 5. Investigator's brochure *(21 CFR 312.23(a)(5))*
- [x] 6. Protocol(s) *(21 CFR 312.23(a)(6))*
  - [x] a. Study protocol(s) *(21 CFR 312.23(a)(6))*
  - [x] b. Investigator data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
  - [x] c. Facilities data *(21 CFR 312.23(a)(6)(iii)(b))* or completed Form(s) FDA 1572
- 6. Protocol(s) *(Continued)*
  - [x] d. Institutional Review Board data (21 CFR 312.23(a)(6)(iii)(b)) or completed Form(s) FDA 1572
- [x] 7. Chemistry, manufacturing, and control data *(21 CFR 312.23(a)(7))*
  - [ ] Environmental assessment or claim for exclusion *(21 CFR 312.23(a)(7)(iv)(e))*
- [x] 8. Pharmacology and toxicology data (21 CFR 312.23(a)(8))
- [x] 9. Previous human experience *(21 CFR 312.23(a)(9))*
- [x] 10. Additional information *(21 CFR 312.23(a)(10))*
- [ ] 11. Biosimilar User Fee Cover Sheet *(Form FDA 3792)*
- [x] 12. Clinical Trials Certification of Compliance *(Form FDA 3674)*

14. Is any part of the clinical study to be conducted by a contract research organization? [x] Yes [ ] No

If Yes, will any sponsor obligations be transferred to the contract research organization? [ ] Yes [x] No

If Yes, provide a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred *(use continuation page)*. **Continuation Page for #14**

15. Name and Title of the person responsible for monitoring the conduct and progress of the clinical investigations

Charles S. Cox, Jr., M.D.

16. Name(s) and Title(s) of the person(s) responsible for review and evaluation of information relevant to the safety of the drug

Charles S. Cox, Jr., M.D.

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold or financial hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set forth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

17. Name of Sponsor or Sponsor's Authorized Representative

Charles S. Cox, Jr., M.D.

18. Telephone Number *(Include country code if applicable and area code)*

713-500-7300

19. Facsimile (FAX) Number *(Include country code if applicable and area code)*

713-500-0714

20. Address

Address 1 *(Street address, P.O. box, company name c/o)*
UTHealth McGovern Medical School, 6431 Fannin Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*
Department of Pediatric Surgery, MSB 5.246

City: Houston
State/Province/Region: Texas
Country: USA
ZIP or Postal Code: 77030

21. Email Address

charles.s.cox@uth.tmc.edu

22. Date of Sponsor's Signature *(mm/dd/yyyy)*

10/19/2016

23. Name of Countersigner

24. Address of Countersigner

Address 1 *(Street address, P.O. box, company name c/o)*

Address 2 *(Apartment, suite, unit, building, floor, etc.)*

City
State/Province/Region
Country: United States of America
ZIP or Postal Code

WARNING : A willfully false statement is a criminal offense (U.S.C. Title 18, Sec. 1001).

25. Signature of Sponsor or Sponsor's Authorized Representative

Charles S. Cox Jr. **Sign**

26. Signature of Countersigner **Sign**

The information below applies only to requirements of the Paperwork Reduction Act of 1995.

The burden time for this collection of information is estimated to average 100 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden to the address to the right:

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

Department of Health and Human Services
Food and Drug Administration
Office of Operations
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

Please do NOT send your completed form to this PRA Staff email address.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

STATEMENT OF INVESTIGATOR

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)

(See instructions on reverse side.)

Form Approved: OMB No. 0910-0014
Expiration Date: February 28, 2019
See OMB Statement on Reverse.

NOTE: No investigator may participate in an investigation until he/she provides the sponsor with a completed, signed Statement of Investigator, Form FDA 1572 (21 CFR 312.53(c)).

1. NAME AND ADDRESS OF INVESTIGATOR

Name of Clinical Investigator			
Charles S. Cox, Jr., M.D.			
Address 1		Address 2	
UTHealth McGovern Medical School, 6431 Fannin Street		Department of Pediatric Surgery, MSB 5.246	
City	State/Province/Region	Country	ZIP or Postal Code
Houston	Texas	USA	77030

2. EDUCATION, TRAINING, AND EXPERIENCE THAT QUALIFY THE INVESTIGATOR AS AN EXPERT IN THE CLINICAL INVESTIGATION OF THE DRUG FOR THE USE UNDER INVESTIGATION. ONE OF THE FOLLOWING IS PROVIDED *(Select **one** of the following.)*

☒ Curriculum Vitae ☐ Other Statement of Qualifications

3. NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED

CONTINUATION PAGE for Item 3

Name of Medical School, Hospital, or Other Research Facility			
Memorial Hermann Hospital- Texas Medical Center			
Address 1		Address 2	
6411 Fannin Street			
City	State/Province/Region	Country	ZIP or Postal Code
Houston	Texas	USA	77030

4. NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY

CONTINUATION PAGE for Item 4

Name of Clinical Laboratory Facility			
UTHealth- Medical School, Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory			
Address 1		Address 2	
UTHealth Biomedical & Behavioral Science Building		1941 East Road, 6th Floor	
City	State/Province/Region	Country	ZIP or Postal Code
Houston	Texas	USA	77030

5. NAME AND ADDRESS OF THE INSTITUTIONAL REVIEW BOARD (IRB) THAT IS RESPONSIBLE FOR REVIEW AND APPROVAL OF THE STUDY(IES)

CONTINUATION PAGE for Item 5

Name of IRB			
UTHealth, Committee for the Protection of Human Subjects			
Address 1		Address 2	
6410 Fannin Street		Univ. Professional Building 11.00	
City	State/Province/Region	Country	ZIP or Postal Code
Houston	Texas	USA	77030

6. NAMES OF SUBINVESTIGATORS *(If not applicable, enter "None")*

Matthew Greives, MD, John Teichgraeber, MD, Fabio Triolo, PhD, Jenifer Juranek, PhD, Claudia Pedroza, PhD, Margaret Jackson, MD, Christopher Schneider, MD, Steven Kosmach, MSN, RN, Joiya Arrington, MSN, RN

CONTINUATION PAGE – for Item 6

7. NAME AND CODE NUMBER, IF ANY, OF THE PROTOCOL(S) IN THE IND FOR THE STUDY(IES) TO BE CONDUCTED BY THE INVESTIGATOR

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. UTHealth IRB# HSC-MS-16-0738

8. PROVIDE THE FOLLOWING CLINICAL PROTOCOL INFORMATION. *(Select **one** of the following.)*

☒ For Phase 1 investigations, a general outline of the planned investigation including the estimated duration of the study and the maximum number of subjects that will be involved.

☐ For Phase 2 or 3 investigations, an outline of the study protocol including an approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; the clinical uses to be investigated; characteristics of subjects by age, sex, and condition; the kind of clinical observations and laboratory tests to be conducted; the estimated duration of the study; and copies or a description of case report forms to be used.

9. COMMITMENTS

I agree to conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.

I agree to report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64. I have read and understand the information in the investigator's brochure, including the potential risks and side effects of the drug.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

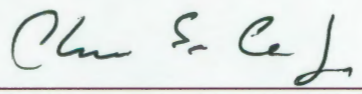
I agree to maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68.

I will ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

INSTRUCTIONS FOR COMPLETING FORM FDA 1572
STATEMENT OF INVESTIGATOR

1. Complete all sections. Provide a separate page if additional space is needed.
2. Provide curriculum vitae or other statement of qualifications as described in Section 2.
3. Provide protocol outline as described in Section 8.
4. Sign and date below.
5. FORWARD THE COMPLETED FORM AND OTHER DOCUMENTS BEING PROVIDED TO THE SPONSOR. The sponsor will incorporate this information along with other technical data into an Investigational New Drug Application (IND). INVESTIGATORS SHOULD NOT SEND THIS FORM DIRECTLY TO THE FOOD AND DRUG ADMINISTRATION.

10. DATE *(mm/dd/yyyy)*	11. SIGNATURE OF INVESTIGATOR
10/19/2016	Sign 

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Department of Health and Human Services
Food and Drug Administration
Office of Operations
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

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DO NOT SEND YOUR COMPLETED FORM TO THIS PRA STAFF EMAIL ADDRESS.

FIRST CONTINUATION PAGE FOR ITEM 3

NAME AND ADDRESS OF ANY MEDICAL SCHOOL, HOSPITAL, OR OTHER RESEARCH FACILITY WHERE THE CLINICAL INVESTIGATION(S) WILL BE CONDUCTED ***(Enter additional names and addresses below.)***

Name of Medical School, Hospital, or Other Research Facility			
Pediatric Out-Patient Surgery Clinic			
Address 1: 6410 Fannin Street		Address 2: Univ. Professional Building, Suite 900	
City: Houston	State/Province/Region: Texas	Country: USA	ZIP or Postal Code: 77030

Name of Medical School, Hospital, or Other Research Facility			
Address 1		Address 2	
City	State/Province/Region	Country	ZIP or Postal Code

Name of Medical School, Hospital, or Other Research Facility			
Address 1		Address 2	
City	State/Province/Region	Country	ZIP or Postal Code

Name of Medical School, Hospital, or Other Research Facility			
Address 1		Address 2	
City	State/Province/Region	Country	ZIP or Postal Code

Name of Medical School, Hospital, or Other Research Facility			
Address 1		Address 2	
City	State/Province/Region	Country	ZIP or Postal Code

Name of Medical School, Hospital, or Other Research Facility			
Address 1		Address 2	
City	State/Province/Region	Country	ZIP or Postal Code

Name of Medical School, Hospital, or Other Research Facility			
Address 1		Address 2	
City	State/Province/Region	Country	ZIP or Postal Code

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FIRST CONTINUATION PAGE FOR ITEM 4

NAME AND ADDRESS OF ANY CLINICAL LABORATORY FACILITIES TO BE USED IN THE STUDY
(Enter additional names and addresses below.)

Name of Clinical Laboratory Facility
Memorial Hermann- Texas Medical Center Laboratory Services

Address 1	Address 2
6411 Fannin Street	

City	State/Province/Region	Country	ZIP or Postal Code
Houston	Texas	USA	77030

Name of Clinical Laboratory Facility

Address 1	Address 2

City	State/Province/Region	Country	ZIP or Postal Code

Name of Clinical Laboratory Facility

Address 1	Address 2

City	State/Province/Region	Country	ZIP or Postal Code

Name of Clinical Laboratory Facility

Address 1	Address 2

City	State/Province/Region	Country	ZIP or Postal Code

Name of Clinical Laboratory Facility

Address 1	Address 2

City	State/Province/Region	Country	ZIP or Postal Code

Name of Clinical Laboratory Facility

Address 1	Address 2

City	State/Province/Region	Country	ZIP or Postal Code

Name of Clinical Laboratory Facility

Address 1	Address 2

City	State/Province/Region	Country	ZIP or Postal Code

Add Second Continuation Page for Item 4 | Remove Continuation Page | Return to Form

19 August 2016

CURRICULUM VITAE

Charles Samuel Cox, Jr.

Present Position:

George and Cynthia Mitchell Distinguished Chair in Neurosciences, Professor of Pediatric Surgery, Pediatrics and Biomedical Engineering, The University of Texas Medical School at Houston
Director of University of Texas Health Science Center Program in Pediatric Regenerative Medicine, Judith Hoffberger Cellular Therapeutics Laboratory and Evelyn Griffin Stem Cell Therapeutics Laboratory; Bentsen Stroke Center Investigator- Brown Foundation Institute for Molecular Medicine
Co-Director, Texas Trauma Institute

Address:

Academic office
Department of Pediatric Surgery
MSB Suite 5.236
6431 Fannin Street
Houston, Texas 77030

Home address
4425 Lula
Bellaire, Texas 77401
Phone: 713-665-4485

Phone: 713-500-7307
Fax: 713-500-7296

Email: charles.s.cox@uth.tmc.edu

Websites:

Department of Pediatric Surgery: http://utsurg.uth.tmc.edu/pedisurgery/index.htm
Translational Laboratories: http://utsurg.uth.tmc.edu/pedisurgery/translational-labs/index.html

Birth date: February 26, 1962

Citizenship: USA

Undergraduate Education:

1980-1984 University of Texas at Austin - BA

Graduate Education:

1984-1988 University of Texas Medical Branch at Galveston - MD

Postgraduate Training:

Internship in Surgery
1988-1989 University of Texas Health Science Center at Houston
Residency in Surgery
1989-1990 University of Texas Health Science Center at Houston
1992-1993 University of Texas Health Science Center at Houston
1993-1994 University of Texas Health Science Center at Houston

Fellowship

1990-1992 NIH Research Fellow in Trauma and Burns
Shriner's Burns Institute, University of Texas Medical Branch
1994-1996 Pediatric Surgery, University of Michigan
1996-1997 Surgical Critical Care, University of Texas-Houston

Military:

Captain, Medical Corps United States Army Reserve - 1990 to 1996
Major, Medical Corps United States Army Reserve - 1996 to 2002
Lieutenant Colonel, Medical Corps United States Army Reserve - 2002 to 2004
Awards: Meritorious Unit Citation; Joint Meritorious Unit Award; Army Service Ribbon; National Defense Service Medal with Bronze Star Device; Army Commendation Medal; Armed Forces Reserve Medal with M device, Bronze Hourglass, and numeral 2 device; Reserve Component Achievement Medal with 3 Oak Leaf Clusters; Global War on Terror Expeditionary Medal; Global War on Terror Medal; Afghanistan Campaign Medal; Expert-Pistol; Combat Medical Badge; Overseas Service Bar; 82nd Airborne Combat Patch- 2002-2003 – 909th Forward Surgical Team; 44th Medical Command; 82nd Airborne Division; FOB Salerno Afghanistan.
Other: Invited Panelist: HLS C2 Prioritization Workshop/USAMRMC-TATRC. 27 April 2003, Orlando, Florida. Gary P. Wratten Memorial Visiting Professor, 20 May 2003, San Antonio, Texas. Invited Panelist: 10th Annual Trauma Symposium – Brooke Army Medical Center, 10 August 2004, San Antonio, Texas. American Association for the Surgery of Trauma Honorary Combat Surgery Medal, 2004.
The Association of Military Surgeons-Texas Medical Center Chapter Faculty Advisor.
The University of Texas Center for Biosecurity and Public Health Preparedness-Associate Director, Hospital and Trauma Preparedness (2004-2007).
Invited panelist: Neural Restoration Workshop: Cognitive and Optical Military Systems, Sandia National Laboratories/DOE; 12 November 2009. Problem Based Learning Curriculum in Military Medicine (2012).
Civilian Liaison: BAMC/SAMC Pediatric Trauma/Critical Care Fellowship Rotation (2010-date)

Academic Appointments:

1995-1996	Instructor of Pediatric Surgery University of Michigan Medical School
1996-1997	Instructor of Pediatric Surgery University of Texas-Houston Medical School
1997-2001	Assistant Professor of Surgery and Pediatrics University of Texas-Houston Medical School
2001-2005	Associate Professor of Surgery and Pediatrics University of Texas-Houston Medical School
2005-Present	Professor of Surgery and Pediatrics University of Texas-Houston Medical School
2008-2012	Adjoint Professor of Biomedical Engineering University of Texas-Austin, Cockrell School of Engineering
2009-Present	Adjunct Professor of Nanomedicine and Biomedical Engineering University of Texas-Houston Medical School

Hospital Appointments:

Children's Memorial Hermann Hospital
LBJ/Ben Taub General Hospitals
M.D. Anderson Cancer Center
Texas Children's Hospital
1996- 2011 Co-Director UT-HMS/Hermann Hospital ECMO Program
1996-Present Director UT-HMS/Hermann Hospital Pediatric Trauma Program

Licensure:

Texas State Board of Medical Examiners: H 6136
Texas DPS: L0086846; DEA: BC 4844634
Michigan State Board of Medical Examiners: 4301063982
Texas Medical Board- Expert Panelist: Surgery, Pediatric Surgery, Surgical Critical Care

Board Certification:

Diplomate, American Board of Surgery #40103, May 2, 1995.
Special Qualifications in Pediatric Surgery #755, April 20, 1998.
Added Qualifications in Surgical Critical Care #1531, October 24, 1997.
Recertification in Surgery 2003 #40103, July 1, 2015 (Exp)
Recertification in Surgical Critical Care #1531, July 1, 2018 (Exp)
Recertification in Pediatric Surgery #755, July 1, 2018 (Exp)
Recertification in Surgery 2013 #40103, December 1, 2025 (Exp)

Other Certifications:

Advanced Trauma Life Support – Provider and Instructor
Basic Cardiac Life Support
Pediatric Advanced Life Support

Professional Organizations:

American Surgical Association
Society of University Surgeons
American Pediatric Surgical Association
American College of Surgeons
American College of Surgeons South Texas Chapter
American Association for the Surgery of Trauma
Shock Society
American Academy of Pediatrics
American Physiological Society-Cardiovascular Section
American Society for Artificial Internal Organs
Association of Academic Surgery
Extracorporeal Life Support Organization
DeBakey Institute
Society of Critical Care Medicine
Microcirculatory Society
Surgical Biology Club III
Mission Connect
Cord Blood Association

Honors and Awards:

1984 Graduate, University of Texas Plan II Liberal Arts Honors Program
1986 Dean's List, University of Texas Medical Branch
1987 University of Texas Medical Branch at Galveston representative to American College of Surgeons Clinical Congress, Medical Student Program.
1990 Resident Award, American College of Surgeons - Committee on Trauma, South Texas Chapter Resident's Competition
1991 American Society of Artificial Internal Organs Traveling Fellowship Award
1992 Alpha Omega Alpha
1992 Resident Award, American College of Surgeons, South Texas Chapter Scientific Session
1993 Co-winner Carl A. Moyer Award for Burn Research (American Burn Association)
1994 George W. Waldron Surgical Award (Outstanding Chief Resident, University of Texas Health Science Center at Houston)
1997 Dean's Excellence Award, University of Texas Medical School-Houston
1998 Dean's Excellence Award, University of Texas Medical School-Houston
2000 Physician of the Year – Memorial Hermann Children's Hospital
2002 Charter Fellow, Michael E. DeBakey Institute for Comparative Cardiovascular Science and Biomedical Devices
2002 Faculty Advisor – The Association of Military Surgeons of the United States: Texas Medical Center Chapter
2003 Microcirculatory Award for Excellence in Lymphatic Research
2006 William Pokorny Surgical Science Lectureship
2007 Michael E. DeBakey Institute Master Lecturer in Cardiovascular Physiology
2007 Surgery Chief Resident Surgical Teaching Award, University of Texas Medical School-Houston
2010 Frank G. Moody Academic Surgeon Award, University of Texas Medical School-Houston
2013 Ralph E. Ligon Award (Formerly Surgery Chief Resident Surgical Teaching Award), University of Texas Medical School-Houston
2014 UT Health Resident Alumni Award: Moody Lectureship
2015 American College of Surgeons, South Texas Chapter, William Pokorny Memorial Lecture, Western Trauma Society Founders Lecture
2015 Robert D. Hart Endowed Lectureship Series – University of Illinois
2015 Jesse Ternberg Endowed Lecture – Washington University Medical School

Editorial Positions (40 manuscripts/year):

1. Clinical

Annals of Surgery - reviewer
Clinical Science - reviewer
Journal of Trauma – reviewer
Intensive Care Medicine - reviewer
Journal of Surgical Endoscopy - reviewer
Surgery - reviewer
Critical Care Medicine – reviewer
ACS Surgical Forum (Pediatric Surgery) – reviewer (2007-Present)
Journal of Pediatric Surgery – reviewer

2. Stem Cells/Tissue Engineering
   - Stem Cells - reviewer
   - Stem Cells and Development – reviewer
   - Brain - reviewer
   - Cytotherapy – reviewer
   - Drug Discovery Today – reviewer
   - Recent Patents on CNS Drug Discovery – reviewer
   - Bentham Science Publishers – book reviewer
   - Tissue Engineering – reviewer
   - Analyst – reviewer
   - Brain Research – reviewer
   - World Journal of Stem Cells – Editorial Board
   - Rejuvenation Research – reviewer
   - Current Medicinal Chemistry – reviewer
   - Journal of Neuroinflammation– reviewer
   - Journal of Neurological Sciences – reviewer
   - Neurobiology of Disease – reviewer
   - Translational Stroke Research – reviewer
   - NMR Medicine – reviewer
   - Brain, Behavior & Immunity – reviewer
   - Biomed Research International – reviewer
   - Stroke – reviewer
   - Stem Cells International – reviewer
3. Medical Devices
   - Journal of Neurotrauma - reviewer
   - Journal of Thoracic and Cardiovascular Surgery – reviewer
   - ASAIO Journal – Section Editor: Gas Exchange/Pediatric Circulatory Support and Perfusion Sections (2009-2011)
4. Physiology
   - Brazilian Journal of Medical and Biological Sciences – reviewer
   - Journal of Applied Physiology – reviewer
   - BMC Gastroenterology - reviewer
5. Editorial Consultant
   - American Journal Experts
6. Springer Publishers
   - Book Proposal Reviewer

Service on National Grant Review Panels, Study Sections, Committees:

1998-Present	State of Florida Emergency Medical Services Trauma Center Site Reviewer
2000-Present	Reviewer, National Student Research Forum
2001-Present	State of Missouri Department of Health Trauma Center Site Reviewer
2001-2007	State of Pennsylvania Department of Health Trauma Center Site Reviewer
2002-2002	Canadian Institutes of Health Research: Cardiovascular Systems Study Section
2002-2002	US Civilian Research and Development Foundation Bilateral Grants Program-III Reviewer (ID #16331)
2000-2003	Society of University Surgeons: Chairman, local arrangements for 2003 meeting

2003-2003	Medical Advisory Council: Congress of the United States House of Representatives; 25th District, Texas
2003-Present	Southeast Texas Regional Advisory Council – Pediatric Trauma Subcommittee Chairman
2004-2004	Centers for Disease Control Injury Center: Measurement of Quality Outcomes Work Group
2004-Present	American College of Surgeons Southern Texas District #1 Committee on Applicants
2005-2008	Society of University Surgeons Membership Committee
2005-2006	Association of Academic Surgery: Fundamentals of Surgical Research Course Faculty
2005-2006	National Medical Research Council (Singapore): Sepsis Study Section
2005-2009	American Heart Association, Western Review Consortium - (Lung, Immunology, Surgery) Study Section
2006-2009	American Heart Association, Western States Affiliate - Research Advisory Committee
2006-2012	American College of Surgeons Committee on Trauma Subcommittees: - Pediatric Trauma - Panelist Trauma Video Session - Publications - Reviewer: SSET Syllabus - Disaster Management - South Texas Chapter
2007-2009	RAPTOR TRIAL: Data Safety Monitoring Board Member
2007-2010	Department of Veterans Affairs Joint Biomedical Laboratory Research and Development Scientific MERIT Review Board (Subcommittee for Surgery)
2008-2010	Chairman: Study Section - Department of Veterans Affairs Joint Biomedical Research A: Integrated Review Group; Office of Research and Development (Subcommittee for Surgery)
2007-Present	American Pediatric Surgical Association Committee on Trauma
2007-Present	American Institute of Biological Sciences (DOD/USAMRMC) Ad Hoc Grant/Program Reviewer
2007	National Institutes of Health - ZHD1 DSR-A (18) Special Emphasis Review Committee: "New Approaches for the Prevention and Treatment of NEC"
2007	National Institute on Disability and Rehabilitation Research (Department of Education): Traumatic Brain Injury Model Systems Centers Collaborative Research Projects
2008-Present	American College of Surgeons: Surgical Forum (Pediatric Surgery) - Reviewer/Judge
2008	Medical Monitor: Whole blood resuscitation trial (Gonzalez, E: PI)
2008	Czech Science Foundation: International Project Reviewer
2008	Department of Defense Deployment Related Medical Research Program: Psychological Health and Traumatic Brain injury – 4 Clinical Trial Award – Scientific Review Panelist
2008	National Medical Research Council (Singapore): Surgery Study Section

2008	LeBonheur Children's Hospital/University of Tennessee-Division of Pediatric Surgery – External Reviewer
2008	Sarah Jane Brain Foundation – Scientific Advisory Board
2009	Georgia Stem Cell Initiative: Conference Panelist: Intellectual property and scientific barriers to clinical trials using progenitor cells
2009	California Institute for Regenerative Medicine Grants Working Group
2009	Moderator Pediatric Surgical Forum
2009-2010	8th World Congress of Trauma, Shock Inflammation and Sepsis: Scientific Committee and Faculty – Stem Cells and Neurological Injury
2009-	Department of Defense Deployment Related Medical Research Program: Traumatic Brain Injury: Scientific Review Panelist
2010-	National Medical Research Council (Singapore): Biomedical Engineering Study Section
2010-	Emergency Medical Services Commission Advisory Board: CHOLA Grant "Pediatric Emergency Logistics of Care Regionalization"
2010-	Stem Cell Therapies as an Emerging Paradigm in Stroke-2. Panelist
2010-	Data Safety Monitoring Board: A randomized clinical trial of pravastatin and atorvastatin in severe traumatic brain injury.
2010-	Department of Veterans Affairs: Rehabilitation Research & Development Merit Review Board (2 Study Sections/Year)
2010-	American Association for the Surgery of Trauma – Pediatric Trauma Surgery Ad Hoc Committee
2010-	AATV International Grant Reviewer
2011-	National Institutes of Health (NHLBI-PACT) Cellular Therapies for Pediatric Diseases Workshop: Panelist / Speaker
2012	CIRM: California Institute for Regenerative Medicine: Chairman: Early Translational Research Award Study Section; Disease Team Award Program; Research Leadership Award; Chairman: New Faculty Clinician Scientist Award; CIRM Strategic Partnership; Translational Award IV
2012	APSA Publications Committee
2012	BSRI/CIRM Workshop: Cellular Therapies in Trauma Critical Care – Organizing Committee and Lecturer
2012	Thrasher Research Fund Grant Reviewer
2013	CIRM: Translational Award IV; CIRM IOM/Grantee Network
2013	Expert Panel: Automated Critical Care Systems Office of Naval Research; PI: George Kramer, Ph.D.
2013-	Government of Hong Kong Health/Medical Research Fund: Grant Reviewer (Annual Reviews)
2013	National Institutes of Health/NINDS: BDCN N58 Special Emphasis Panel; Sports Related TBI; SCI: Reviewer
2013	UMKC Trauma Research Center External Evaluation Committee
2013	Center for Advancement of Science in Space (CASIS)/NASA: Impact of microgravity on Fundamental Stem Cell Properties; Grant Reviewer
2014	Columbia University Specialized Research Center in Injury and Perioperative Sciences: External Advisory Committee
2014	Le Bonheur Children's Hospital, Department of Pediatric Surgery; External Reviewer
2014	CIRM: Research Leadership Awards; Tools and Technologies Awards; Alpha Stem Cell Clinic Awards; Chairman: External Innovation Awards;

	Bridging Supplements/Early Translation Awards
2014	National Institutes of Health: Loan Repayment Program Special Emphasis Panel 2014/08 ZTR1 CI (01); Reviewer
2014	PSI Foundation; Ad hoc Grant Reviewer
2014	National Institute of Health – NINDS, NSD-K (G78) Special Emphasis Panel Study Section Ad Hoc Reviewer
2014	National Institutes of Health – NINDS, BINP Study Section (Brain injury and Neurovascular Pathologies) Ad Hoc Reviewer
2014	CIRM: Pre-Clinical Development Award – Grants Working Group Chairman
2015	CIRM 2.0 Grants Working Group/Study Chair
2015	National Institutes of Health – MODS Conference
2015	National Institutes of Health – NINDS, 2RGI BDCN-Y Study Section; Special Emphasis Panel – TBI
2016	National Institutes of Health – NINDS, BINP Study Section (Brain Injury and Neuroprotection) Ad Hoc Reviewer
2016	CIRM: Clinical Trials Grants Working Group Chairman; Translational Discovery Working Group Chairman; Quest Awards Reviewer
2016	American Board of Surgery – Pediatric Surgery Examiner

Service on the University of Texas Medical School at Houston Committees:

1. Residency Advisory Council, Department of Surgery, University of Texas Health Science Center at Houston, 1993-1994.
2. House Officer Association Representative, University of Texas Health Science Center at Houston 1993-1994.
3. Faculty Senate, University of Texas Medical School-Houston 1996-1997; 2001-2002; 2004-2006.
4. ACGME Residency review committee for Department of Pediatrics, University of Texas Health Science Center at Houston, 1994.
5. Student Evaluation and Promotions Committee, 2001-2005.
6. Research Conflict of Interest Committee, 2006-Present.
7. Faculty Appointments, Promotion & Tenure Committee: Ad Hoc Member 2007-Present
8. Advisory Committee on Resource Development and Utilization. 2008.
9. Center for Clinical and Translational Sciences
   a. Institutional Data Safety Monitoring Board Member
   b. CCTS Engine of Innovation
   c. CCTS Investigator/Mentor
   d. KL2 Scholar Mentor
10. Faculty Grievance Committee – 2010
11. KL2 CCTS Grant Reviewer - 2014

Service on the University of Texas Medical School at Houston Affiliated Hospital Committees:

1996-Present	Director-PAT for Pediatric Trauma Center Development (Children's Memorial Hermann Hospital) Directorship of the Pediatric Trauma Program entailed developing the processes and material, and personnel required to meet the standard of the American College of Surgeons Level I Pediatric Trauma Center. The evolution began with no personnel/resources to what is now has a dedicated Pediatric Trauma Program Manager, Registrars, and a full service Acute Care Nurse Practitioner run Pediatric Trauma Service. During this time, the Pediatric Trauma volume doubled in size. Benchmarked outcomes to NTDB standards have demonstrated lower injury adjusted mortality rates compared to other Level I Trauma Centers and excellent solid organ preservation rates. Venue for quarterly pre hospital airway course; annual pre-hospital pediatric Trauma conference. There have been three successful verification visits during this time frame.
1996-Present	Pediatric Operating Room Subcommittee (Memorial Hermann Hospital)
1996-1998	Pharmacy and Therapeutics Committee (Memorial Hermann Hospital)
1999-Present	Medical Staff Peer Review Committee (Memorial Hermann Hospital)
2003-2005	Chair – Medical Staff Quality Assurance (Memorial Hermann Hospital)
2005-2008	Co-Chair Pediatric Medical Staff Quality Assurance (Children's Memorial Hermann Hospital)
2009	Ophthalmology Chair Search Committee
2012	Transfer Committee
2015-2016	Texas Trauma Institute Chief Search Committee

Sponsorship of Candidates for Postgraduate degree (Includes undergraduate, graduate and post-doctoral candidates): Majority funded via: National Institutes of Health: 2T35DK07676-22; 2014-2019 short term research training (Faculty Mentor; Kone-PI)

1. Sheena Kim: (NIH sponsored summer medical student research program, 1991); Project: Hollow fiber membrane removal of circulating endotoxin.
2. David Butler: (NMF/Bristol-Myers-Squibb Scholarship, 1999) Project: Endotoxin increases microvascular permeability associated with cardiopulmonary bypass. Manuscript Published/Abstract Published (J Surg Rsch) Pediatrics Residency
3. Laura Johnson, MD: (Neonatology post-doctoral fellow, 2000). Project: PMN priming by gut lymph after ECMO. (Pediatrics Faculty-UT Houston)
4. Jarrod Little: (American Heart Association Cardiovascular Diseases Scholarship, 2000). Project: Mechanisms of PMN priming by gut lymph. Otolaryngology Residency; Assistant Professor of Plastic & Reconstructive/Head & Neck Surgery, University of Louisville.
5. Jeff Padalecki: (NIH sponsored summer medical student research program, 2003); Project: Does enteral feeding improve resuscitation induced ileus? (NIH T35 DK 007676-14) Manuscript Published/Abstract Published (J Surg Rsch) Orthopaedic Surgeon – Private practice, Austin, TX.
6. Samuel Lance: (NIH sponsored summer medical student research program, 2005); Project: Role of aquaporins in the resolution of resuscitation induced intestinal edema. (NIH T35 DK 007676-14) Manuscript In Press/Abstract Published (Crit Care Med) Plastic Surgery Residency, UC-Davis.

7. Zachary Wright: (NIH sponsored summer medical student research program, 2005); Project: NF-Kß and resuscitation induced gut edema. (NIH T35 DK 007676-14) Manuscript In Press/Abstract Published. Internal Medicine Residency
8. LeeAnn Sloan: (NIH sponsored summer medical student research program, 2007); Project: Neural Stem Cell Transplantation for Traumatic Brain Injury. (NIH T35 DK 007676-14); Winner Student Travel Award for Presentation at Academic Surgical Congress, Huntington Beach, CA, 2008. Manuscript in J Surg Res; Oral presentation at American College of Surgeons, South Texas Chapter, Houston, TX, 2008. 2010-UTSW General Surgery Resident.
9. Scott Collins: UT BME Graduate Student – 2008-2009: Microfluidics and Tissue Scaffolding Designs. Thesis: "Guided vascular growth by mesenchymal stem cells". (Industry) CSO TeVido, Inc.
10. Lindsey Fogle: (NIH sponsored summer medical student summer research program, 2008); Project: Mechanotransductive forces mimic intestinal edema induced STAT-3 activation. (NIH T35 DK 007676-14); Manuscript Accepted; Abstract Published (Surgical Forum/JACS, 2009). Resident in Pathology 2011; Internal Medicine Faculty, UT Health.
11. Brooke Huffsmith: (NIH sponsored summer medical student summer research program, 2009); Project: Inflammatory response after traumatic brain injury. (NIH T35 DK 007676-16)
12. Ngoc Pham: (Scholarly Concentration Program: Molecular Basis of Disease Program, 2010-2012) Project: Degenerate cloning of E-Cadherin in intestinal tissue.
13. Chelsea Thomas: (NIH Sponsored summer medical student research program, 2011). Project: Placental derived stem cell effect on TBI. (NIH T35 DK 007676-18). Webber Research Award (2nd place), 2011. Scholarly Concentration Program: Microglial activation after TBI. Abstract published (Surgical Forum/JACS 2012).
14. Stephen Williams: (Summer Research Program, 2012). Project: Morphology of activated microglia after TBI. UT-Houston Medical Student.
15. Henry Caplan: (Summer Research Program, 2013). Project: Effects of MAPC on microglial phototype after TBI. Extramural presentation at TAMU Institute for Neuroscience; Manuscript Accepted. UT Health Neurosciences Research Center 2016 Distinguished Medical Student in Neuroscience. (NIH T35 DK 007676-18). General Surgery Resident, UT Health.
16. Grace Philip: (Summer Research Program, 2013). Project: Microglia response to TBI. Extramural presentation at TAMU Institute for Neuroscience.
17. Matt Mitchell: (Summer Research Program, 2014). Project: DT MRI evaluation of TBI – effects of MAPC treatment on imaging biomarkers. 2nd Place Mission Connect Poster Competition.
18. Pam Zelnick: (Summer Research Program, 2014). Project: TSPO as an immunohistochemistry marker for activated microglia after TBI.
19. Margaret Johnson: (Summer Research Program, 2015). Project: Microglial activation after TBI. Dean's Research Scholarship Award.

Post-Doctoral Fellows:

NIH T32 GM 0879201: Trauma Research Training Program

1. Stacey Moore-Olufemi, MD (2002-2004); Developed novel model of resuscitation induced intestinal edema. Began work on mechanisms of edema induced gut dysfunction, describing iNOS mediated smooth muscle dysfunction. Developed model of intestinal ischemic preconditioning and mechanisms of organ protection. Matriculated to Pediatric Surgery Residency, University of Texas-Houston (2007-2009). Assistant Professor of Pediatric Surgery (2009-Present). Recipient Robert Wood Johnson/Harold Amos Foundation Career Development Award (2010-2014).
2. Ravi Radhakrishnan, MD (2004-2006); Described physical characteristics of edema-induced intestinal dysfunction using engineering methods. Subsequently defined the cytoskeletal/subcellular events that cause the tissue level changes. Re-entered General Surgery Residency at University of Texas-Houston Medical School, (2006-2009). Pediatric Surgery Residency – Northwestern McGaw Medical School/Children's Memorial Hospital; Chicago, IL, (2009-2011). Assistant Professor of Pediatric Surgery, UTMB, Galveston, Texas (2011-2014). Associate Professor of Pediatric Surgery and Leonard & Marie Rosoff Professorship in Surgery, UTMB, Galveston, Texas (2015-Present).
3. Matthew Harting, MD (2006-2008); Developed a controlled-cortical impact model of TBI with treatment using GFP (+) bone-marrow progenitor cells and mesenchymal stem cells. This included a novel flow-cytometry based method to regionally quantify cell migration in the brain. Described the regional pro-inflammatory microenvironments of TBI. Used mesenchymal stem cells, neural progenitor cells, and bone marrow derived mononuclear cells in models of TBI and stroke. Completing General Surgery Residency at University of Michigan (2008-2011). Pediatric Surgery Resident- University of Washington/Seattle Children's Hospital (2011-2013). Assistant Professor Pediatric Surgery – UT Health (2013). CTSA/CCTS Scholar (2015).
4. Peter Walker, MD (2008-2010); Use of multipotent adult progenitor cell treatment of traumatic brain injury. Modulation of the inflammatory response to TBI using progenitor cell transplantation. Defined novel role of MAPC-Spleen interactions to alter the intra-cerebral macrophage phenotype after TBI. Winner: President's Award for outstanding basic science research, South Texas Chapter ACS (2008). Independent grant funding: BD Biosciences: T-regulatory cells as a therapeutic target for progenitor cells in traumatic brain injury. General Surgery Resident 2010-2013. MIS Fellowship – UT Health (2013-2014). Assistant Professor of Surgery – UT Health (2014).
5. Shinil Shah, MD (2008-2010); Developed advanced models of abdominal compartment syndrome and intestinal edema. Mechanisms of edema induced intestinal dysfunction. Identified mechanotransductive mechanism of signal transduction that initiates/amplifies edema induced organ dysfunction. Winner: Outstanding basic science research award, South Texas Regional Committee on Trauma (2009),Outstanding basic science research award, Region VI (2010); Winner: Best manuscript from new Association of Academic Surgeons for, "Evaluating the potential role of nitric oxide as a mediator of hydrostatic edema mediated intestinal contractile dysfunction,"(2010). General Surgery Resident Award (2010); General Surgery Resident (2010-2013). Raleigh Ross Scholar, UT Health (2012). Chief Resident of the Year. MIS Fellowship – UT Health (2013-2014). Assistant Professor of Surgery – UT Health (2014).

6. Supinder Bedi, PhD (2010-2012); Progenitor cell therapies for neurological injury. Role of microglial phenotype/activation in TBI and effects of progenitor cells as an adoptive immunotherapy targeting microglial response to TBI. Winner: University of Texas- Houston Post-Doc Travel Award (2010). Instructor in Pediatric Surgery – Research (2012).
7. George Liao, MD (2013-2015); Use of adult adherent stem cells (MSCs, MAPCs, hafMSCs) in immunomodulation of TBI and tissue engineering applications. American College of Surgeons 2014 Excellence in Research Award Recipient.
8. Margaret Jackson, MD (2015-2017); Development of a model of CPB induced neurological injury and cellular therapies. Microglial knockout mouse model of TBI. SUS Scholarship/Storz Fellowship Award Finalist (2015).
9. Mitchell George, MD (2016-2018); Bioreactor designs development for mechanotransduction of stem cells/induction of gene programs.

Dr. F. Kohler Chemie GmGH Scholars:

1. Henning Sauer, MD (1998-2000); Focused on role of varying myocardial protection strategies with emphasis on relationship of myocardial edema and myocardial performance.
2. Uwe Fischer, MD (2000-2002); Extended work on PMN/oxidative injury of myocardium during cardioplegic arrest. Demonstrated mechanisms of apoptosis associated with cardioplegic arrest. Defined relative roles of hypothermia and edema on myocardial dysfunction.

Alexander von Humboldt Foundation Fellow:

1. Uwe Fischer, MD (2007-2009); Developed novel therapeutics for minimizing myocardial dysfunction associated with various myocardial injuries. Defined the effect of NF-Kß blockade on gut I/R induced myocardial dysfunction. Used AAV-GFP tagged gene transfer as a strategy for myocardial protection. Was the primary author on one of the most highly cited articles (over 50 citations in under 2 years) that was listed as a "seminal article/core article" in Stem Cells and Development. General Surgery Resident, University of Texas-Houston (2009-2014). Vascular Surgery Residency – The Methodist Hospital (2014-2016).

DeBakey Institute Post-Doctoral Fellow:

1. Ji Chu, PhD (2010-2012); Studied the molecular mechanisms of myosin light chain phosphorylation and regulation as related to intestinal contractility in setting of edema.

Brown Foundation Fellows:

1. Robert Hetz, M.D. (2011-2013); Studied the effects of cell based therapies on traumatic brain injury. Developed a modified splenocyte MLR to monitor MAPC potency; novel potency assays of MSC. Developed and characterized a novel & good grade human amniotic fluid MSC working cell bank. General Surgery Resident, UT Health. Thoracic Surgery Fellow, MD Anderson Cancer Center (2016).
2. Alex Olsen, M.D. (2011-2012); Studied the immune modulatory effects of autonomic nervous system on innate immune system after injury. Evaluated neuroinflammatory reflex arc after TBI and effects on intestinal function. General

Surgery Resident, St. Joseph's Hospital - Phoenix (2014). Critical Care Fellowship, Baylor College of Medicine/Texas Children's Hospital (2014-2015).

Glassell Foundation Fellow:

1. Christopher Corkin (2014); Studied the effects of various stem cell/scaffold combinations on diaphragm – tissue engineered constructs.
2. Benjamin Aetker (2014); Effects of stress induced gene programs in MSC function as a therapy for TBI. Neurology Resident, UT Health (2016).
3. Christopher Schneider (2016-2018); Immune mechanisms of post-TBI inflammation/secondary brain injury.

Pediatric Critical Care Medicine Fellows:

1. Sowmaya Kallur, M.D. (2014-2016); Scholarly oversight committee in conjunction with MSC biology program. Use of alginate beads/MSCs as a local-regional delivery system of MSCs for TBI.

CETIR (Center for Translational Injury Research) Fellows:

1. Phillip Le Tourneau, M.D. (2009-2011); Immunomodulatory effects of MSCs in TBI. Inflammatory response to resuscitation. Instrumental in a number of mechanistic studies in MSCs: TBI resulting in very high impact journal publications – Science/Translational Medicine.Trauma/Critical Care Residency – MPH/Harvard.
2. Elaheh Rahbar, Ph.D. (2012-2014); Resuscitation strategies to minimize edema formation. K99-R00 application.

Faculty:

1. Stacey Moore-Olufemi, MD (2009-Date): Modeling of NEC/gastroschisis and short bowel syndrome with a focus on epigenetic regulation of phenotypic response to pro-inflammatory stimuli. Clinical correlative studies exploring functional genomics and biomarkers of NEC are being developed. Recipient of Robert Wood Johnson – Harold Amos Career Development Award: "Molecular Mechanisms of Intestinal Dysfunction in Gastroschisis" (2010-2014).
2. Shibani Pati, MD, PhD (2009-Date): Grant sponsor/mentor for translational component of career enhancement award in stem cell research with a focus on adult stem cell populations as applied to neurological diseases. K18 Award recipient in stem cell research training. Assistant Investigator – BSRI, San Francisco, CA and Assistant Professor Laboratory Medicine – UCSF.
3. Shiraz Younas, MD (2009-Date): Clinical studies in traumatic pediatric femur fractures. Development of clinical research infrastructure for future clinical trials.
4. John Hagan, PhD (2012-Date): Studies using inhibitory RNA strategies in cancer. KL2 mentor for "LIN28/let-7 Pathway and 3' RNA uridylation in ovarian cancer."
5. Matthew Harting, MD (2013-Date): Development of novel therapeutic strategies to treat CDH, including stem cell based treatments. CTSA/CCTS Scholar Award (2015-2018).
6. Manish Shah, MD (2015-Date): Cortical plasticity in spastic diplegic children after selective dorsal rhizotomy.

Biomedical Engineering Research Program:

A. UTCBME Internship
1. Natalia Velez (2009) – The role of iNOS in intestinal edema. (Manuscript Published: J Surg Res.)
2. Huram Mok (2009) – Intravenous multipotent adult progenitor cell therapy for traumatic brain injury. (Manuscript Submitted)
3. Michael Yim (2008) – Effects of catheter-based delivery systems on progenitor cell survival and function. (Manuscript Submitted)
4. Sean Paschall (2008) – Electrospun nanofiber tissue scaffolds for organ replacement/repair.
5. Greg Minwell (2007) – Circulatory system modeling for intravascular catheter navigation system.
6. Tanmay Gokhale (2007) – Mechanotransductive forces in small intestine. (Manuscript Submitted)
7. Lindsey Villarrubia (2006) – Interstitial pressure-volume relationship with resuscitation induced gut edema. (Manuscript Published). Resident Pediatrics; Washington University.
8. Lindsey Fogle (2006) – Dynamic fluid flow modeling of an arteriovenous resuscitation device. (Manuscript Published). Resident Pathology; UTSA
9. Kunal Shah (2005) – Bioimpedance analysis quantifies level of edema and capacitance of small intestine. (Manuscript Published)
10. Sima Yazdani (2005) – In vitro pressure – flow modeling of ATR device.
11. David Rainosek (2005) – Cardiovascular variable display project.

B. Bioengineering Research Partnership
1. Nate Kemp, PhD: Assisted in developing contral system feedback for Active Thermal Resuscitation.
2. Kevin Aroom, MS: Integrated combustion/manufacturing and control systems for GEN 3 ATR device. (Master's thesis)
3. Ozgur Ekici, BS: First principle heat exchanger calculations, including materials testing.
4. Alex Bjelica, BS: Initial translation of optical/ultrasound guided automated thoracic access.
5. Albert Espinosa, BS: Automated vascular access device. (Master's Thesis)

C. Senior Tutorial
1. Sheena Black: Real time calorimetric determination of HCT.
2. Santhisri Kodali: Real time calorimetric determination of HCT.
3. Chris King: Real time calorimetric determination of HCT.
4. Kaiwun Lee: Real time calorimetric determination of HCT.

D. Summer College Internships
1. Amanda Hayley (2008) (USC): Osmolar effects on progenitor cell size/function.
2. Adam Baumgartner (2008) (UT-Austin): Neural progenitor cell culture optimization.
3. Stephen Williams (2010) (UT-Austin): Scaffolds/Cell Interaction. UT Medical School - 2011.

4. Modupe Olufemi (2011) (DeBakey High School for Health Professions): Microglial Morphometrics in TBI. Bryn Mawr College - 2013.
5. Fanni Mandy (2013-2014) (Cornell): Microglial activation after TBI.
6. Franciska Mandy (2013-2014) (Cornell): Microglial activation after TBI.

Current Teaching Responsibilities:

1. Problem based learning instructor: Junior student surgery rotations. (1996-1998)
2. Oral examinations: Junior student surgery rotations. (1996-1998)
3. Surgery House Officer Clinical Teaching: (1996-Present)
    i. Departmental Morbidity and Mortality
    ii. Divisional Morbidity and Mortality
    iii. Teaching rounds (patient care teaching)
    iv. Intraoperative teaching (technical components of surgery)
    v. Pediatric house officer, clinical teaching – didactic lecture series
4. Teaching of Surgical Tutorial for Senior Medical students. (1997-1998)
5. Chairman CME course: "Pediatric Review and Update, 1998", University of Texas-Houston Medical School (1998)
6. Instructor: Pediatric Advanced Life Support (PALS) (1996-2000)
7. Instructor: Advanced Pediatric Life Support (APLS) (1996-2000)
8. Instructor: Hermann Hospital Life Flight cadaver procedure lab; update on TBI management
9. Pediatric Transport Team curriculum
10. Instructor: Advanced Trauma Life Support (ATLS) – 2-4 Courses/Year (1998-date)
11. Lecturer: Neonatal Fellows Conference: Surgical Problems of the Neonate.
12. Lecturer: ECMO Team Training Conference (Annual) (1996-Present) – 1 Course/Year
13. The Center for Biomedical Engineering: University of Texas-Houston and MD Anderson Cancer Center (2004-Present)
14. Instructor: Seminar-Topics in Translational Research (2006-Present)
    a. Innovation by Surgeons
    b. Translational Surgical Research and Regenmed 2.0
15. Developer: Pediatric Trauma Airway Management Course for pre-hospital providers. 3 Courses/Year
16. C-STEP: Teaching Faculty. 1 Seminar/Year
17. Joint Admissions Medical Program Seminar- 1 Lecture/Year.
18. Lecturer: Graduate School of Biomedical Sciences: Topics in Neurobiology of Disease (GS140021) – Stem cells and Regenerative Medicine.
19. Lecturer: Graduate School of Biomedical Sciences: Stem Cells for Neurological Injury; 1 Lecture/Year.
20. Uniformed Services University of Health Sciences: eCurriculum for Pediatric Trauma Care/Medical Services for Children Program

Visiting Professor Lectures:

2003 University of Chicago Medical School
2005 University of Michigan Medical School
2006 University of Texas-Southwestern Medical School
2006 University of Iowa Medical School
2006 Ohio State University/Columbus Children's Hospital
2007 Texas A&M University College of Veterinary Medicine

2009 University of Louisville College of Medicine
2011 Monash University Medical School
2011 Tufts University Medical School
2012 National Institutes of Health
2013 University of Nebraska Medical School
2014 Uniformed Services University of the Health Sciences – National Institutes of Health
2015 University of Illinois College of Medicine Robert D. Hart, MD Endowed Lectureship Series
2015 Washington University Medical School; Jesse Ternberg, MD Endowed Lecture

Current Grant Support:

1. National Institutes of Health: 2T32 GM 0879201-11A1: $1,835,646 – 2001-2017. *NRSA Institutional Training Grant. Trauma Research Training Program.* Associate Program Director (Holcomb).
   Fellow – Stacey Moore-Olufemi, M.D. (2002-2004)
   Fellow – Ravi Radhakrishnan, M.D. (2004-2006)
   Fellow – Matt Harting, M.D. (2006-2008)
   Fellow – Peter Walker, M.D. (2008-2010)
   Fellow – Supinder Bedi, Ph.D. (2010-2012)
   Fellow – George Liao, M.D. (2013-2015)
   Fellow – Margaret Jackson, M.D. (2015-2017)
   Fellow – Mitchell George, M.D. (2016-2018)

2. National Institutes of Health: 1RO1 NS077963-01A1: $3,432,964. 2013 -2018. *Phase 2 Pediatric Autologous BMMNC for Severe Traumatic Brain Injury.* Principal Investigator.

3. National Institutes of Health: 1RO1 NS090935-01A1: $2,588,836. 2015-2020. *Reducing neuronal loss after traumatic brain injury.* Co-Principal Investigator.

4. National Institutes of Health: 1 U44-NS-077511-01: $1,925,263 – 2012-2016. SBIR Subcontract – Academic Component: Athersys, Inc.; *Cell Based Therapy for Traumatic Brain Injury.* Co-Principal Investigator (Cox/Mays).

5. CDMRP/MRMC/Joint Warfighter Medical Research Program: JW150014: $6,800,000. 2016-2020. *Joint Warfighter Medical Research Program. Cellular Therapy for TBI: Phase 2a/2b.* Principal Investigator.

6. National Institutes of Health: 2R01NS046308-10A: $3,622,165 – 2009-2016. *Traumatic Stress After Pediatric Injury: Neurological Influences.* Co-Investigator (Ewing-Cobbs).

7. National Institutes of Health: R43HD086076-01: 2015-2016. *NEOSAFE: Novel Materials for Manufacturing of Neonatal Devices.* Consultant.

8. State of Texas Emerging Technology Fund: $3,150,000 – 2011-2016. *Program in Children's Regenerative Medicine*. Principal Investigator.

9. Center for Disease Control: 1 U01 CE 002188-01: $2,749,792 – 2012-2017. *Developmental consequences of Pediatric TBI.* Co-Principal Investigator (Ewing-Cobbs).

10. Celgene, Inc.: $237,219 – 2011-2016. *Efficacy of PDA001 and HPDSC for TBI.* Principal Investigator.

11. CBR, Inc.: $500,000 – 2013-2016. *Autologous cellular therapy for cerebral palsy.* Principal Investigator.

12. Athersys, Inc.: $65,000 - 2012-2016. *Phase III Pre-Clinical Studies in Cellular Therapy for TBI.* Principal Investigator.

13. Bentsen Foundation: $486,684 - 2012-2017. *Amniotic fluid derived MSCs for neurological injury.* Principal Investigator. Competitive Renewal: $300,000 – 2015-2017.

14. Kinetic Concepts, Inc.: $390,287 – 2009-2015. *Negative pressure therapy modulation of intestinal lymph flow during resuscitation induced intestinal edema*. Principal Investigator.

15. Men of Distinction Foundation: $125,000 – 2013-2018. *Pediatric traumatic brain injury Impact of the inflammatory response to injury.* Principal Investigator.

16. National Science Foundation: 1312391: $250,000 – 2013-2018. *Collaborative Research: Mathematical modeling of biological processes in edematous tissue.* Consultant (Uray).

17. Let's Cure CP: $110,000 – 2014-2016. *ACT for CP Clinical Trial Support.* Principal Investigator.

18. Coagulex, Inc.: $200,000 – 2014-2016. *MEMS-Based Coagulometer Development*. Principal Investigator.

19. Dunn Foundation: $8,000 – 2014-2016. *TMC-GCC Collaborative Workshop: Regenerative Medicine in Neuroscience & Neuroengineering.* Principal Investigator.

20. CBR, Inc.: $80,000 – 2015-2016. *Assaying the potency of hUCB on BBB permeability.* Principal Investigator.

21. Ladybug Foundation: $20,000, 2015-2017. *Studies in Pulmonary Hypertension.*

22. CBR, Inc.: $620,622 – 2016-2018. *Tools and Technologies for the harvest/storage/deployment of Wharton's Jelly in Pediatric Craniofacial Surgery.* Principal Investigator.

23. Biostage, Inc: $312,300 – 2016-2018. *Manufacturing of tissue engineered esophagus.* Principal Investigator.

24. Hope Biosciences, LLC: $101,129 – 2016-2017. *Assaying the potency of AMSC on BBB permeability after TBI.* Principal Investigator.

Endowments:

1. George and Cynthia Mitchell Distinguished Chair in Neurosciences. Established 2003. ($1,700,000)

2. Judith Hoffberger Cellular Therapeutics Laboratory. Established September 2009. ($500,000)

3. Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory. Established September 2009. ($2,000,000) FDA Establishment Identifier: 3009561521. Supplement: William & Madeline Welder Smith Foundation. ($10,000); Supplement 2014 ($5,000,000)

4. Glassell Foundation Stem Cell Research Working Group 1. Established April 2011. ($1,500,000)

5. Dewar Pediatric Head Injury Research Fund. Established July 2012. ($50,000)

6. Glassell Foundation Stem Cell Research Program. Established September 2013. ($2,500,000 Active; $2,000,000 Endowment)

Pending Grant Support:

1. National Institutes of Health 1RO1 NS073658-01A1: $2,913,448. 2012-2017. *Vulnerability of frontal lobe networks after traumatic brain injury in young children*. Co-Investigator (Ewing-Cobbs) (Impact/Priority Score 30; Percentile 25); In Revision.

2. University of Texas System Safety & Effectiveness Grant Program: *Trauma Quality Improvement Interprofessional Collaborative*. Consultant (Drake).

3. National Institutes of Health/NICHD: $3,312,853. 2015-2020. *Thrombelastography-guided resuscitation of pediatric trauma and associated coagulopathy.* Program Director. (Priority Score 42:11/2015)

4. National Institutes of Health: $1,574,474. 2016-2021. *Pediatric Injury: Modules to Manage Stress.* Co-Investigator (Ewing-Cobbs).

Past Grant Support:

1. Shriner's Burns Institute: $288,444 - 1991-1994; *Pathophysiology of ovine smoke inhalation injury treated with intravascular oxygenation and carbon dioxide removal device and intratracheal pulmonary ventilation.* Co-Investigator. (Zwischenberger)

2. Children's Miracle Network Telethon Grant: $25,850, 1997-1998. *Peripheral microvascular permeability in the systemic inflammatory response syndrome*. Principal Investigator.

3. Children's Miracle Network Telethon Grant: $8,300, 1997-1998. *Equipment funding for Pediatric lung injury and inflammation research group*. Co-Principal Investigator. (Okhuysen)

4. Merck KGaA: $125,500, 1999-2000. *Effects of Na^+/H^+ exchange inhibition on cardiac function*. Principal Investigator.

5. National Highway Transportation Safety Administration: $50,000 – 1999-2001. *Development of a pediatric functional capacity index*. Consultant.

6. University of Texas JSP Grant $1,500 – 2000. *Development of PMN superoxide assay*. Principal Investigator.

7. National Institutes of Health 1 K08 GM 00675-01: $596,430 – 1999-2005. *Mentored Clinical Scientist Award. Microvascular injury during extracorporeal life support*. Principal Investigator.

8. United States Army Medical Research and Material Command/TATRC 04343002: $98,369 – 2004-2005. *A portable fluid warming system*. Co Principal Investigator. (Gill)

9. National Institutes of Health 1 R21 HD042659-01A1; $222,750 – 2003-2006. *Multi-center Traumatic Brain Injury Network*. Principal Investigator.

10. National Institutes of Health P30 DK 56338; $10,000 – 2005-2006. *Digestive Diseases Center Award – Pilot/Feasibility. Role of iNOS in Gut Edema Induced Ileus.* Principal Investigator (Subcontract).

11. University of Texas Center for Biomedical Engineering/Charles W. Tate and Judy Spencer Tate Charitable Foundation; $20,000- 2005-2006. *Engineering problems of portable biologic fluid warmers.* Co-Principal Investigator (Gill).

12. American Heart Association Grant-in-Aid; $124,000 – 2005-2008. *Mechanisms of myocardial dysfunction after gut ischemia/reperfusion.* Principal Investigator.

13. Charles W. Tate and Judy Spencer Tate Charitable Foundation; $30,000 – 2006-2007. *A System for Automated Field Tube Thoracostomy*. Co-Principal Investigator (Gill).

14. Congressional Mandated Program/United States Army Medical Research and Material Command. T5 (Texas Training and Technology against Trauma and Terrorism); $748,609 – 2004-2008. *Portable veno-venous rewarming for hypothermia.* Principal Investigator.

15. National Institutes of Health/NHLBI P018/N01-HB-37163 (Gee PI/Baylor College of Medicine Subcontract); $29,468.80 – 2006-2008. *Production assistance for cellular therapies*. Principal Investigator. (Subcontract)

16. National Institutes of Health M01 RR 02558; $30,000 – 2006-2008. *Safety of autologous stem cell treatment for traumatic brain injury in children*. Principal Investigator.

17. National Institutes of Health ULI RR024148; $21,000 – 2006-2008. *Supplement: Safety of autologous stem cell treatment for traumatic brain injury in children*.

18. National Institutes of Health 5 T35 DK 007676-14; $10,000-2003-2007. *Short-term Research Training*. Subcontract (Rosenfeld).

19. National Institutes of Health 1 R01 NS 046308-01A1; $1,307,374 – 2004-2009. *Academic Outcomes After Pediatric Traumatic Brain Injury*. Co-Investigator. (Ewing-Cobbs)

20. Texas Higher Education Coordinating Board; $1,800,000 – 2005-2009. *Center for Engineered Microtherapeutics*. Principal Investigator.

21. National Instruments; $50,000 – 2007-2009. *Mechanics and control for an automated field tube thoracostomy*. Co-Investigator (Longoria)

22. National Science Foundation; $90,000 – 2007-2009. *Mechanics and control for an automated field tube thoracostomy*. Co-Investigator (Longoria)

23. USAMRMC/TATRC/DOD: $872,504 – 2006-2009. *Active thermal resuscitation*. Co-Principal Investigator (Gill)

24. National Institutes of Health: P50 GM 38529: Project 2; $213,313 (year 5 budget); 2008-2009. *Molecular pathogenesis of gut injury in multiple organ failure*. Co-Investigator (Ko, T)

25. Kinetic Concepts, Inc.: $20,000- 2009. *Inflammatory profiling of intra-abdominal sepsis.* (Subcontract).

26. National Institutes of Health: P30 DK 56338: $25,000 – 2009-2010. *Intestinal Stem Cell Regeneration in Necrotizing Enterocolitis*, Mentor/Sponsor. (Moore-Olufemi).

27. Kinetic Concepts, Inc.: $275,151 – 2008-2011. *Negative pressure therapy modulates the inflammatory response to intraabdominal hypertension*. Principal Investigator.

28. BD Biosciences: $10,000 – 2010-2011. T-regulatory cells as a therapeutic target for progenitor cells in traumatic brain injury. Co-Investigator (Walker).

29. National Institutes of Health: 1RO1 NS 21189: $572,403 – 2010-2012. *Social Cognitive outcome of head injury in children.* Co-Investigator (Levin).

30. National Institutes of Health: 1K01 DK 070758: $515,008 – 2005-2012. *Effects of edema on cadherins in small intestine.* Co-Sponsor (Uray).

31. USAMRMC-DOD: $561,012 – 2010-2012. *Active thermal resuscitation*. Co-PI (Gill).

32. California Institute of Regenerative Medicine $41,069. Cell Therapies in Trauma & Critical Care: Barriers in Translation from Pre-Clinical to Clinical Development. (Pati-PI).

33. Mission Connect: $150,000 – 2011-2012. *Immunomodulatory Effects of Stem Cell Therapy in Traumatic Brain Injury.* Principal Investigator.

34. The Brown Foundation, Inc.: $666,667 – 2008-2016: *Program in Regenerative Medicine*. Principal Investigator.

35. National Institutes of Health: 1 RO1 HL 092916-O1A1: $437,179 – 2009-2013. *Short-term mesenteric lymphatic adaptation to trauma related intestinal edema*. Consultant (Stewart).

36. CBR, Inc.: $80,000 – 2007-2013. *Protocol development for UCB treatment of ischemic encephalopathy of the newborn*. Principal Investigator.

37. National Institutes of Health: 1K18HL-102256-01A1: $105,866 – 2011-2013. *Systemic Effects of Bone Marrow Derived MSCs on Vascular Stability*. Mentor (Pati).

38. EMIT, Corp.: $180,000 – 2009-2014: *Design modifications for active thermal resuscitation.* Principal Investigator.

39. Department of Defense: Combat Casualty Care: W81XWH-11-1-0460: $1,731,307 – 2011-2015. *Treatment of severe, adult traumatic brain injury using autologous bone marrow mononuclear cells*. Principal Investigator.

40. Robert Wood Johnson/Harold Amos Medical Faculty Development Program Award: $500,000 – 2010-2015. *Molecular Pathophysiology of Gastroschisis Related Intestinal Dysfunction.* Mentor (Moore-Olufemi).

41. Ladybug Foundation: $10,000 – 2014-2015. *Mesenchymal stromal exosome isolation for the treatment of pulmonary hypertension.* Principal Investigator.

42. Athersys, Inc.: $60,000 – 2008-2015. *Multipotent adult progenitor cell therapy for traumatic brain injury*. *Addendum: Assaying changes in immunobiology select MAPC products.* Principal Investigator.

Inventions/Patents/Intellectual Property:

Patents:

1. Gill, Brijesh; **Cox, Charles S**.; "Portable Fluid Warming System." US Patent 7,261,557; August 28, 2007. Assignee: The Board of Regents of the University of Texas System (Austin, TX). 7,891,974; 8,753,382, June 7, 2014. [PCT/USO5/047209;PCT/US08/070812] Continuation-in-Part, 2007. 11/832,41505855722.4;192404,10/886,191. European Patent Office N..08796439.L-1269; EP Patent #1966546 (Licensed- EMIT Corp); International patent: WO 2014/066370A1; FDA 510(K) submission #K103801.

2. Gill BS, **Cox CS**, Aroom KR; "Centrifugal Pump". US Provisional Patent Pending. Assignee: The Board of Regents of the University of Texas System (Austin, TX). 61/074,053.; US Patent 12/483,287. Assignee: The Board of Regents of the University of Texas System (Austin, TX) [PCT/US09/04732]. (Licensed- EMIT Corp)

3. Aroom K, Bjelica A, **Cox CS**, Espinoza A, Gil B, Longoria R; "Automated needle insertion mechanism." US Provisional Patent Pending. Assignee: The Board of Regents of the University of Texas System (Austin, TX) [PCT 13/392, 185].

4. Gill BS, **Cox CS**, Aroom KR, Sheldon JJ, Westerbeck TL. "Portable Body Warming Device." US Patent US 2012/0065716A1. Assignee: The Board of Regents of the University of Texas System (Austin, TX) (Licensed - EMIT Corp)

5. **Cox CS**, Mays RW; "Modulation of splenocytes in cell therapy for traumatic brain injury." US Patent Pending. Assignee: The Board of Regents of the University of Texas System (Austin, TX) and Athersys, Inc. (Cleveland, OH). 13/150,481; [PCT/US 2011/036231]; 151064180.

6. Gill BS, Aroom K, **Cox CS**. "Blood Coagulometer and Method." US Provisional Patent Pending. Assignee: The Board of Regents of The University of Texas System (Austin, TX). 61/699,494[PCT/US2013/059286] (Licensed – Coagulex, Inc.)

7. Gill BS, Aroom K, **Cox CS**. "Compact fluid warmer." US Provisional Patent Pending. Assignee: The Board of Regents of The University of Texas System (Austin, TX). 61/716,752 International Patent Publication Number: WO 2014/066370A1 (Licensed – EMIT Corp)

8. Gill BS, Aroom K, **Cox CS**. "Submersible Warming Device." US Provisional Patent 62/065,111[PCT/US2015/55948] International Publication Number: WO 2016/061459A1 Assignee: The Board of Regents of The University of Texas System (Austin, TX). (Licensed-EMIT Corp)

9. **Cox CS**, Gill BS, Aroom K, Wenzel P. "Methods and Apparatus for conditioning cell populations for cell therapies." US Provisional Patent 62/183,273; International Patent Application Number PCT/US2012/039044; Assignee: The Board of Regents of The University of Texas System (Austin, TX). (License option executed by Helocyte 2/2016)

Disclosures-(Not yet Patented/Licensed):

1. UTOTM ID: 2006-0053; Method and apparatus for calorimetric determination of hematocrit in an extracorporeal circuit. (Active).
2. UTOTM ID: 2007-0023; Apparatus for thermoelectric generation. (Active).
3. UTOTM ID: 2008-0054; Immunomodulation by affinity hemofiltration. (Active).
4. UTOTM ID: 2008-0061; Neural progenitor/mesenchymal or MAPC stem cell composite graft. (Closed/Transitioned).
5. UTOTM ID: 2009-0005; Impedance mapping for measurement of bowel edema. (Active).
6. UTOTM ID: 2009-0011; Endovascular detection and control of hemorrhage. (Active).
7. UTOTM ID: 2009-0030; Closed-cycle electronic butane combustion system. (Active).
8. UTOTMID: 2009-XX; Integrated scanner/bioprinter for tissue repair. (Active).
9. UTOTMID: 2010-XX; Negative pressure silo for gastroschisis. (Active).
10. UTOTMID: 2010-XX; Pelvic hemorrhage control catheter. (Active).

11. UTOTMID: 2012-XX; Use of a PAK inhibitor for treatment of gastrointestinal motility disorders. (Active).
12. UTOTMID: 2014-0017; Modular cellular bioreactor. (Active)
13. UTOTMID: 2014-0018; Scaffold management system. (Active).
14. UTOTMID: 2013-0040; Activation of MSC immunomodulatory function by mechanical force. (Active).
15. UTOTMID: 2014-0003; Use of mechanical infusion to spread donor cells into a scaffold to build a functional 3D compound tissue. (Active).
16. UTOTMID: 2015-02; Electrical manipulation of coagulation
17. UTOTMID: 2015-03; Shear decay of coagulation

Consulting:

1. Cord Blood Registry, Inc. (2006-Present) Relationship includes membership on scientific advisory board and speaker's bureau. We have a sponsored research agreement with CBR that assists in clinical trial protocol development. A research conflict of interest plan is in place in the Office of Institutional Compliance.

2. VidaCare, Intraosseous Infusion Device (2004-2006) VidaCare produces intra-osseous access devices for fluid resuscitation in austere/difficult vascular access conditions. The engagement with this company has been to provide feedback on device design/utility. The relationship is currently dormant.

3. Gerson Lehman Group: Council of HealthCare Advisors (2003-Present) This is an internet based "connectivity" organization that links subject matter experts with industry to provide forced consultations on topics of interest and/or surveys regarding market development of medical products/biotechnology.

4. Kinetic Concepts, Inc. (2008-2013) KCI produces the wound VAC and has a long standing interest in the application of negative pressure therapy in abdominal compartment syndrome. I serve as a consultant (paid) and we have funded large animal projects exploring mechanisms of NPT modulation of the inflammatory response. Our group also works with KCI in developing advancements in their existing technology.

5. The Sarah Jane Brain Project – National Advisory Board (2008-Present) This is a private effort to develop a comprehensive pediatric acquired brain injury treatment plan. I served on the National Advisory Board; this project is functionally inactive.

6. Athersys, Inc. – Scientific Advisory Board (2007-present) Athersys, Inc. is a publicly traded biotechnology company based in Cleveland, OH. They have licensed the IP of MAPC technology from the University of Minnesota. We are collaborating on the use of MAPC in TBI and stroke. I serve on their CNS Scientific Advisory Board (compensated). Athersys has sponsored research agreements with our group. I am the PI on the academic component of a SBIR grant with Athersys as the industry partner.

7. Auxocell, Inc. (2009-2011) - Collaborative research agreement including a Materials Transfer Agreement of progenitor cells for pre-clinical use in models of traumatic brain injury. This includes a tri-member collaborative relationship with a cell-labeling company (Cell Sense). Auxocell was acquired by Perkins-Elmer, and we have no active collaborations.

8. Proteus Ventures (2009-Present) – Ad hoc consulting regarding clinical grade stem cell production and Go-To-Market strategies.

9. Patton Surgical, Inc. (2008-2011) – Collaborative research agreement to provide consultation on novel medical device development. This company successfully exited/was acquired and relationship concluded.

10. International Society for Cellular Therapy- Industry Task Force (2010- Present) - Engage industry stakeholders to review barriers to commercialization and make recommendations on priority issues facing translation of cell therapies.

11. Celgene, Inc. (2010-Present) – Collaborative research agreement regarding translational therapeutic use of cellular treatments for neurological injury.

12. Back Bay Life Science Advisors (2012-Present) – Ad hoc consulting regarding drug translation relative to our work in intestinal smooth muscle dysfunction.

13. Pfizer Neuroscience (2015-Present) – Consulting regarding Neuroinflammatory modulation and the development of new agents to target inflammation after TBI.

14. HLI, Inc./Celgene Cellular Therapeutics, Inc. (2015-Present) – Consulting and collaboration regarding genomic profiling of traumatic injury patients and response to treatment.

15. Lynntech, Inc. (2014-Present) – Consultant on SBIR award for "NEOSAFE" – A Novel Material for Manufacturing Pediatric Medical Devices.

16. GTC Bio (2015-Present) – Consultant Stem Cell Biology Meeting Development.

Biotechnology Small Business:

1. EMIT, Inc., Scientific Advisory Board: (Evolution/Successor to ThermaLabs, Inc.); Shareholder; Royalty Interest. (2009-present) EMIT, Inc. is a biotechnology start-up based on IP developed in our laboratory (see patents above). The company was founded in 2009 and initially capitalized with $4 million from a regional biotechnology venture fund CitareTEX. EMIT has developed the existing technology through the FDA 510K process to successfully manufacture/sales & royalty generation. Series B financing via Horizon Fund.

2. Coagulex, Inc. – Founder/shareholder & royalty interest. Coagulex is a biotech start-up that is closing venture funding after founding in 2013. Coagulex uses IP surrounding the use of MEMS based coagulation testing. Coagulex is the corporate entity of Coagulation Investment Partners, LLP.

3. Helocyte, Inc. – Scientific Advisory Board Chairman; Shareholder. Helocyte is a biotechnology company that is a wholly owned subsidiary of Fortress Biotechnology (FBIO: NASDAQ). Helocyte develops immunotherapies and has a license option for cell based therapies in TBI.

4. Alation, Inc. – Founder; Secretary (2006-present) Alation is a biotechnology shell corporation available for use as an SBIR/STRR entity for newly developed biotechnologies.

5. Immatics, Inc. – (2015-2019) Collaborative cGMP production of T-cell products for multiple clinical trials at MDACC, as component of 80 million USD program project.

6. Verde Environmental Inc. – Royalty Interest VEI is a small biotechnology firm in Houston, TX that uses IP protected processes of re-animation of sporulated bacteria with surfactant fire-fighting foams to mitigate the environmental impact of hydrocarbon spills/fires using bacteria mediated bio-remediation. We maintain a royalty interest and non-controlling equity interest in the company.

7. Texas Medical Accelerator – The TMDA is a consortium of innovations from UCSF, Stanford, CHOP, and UT-Houston focusing on partnering with venture capital and industry to develop novel pediatric surgical/critical care based medical devices. The accelerator is based in Austin, Texas, and linked to UT-Austin and private industry.

8. United States Department of Justice – Expert for prosecution regarding Stem Cell therapy fraud.

Investigational New Drugs (FDA – CBER/OCTGT):

1. BB-IND-12620 (2005): Safety of autologous bone marrow progenitor cell treatment for traumatic brain injury. (Active)
2. BB-IND-13127 (2006): Autologous stem cell treatment for hypoxic-ischemic encephalopathy in the newborn. (Closed)
3. BB-IND-14214 (2009): Autologous hUCB for the treatment of traumatic brain injury. (Active)
4. BB-IND-14281 (2010): Safety of autologous stem cell treatment for spinal cord injury in children. (Active) (Co-Investigator) (Baumgartner-PI)
5. BB-IND-76772 (2005) - MD Anderson Protocol 2005-0917: A phase I study of continuous hyperthermic peritoneal perfusion with escalating doses of cisplatin for children with peritoneal carcinomatosis or advanced peritoneal and retroperitoneal disease. (Active): Collaborator.
6. BB-IND-13775 (2009): Safety of autologous bone marrow treatment for ischemic stroke. (Active) (Co-Investigator) (Savitz-PI)
7. BB-IND-15246 (2012): Autologous cell therapies for cerebral palsy-chronic (ACT for CP). (Active)

Publications:

Abstracts:

1. **Cox CS**, James E, McClellan DG, Townsend CM, Thompson JC: Calmodulin antagonist trifluoperazine inhibits growth and survival of pancreatic cancer *in vitro*. Dig Dis Sci 1986; 31(10):1186. (Poster presentation)

2. **Cox CS**, Zwischenberger JB, Traber LD, Traber DL, Herndon DN: Application of an intravenous mechanical blood oxygen/carbon dioxide exchange device (IVOX) for smoke inhalation injury in an *ovine* model. Cardiovascular Science and Technology: Basic and Applied, II 1:391, 1990.

3. Wang CZ, **Cox CS**, Barrow RE, Herndon DN. Detergent induced change in pulmonary microvascular permeability. FASEB J 5:A769, 1991.

4. Fleming RYD, **Cox CS**, Gore D, Honeycutt DA, Jahoor F, Wolfe RR, Herndon DN. Effect of propranolol on glucose uptake in hypermetabolic burned patients. Proc Am Burn Assoc, 124:125, 1992.

5. **Cox CS**, Fleming RYD, Zwischenberger JB, Myers T., Kurusz M, Alison WE, Herndon DN. Ileal mucosal hypoperfusion during cardiopulmonary bypass. Crit Care Med 20:S64, 1992. (Poster presentation)

6. **Cox CS**, Zwischenberger JB, Traber DL, Traber LD, Herndon, DN. Intravascular oxygenation/CO_2 removal allows reduction of mechanical ventilation support in an *ovine* model of acute lung injury. Crit Care Med 20:S60, 1992. (Poster presentation)

7. **Cox CS**, Zwischenberger JB, Graves D, Raymond G. Mortality from severe ARDS remains unchanged. Am Rev Resp Dis 145:A83, 1992. (Poster presentation)

8. **Cox CS**, Zwischenberger JB, Graves D, Cardenas, V. Permissive hypercapnia with IVOX: A ventilation technique to reduce barotrauma. ASAIO Abstracts 21:65, 1992. (Poster presentation)

9. **Cox CS**, Traber DL, Gasser H, Redl H, Schlag G, Herndon DN. Interleukin 6 levels in burned patients: Effect of inhalation injury. FASEB J 6:A2042, 1992. (Poster presentation)

10. **Cox CS**, Zwischenberger JB, Traber DL, Traber LD, Haque AK, Herndon DN. Heparin improves oxygenation and minimizes barotrauma after severe smoke inhalation in an ovine model. Proc Am Burn Assoc 124:20, 1992. (Oral presentation)

11. **Cox CS**, Zwischenberger JB, Traber LD, Traber DL, Herndon DN. Beneficial effects of heparin treatment of smoke inhalation injury not due to reduced oxygen free-radical activity. Am Rev Resp Dis 145:A80, 1992. (Poster presentation)

12. Zeigler ST, **Cox CS**, Meyer J, Traber DL, Herndon DN. The role of nitric oxide in the pulmonary pathophysiology of burn/sepsis. Am Rev Resp Dis A45:A313, 1992.

13. Zwischenberger JB, **Cox CS**, Graves DF. Initial clinical experience with an intravascular oxygenator and carbon dioxide removal device (IVOX). Thorac Cardiovasc Surg 40:104, 1992.

14. Meyer J, **Cox CS**, Traber DL, Nakazawa H, Herndon DN. Cardiopulmonary effects of heparin in endotoxemia. Circ Shock 37:40, 1992.

15. Meyer J, Kramer GC, **Cox CS**, Traber DL, Nakazawa H, Herndon DN. Continuous small volume resuscitation with hypertonic saline/dextran solution improves survival in experimental sepsis. Circ Shock 39:12.49, 1993.

16. **Cox CS**, Zwischenberger JB, Fleming RYD, Myers T, Kurusz M, Herndon DN, Kramer GC. Hypertonic saline-dextran does not improve regional perfusion in a *porcine* model of cardiopulmonary bypass. Proc Intl Conf on Hypertonic Resuscitation 5:4, 1992. (Oral presentation)

17. **Cox CS**, Biondo N, Traber LD, Lentz CW, Herndon DN, Traber DL. Effects of superoxide dismutase (SOD) on pulmonary microvascular response to endotoxin. Proc Intl Endotoxin Society. 2:245, 1992. (Poster presentation)

18. McDaniel LB, **Cox CS**, Zwischenberger JB, Kurusz M, Kramer GC. Effects of hypertonic saline dextran on intracranial pressure and brain water during CPB. Anesthesiology 77:A661, 1992.

19. Nguyen TT, Herndon DN, **Cox CS**, Biondo N, Traber LD, Bush PE, Traber DL. Effects of MnSOD on lung fluid balance after smoke inhalation injury. Proc Am Burn Assoc 75:31, 1993.

20. Nguyen TT, **Cox CS**, Fleming RYD, Traber DL, Gasser H, Redl H, Schlag G, Herndon DN. Free radical activity and loss of plasma antioxidants, Vitamin E, and sulfhydryl groups in burned patients. Proc Am Burn Assoc 75:146, 1993.

21. Nguyen TT, **Cox CS**, Matheis E, Bush P, Traber DL, Herndon DN, Bidani A, Zwischenberger JB. Marked enhancement of carbon dioxide removal by second generation IVOX. ASAIO Abstracts, 1993.

22. Nguyen TT, **Cox CS**, Tzounakis A, Bush P, Traber DL, Herndon DN, Bidani A, Zwischenberger JB. Carotid blood flow during permissive hypercapnia in an ovine model. Crit Care Med 21:S190, 1993.

23. Zwischenberger JB, Nguyen T, Upp JR, Bush P, **Cox CS**, Delosh T., Broemling L. Complications of neonatal extracorporeal membrane oxygenation (ECMO): Experience from the Extracorporeal Life Support Organization. Proc Am Assoc Thorac Surg. (In Press)

24. Nguyen TT, Zwischenberger JB, **Cox CS**, Bush P, Matheis E, Bidani A, Traber DL, Herndon DN. IVOX with permissive hypercapnia: A new management technique in an *ovine* smoke inhalation injury model. Proc Assoc Academic Surgery 27:136, 1993.

25. Meyer J, Stothert JC, Pollard V, Potter R, **Cox CS**, Traber LD, Traber DL. Nitric oxide synthesis inhibition in *ovine* endotoxemia: Effects on brain blood flow. Anesth Analg 76:S264, 1993.

26. Reickert CA, Hirschl RB, Schumacher R, Geiger JD, **Cox CS**, Teitelbaum DH, Polley TZ, Harmon CM, Lelli JL, Coran AG. The effect of delayed repair of congenital diaphragmatic hernia on survival. Proc Central Surgical Association 53:73, 1996.

27. Nguyen T, **Cox CS,** Biondo N, Traber LD, Herndon DN, Bush PE, Traber DL. Effects of superoxide dismutase on the pulmonary response to endotoxin. Circ Shock 39 (Suppl 2):36, 1993.

28. Allen GS, **Cox CS**, Cocanour CS, Duke JH, Moore FA, Andrassy RJ. Pulmonary contusions: Are children different? Proc SW Surgical Congress. (In Press)

29. **Cox CS**, Black CT, Duke JH, Cocanour CS, Moore FA, Lally KP, Andrassy RJ. Operative management of truncal vascular injuries in children and adolescents. Proc Pacific Assoc of Pediatr Surg 30:109, 1997. (Poster presentation)

30. Allen GS, **Cox CS**, White NW, Khalil S, Rabb M, Lally KP. Postoperative respiratory complications in ex-premature infants after inguinal herniorrhaphy. Proc Am Acad Pediatrics 49:75, 1997.

31. Allen GS, Moore FA, **Cox CS**, Wilson JT, Cohn JM, Duke JH. Hollow visceral injury in blunt trauma. J Trauma 43:196, 1997.

32. **Cox CS**, Allen SJ, Brennan MS. Analysis of microvascular permeability with cardiopulmonary bypass. Proc Assoc Acad Surg. 32:252, 1998. (Poster presentation)

33. Sauer H, **Cox CS**, Laine GA, deVivie ER, Frederick JR, Allen SJ. Effects of TBC 1269, a novel sialyl Lewis^x selectin antagonist, on the coronary microvasculature during myocardial reperfusion in a dog model. Proc FASEB. (In Press)

34. **Cox CS**, Allen SJ, Brennan M. Colloids minimize intestinal edema after ongoing resuscitation. Proc Am Assoc Surg Trauma, 1999.

35. Neville H, Lally KP, **Cox CS**. Emergent abdominal decompression with patch abdominoplasty in the pediatric patient. Proc Can Assoc Pediatr Surg 31:34, 1999.

36. **Cox CS,** Allen SJ, Butler D, Sauer H, Frederick J. Extracorporeal circulation exacerbates microvascular permeability after endotoxemia. J Surg Res 86:283, 1999. (Oral presentation)

37. Gill BS, Neville HL, Khan AM, **Cox CS**, Lally, KP. Delayed Institution of ECMO increases morbidity in meconium aspiration syndrome. Proc ELSO 11:1, 2000.

38. Mehall JR, Cook P, Andrassy RJ, **Cox CS**. Incidence of missed injuries in pediatric blunt trauma patients with GCS <9. J Trauma 52:197, 2002.

39. Stewart R, Rohn D, Fischer U, **Cox CS**, Allen SJ, Criscione J, Laine GA. Alterations in cardiac and vascular function curves following resolution of acute myocardial edema. FASEB J 16:A1128, 2002.

40. **Cox CS**, Fischer U, Allen SJ. Is mesenteric lymph toxic to endothelial cells after mesenteric ischemia/reperfusion? FASEB J 16:A442, 2002.

41. Fischer U, **Cox CS**, Mehlhorn U, deVive ER, Stewart RH, Laine GA, Allen SJ. Impact of acute myocardial edema on left ventricular function. FASEB J 16:A1128, 2002.

42. Fischer U, Allen ST, **Cox CS**, Stewart RH, Laine GA, Allen SJ. Effect of chronic and acute myocardial edema on left ventricular interstitial pressure and chamber compliance. FASEB J 16:A1128, 2002.

43. **Cox CS**, Allen SJ, Murray M. Improved myocardial function using a Na^+/H^+ exchange inhibitor during cardioplegic arrest and cardiopulmonary bypass. Surg Forum 52:74-77, 2001. (Oral presentation)

44. Blakely ML, Neville HL, Mehall J, Smith S, Warner BW, Mantor PC, **Cox CS,** Lally, KP. A Comparison of early versus late inguinal hernia repair in premature infants. Proc Pacific Assoc. Pediatr Surg 35:48, 2002.

45. **Cox CS**, Fischer UM, Allen SJ, Laine GA. Mesenteric lymph diversion prevents myocardial edema and dysfunction after gut ischemia/reperfusion (I/R). Surg Forum 195:S82, 2002. (Oral presentation)

46. Hassoun H, Fischer U, Allen SJ, Moore FA, Laine GA, **Cox CS**. Regional hypothermia reduces mucosal NF-K□ DNA binding activity and PMN priming via gut lymph during mesenteric ischemia/reperfusion. J Surg Res 107:316, 2002.

47. **Cox CS**, Allen SA, Fischer U, Xue H, Stewart R, Laine GL, Moore FA. Gut edema causes ileus. Crit Care Med 30:A148 (590), 2002. (Poster presentation)

48. Stewart R, Quick C, Criscione J, **Cox CS**, Allen S, Laine G. The effect of nitric oxide on lymphatic function depends on the axial pressure gradient. FASEB J 17:A128, 2003.

49. Miller S, Thacker L, Raney RB, Andrassy RJ, Herzog C, Lally KP, **Cox CS**, Blakely ML. Long-term outcome of children and adolescents with tumors of fibrous etiology. Proc ASCO A3230 (www.asco.org/ac), 2003.

50. Fischer UM, **Cox CS**, Laine GA, Mehlhorn U, Allen SJ. Mild hypothermia impairs left ventricular diastolic but not systolic function. Thorac Cardiovasc Surg V192, 2003.

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28. Liao GP, **Cox CS**. Pathophysiology of Traumatic Brain Injury: Rationale and Role of Cellular Therapies in Hess D (eds.); Cell Based Therapeutics in Acute Brain Injury, Springer, New York, pp. 267-306, 2015.

Films/Other Media:

1. Coran AG, **Cox CS.** Partial gastric transposition for esophageal atresia as a primary procedure. American College of Surgeons Clinical Congress Video presentation, 1996.

2. iCritical Care Podcast: George P. Liao/Charles S. Cox, Jr. "Autologous bone marrow cells reduce therapeutic intensity level for severe TBI in children." www.sccm.org/communcations/iCritical-Care/Pages/iCritical-Care/aspx.

3. Hamilton E, Rivera P, Lazar D, Tsao KJ, **Cox CS**, Austin MA. ECMO for severe tracheobronchial injuries. Pacific Association of Pediatric Surgeons. Video Presentation, 2016.

Letters-to-Editor:

1. **Cox CS**. Epiphenomenology of feeding access of the neurologically impaired child. Pediatrics 100:899, 1997.

2. **Cox CS.** Same day ileostomy closure. Not for children! Am J Man Care 3:1444, 1997.

3. **Cox CS**. Heparin for smoke inhalation injury. Crit Care Med 31:1291, 2003.

4. Harting MT, Jimenez F, **Cox CS**. The pulmonary first-pass effect, xenotransplantation, and translation to clinical trials – a commentary. Brain doi 10.1093/brain/awn142, 2008.

5. **Cox, CS**., Harting MT. Editorial response to: Chopp M, et al. J Neurosurgery, 110: 1186-1189, 2009.

6. Green H, Rytting ME, **Cox CS**. A rapidly growing lymphoma and tumor lysis syndrome in a toddler. JAAPA. 23:30-34, 2010.

Other Professional Communications:

Presentations (Invited)

1. "Hemostasis, Coagulation and Blood-Surface Interactions during ECMO", University of Texas Medical Branch at Galveston, ECMO Training Course, Galveston, Texas, 10/19/90.

2. "Respiratory Assist Devices: Future Trends", University of Texas Medical Branch at Galveston, Galveston, Texas, 12/12/90.

3. "Extracorporeal membrane oxygenation (ECMO) and Intravascular Oxygenation for Acute Respiratory Failure in an Ovine Model", University of Texas Health Science Center at Houston Surgical Research Symposium, Houston, Texas, 6/1/91.

4. "Acute Respiratory Failure", Shriner's Burns Institute Basic Science Lecture Series, Galveston, Texas, 10/25/91.

5. "ECMO for neonatal and pediatric respiratory failure", Houston Area Respiratory Care Conference, Houston, Texas, 8/5/92.

6. "Pathophysiology of smoke inhalation injury", University of Texas Health Science Center at Houston, Houston, Texas, 10/25/92.

7. "New treatments of smoke inhalation injury", University of Texas Health Science Center at Houston, Houston, Texas, 6/24/94.

8. "Pediatric Shock Update", University of Texas Health Science Center at Houston, 7/7/97.

9. "Pediatric Trauma: ABC's-Abuse, Burns and Closed Head injury", Hermann Life Flight Roseann Waindell Memorial Emergency Care Symposium. 8/13/96.

10. "Oxygen Kinetics of ECMO", University of Texas Medical School-Houston-ECMO and Neonatal Transport Course, Houston, Texas, 7/23/97.

11. "Venoarterial and Venovenous ECMO", University of Texas Medical School-Houston-ECMO and Neonatal Transport Course, Houston, Texas, 7/24/97.

12. "ECMO Cannulation", University of Texas Medical School-Houston-ECMO and Neonatal Transport Course, 10/25/00.

Presentations (Scientific; not represented by abstracts)

1. "Malignant fibrous histiocytoma arising from the superficial femoral artery", San Antonio Vascular Surgery Society, San Antonio, Texas, 9/29/89. (Oral)

2. "Subclavian Artery Trauma", American College of Surgeons Committee on Trauma Meeting, Dallas, Texas, 11/2/90. (Oral)

3. "Consumptive Coagulopathy during ECMO?", Extracorporeal Life Support Organization, Ann Arbor, Michigan, 11/30/90. (Poster)

4. "Use of an Intravascular Oxygenator in an *Ovine* Smoke Inhalation Injury Model", Society of University Surgeons, Galveston, Texas, 2/7/91. (Resident's Program) (Oral)

5. "Mechanical and Patient Complications of Extracorporeal membrane oxygenation for respiratory failure", Ninth Biomedical Engineering Research Conference, Houston, Texas, 2/12/91. (Oral)

6. "Airtrapping capability of the SCIMED Ecmotherm heat exchanger", Extracorporeal Life Support Organization Meeting, Ann Arbor, Michigan, 9/12/91. (Poster/Device Demonstration)

7. "Heparin improves oxygenation and minimizes barotrauma after smoke inhalation in an *ovine* model", American College of Surgeons, So. TX. Ch. Meeting, San Antonio, Texas, 2/17/92. (Oral)

8. "Ileal mucosal hypoperfusion during cardiopulmonary bypass", Society of University Surgeons, Cincinnati, Ohio, 2/15/92. (Resident's Program) (Oral)

9. "Intratracheal pulmonary ventilation - a new technique of mechanical ventilation", Biomedical Engineering Research Meeting, Houston, Texas, 3/19/92. (Oral)

10. "Reduction of ventilator support with IVOX", Biomedical Engineering Research Meeting, Houston, Texas, 3/19/92. (Oral)

11. "Effects of heparin on smoke-induced pulmonary injury: A review of potential mechanisms of action", University of California, San Diego; Effects of heparin in burns Meeting, San Diego, California, 2/25/94. (Oral)

12. "Heparin and experimental sepsis", University of California, San Diego; Effects of heparin in burns Meeting, San Diego, California, 2/25/94. (Oral)

13. "Surgical management of ambiguous genitalia: An update", University of Oklahoma, Pediatric Surgery Resident's Conference, Oklahoma City, Oklahoma, 11/15/95. (Oral)

14. "Blunt versus penetrating subclavian artery injury: Presentation, injury pattern, and outcome", American Association for Surgery of Trauma, Waikoloa, Hawaii, 9/25/97. (Poster)

15. "Imaging and decision making in pediatric blunt abdominal trauma", University of Texas-Houston Medical School Department of Surgery Grand Rounds, Houston, Texas, 11/24/97. (Oral)

16. "Fundamentals of Pediatric Trauma Resuscitation", University of Texas-Houston Medical School Department of Surgery Grand Rounds, Houston, Texas, 1/20/97. (Oral)

17. "PMN mediated microvascular injury with extracorporeal circulation", Rice University Bioengineering Seminar, Houston, Texas 9/15/99. (Oral).

18. "Evaluation of Pediatric Blunt Abdominal Trauma", East Texas Medical Conference Continuing Medical Education/Grand Rounds, Tyler, Texas, 5/19/00. (Oral)

19. "ECMO for Trauma-Related Cardiopulmonary Failure", East Texas Medical Conference Continuing Medical Education/Grand Rounds, Tyler, Texas, 5/19/00. (Oral)

20. "Evaluation of Pediatric Blunt Abdominal Trauma", Trauma Regional Advisory Council V Symposium, South Padre Island, Texas, 6/15/00. (Oral)

21. "Update on Smoke Inhalation Injury", Southeast Texas Trauma Regional Advisory Council, Houston, Texas, 7/10/00. (Oral)

22. "Decision making in Pediatric Blunt Abdominal Trauma", Southeast Memorial Hospital Grand Rounds, Houston, Texas, 9/16/00. (Oral)

23. "Management of Pediatric Blunt Abdominal Trauma", Valley Baptist Medical Center, Harlingen, Texas, 12/7/00. (Oral)

24. "Unconventional adjuncts to mechanical ventilation in ARDS", American College of Surgeons, New Orleans, Louisiana 10/11/01. www.facs.org/clinical2001/cox.pdf (Oral)

25. "Intestinal edema – effect on function", Red Duke Professorship Day, University of Texas – Houston, Houston, Texas, 1/15/02. (Oral)

26. "Pediatric Trauma Resuscitation", East Texas Medical Center Trauma Symposium, Tyler Texas, 08/23/02. (Oral).

27. "Trauma Surgery in Afghanistan", Valley Baptist Medical Center, Harlingen, Texas, 3/25/03. (Oral)

28. "Terror in Afghanistan", University of Texas-Houston Grand Rounds, Houston, Texas, 4/01/03. (Oral)

29. "A Semester Abroad", University of Chicago Grand Rounds, Chicago, Illinois, 5/22/03. (Oral)

30. "Pediatric Trauma: Relevance for the Military Surgeon", Gary Wratten Memorial Surgical Conference, San Antonio, Texas, 5/20/03. (Oral)

31. "Fluid Balance and ECMO", Extracorporeal Life Support Organization, Chicago, Illinois, 9/13/03. (Oral-Plenary)

32. "Military Surgery as a Reservist", Surgical Student Association, Houston, Texas, 10/4/03.

33. "Pediatric Surgery in a Combat Zone", Department of Pediatrics Grand Rounds, Houston, Texas, 12/7/03. (Oral)

34. "AMEDD Recruiting Strategies", AMEDD Advertising and Marketing Workshop, Louisville, Kentucky, 4/12/04. (Oral)

35. "Military Medical Careers", Joint Admission Medical Program, University of Texas-Houston Medical School, Houston, Texas, 6/25/04. (Oral)

36. "Pediatric Trauma Management: FST or Tertiary Trauma Center", BAMC 10th Annual Trauma Symposium, San Antonio, Texas, 8/10/04. (Oral)

37. "Mechanisms of Myocardial Dysfunction after Gut Ischemia/Reperfusion." Red Duke Professorship Day, University of Texas-Houston, Houston, Texas, 1/22/05. (Oral)

38. "Immune Function During ECMO", Arnold G. Coran, M.D. Symposium, University of Michigan, Ann Arbor, Michigan, 6/5/05. (Oral)

39. "Thoracic Trauma", Cook Children's Hospital Trauma Symposium, Fort Worth, Texas, 6/10/05. (Oral)

40. "Dynamic Fluid Flow Modeling of an Arteriovenous Resuscitation Device", UTCBME Symposium, 4/29/05. (Poster)

41. "Bioimpedance Analysis Quantifies Level of Edema and Capacitance of Rat Small Intestine", UTCBME Symposium, 7/29/05. (Poster)

42. State of the Art Presentations: Meconium Aspiration - "ECMO for MAS – Do we use the right criteria?" Extracorporeal Life Support Organization Meeting, 9/17/05. (Oral-Panel)

43. "Development of Stem-Cell Therapy for Brain Injury", University of Texas-Houston Medical School, Houston, Texas, 10/28/05. (Oral)

44. "Bone-marrow Progenitor Cells and Traumatic Brain Injury", South Texas Chapter of the American College of Surgeons, Houston, Texas, 2/24/06. (Oral)

45. "Cellular Therapy for Traumatic Brain Injury", Pediatric Brain Injury Research Symposium, University of Texas Southwestern Medical School, Dallas, Texas, 3/31/06. (Oral)

46. "Human Umbilical Cord Blood as Cellular Progenitor Therapy for Brain Injury", Cord Blood Stem Cell Advisory Meeting, Tucson, Arizona, 4/6/06. (Oral)

47. "Trauma and Child Abuse", University of Texas Child Abuse Symposium, Houston, Texas, 5/12/06.

48. "Novel Cellular Therapies for Neurologic Diseases", University of Iowa Department of Surgery Grand Rounds, Iowa City, Iowa, 7/18/06.

49. "Edema-induced Intestinal Dysfunction", Columbus Children's Hospital/Ohio State University, Columbus, Ohio, 2/20/07.

50. "Mechanisms of edema-induced organ dysfunction", Michael E. DeBakey Institute, Texas A&M University, College Station, Texas, 7/26/07.

51. "Modern approach to pediatric TBI", Children's Hospital of Los Angeles Pediatric Disaster Summit. Los Angeles, California, 9/11/08.

52. "Progenitor cell therapy for TBI", MD Anderson Cancer Center Grand Rounds, Houston, Texas, 11/24/08.

53. "Pediatric Traumatic Brain Injury Clinical Trials using Progenitor Cells" Georgia Stem Cell Institute, Augusta, Georgia, 2/10/2009.

54. "Advances in umbilical cord blood therapies for traumatic brain injury." University of Texas Medical School at Houston, Obstetrics and Gynecology Grand Rounds, Houston, Texas, 2/17/09.

55. "Human umbilical cord blood treatment for traumatic brain injury." CBR, Inc., Invited lecture: Molecular Medicine Tri-Conference, San Francisco, California, 2/25/09.

56. "Cerebral Resuscitation for Traumatic Brain Injury" Kiwanis Pediatric Trauma Institute. San Juan, Puerto Rico, 5/27/09.

57. "Stem Cells in Trauma", Shock Society, San Antonio, Texas, 6/6/09.

58. "Cellular Therapeutics for TBI", American Pediatric Surgical Association, San Juan, Puerto Rico, 5/23/09.

59. "cGMP processes/clean rooms: Prerequisites to cell tissue and biologics translational therapeutics". Regenerative Medicine Grand Rounds, Houston, TX, 8/3/09.

60. "Evolving concepts in cell therapy for TBI", UC-Davis, Sacramento, CA, 8/7/09.

61. "Cerebral Resuscitation". Kentucky Statewide Trauma and Emergency Medicine Symposium, Louisville, KY, 10/29/09.

62. "Progenitor Cell Therapeutics for TBI", University of Louisville Grand Rounds/Visiting Professor, Louisville, KY, 10/30/09.

63. "Perspectives on Stem Cell Therapies for TBI", Sandia National Laboratories/DARPA. Albuquerque, NM, 11/12/09.

64. "Cord Blood to Treat Traumatic Brain Injury". CBR, Inc., Invited Lecture; Fort Worth, TX, Obstetrics and Gynecology Society Meeting, 12/7/09.

65. "Cellular therapy for Neuroprotection during ECLS: Potential approaches." 26th Annual CNMC Symposium: ECMO and Advanced Therapies for Respiratory Failure. Keystone, Colorado, 2/21/10.

66. "Cellular Therapies for TBI in Children" Eighth World Congress on Trauma, Shock, Inflammation and Sepsis- TSIS 2010. Munich, Germany, 3/10/2010.

67. "Endogenous Bioreactors" Eighth World Congress on Trauma, Shock, Inflammation and Sepsis- TSIS 2010. Munich, Germany, 3/10/2010.

68. "Progenitor Cell Biodistribution after Intravenous Delivery" STEPS-2 Conference, Houston, Texas, 3/26/2010.

69. Emerging Indications for hUCB Therapies: Bay Area OB-GYN, Clear Lake, Texas, 4/28/2010.

70. "Progenitor Cell Therapies for TBI: Emerging Mechanisms of Action. The University of Texas-Houston Medical School; Department of Surgery Grand Rounds, Houston, Texas, 4/29/2010.

71. "Mechanisms of action of Multistem for TBI." Athersys Investor Day Conference. New York, New York, 5/14/2010.

72. "Update on cellular therapies." MEDNAX Research conference. Washington, DC, 6/29/2010.

73. "Implication of Progenitor cell: Spleen interactions for TBI." Neurosurgery Grand Rounds, University of Texas Medical School at Houston, Houston, Texas, 9/2/2010.

74. "Management of the Open Abdomen Inflammatory Component." American College of Surgeons/KCI, Inc., Open Abdomen Symposium, Washington, D.C., 10/4/2010.

75. "Developing & maintaining a DSMB." GCP lecture series: CCTS, Houston, Texas, 12/7/2010.

76. "Cerebral Resuscitation." 2nd Annual Pediatric Pre-Hospital Trauma Conference. Houston, Texas, 5/16/2011.

77. "Barriers in Pediatric Cell Therapy." NIH/NHLBI Workshop in Pediatric Cell Therapy. Bethesda, MD, 9/14/2011.

78. "Umbilical Cord Blood for Traumatic Brain Injury." US Naval Hospital-Bethesda Department of Obstetrics and Gynecology Grand Rounds. Bethesda, MD, 9/15/2011.

79. Afghanistan Trauma Surgery 2002. Association of Surgical Technology Meeting. Houston, TX, 10/16/2011.

80. "Cell Therapy for Traumatic Brain Injury: Potential Mechanisms and Novel Approaches." Monash Institute for Medical Research: The Ritchie Center. Melbourne, Australia, 12/1/2011.

81. "Autologous bone marrow mononuclear cells for traumatic brain injury: Mechanisms of action." University of Texas – Houston Department of Neurosurgery Grand Rounds, Houston, TX, 04/15/2012.

82. "Update: Cell Therapies for TBI – Emerging Mechanisms." University of Texas – Houston Department of Surgery Grand Rounds, Houston, TX, 07/12/2012.

83. "Evolution of Concepts in Autologous Cell Therapy for TBI." Surgical Biology III, Chicago, IL, 09/29/2012.

84. "Stem Cell Therapies for Neurological Injury: Fast, Cheap or Good – Pick Two." Mission Connect Symposium, Houston, TX, 10/23/2012.

85. "Cellular Therapies for Neurological Injuries: Emerging Mechanisms." Cellular Therapies in Trauma and Critical Care, San Francisco, CA, 12/3/2012.

86. "Regenerative Medicine: Update on Amniotic Fluid-derived MSC Program" UT Health Fetal Conference, Houston, TX, 1/22/2013.

87. "Adult Stem Cells and Traumatic Brain Injury." The Second International Vatican Adult Stem Cell Conference: Regenerative Medicine – A Fundamental Shift in Science and Culture. Vatican, Rome, Italy, 4/12/2013.

88. "Spleen – Stem Cells – Neurological Injury." Gulf Coast Consortium Cluster on Regenerative Medicine. Houston, Texas (Rice University), 6/20/13.

89. "Clinical Trials: Tribulations using Cell Therapy for Neurological Injury." MSC 2013. Cleveland, Ohio, 8/21/13.

90. "Advanced Therapies in TBI." Mischer Neurosciences Institute NeuroUpdate 2014. Houston, Texas, 3/10/14.

91. "Evolution of cell therapies for traumatic brain injury." Baylor – University of Texas – Houston. Physical Medicine & Rehabilitation Grand Rounds. Houston, Texas, 4/24/14.

92. "Next phase clinical trials in Neurological Injury – Cellular Therapies; Prerequisites – Opportunities." GTC Biotechnology Conference. Boston, Massachusetts, 4/23/14.

93. "Cell Therapy & Clinical Trials in Neurological Injury: Targets, Results and Rationale." World Stem Cell Summit. San Antonio, Texas, 12/3/14.

94. "Cellular Therapies for TBI: Update & Future State." DOD Meeting on Cellular Therapies in Trauma/Critical Care. Ft. Detrick, Maryland, 2/5/15.

95. "Advanced Cellular Therapies for TBI." Western Trauma Association Founders Lecture. Telluride, Colorado, 3/5/15.

96. "Pediatric Trauma Resuscitation Strategies: Opportunity to Impact Multiple Organ Systems (Gut & Brain)" MODS Workshop. Washington, DC, 3/26/15.

97. "Cellular Therapies for Neurological Injuries: Update on ACT for CP" International Cord Blood Symposium. San Francisco, CA, 6/13/15.

98. "Progenitor Cell Therapy for TBI – Session" National Neurotrauma Symposium. Santa Fe, NM, 6/30/15.

99. "What did I sign up for" University of Texas Medical School. Houston, TX, 11/12/15.

100. "How will we know it works? Potency assays for cell therapies" Perinatal Stem Cell Society – The Aspen Institute. Aspen, CO, 3/6/16.

101. "Autologous cell therapy reduces therapeutic intensity for TBI" Neuro ICU Symposium – Mischer Neurosciences Institute. Houston, TX, 3/24/16.

102. "Autologous cellular therapies for TBI" GTC Bio Conference. Boston, MA, 4/25/16.

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COURSEWORK REQUIREMENTS REPORT*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- **Name:** Charles Cox (ID: 2269259)
- **Email:** charles.s.cox@uth.tmc.edu
- **Institution Affiliation:** University of Texas Health Science Center at Houston (ID: 661)
- **Institution Unit:** Pediatric Surgery
- **Phone:** 7135007300

- **Curriculum Group:** Human Research
- **Course Learner Group:** Group 1 Biomedical Researcher and Key Personnel
- **Stage:** Stage 2 - Refresher Course

- **Report ID:** 19675796
- **Completion Date:** 06/14/2016
- **Expiration Date:** 06/14/2019
- **Minimum Passing:** 80
- **Reported Score*:** 97

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED
Biomed Refresher 2 - Instructions (ID: 764)	06/14/16
Biomed Refresher 2 – History and Ethical Principles (ID: 511)	06/14/16
Biomed Refresher 2 – Regulations and Process (ID: 512)	06/14/16
Biomed Refresher 2 – Informed Consent (ID: 514)	06/14/16
Biomed Refresher 2 – SBR Methodologies in Biomedical Research (ID: 515)	06/14/16
Biomed Refresher 2 – Genetics Research (ID: 518)	06/14/16
Biomed Refresher 2 – Records-Based Research (ID: 516)	06/14/16
Biomed Refresher 2 - Populations in Research Requiring Additional Considerations and/or Protections (ID: 519)	06/14/16
Biomed Refresher 2 – Vulnerable Subjects – Prisoners (ID: 520)	06/14/16
Biomed Refresher 2 – Vulnerable Subjects – Children (ID: 521)	06/14/16
Biomed Refresher 2 – Vulnerable Subjects – Pregnant Women, Human Fetuses, Neonates (ID: 522)	06/14/16
Biomed Refresher 2 – FDA-Regulated Research (ID: 524)	06/14/16
Biomed Refresher 2 – HIPAA and Human Subjects Research (ID: 526)	06/14/16
Biomed Refresher 2 – Conflicts of Interest in Research Involving Human Subjects (ID: 681)	06/14/16
How to Complete the CITI Refresher Course and Receive a Completion Report (ID: 922)	06/14/16

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

CITI Program
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CURRICULUM VITAE

September 3, 2015

NAME: Matthew Robert Greives, MD

PRESENT TITLE: Assistant Professor

BUSINESS ADDRESS: University of Texas Health Science Center at Houston
Department of Pediatric Surgery
6431 Fannin Street, MSB 5.281
Houston, Texas 77030

BIRTHDATE: April 19, 1980

CITIZENSHIP: United States

UNDERGRADUATE EDUCATION

1998-2001 Washington University in St. Louis
St. Louis, MO
BA with Honors, Biochemistry

GRADUATE EDUCATION

2002-2007 New York University, School of Medicine
New York, NY
MD with Honors

POSTGRADUATE TRAINING:

2007-2013 University of Chicago Hospital, Division of Plastic and Reconstructive Surgery, Chicago, Illinois
Residency, Administrative Chief Resident

2013-2014 Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania
Fellowship in Pediatric and Craniofacial Plastic Surgery

MILITARY SERVICE (IF APPLICABLE): N/A

ACADEMIC APPOINTMENTS:

2014-present Assistant Professor, Department of Pediatric Surgery, Division of Pediatric Plastic Surgery, The University of Texas Health Science Center at Houston

HOSPITAL APPOINTMENTS:

2014-present Memorial Hermann/Children's Memorial Hermann Hospital
2014-present Memorial Hermann- The Woodlands
2014-present The Woman's Hospital of Texas
2014-present The Shriner's Hospital
2014-present Lyndon B. Johnson General Hospital, Harris County Hospital District
2014-present Westside Surgical Hospital

LICENSURE: Texas Medical Board License # Q0760

CERTIFICATION:
Basic Life Support
Advanced Cardiac Life Support
Pediatric Advanced Life Support

PROFESSIONAL ORGANIZATIONS (AND COMMITTEES OF THESE):

LOCAL:
Houston Society of Plastic Surgeons

REGIONAL:
Texas Society of Plastic Surgeons

NATIONAL:
American Society of Maxillofacial Surgeons (Education Committee)
American Society of Craniofacial Surgeons
American Cleft Palate-Craniofacial Association
American Association of Pediatric Plastic Surgeons

HONORS AND AWARDS:

Residency Honors
May 2011 Huggins Symposium Best Clinical Presentation
Dec 2012 Best Microsurgery Paper: ASPS Senior Resident Conference

Medical School Honors
July 2002 Dean's Scholarship
June 2005 Outstanding Essay for Humanism in Medicine (Medicine Dept.)
June 2005-May 2006 Plastic Surgery Research Fellowship
Feb 2006 Honors Program
May 2006 Glorney Raisbeck Fellowship for Cardiovascular Research
June 2006 Albert Ellis Scholar for International Research
April 2007 Research Day Award Recipient: Best presentation - Student

College Honors
1998-2001 Dean's List
May 1998 Dean of Engineering Scholarship
May 1998 Eliot Society Scholarship
2000-2001 Steven Alonzo Jackson Scholarship: Kappa Sigma Fraternity
1998-2001 Varsity Swimming *(All American,* 2001*; Academic All American 1999, 2001)*
2001 Washington University Outstanding Athlete
2001 College Honors Recipient

EDITORIAL POSITIONS: N/A

SERVICE ON NATIONAL GRANT REVIEW PANELS, STUDY SECTIONS, COMMITTEES: N/A

SERVICE ON THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT HOUSTON COMMITTEES:

Department of Pediatric Surgery, Compliance Committee member (2015- current)

SERVICE ON THE UNIVERSITY OF TEXAS MEDICAL SCHOOL AT HOUSTON COMMITTEES:

SERVICE ON GRADUATE SCHOOL COMMITTEES: N/A

SERVICE ON UTMSH AFFILIATED HOSPITAL COMMITTEES: N/A

SERVICE TO THE COMMUNITY: N/A

SPONSORSHIP OF CANDIDATES FOR POSTGRADUATE DEGREE: N/A

SPONSORSHIP OF POSTDOCTORAL FELLOWS: N/A

CURRENT TEACHING RESPONSIBILITIES: N/A

CURRENT CLINICAL AND SERVICE RESPONSIBILITIES:

Plastic Surgeon for Vascular Anomalies Clinic, Department of Pediatric Surgery
Plastic Surgeon for Texas Cleft-Craniofacial Clinic, Department of Pediatric Surgery

PREVIOUS CLINICAL EXPERIENCE:

International
Komedyplast: Craniofacial Mission (Lima, Peru)
February 2014
International Surgical Mission Trip

- Fellow represenative on Mission trip to the Lima, Peru.
- Focus on craniofacial surgery and cleft lip/palate surgery.

Elective Roation: Ear Reconstruction (Paris, France)
May 2013
Clinical Observership

- Shadowed Dr. Francoise Firmin to learn her techniques for complex ear reconstructions for microtia, burn, and trauma patients.

Medical Aid for Children of Latin America (Santo Domingo, Dominican Republic)
Feb 2012- Feb 2013
International Surgical Mission trip

- Resident represenative on Mission trip to the Dominican Republic
- Focus on cleft lip and palate reconstruction

Fundación Gantz, Hospital del Niño Fisurado (Santiago, Chile)
June 2006 –July 2006
Smile Train Medical Student International elective

- Researched the demographics of the cleft population in Chile with Dr. Luis Monasterio, Director of the Fundación Gantz.

- Evaluated labial and nasal outcome scores for a study comparing the Nakajima and Millard Rotation-Advancement techniques.
- Translated research involving median cleft syndromes and a novel technique for nasal molding in cleft patients for English journal publications.
- Gathered data and patient profiles for the Smile Train national headquarters.

Infectious Disease Unit, L'Hôpital de l'Archet (Nice, France)
May 2000–July 2000
Volunteer Assistant to Interns

- Shadowed interns and physicians during daily rounds to assist in patient physical exams.
- Organized charts and updated the files with current lab results prior to the rounds.

PREVIOUS RESEARCH EXPERIENCE:

Section of Plastic and Recontstructive Surgery, **University of Chicago Hospital**
June 2010–June 2013
Research Fellow

- Evaluated critical size cranial defects in a mouse model with Dr. Russell Reid.
- Utilized cranial suture progenitor cells to fill defects based on alloplastic implants for tissue engineering.
- Initiated airway protocols to evaluate patients for mandibular and midface distraction using 3D CT generated volumes to determine surgical outcomes.

Laboratory for Microvascular Research, NYU School of Medicine
May 2005–May 2007
Research Fellow

- Initiated trials of low-dose radiation as a possible therapeutic for vascular disease and its ability to stimulate vasculogenesis with Dr. Jamie Levine, Dr. Pierre Saadeh, and Dr. Alexis Hazen.
- Analyzed endothelial cells responses to hypoxia and radiation, specifically in the induction of HIF-1 upregulation of SDF-1.
- Utilized topical siRNA in a novel mechanism to improve diabetic wound healing through the silencing of cell cycle regulators.

Department of Pathology, NYU School of Medicine
June 2005–May 2007
Research Fellow

- Researched the effects of Calreticulin (CRT) as a topical therapeutic for wound healing with Dr. Leslie Gold, Associate Professor of Medicine and Pathology, using a diabetic mouse model of impaired wound healing.
- Isolated diabetic cells lines to assess their altered migratory and proliferative capacity and analyze improvements in these parameters with administration of CRT.

Laboratory for Microvascular Research, NYU School of Medicine
May 2003–December 2005
Research Fellow

- Researched wound healing and scar formation with Dr. Geoffrey Gurtner.
- Developed a novel model for hypertrophic scar formation under tension in a murine model.
- Gained proficiency in Immunohistochemistry, RNA Isolation, and Animal Surgery.
- Initiated therapeutic arm of scar model to determine efficacy of current treatment modalities.

Washington University School of Medicine
May 2000 –August 2000
Laboratory Technician

- Researched the immune responses in the eye for Dr. Thomas Ferguson, Professor of Ophthalmology.
- Analyzed cytokine levels in murine leukocyte cell cultures after tolerance testing

CURRENT GRANT SUPPORT: N/A

PAST GRANT SUPPORT: N/A

Publications:

Abstracts:

- Anderson C, Doringo LI, Teichgraeber J, **Greives MR.** Survey of Parent Experiences in Prenatal Visit for Infants with Cleft lip and Palate. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, Teichgraeber J, **Greives MR.** An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Chang O, Bailey V, Teichgraeber J, **Greives MR.** Tissue expander indications and complications in the pediatric population. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Mobli K, Patel S, Teichgraeber J, **Greives MR.** Cost analysis of acellular dermal matrix in cleft palate repairs. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Barr R, Shah M, Teichgraeber J, **Greives MR.** Cervical instability in Pierre Robin Sequence: an Addition to the Algorithm. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Gibson A, Lin PY, Teichgraeber J, **Greives MR.** Secondary cleft nasal reconstruction using resorbable nasal stents. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Dean R, Anderson C, Doringo IL, Teichgraeber J, **Greives MR.** Assessing the burden of care in the cleft lip and palate patient: factors influencing success and failure in nasoalveolar molding. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
- Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, Teichgraeber J, **Greives MR.** An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. Housestaff Research Day University of Texas Health Sciences Center at Houston, May 2015.
- Nguyen TQ, Francyzk M, Lee JC, **Greives MR**, O'Connor Annemarie, Gottlieb LJ. Prospective Randomized Controlled Trial Comparing Two Methods of Securing Skin Grafts Using Negative Pressure Wound Therapy: Vacuum-Assisted Closure and Gauze Suction. American College of Surgeons Clinical Congress, San Francisco, CA, October 25, 2011, and at the American Burn Association Annual Meeting, Palm Springs, CA, April 25, 2013

- Katzel, EB; Naran, S; MacIsaac, Z; Camison, L; **Greives, MR**; Golstein, JA; Grunwaldt, LJ; Ford, FD; Losee, JE. Speech Outcomes Following Clinically Indicated Posterior Pharyngeal Flap Takedown. 59th Ohio Valley Society of Plastic Surgeons Annual Meeting. The Greenbrier, WV June 2014. (Best Clinical Paper Award)

- Katzel, EB; Naran, S; MacIsaac, Z; Camison, L; **Greives, MR**; Golstein, JA; Grunwaldt, LJ; Ford, FD; Losee, JE. Speech Outcomes Following Clinically Indicated Posterior Pharyngeal Flap Takedown. The Annual Scientific Meeting of the Robert H. Ivy Society of Plastic Surgeons. Bedford Springs, PA May 2014.

- Nguyen TQ, Francyzk M, Lee JC, **Greives MR**, Gottlieb LJ. Prospective Randomized Controlled Trial Comparing Two Methods of Securing Skin Grafts Using Negative Pressure Wound Therapy: VAC and GSUC. Illinois Society of Plastic Surgeons Senior Resident Paper Competition, Chicago, IL, June 8, 2013.

- Nguyen TQ, Francyzk M, Lee JC, **Greives MR**, Gottlieb LJ. Prospective Randomized Controlled Trial Comparing Two Methods of Securing Skin Grafts Using Negative Pressure Wound Therapy: VAC and GSUC. American Burn Association, Palm Springs, CA, April 25, 2013.

- Nguyen TQ, Francyzk M, Lee JC, **Greives MR**, Gottlieb LJ. Prospective Randomized Controlled Trial Comparing Two Methods of Securing Skin Grafts Using Negative Pressure Wound Therapy: VAC and GSUC. University of Chicago 20th Annual Charles B. Huggins Research Symposium, Chicago, IL, February 20, 2013.

- Teven CM, **Greives MR**, Natale R, He TC, Reid RR. "Differentiation but not Proliferation of Osteoprogenitor Cells is Enhanced by High-Frequency Pulsed Electromagnetic Fields." Pritzker School of Medicine 66th Annual Senior Scientific Session. Chicago, IL. May 2012.

- **Greives MR**, Wimmer-Kunitomo K, Collins J, Poon C, Baroody FM, Reid RR. 3D CT Analysis Demonstrates Improved Airway Volumes Following Mandibular Distraction Osteogenesis. Huggin's Surgical Research Symposium (Poster). Chicago, IL. April 2012.

- Teven CM, **Greives MR**, Natale R, Shenaq D, Rossi M, Chenard K, He TC, Reid RR. Osteoprogenitor Cell Differentiation into Bone is Accelerated by a Novel Delivery System of High-frequency Pulsed Electromagnetic Fields. American Society of Plastic Surgery. Denver, CO. September 2011.

- Lee J, Francyk M, Nguyen TQ, **Greives MR**, Maertens F, Dorafshar AH, Gottlieb LJ. Prospective Randomized Controlled Trial Comparing Two Methods of Securing Skin Grafts Using Negative Pressure Wound Therapy: VAC and GSUC. American College of Surgeons Conference. San Francisco, CA. October 2011.

- Teven CM, **Greives MR**, Natale RB, Shenaq D, Kwan D, He TC, Reid RR. "High-frequency Pulsed Electromagnetic Fields Accelerate Ostoeoprogenitor Cell Differentiation." Huggin's Surgical Research Symposium. Chicago, IL. April 2011

- Teven CM, **Greives M**, Kwan D, Spiguel L, Luu HH, Haydon R, He TC, Reid RR. A novel delivery system of high-frequency pulsed electromagnetic fields accelerates

differentiation of osteoprogenitor cells. Oral presentation at the 68th Annual Meeting of the American Cleft Palate-Craniofacial Association. San Juan, Puerto Rico, April 2011.

- Teven CM, **Greives M**, Natale RB, Kwan D, Spiguel L, Luu HH, Haydon R, He TC, Reid RR. Differentiation of osteoprogenitor cells is accelerated by a novel delivery system of high-frequency pulsed electromagnetic fields. Poster presentation at the 90th Annual Meeting of the American Association of Plastic Surgeons. Boca Raton, FL, April 2011.

- Teven CM, **Greives M**, Collier JH, Natale R, He TC, Reid RR. The use of a novel biocompatible scaffold to produce bone in 3-D cell culture. Oral presentation at the 56th Annual Meeting of the Plastic Surgery Research Council. Louisville, KY, April 2011.

- Samra F, Naylor SM, Gorovets D, Pavlides S, **Greives MR**, Murphy-Ullrich J, Levine JP, Gold LI, Warren SM. Functions of the ER Chaperone Calreticulin in Diabetic Wound Repair. Plastic Surgery Research Council. San Francisco, CA, May 2010.

- Rohde, C**.**, **Greives, MR**, Cetrulo, C., Lerman, OZ., Levine J., and Hazen, A. "Gustilo Grade IIIB Tibial Fractures Requiring Microvascular Free Flaps: External Fixation versus Intramedullary Rod Fixation." American Society of Reconstructive Microsurgery Annual Meeting, Puerto Rico, 2007.

- Lerman OZ, Chang CC, **Greives MR**, Thanik VD, Seiser NS, Hazen OZ, Warren SM, Saadeh PB, Levine JP. "Low-dose radiation improves perfusion and vascularity in an ischemic flap model" Plastic Surgery Research Council. Palo Alto, CA. June 2007.

- Thanik VD, **Greives MR**, Lerman OZ, Hazen A, Warren SM, Levine JP, Saadeh PB. "*In vivo* gene silencing: siRNA as a topical therapeutic." Plastic Surgery Research Council. Palo Alto, CA. June 2007.

- Chang CC, Scharf C, Lerman OZ, **Greives MR**, Thanik VD, Macklin J, Hazen A, Warren SM, Levine JP, Saadeh PB. "Radiation is a key regulator of angiogenic and angiostatic CXC chemokine expression in human endothelial cells." Plastic Surgery Research Council. Palo Alto, CA. June 2007.

- Chang CC, Lerman OZ, **Greives MR**, Thanik VD, Seiser N, Warren SM, Saadeh PB, Levine JP. "Impaired neovascularization after high-dose radiation injury: The role of EPCs." Plastic Surgery Research Council. Palo Alto, CA. June 2007.

- Chang CC, Scharf C, Lerman OZ, **Greives MR**, Thanik VD, Macklin J, Hazen A, Warren SM, Levine JP, Saadeh PB. "Radiation is a key regulator of angiogenic and angiostatic CXC chemokine expression in human endothelial cells." Wound Healing Society. Naples, FL May 2007.

- Thanik VD, **Greives MR**, Lerman OZ, Hazen A, Warren SM, Levine JP, Saadeh PB. "*In vivo* gene silencing: siRNA as a topical therapeutic." Wound Healing Society. Naples, FL May 2007.

- Gobble RM, Parikh MS, **Greives MR**, Ren CJ, Fielding GA. “Gastric Banding as a Salvage Procedure for Patients with Weight Loss Failure after Roux-en-Y Gastric Bypass. The Society of American Gastrointestinal and Endoscopic Surgeons Annual Meeting. Las Vegas, NV. April 2007.

- Lerman OZ, Chang CC, **Greives MR**, Thanik VD, Seiser N, Brown DJ, Galiano RD, Formenti S, Hazen A, Warren SW, Saadeh PB, Levine JP. “Low dose radiation augements endothelial progenitor cell mobilization and neovascularization in an ischemic flap.” Keystone Symposia. Keystone, CO Jan. 28- Feb. 2, 2007.

- Rohde C, **Greives MR**, Lerman OZ, Hazen A, and Levine J. “Gustilo Grade IIIB Fractures Requiring Microvascular Free Flaps: External Fixation versus Intermedullary Rod Fixation.” American Society of Reconstructive Microsurgery Conference. Rio Grande, Puerto Rico. January 2007.

- Rohde C, **Greives MR**, Lerman OZ, Hazen A, and Levine J. “Gustilo Grade IIIB Fractures Requiring Microvascular Free Flaps: External Fixation versus Intermedullary Rod Fixation.” Northeastern Society of Plastic Surgeons Conference. Boston, MA. November 2006.

- Thanik VD, **Greives MR**, Lerman OZ, Hazen A, Saadeh PB, Levine JP. “In-Vivo Gene Silencing Using Topical Delivery of siRNA.” American College of Surgeons Conference. Chicago, IL. October 2006.

- Thanik VD, **Greives MR**, Lerman OZ, Hazen A, Saadeh PB, Levine JP. A Novel Method of Topical Gene Silencing Using siRNA.” American Society of Plastic Surgery. San Francisco, CA. October 2006.

- Le H, Lerman OZ, **Greives MR**, Blechman K, Brown DJ, Thanik VD, Hazen A, Saadeh PB, Gurtner GC, Galiano RD, Levine JP. “Hedgehog signaling disruption inhibits wound healing.” Plastic Surgery Research Council. Dana Point, CA. May 2006.

- Le H, Lerman OZ, **Greives MR**, Blechman K, Brown DJ, Thanik VD, Hazen A, Saadeh PB, Gurtner GC, Galiano RD, Levine JP. “Inhibition of the Hedgehog Signaling Pathway Impairs the Wound Healing Process.” Wound Healing Society. Scottdale, AZ. May 2006.

- Lerman OZ, **Greives MR**, Thanik VD, Brown DJ, Ceradini DJ, Galiano RD, Levine JP, Saadeh PB, Hazen A. “Low and High Dose Ionizing Radiation Induce Differential Proangiogenic and Proinflammatory Chemokine Expression Profiles in Endothelial Cells.” Wound Healing Society. Scottdale, AZ. May 2006.

- Gold LI, **Greives MR**, Blechman KM, Cadacio C, Rahman MR, Levine JP, Michalak M, Nanney LB. “The biological effects of calreticulin on tissue repair.” International Calreticulin Workshop. Niagara Falls, NY. April 2006.

- Bhatt KA, **Greives MR**, Bastidas N, Ghali S, Tabbal G, Jones DM, Lin S, Gurtner GC. "Mechanical Strain Leads to Hypertrophic Scars by Altering Apoptotic Pathways In Vivo." Plastic Surgery Research Council. Toronto, Canada. May 2005.

- Bhatt KA, **Greives MR**, Galiano RD, Ashinoff RL, Bonillas RG, Ceradini DJ, Jones DM, Levine JP, Gurtner GC. "Mechanical Strain Induces Hypertrophic Scars in Mice by Reducing Stromal Cell Apoptosis." Plastic Surgery Research Council. Ann Arbor, MI. May 2005.

- Bhatt KA, Bastidas N, **Greives MR**, Ghalie S, Thibonnier A, Bonillas RG, Jones DM, Ceratini DJ, Gurtner GC. "Genetic Analysis of Mechanical Strain on a Murine Model of Hypertrophic Scar." Northeastern Society of Plastic Surgeons. Naples, FL. November 2004.

- Bhatt KA, **Greives MR**, Galiano RD, Ashinoff RL, Bonillas RG, Ceradini DJ, Jones DM, Levine JP, Gurtner GC. "Mechanical Strain Induces Hypertrohic Scars in Mice by Reducing Stromal Cell Apoptosis." Wound Healing Society. Atlanta, GA. May 2004.

Refereed Original Articles in Journals:

- Gold LI, Rahman M, Blechman KM, **Greives MR**, Churgin S, Michaels J, Callaghan MJ, Gurtner GC, Caldwell NL, Pollins AC, Michalak M, Nanney LB. "Overview of the Role for Calreticulin in Enhancement of Wound Healing Through Multiple Biological Effects." *Journal of Investigative Dermatology.* (2006) 11, 57-65. PMID: 17069011

- Thanik VD, **Greives MR**, Lerman OZ, Seiser N, Dec W, Chang CC, Warren SM, Levine JP, Saadeh PB. "Topical matrix-based siRNA silences local gene exprssion in a murine wound model." *Gene Ther.* (2007) 14(17): 1305-8. PMID: 17625576

- Rhode C, **Greives MR**, Cetrullo C, Lerman OZ, Hazen H, Levine JP. "Gustillo Grade IIIB Tibial Fractures Requiring Microvacular Free Flaps: External Fixation versus Intramedullary Rod Fixation." *Ann Plast Surg* (2007) 59(1): 14-7. PMID: 17589252

- Michaels J 5th, Churgin SS, Blechman KM, **Greives MR**, Aarabi S, Galiano RD, Gurnter GC. "db/db mice exhibit severe wound healing impairments compared with other murine diabetic strains in a silicone-splinted excisional wound model." *Wound Repair Regen.* (2007) 15(5): 665-70. PMID: 17971012

- Kleinman ME, **Greives MR**, Churgin SS, Blechman KM, Chang EI, Ceradini DJ, Tepper OM, Gurtner GC. Hypoxia-induced mediators of stem/progenitor cell trafficking are increased in children with hemangioma." *Arterioscler Thromb Vasc Biol* (2007) 27(12): 2664-70. PMID: 17872454

- Gobble RM, Parikh MS, **Greives MR**, Ren CJ, Fielding GA. "Gastric banding as a salvage procedure for patients with weight loss failure after Roux-en-Y gastric bypass." *Surg Endosc.* (2008) 22(4): 1019-22. PMID: 17943353

- Nanney LB, Woodrell CD, **Greives MR**, Cardwell NL, Pollins AC, Bancroft TA, Chesser A, Michalak M, Rahman M, Siebert JW, Gold LI. "Calreticulin enhances porcine wound repair by diverse biological effects." *Am J Pathol.* (2008) 173(3): 610-30. PMID: 18753412

- Chang CC, Lerman OZ, Thanik VD, Scharf CL, **Greives MR**, Schneider RJ, Formenti SC, Saadeh PB, Warren SM, Levine JP. "Dose-dependent effect of radiation on angiogenic and angiostatic CXC chemokine expression in human endothelial cells." *Cytokine* (2009) 48(3): 295-302. PMID: 19782578.

- Gold, LI, Eggleton P, Sweetwyne MT, Van Duyn LB, **Greives MR**, Naylor SM, Michalak M, Murphy-Ullrich JE. "Calreticulin: non-endoplasmic reticulum functions in physiology and disease." *FASEB J. (*2010) 24(3): 665-83. PMID: 19940256

- Thanik VD, Chang CC, Lerman OZ, **Greives MR**, Le H, Warren SM, Schneider RJ, Formenti SC, Saadeah PB, Levine JP. "Cutaneous low-dose radiation increases tissue vascularity through upregulation of angiogenic and vasculogenic pathways." *J Vasc Res.* (2010) 47(6): 472-80. PMID: 20431296

- Lerman, OZ, **Greives MR**, Singh SP, Thanik VD, Chang CC, Seiser N, Brown DJ, Knobel D, Schneider RJ, Formenti SC, Saadeh PB, Levine JP. "Low-dose radiation augments vasculogensis signaling through HIF-1-dependent and -independent SDF-1 induction. " *Blood* (2010) 116(18): 3669-76. PMID: 20631377

- Teven CM, **Greives MR**, Natale RB, Su Y, Luo Q, He BC, He TC, Reid RR. "Differentiation of Osteoprogenitor Cells is Enhanced by a Novel Delivery System of High-frequency Pulsed Electromagnetic Fields. *J Craniofac Surg* (2012) 23(2): 586-593. PMID: 22446422

- **Greives MR**, Fares S, Woodrel CD, Pavilides S, Blechman KM, Naylor SM, Cadacio CL, Levine JP, Warren SM, Gold LI. "Exogenous Calreticulin improves Diabetic Wound Healing." *Wound Repair and Regen. (*2012) 21: 337. PMID: 22985041

- **Greives MR**, Odessey EA, Waggoner D, Lui DC, Aradhya S, Mitchell A, He TC, Reid RR. "RUNX2 Quadruplication: Additional Evidence Towards a New Form of Syndromic Craniosynostosis." *J Craniofac Surg*. (2013) 24(1):126-9. PMID: 23348268

- Nguyen TQ, Franczyk M, Lee JC, **Greives MR**, O'Connor A, Gottlieb LJ. Prospective Randomized Controlled Trial Comparing Two Methods of Securing Skin Grafts Using Negative Pressure Wound Therapy: Vacuum-Assisted Closure and Gauze Suction. J Burn Care Res. (2015) 36(2): 324-8. PMID: 25162948

- **Greives MR**, Ware BW, Tian AG, Taylor JA, Pollack IF, Losee JE. Complications in Posterior Cranial Vault Distraction. *Ann Plast Surg*.(2015 May). [Epub ahead of print] PubMed PMID: 25954848

- Shenaq DS, Teven TC, Seitz IA, Rastegar F, **Greives MR**, He TC, Reid RR. Characterization of Reversibly Immortilized Calvarial Mesenchymal Progenitor Cells. *J Craniofac Surg.* (2015): 26 (4): 1207-13. Pubmed PMID: 26080159

- **Greives MR,** Figueroa AA, Reid RR. Successful Treatment of Postoperative Mouth Opening Limitation Following Lefort III Distraction with Bilateral Coronoidectomies. *J Max Oral Surg* (2015): Epub ahead of print.

- Katzel, EB; Naran, S; MacIsaac, Z; Camison, L; **Greives, MR**; Golstein, JA; Grunwaldt, LJ; Ford, FD; Losee, JE. Speech Outcomes Following Clinically Indicated Posterior Pharyngeal Flap Takedown. *Ann Plast Surg.* Sept 2015. [Epub ahead of print]

- Fu K, Teichgraeber JF, **Greives MR**. Botulinum Use in Pediatric Plastic Surgery*. Ann Plast. Surg*. Sept 2015. [Epub ahead of print]

- Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, Teichgraeber J, **Greives MR.** An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. Accepted for publication. *J Craniofac Surg*. Accepted for publication.

Invited Articles in Journals:

- **Greives MR**, Song DS. *Essential Tissue Healing of the Head and Neck*. Guyrion. Book review. *Plastic and Reconstructive Surgery Journal* (2011) 127(2): 1385.

- **Greives MR**, Losee JE. "Discussion: Factors Influencing Fellowship Selection, Career Trajectory and Academic Productivity. *Plast Reconstr Surg*. (2014) 133(3): 737-9. PMID: 24572863

- **Greives MR**, Camison L., Losee JE. “Evidence Based Medicine: Unilateral Cleft Lip and Nose Repair. *Plast Reconstr Surg*. (Dec 2014), 134(6):1372-80. PMID: 25414100

- **Greives MR,** Camison L, Losee JE. Reply: Evidence-Based Medicine: Unilateral Cleft Lip and Nose Repair. *Plast Reconstr Surg.* (Jul 2015), 136(1): 119e-120e. PMID: 25839170

Chapters:

- Ghavami A, Alkureshi LWT, **Greives MR**, Genioplasty. *Essentials of Plastic Surgery 2nd Edition.* (submitted).
- **Greives MR,** Reid RR. Posterior Pharyngeal Flap. *Atlas of Craniofacial Surgery* (submitted)
- **Greives MR.** Pierre Robin Sequence. *Comprehensive Cleft Care Family Companion (2015)*

Books: N/A

Other Professional Communications:

Presentations:

- **Greives MR**, Lerman OZ, Seiser N, Thanik VD, Draper L, Blechman K, Brown D, Hazen A, Saadeh PB, Levine JP "Low-dose radiation augments wound healing by stimulating
EPC migration through HIF-1/SDF-1 induction." Wound Healing Society. Scottdale, AZ. May 2006.

- **Greives MR**, Lerman OZ, Thanik VD, Seiser N, Draper L, Blechman K, Brown D, Ceradini DJ, Galiano RD, Formenti S, Hazen A, Saadeh PB, Levine JP. "Low-dose radiation stimulates EPC migration through HIF-1/SDF-1 induction." Plastic Surgery Research Council.
Dana Point, CA. May 2006.

- **Greives MR**, Cadacio CL, Woodrel C, Blechman KM, Levine JP, Gold LI. In Vitro Effects of Calreticulin Corroborates its Role in Healing of Diabetic Wounds. New York University School of Medicine Research Day Awards. New York, NY. May 2007. ***Best Medical Student Paper Award*

- **Greives MR**, Teven C, Collier J, Rudra J, Natale R, He TC, Reid RR. Self-assembling peptide scaffolding as a three-dimensional matrix for osteoprogenitor cell proliferation and differentiation. Huggin's Surgical Research Symposium. Chicago, IL. April 2011. ** *Best Clinical Paper Award*

- **Greives MR**, Teven CM, Luu HH, Reid RR. The Use of a Novel, Three-Dimensional Biocompatible Scaffold to Deliver Osteoprogenitor Cells to a Critical-Sized Calvarial Defect. Midwestern Association of Plastic Surgeons. Chicago, IL. May 2011.

- **Greives MR**, Dickie S, Gottlieb L, Park J. External Oblique Rotational Flap for Chest Wall Reconstruction for Radiation induced wounds. Midwestern Association of Plastic Surgeons. Chicago, IL. May 2011.

- **Greives MR**, Bank J, Ledbetter K, Gottlieb L. Phalloplasty Revisited: Additional Venous Drainage Reduces Flap Loss and Secondary Complications. American Society of Plastic Surgery Senior Resident Conference. Chicago, IL. December 2012. ** *Best Microsurgical Paper Award*

- **Greives MR**, Bank J, Ledbetter K, Gottlieb L. Additional Venous Drainage Improves Flap Survial in the Radial Forearm Free Flap Phalloplasty. American Society for Reconstructive Microsurgery. Naples, FL. January 2013.

- **Greives MR**, Bank J, Ledbetter K, Gottlieb L. Phalloplasty Revisited: Additional Venous Drainage Reduces Flap Loss and Secondary Complications. Illinois Society of Plastic Surgery Senior Resident Research Competition. Chicago, IL. June 2013

- **Greives MR**. Mandibular Distraction in the Pierre Robin Patient. Division of Plastic Surgery Grand Rounds. University of Texas Health Sciences Center at Houston. Houston, TX. October 2014.

- **Greives MR,** Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, Teichgraeber J, An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. International Society of Craniofacial Surgery, Tokyo Japan, September 2015.

- **Greives MR,** Teichgraeber J. Abnormalities in Head Shape: Deformational Plagiocephaly and Craniosynostosis. Houston Pediatric Society, Houston, TX. September 2015.

- **Greives MR**. Mandibular Distraction in the Pierre Robin Patient. Department of Otolaryngology Grand Rounds. University of Texas Health Sciences Center at Houston. Houston, TX. October 2015.

- **Greives MR**. Mandibular Distraction in the Pierre Robin Patient. Neonatal Medicine Grand Rounds. University of Texas Health Sciences Center at Houston. Houston, TX. October 2015.

Non-refereed Publications: N/A

Letters to the Editor: N/A

Scientific Exhibits: N/A

Videos: N/A

Visiting Professorships: N/A

G. Visiting Professorships

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

HUMAN RESEARCH CURRICULUM COMPLETION REPORT

Printed on 09/12/2014

LEARNER	Matthew Greives (ID: 4382165) University of Texas Health Science Center at Houston 6431 Fannin St. MSB 5.281 Houston Texas 77030 USA
DEPARTMENT	Pediatric Surgery
PHONE	7135007285
EMAIL	Matthew.R.Greives@uth.tmc.edu
INSTITUTION	University of Texas Health Science Center at Houston
EXPIRATION DATE	09/11/2017

GROUP 1 BIOMEDICAL RESEARCHER AND KEY PERSONNEL

COURSE/STAGE:	Basic Course/1
PASSED ON:	09/12/2014
REFERENCE ID:	14009869

REQUIRED MODULES	DATE COMPLETED
Avoiding Group Harms - U.S. Research Perspectives	09/12/14
Belmont Report and CITI Course Introduction	09/12/14
History and Ethics of Human Subjects Research	09/12/14
Basic Institutional Review Board (IRB) Regulations and Review Process	09/12/14
Informed Consent	09/12/14
Records-Based Research	09/12/14
Genetic Research in Human Populations	09/12/14
Vulnerable Subjects - Research Involving Children	09/12/14
Vulnerable Subjects - Research Involving Pregnant Women, Human Fetuses, and Neonates	09/12/14
FDA-Regulated Research	09/12/14
Research and HIPAA Privacy Protections	09/12/14
Conflicts of Interest in Research Involving Human Subjects	09/12/14
University of Texas Health Science Center at Houston	09/12/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid Independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

DATA ACQUISITION AND MANAGEMENT CURRICULUM COMPLETION REPORT

Printed on 09/12/2014

LEARNER	Matthew Greives (ID: 4382165) University of Texas Health Science Center at Houston 6431 Fannin St. MSB 5.281 Houston Texas 77030 USA
DEPARTMENT	Pediatric Surgery
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EMAIL	Matthew.R.Greives@uth.tmc.edu
INSTITUTION	University of Texas Health Science Center at Houston
EXPIRATION DATE	

DATA ACQUISITION AND MANAGEMENT :

COURSE/STAGE:	RCR/1
PASSED ON:	09/12/2014
REFERENCE ID:	14009873

REQUIRED MODULES	**DATE COMPLETED**
Data Management (RCR-Biomed)	09/12/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid Independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

CITI GOOD CLINICAL PRACTICE GRADEBOOK CURRICULUM COMPLETION REPORT

Printed on 09/12/2014

LEARNER	Matthew Greives (ID: 4382165) University of Texas Health Science Center at Houston 6431 Fannin St. MSB 5.281 Houston Texas 77030 USA
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PHONE	7135007285
EMAIL	Matthew.R.Greives@uth.tmc.edu
INSTITUTION	University of Texas Health Science Center at Houston
EXPIRATION DATE	09/11/2016

GCP

COURSE/STAGE:	Basic Course/1
PASSED ON:	09/12/2014
REFERENCE ID:	14009871

REQUIRED MODULES	DATE COMPLETED
The CITI Good Clinical Practice Course for Clinical Trials Involving Drugs and Devices	09/12/14
Overview of New Drug Development	09/12/14
Overview of ICH GCP	09/12/14
ICH - Comparison Between ICH GCP E6 and U.S. FDA Regulations	09/12/14
Conducting Investigator-Initiated Studies According to FDA Regulations and GCP	09/12/14
Investigator Obligations in FDA-Regulated Clinical Research	09/12/14
Managing Investigational Agents According to GCP Requirements	09/12/14
Overview of U.S. FDA Regulations for Medical Devices	09/12/14
Informed Consent in Clinical Trials of Drugs, Biologics, and Devices	09/12/14
Detecting and Evaluating Adverse Events	09/12/14
Reporting Serious Adverse Events	09/12/14
Audits and Inspections of Clinical Trials	09/12/14
Monitoring of Clinical Trials by Industry Sponsors	09/12/14
Completing the CITI GCP Course	09/12/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid Independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

CONFLICTS OF INTERESTS IN RESEARCH TRAINING CURRICULUM COMPLETION REPORT

Printed on 09/12/2014

LEARNER	Matthew Greives (ID: 4382165) University of Texas Health Science Center at Houston 6431 Fannin St. MSB 5.281 Houston Texas 77030 USA
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INSTITUTION	University of Texas Health Science Center at Houston
EXPIRATION DATE	

CONFLICTS OF INTERESTS IN RESEARCH TRAINING

COURSE/STAGE:	RCR/1
PASSED ON:	09/12/2014
REFERENCE ID:	14009872

REQUIRED MODULES	DATE COMPLETED
Conflicts of Interests in Research Training	09/12/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid Independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

Curriculum Vitae

Name:
John F. Teichgraeber, M.D.

Present Title:
Professor and Chief, Department of Pediatric Surgery, Division of Pediatric Plastic Surgery

Business Address:
University of Texas Health Science Center at Houston
Department of Pediatric Surgery
6431 Fannin Street, Suite 5.282
Houston, Texas 77030
713-500-7361 (office)
713-500-7296 (fax)
John.F.Teichgraeber@uth.tmc.edu

Undergraduate Education:
1966-1967 Stanford University

1967-1970 Williams College, Williamstown, MA – **Bachelor of Arts**, History, cum laude

Graduate Education:
1971-1972 Harvard Graduate School of Business Administration
Cambridge, MA – **Completed first year of MBA program**

1972-1974 Harvard University – **Pre-medical Curriculum**

1974-1978 Emory University Medical School – Atlanta, GA. **Doctorate of Medicine**

Postgraduate Training:
1978-1979 Emory University Hospitals, Atlanta, GA. **General Surgery Intern**

1979-1982 Emory University Hospitals – Atlanta, GA. **Otolaryngology Resident**

1982-1983 M.D. Anderson Cancer Center – Houston, TX. **Head and Neck Surgery Fellow**

1984-1986 University of Texas-Houston Medical School – Houston, TX. **Plastic and Reconstructive Surgery Resident**

1994-1995 Medical City Hospital – Ian Munro, M.D., Dallas, TX. **Craniofacial Surgery Externship**

Academic Appointments:
2000 – Professor of Surgery
Department of Pediatric Surgery
Division of Pediatric Plastic Surgery
University of Texas-Houston Medical School

1997 – 2000 Associate Professor of Surgery
Division of Pediatric Surgery
University of Texas-Houston Medical School

1996 – Director – TX Cleft and Craniofacial Clinic
University of Texas-Houston Medical School

2000 – Associate Professor of Surgery
Division of Plastic and Reconstructive Surgery
University of Texas-Houston Medical School

1987 – 1992 Assistant Professor of Surgery
Division of Plastic and Reconstructive Surgery
University of Texas-Houston Medical School

1987 – Assistant Professor of Oral/Maxillofacial Surgery (Clinical)
University of Texas Dental Branch

1987 – Assistant Professor of Otolaryngology (Clinical)
Department of Otolaryngology
University of Texas-Houston Medical School

Hospital Appointments:
Harris County Hospital District
Memorial Hermann/Children's Memorial Hermann Hospital
Texas Children's Hospital

Licensure:
Texas License # **G4061**

Certifications:
1983 American Board of Otolaryngology
1988 American Board of Plastic Surgery

Professional Organizations:
Local:
Harris County Medical Society
Houston Society of Plastic and Reconstructive Surgeons

Regional:
Texas Medical Association
Texas Society of Plastic Surgeons

National:
American Academy of Facial, Plastics and Reconstructive Surgery
American Academy of Otolaryngology and Head and Neck Surgery
American Cleft Palate-Craniofacial Association
American College of Surgeons
American Medical Association
American Society of Plastic and Reconstructive Surgeons

Honor and Awards:

1982 – 1983 Jesse H. Jones Fellowship in Cancer Education, Honoring W. Bertner, M.D. Anderson Cancer Center
1990 F.A.C.S. – Fellow of the American College of Surgeons
1990 – 1993 Dean's Teaching Excellence Award
2002 Senior Residents Choice Award for Plastic Surgery
2005 House of Charity-Humanitarian of the Year Award
2005 – 2006 Dean's Teaching Excellence Award
2005 – 2006 Best Doctors in America
2011 – 2012 Best Doctors in America

Editorial Positions: N/A

Services on National Grant Review Panels, Study Sections, Committees: N/A

Service on the University of Texas Health Science Center at Houston Committees: N/A

Service on the University of Texas Medical School at Houston Committees:
1987-Present Medical School Admissions Committee
University of Texas-Houston Medical School

1987-Present Medical Student Advisor
University of Texas-Houston Medical School

1987-Present Medical Student Mentor Program
University of Texas-Houston Medical School

Service on Graduate School Committees: N/A

Service on UTMSH Affiliated Hospital Committees:
Infection Control - Memorial Hermann Hospital

Service to the Community:
1994 - 2005 Austin Smiles, volunteer medical missions to Latin America to repair cleft lips and cleft palates. Latin America.

1991-Present National Youth Leadership Forum, pre-medical educational program guiding high school and university students by shadowing clinical and surgical techniques. Houston, Texas.

1997-Present House of Charity advisor and volunteer bringing medical care to children from across the world. Houston, Texas.

2006-Present Operation Rainbow, volunteer medical missions to Trinidad to repair cleft lips and cleft palates. Trinidad

Sponsorship of Candidates for Postgraduate Degree:
The effects of presurgical nasoalveolar molding in the bilateral cleft. Adam Spengler, Medical Student, MS-1 Summer Research Program (NIH). University of Texas Medical School at Houston. 06/2004-08/2004

Sponsorship for Postdoctoral Fellows: N/A

Current Teaching Responsibilities:
Plastic Surgery Rounds – Residents and Medical Students

Cleft Lip and Craniofacial Rounds – Residents and Students

Current Grant Support:

2009-2011 NIH RO1 Digital Dental Alignment Grant, Amount: $1,250,000, Co-Investigator
University of Texas Medical School at Houston, Houston, Texas.

Previous Grant Support:

1. *Use of Topical Atropine in Acute Rhinitis*: $3,000 – Jan. 1980 – May 1980. Principal Investigator

2. *Evaluation of Prosthetic materials in the Cat Middle Ear*: $20,000 Aug. 1980 – Oct. 1980. Principal Investigator

3. *Evaluation of the Oral Cavity Functional Results for Head and Neck Surgery*: $1,000 – July 1982 – June 1983. Principal Investigator

4. Thomas D. Cronin, M.D. Chair of Plastic Surgery – *Experimental Craniosynostosis in Rabbits*: $3,000 – Oct. 1989 – Oct. 1993. Principal Investigator.

5. *Development of Surgical Planning and Navigation Software for Cranio-Maxillofacial Surgery*. Gateno J, Xia J, Teichgraeber JF. Stryker-Leibinger Co. $30,000. October 2001-September 2002. Co-Investigator.

6. *Use of Computer Modeling in Craniofacial Reconstruction*: $20,000 – Current Principal Investigator – Jamie Gateno, M.D.

Publications:

Abstracts:

1. Parks DH, **Teichgraeber JF**. Use of silastic sheeting for temporary abdominal wound closure. *J Trauma* 25:7, 1985.
2. **Teichgraeber JF**, Parks DH. Current role of tracheostomy in major maxillofacial trauma. *J Trauma* 25:7, 1985.
3. **Teichgraeber JF**, Parks DH. The radiographic diagnosis of upper airway obstruction on maxillofacial trauma. *J Trauma* 25:7, 1985.
4. Xia J, Gateno J, **Teichgraeber J**. Virtual reality surgical planning for craniofacial and maxillofacial deformities. *J Oral Maxillofac Surg* 59:77 (Suppl), 2001.
5. Xia J, Gliddon MJ, Gateno J, **Teichgraeber JF**, Wong HTF, Liebschner MAK. The accuracy of cephalometric tracing superimposition. *J Oral Maxillofac Surg* 61:78 (Suppl), 2003.
6. N Bodily, M Lypka, **JF Teichgraeber**, S Yuksel, S Oy. Mandibular Distraction Osteogenesis in Patients with Pierre Robin Sequence: Preoperative Assessment Determines Success. Otolaryngology Head and Neck Surgery (Impact Factor: 1.72). 09/2014; 151(1 Suppl):P239-P239. DOI: 10.1177/0194599814541629a322

Refereed Original Articles in Journals:

1. Jackson RT, **Teichgraeber JF**. Low dose topical atropine for rhinorrhea. *Arch Otolaryngol* 107:288-289, 1981.
2. **Teichgraeber JF**, Per-Lee JH, Turner JS. Lateral sinus thrombosis: a Modern perspective. *Laryngoscope* 92:744-751, 1982.
3. McConnell FS, **Teichgraeber JF**. Neoglottis reconstruction following total laryngectomy: Emory experience. *Otolaryngol Head and Neck Surg* 90:569-575, 1982.
4. **Teichgraeber JF**, Spector M, Per-Lee JH, Jackson RT. Tissue response

to Plasti-pore and Proplast otologic implants in the middle ear of cats. *Am J Otol* 5:127-136, 1983.
5. **Teichgraeber JF**, Clairmont AA. The incidence of occult metastases for cancer of the oral tongue and floor of the mouth: treatment rationale. *Head Neck Surg* 7:15-21, 1984.
6. **Teichgraeber JF**, Larson DL, Castenada O, Martin JW. Skin grafts in intra-oral reconstruction: indications and presentation of a new stenting method. *Arch Otolaryngol* 110:463-467, 1984.
7. **Teichgraeber JF**, Bowman J, Goepfert H. New test series for functional evaluation of oral cavity cancer. *Head and Neck Surg* 8:9-20, 1985.
8. Henderson L, Denneny J, **Teichgraeber JF**. Airway-obstructing epiglottic cyst. *Ann Otorhinolaryngol* 94:473-476, 1985.
9. McConnell FS, **Teichgraeber JF**. Treatment of posterior pharyngeal wall carcinoma. *Otolaryngol Head and Neck Surg* 94:287-290, 1986.
10. **Teichgraeber JF**, Bowman J, Goepfert H. Functional analysis of oral Cavity cancer treatment. *Arch Otolaryngol* 112:959-965, 1986.
11. McConnell FS, **Teichgraeber JF**, Adler RD. A comparison of three methods of oral reconstruction. *Arch Otolaryngology* 113:496-500, 1987.
12. **Teichgraeber JF**, Larson DL. Some oncologic considerations in the treatment of lip cancer. *Otolaryngol Head and Neck Surg* 98(6):589-592, 1988.
13. **Teichgraeber JF**, Riley WB, Parks DH. Primary skin closure in large myelomeningoceles. *Pediatric Neuroscience* 15:18-22, 1989.
14. **Teichgraeber JF**, Goepfert H. Rhinectomy: timing and reconstruction. *Otolaryngol Head and Neck Surg* 102(4):362-369, 1990.
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17. **Teichgraeber JF**, Rappaport NH, Harris JH. The radiology of upper airway obstruction in maxillofacial trauma. *Ann Plastic Surg* 27:103-109, 1991.
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68. Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, **Teichgraeber J**, Greives MR. An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. Submitted to Journal Of Craniofacial Surgery.

Invited Articles in Journals:

1. **Teichgraeber JF**, Russo RC, Riley WR. External rhinoplasty technique. Yearbook of Otolaryngology and Head and Neck Surgery, 1991.
2. **Teichgraeber JF**, Riley WB, Parks DH. Nasal surgery complications. Yearbook of Otolaryngology and Head and Neck Surgery, 1991.
3. **Teichgraeber JF**, Larson DI, Castenada O, Martin JW. Skin grafts in intra-oral reconstruction. Yearbook of Plastic, Reconstructive, and Aesthetic Surgery, 1993.

Chapters:

1. **Teichgraeber JF**. Biomaterials in Ototoloty. IN: Grote JJ, Nijhoff M (eds.) Proceedings of the First International Symposium, Boston, 1984.
2. McConnell FS, **Teichgraeber JF**. Speech and swallowing function following surgery of the oral cavity. IN: Myeren, Barofsky, Yates (eds.) Rehabilitation and Treatment of Head and Neck Cancer, NIH Publication 86-2762, Washington, D.C., 1986.
3. Xia J, Gateno J, **Teichgraeber J**, Rosen A. Methodology of precise skull model creation. In: Viergever M, Dohi T and Vannier Meds: Lecture Notes in Computer Science: Medical imaging computing and computer-assisted intervention-MICCAI 2001. Springer-Verlag pp. 434-440, 2001.
4. Gateno J, **Teichgraeber JF**, Xia J. Surgical planning for distraction osteogenesis. In: Intraoral Distraction Osteogenesis of the Facial Skeleton. Bell WH, Guerrero C, Chin M. (Eds) BC Decker, Elsevier: Ontario, Canada. pp. 131-9, 2007.
5. Xia JJ, Gateno J, **Teichgraeber JF**. Secrets in computer-aided surgical simulation for complex craniomaxillofacial surgery. In English JD, Peltomaki T, Pham-Litschel K (Eds): Mosby's Orthodontic Review. pp. 277-87, 2008.
6. Gateno J, Xia JJ, **Teichgraeber JF**. New Methods to Evaluate Craniofacial Deformity and to Plan Surgical Correction. In: Grayson B (ed).
7. Xia JJ, Gateno J, **Teichgraeber JF**. A New Clinical Protocol to Plan Cranio-maxillofacial (CMF) Surgery Using Computer-Aided Surgical Simulation (CASS). In Kau CH (ed).
8. Xia JJ, Gateno J, **Teichgraeber JF**. Controversial Issues in Computer-Aided Surgical Planning for Cranio-Maxillofacial (CMF) Surgery. In: Kau CH (ed).

Books: N/A

Other Professional Communications:

Presentations:

1. Xia JJ, Gateno J, Anderson P, Horng J, **Teichgraeber JF**, Liebschner MAK. *A comparative assessment of mandibular condylar position in patients with anterior disc displacement of the temporomandibular joint*. 84th Annual Meeting of American Association of Oral & Maxillofacial Surgeons. Chicago, Illinois. October 2-5, 2002. (poster)

2. Xia JJ, Gateno J, **Teichgraeber JF**, Rosen R, Hultgren B, Vadnais T. *The precision of computer-generated surgical splints*. 84th Annual Meeting of American Association of Oral & Maxillofacial Surgeons. Chicago, Illinois. October 2-5, 2002. (15 min)
3. Xia J, Gateno J, Kim KW, Lalani Z, **Teichgraeber JF**. *Tissue resistance to forces in distraction osteogenesis of the mandible: cadaver study*. 84th Annual Meeting of American Association of Oral & Maxillofacial Surgeons. Chicago, Illinois. October 2-5, 2002. (15 min)
4. Xia J, Gateno J, **Teichgraeber J**. *Virtual reality surgical planning for cranio and maxillofacial deformities*. 84th Annual Meeting of American Association of Oral & Maxillofacial Surgeons. Chicago, Illinois. October 2-5, 2002. (15 min)
5. Gliddon MJ, Xia JJ, Gateno J, Wong HTF, **Teichgraeber JF**. *The accuracy of cephalometric tracing superimposition*. 84th Annual Meeting of American Association of Oral & Maxillofacial Surgeons. Chicago, Illinois. October 2-5, 2002. (15 min)
6. Gateno J, Xia JJ, Engel E, **Teichgraeber JF**, Yamaji K. *The use of a new Le Fort I internal distraction device in the treatment of severe maxillary hypoplasia.* 2004 American Cleft Palate-Craniofacial Association Annual Meeting, Chapel Hill, NC. March 13, 2004. (5 min)
7. **Teichgraeber JF**, Ezzat C, Chavarria C, Stratmann R, Chen JW, Xia JJ. *Pre-surgical nasoalveolar molding in unilateral cleft patients*. 2004 American Cleft Palate-Craniofacial Association Annual Meeting, Chapel Hill, NC. March 20, 2004. (10 min)
8. **Teichgraeber JF**. *Hemifacial Microsomia: Evaluation and Staging of Reconstruction.* Controversies in Pediatric Maxillofacial Surgery Diagnosis and Treatment, Healthstream Symposium, Houston, TX. May 8th, 2004. (1 hour 15 min)
9. **Teichgraeber JF**. Microscopic Approach to Craniosynostosis. 2003 Annual Meeting of the Texas Society of Plastic Surgeons. September 11, 2004. (10 min)
10. Spengler A, Chavarria C, **Teichgraeber JF**, Gateno J, Xia JJ. *Pre-surgical nasoalveolar molding (PNAM) therapy for the treatment of bilateral cleft lip and palate*. 2005 American Cleft Palate-Craniofacial Association Annual Meeting, Myrtle Beach, SC. April 4-9th, 2005. (10 min)
11. Spengler A, Chavarria C, **Teichgraeber JF**, Gateno J, Xia JJ. *Pre-surgical nasoalveolar molding (PNAM) therapy for the treatment of bilateral cleft lip and palate*. Texas Society of Plastic Surgeons 2005 Annual Meeting, San Antonio, TX. October 21-23rd, 2005. (10 min) Received 1st Place Award.
12. **Teichgraeber JF**, Baumgartner JE. *Recent Advances in Diagnosis and Treatment of Craniosynostosis and Plagiocephaly*. Memorial Hermann Katy Hospital CME Presentation, Katy, Texas, January 25, 2006. (1 hour)
13. Xia JJ, Gateno J, **Teichgraeber JF**. Method and apparatus for fabricating orthognathic surgical splints. 2006 BioHouston Orthopedic Medical Device Emerging Technology Showcase, Houston, TX, April 5, 2006 (20 min)
14. Xia JJ, Gateno J, **Teichgraeber JF**, Christensen AM, Lemoine JJ, Leibschner MAK, Lasky RE. *Clinical feasibility of computer-aided surgical simulation for complex cranio-maxillofacial surgery*. 2006 Annual Meeting of American Cleft Palate-Craniofacial Association, Vancouver, Canada. April 7, 2006. (10 min)
15. Baumgartner JE, **Teichgraeber JF**, Waller A, Xia JJ, Gateno J. *A protocol for the microscopic minimally invasive approach to sagittal craniosynostosis*. 2006 Annual Meeting of American Cleft Palate-Craniofacial Association, Vancouver, Canada. April 7, 2006. (10 min)
16. Lee RPS, **Teichgraeber JF**, Baumgartner JE, Waller A, Xia JJ, English JD, Gateno J. *Long-term treatment effectiveness of molding helmet therapy in the correction of posterior deformational plagiocephaly: A five year follow-up*. 2006 Annual Meeting of American Cleft Palate-Craniofacial Association, Vancouver, Canada. April 5, 2006. (10 min)
17. Perez SD, **Teichgraeber JF**, Xia JJ, Day MC, Fleshman RG, Gateno J. *The effectiveness of a resorbable supporter in nasal reconstructive surgery*. 2006 Annual Meeting of American Cleft Palate-Craniofacial Association, Vancouver, Canada. April 5, 2006. (4 min, Show and Tell Section)

18. Xia JJ, Gateno J, **Teichgraeber JF**, Christensen AM. Computer-aided surgical simulation for complex cranio-maxillofacial deformities. (Invited keynote speaker). 7th Asian Congress on Oral and Maxillofacial Surgery, Hong Kong, China, November 6-9, 2006 (30 min) (invited and scheduled)
19. Gateno J, Xia JJ, **Teichgraeber JF**. Distraction osteogenesis for complex cranio-maxillofacial deformities. (Invited keynote speaker). 7th Asian Congress on Oral and Maxillofacial Surgery, Hong Kong, China, November 6-9, 2006 (30 min) (invited and scheduled)
20. Greives MR, Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, **Teichgraeber J**, An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. International Society of Craniofacial Surgery, Tokyo Japan, September 2015.
21. Greives MR, **Teichgraeber J**. Abnormalities in Head Shape: Deformational Plagiocephaly and Craniosynostosis. Houston Pediatric Society, Houston, TX. September 2015.
22. Anderson C, Doringo LI, **Teichgraeber J**, Greives MR. Survey of Parent Experiences in Prenatal Visit for Infants with Cleft lip and Palate. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
23. Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, **Teichgraeber J**, Greives MR. An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
24. Mobli K, Patel S, **Teichgraeber J**, Greives MR. Cost analysis of acellular dermal matrix in cleft palate repairs. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
25. Barr R, Shah M, **Teichgraeber J**, Greives MR. Cervical instability in Pierre Robin Sequence: an Addition to the Algorithm. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
26. Gibson A, Lin PY, **Teichgraeber J**, Greives MR. Secondary cleft nasal reconstruction using resorbable nasal stents. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
27. Dean R, Anderson C, Doringo IL, **Teichgraeber J**, Greives MR. Assessing the burden of care in the cleft lip and palate patient: factors influencing success and failure in nasoalveolar molding. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.
28. Fu K, Barr R, Kerr M, Shah M, Fletcher S, Sandberg D, **Teichgraeber J**, Greives MR. An outcomes comparison between autologous and alloplastic cranioplasty in the pediatric population. Housestaff Research Day University of Texas Health Science Center at Houston, May 2015.
29. Chang O, Bailey V, Teichgraeber J, Greives MR. Tissue expander indications and complications in the pediatric population. Texas Society of Plastic Surgeons, San Antonio, TX September 2015.

Non-refereed Publications: N/A

Letters to the Editor: N/A

Scientific Exhibits: N/A

Videos: N/A

Visiting Professorships: N/A

Patents:
*Gateno J, **Teichgraeber JF**, Xia J. Method and apparatus for fabricating orthognathic surgical splints. October 3, 2001. Patent No: US 6,671,539 in USPTO Patent Full-Text and Image Database. 2001, United States Patent and Trademark Office.*

Films:
"Unmasked: Treacher Collins Syndrome" documentary, the Discovery Channel, 2004.

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COURSEWORK REQUIREMENTS REPORT*

* NOTE: Scores on this Requirements Report reflect quiz completions at the time all requirements for the course were met. See list below for details. See separate Transcript Report for more recent quiz scores, including those on optional (supplemental) course elements.

- **Name:** John Teichgraeber (ID: 159325)
- **Institution Affiliation:** University of Texas Health Science Center at Houston (ID: 661)
- **Institution Unit:** Surgery
- **Phone:** 713 500 7300

- **Curriculum Group:** Human Research
- **Course Learner Group:** Group 1 Biomedical Researcher and Key Personnel
- **Stage:** Stage 2 - Refresher Course

- **Report ID:** 3311249
- **Completion Date:** 29-Apr-2014
- **Expiration Date:** 28-Apr-2017
- **Minimum Passing:** 80
- **Reported Score*:** 83

REQUIRED AND ELECTIVE MODULES ONLY	DATE COMPLETED
Biomed Refresher 2 - Instructions (ID: 764)	29-Apr-2014
Biomed Refresher 2 – History and Ethical Principles (ID: 511)	29-Apr-2014
Biomed Refresher 2 – Regulations and Process (ID: 512)	29-Apr-2014
Biomed Refresher 2 – Informed Consent (ID: 514)	29-Apr-2014
Biomed Refresher 2 – SBR Methodologies in Biomedical Research (ID: 515)	29-Apr-2014
Biomed Refresher 2 – Genetics Research (ID: 518)	29-Apr-2014
Biomed Refresher 2 – Records-Based Research (ID: 516)	29-Apr-2014
Biomed Refresher 2 - Populations in Research Requiring Additional Considerations and/or Protections (ID: 519)	29-Apr-2014
Biomed Refresher 2 – Vulnerable Subjects – Prisoners (ID: 520)	29-Apr-2014
Biomed Refresher 2 – Vulnerable Subjects – Children (ID: 521)	29-Apr-2014
Biomed Refresher 2 – Vulnerable Subjects – Pregnant Women, Human Fetuses, Neonates (ID: 522)	29-Apr-2014
Biomed Refresher 2 – FDA-Regulated Research (ID: 524)	29-Apr-2014
Biomed Refresher 2 – HIPAA and Human Subjects Research (ID: 526)	29-Apr-2014
Biomed Refresher 2 – Conflicts of Interest in Research Involving Human Subjects (ID: 681)	29-Apr-2014
How to Complete the CITI Refresher Course and Receive a Completion Report (ID: 922)	29-Apr-2014

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

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COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI PROGRAM)

COURSEWORK TRANSCRIPT REPORT**

** NOTE: Scores on this Transcript Report reflect the most current quiz completions, including quizzes on optional (supplemental) elements of the course. See list below for details. See separate Requirements Report for the reported scores at the time all requirements for the course were met.

- **Name:** John Teichgraeber (ID: 159325)
- **Institution Affiliation:** University of Texas Health Science Center at Houston (ID: 661)
- **Institution Unit:** Surgery
- **Phone:** 713 500 7300

- **Curriculum Group:** Human Research
- **Course Learner Group:** Group 1 Biomedical Researcher and Key Personnel
- **Stage:** Stage 2 - Refresher Course

- **Report ID:** 3311249
- **Report Date:** 05-Aug-2016
- **Current Score**:** 87

REQUIRED, ELECTIVE, AND SUPPLEMENTAL MODULES	MOST RECENT
Biomed Refresher 2 - Instructions (ID: 764)	29-Apr-2014
Biomed Refresher 2 – History and Ethical Principles (ID: 511)	29-Apr-2014
Biomed Refresher 2 – Regulations and Process (ID: 512)	29-Apr-2014
Biomed Refresher 2 – Informed Consent (ID: 514)	29-Apr-2014
Biomed Refresher 2 – SBR Methodologies in Biomedical Research (ID: 515)	29-Apr-2014
Biomed Refresher 2 – Records-Based Research (ID: 516)	29-Apr-2014
Biomed Refresher 2 – Genetics Research (ID: 518)	29-Apr-2014
Biomed Refresher 2 - Populations in Research Requiring Additional Considerations and/or Protections (ID: 519)	29-Apr-2014
Biomed Refresher 2 – Records-Based Research (ID: 670)	30-Mar-2009
Biomed Refresher 2 – Vulnerable Subjects – Prisoners (ID: 520)	29-Apr-2014
Biomed Refresher 2 – Records-Based Research (ID: 671)	30-Mar-2009
Biomed Refresher 2 – Vulnerable Subjects – Children (ID: 521)	29-Apr-2014
Biomed Refresher 2 – Vulnerable Subjects – Pregnant Women, Human Fetuses, Neonates (ID: 522)	29-Apr-2014
Biomed Refresher 2 – Genetics Research (ID: 672)	30-Mar-2009
Biomed Refresher 2 – FDA-Regulated Research (ID: 524)	29-Apr-2014
Biomed Refresher 2 – HIPAA and Human Subjects Research (ID: 526)	29-Apr-2014
Biomed Refresher 2 – Conflicts of Interest in Research Involving Human Subjects (ID: 681)	29-Apr-2014
Biomed Refresher 2 – Vulnerable Subjects – Children (ID: 550)	30-Mar-2009
Biomed Refresher 2 – Vulnerable Subjects – Children (ID: 551)	30-Mar-2009
How to Complete the CITI Refresher Course and Receive a Completion Report (ID: 922)	29-Apr-2014
Biomed Refresher 2 – Vulnerable Subjects – Pregnant Women, Human Fetuses, Neonates (ID: 552)	30-Mar-2009
Biomed Refresher 2 – Conflicts of Interest in Research Involving Human Subjects (ID: 523)	30-Mar-2009
Biomed Refresher 2 – FDA-Regulated Research (ID: 553)	30-Mar-2009

For this Report to be valid, the learner identified above must have had a valid affiliation with the CITI Program subscribing institution identified above or have been a paid Independent Learner.

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CURRICULUM VITAE

October 17, 2016

NAME: Fabio Triolo, PhD

PRESENT TITLE: Associate Professor, Department of Pediatric Surgery
Director, Cellular Therapy Core
The University of Texas Medical School at Houston
Houston, TX

ADDRESS: The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory
BBS 6106
1941 East Rd.
Houston, TX, 77054
Ph. 713-486-2542
HIPAA Secure Fax: 713-383-3704
E-mail: fabio.triolo@uth.tmc.edu

UNDERGRADUATE EDUCATION:

1994 Italian *Laurea* in Biological Sciences
University of Palermo
Palermo, Italy

GRADUATE EDUCATION:

1999 Ph.D. in Chemical Sciences
University of Palermo
Palermo, Italy

2000 M.Phil. in Biomedical Sciences
Mount Sinai School of Medicine of New York University
New York, NY

2002 Ph.D. in Biomedical Sciences
Mount Sinai School of Medicine of New York University
New York, NY

ACADEMIC APPOINTMENTS:

2005-2008 Adjunct Assistant Professor of Surgery
Department of Surgery
University of Pittsburgh School of Medicine
Pittsburgh, PA

2009-2011 Affiliate Faculty Member

McGowan Institute for Regenerative Medicine
University of Pittsburgh
Pittsburgh, PA

2011-2014 Assistant Professor of Clinical and Translational Sciences
Center for Clinical and Translational Sciences
UTHealth, Houston, TX

2011-2014 Assistant Professor
Department of Pediatric Surgery
The University of Texas Medical School at Houston
Houston, TX

2014-present Associate Member
University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX
Program Affiliation: Clinical and Translational Sciences

2014-present Associate Professor
Department of Pediatric Surgery
The University of Texas Medical School at Houston
Houston, TX

2014-present Associate Professor of Clinical and Translational Sciences
Center for Clinical and Translational Sciences
UTHealth, Houston, TX

2014-present Faculty Member
Gulf Coast Cluster for Regenerative Medicine
Gulf Coast Consortia for Quantitative Biomedical Sciences
Houston, TX

HOSPITAL AND ADMINISTRATIVE APPOINTMENTS:

2003-2004 Coordinator of Biological and Biomedical Research
Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT)
University of Pittsburgh Medical Center, Palermo, Italy

2004-2010 Founder and Director
Office of Research, Health and Biomedical Sciences
Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT)
University of Pittsburgh Medical Center
Palermo, Italy

2004-2010	Director, Experimental Cell Therapy and Cell Transplantation Laboratory Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) University of Pittsburgh Medical Center Palermo, Italy
2006-2010	Technical Director Regenerative Medicine and Cell Therapy Unit Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) University of Pittsburgh Medical Center Palermo, Italy
2006-2008	Interim Head of Production Human Cell Processing cGMP Facility Regenerative Medicine and Cell Therapy Unit Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) University of Pittsburgh Medical Center Palermo, Italy
2010	Director of the Human Cell Processing cGMP Facility and of Cell Therapy R&D Regenerative Medicine and Biomedical Technologies Unit Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) University of Pittsburgh Medical Center Palermo, Italy
2011-present	Director, Human Cell Processing cGMP Facilities Program of Regenerative Medicine The University of Texas Medical School at Houston Houston, TX
2014-present	Director, Cellular Therapy Core (UTHealth Service Center)

LICENSURE:

Biologist Licensing examination, 2001
University of Palermo (Palermo, Italy) with a grade of 150/150
Member of the Italian National Board of Biologists since 2001

CERTIFICATION:

Authorized by the Italian Drug Agency and by the Italian Ministry of Education,

University and Research, to act as *Qualified Person* of Pharmaceutical cGMP facilities authorized to produce biological products for cell therapy, according to Italian law n.219 of April 24, 2006, implementation of European directive 2001/83/EC

PROFESSIONAL ORGANIZATIONS:

National:

American Society of Gene and Cell Therapy

Italian Scientists and Scholars of North America Foundation

- Treasurer, and member of the biosciences subcommittee, of the US Southwest Chapter of the Italian Scientists and Scholars of North America Foundation in 2012

International:

European Qualified Person Association

International Society for Cellular Therapy

The Cell Transplant Society

Tissue Engineering International and Regenerative Medicine Society

US Department of State International Exchange Alumni

HONORS AND AWARDS:

1982	Honorary Citizenship of the State of Tennessee
1986-87	University of Palermo (Italy) Competitive Student Scholarship
1994	University of Palermo (Italy) Experimental Thesis Special Mention
1994-95	University of Palermo (Italy) Competitive Fellowship
1996-97	Mount Sinai Graduate School of Biological Sciences of CUNY Fellowship
1996-99	University of Palermo (Italy) Competitive Doctoral Fellowship
1996-2001	Fulbright Fellowship
1999	American Society for Cell Biology Competitive Travel Award
2000	Mount Sinai Graduate School of Biological Sciences of NYU Competitive Travel Award
2000	European Union Competitive Travel Award

2009 Best Poster Award at the 6th National Congress of the Italian Society for Clinical and Experimental Cytometry

2010 Best Poster Award at the EMBO Workshop "From Fetomaternal Tolerance to Immunomodulatory Properties of Placenta-derived Cells in Cell Therapy"

2014 Italian Flame Award – One of 21 distinguished medical professional honorees of Italian descent whose personal and professional achievements were recognized at the Italian Cultural and Community Center of Houston's 2014 Gala focused on Italian Influence on Medicine.

2016 One of 14 Italian scientists and physicians selected by the Italian Ministry of International Affairs to meet and update Sergio Mattarella, President of Italy, on research activities involving Italians in Texas.

EDITORIAL POSITIONS:

2005 Guest Co-Editor of Cell Transplantation Journal, Vol. 15, Supplement 1 (2006), special supplement dedicated to Regenerative Medicine

2013-present Academic Editor, Translational Science Section of *Progressive Science* peer-reviewed online research journal

SERVICE ON NATIONAL AND INTERNATIONAL GRANT REVIEW PANELS, STUDY SECTIONS, COMMITTEES:

2004-2010 Founding member of the Institutional Research Review Board at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT), Palermo, Italy

2004-2006 Member of the Coordinating Committee of the Immunology and Transplantation Study Group of the Italian Society of Diabetology

2005-2006 Member of the Scientific Organizing Committee of the 3rd International Conference on Functional Genomics of Ageing held in Palermo, Italy from March 29 to April 1, 2006

2005 Member of the Scientific Organizing Committee of the International Workshop on Regenerative Medicine held in Palermo, Italy on April 21, 2005

2005	Member, representing ISMETT, of the social-economic partnership identified by the Regional Province of Palermo, Italy for the establishment of the 2007-2013 strategic plans
2005-2010	Member (Technical Director), nominated by the Region of Sicily, Italy of the Steering Committee for the implementation of the Regenerative Medicine: from Research to Enterprises through ICT Technology - Province of Palermo (ICT-E2) grant, the goal of which was to establish a state-of-the-art Human Cell Processing cGMP Facility
2006	Member of the Scientific Organizing Committee of the Innovative Techniques for the Treatment of Diabetes: Pancreatic Islet Transplantation Forum held in Palermo, Italy on March 17, 2006
2006-2007	Member of the Working Group, nominated by the Italian National Transplant Center, for the definition of the national guidelines for procurement, processing, preservation, storage and distribution of pancreatic islets and hepatocytes
2008	Member of the Working Group for the organization of Bioforum 2008, a leading Italian biotechnology conference held in Milan, Italy on October 1-2, 2008
2008	Director of the first edition of the "Introduction to Regenerative Medicine" workshop, held in Palermo, Italy from November to December 2008, within the "FARO – Multidisciplinary Patient-Oriented Education/Learning" Training Grant
2008	Reviewer of the Italian Ministry of Health National Guidelines for Procurement, Processing, Storage and Distribution of Cells and Tissues for Clinical Use
2008-present	Expert Member of the Italian National Reference Pole for the Coordination of Biological Resource Centers and Biobanks, nominated by the National Committee for Biosafety, Biotechnology and Life Sciences of the Italian Presidency of the Council of Ministers
2008-2011	Member (Technical Director), nominated by the Region of Sicily, Italy of the Steering Committee for the implementation of the ICT-E2 STEP 2: The Cell Factory Collaboratory grant, the goal of which was to establish a state-of-the-art virtual collaboratory for cell therapy

2008-2010	Member of the Strategic Planning Working Group of the Pharmaceutical-Medical Sector Circle of Knowledge of the "Resint – Sicilian Network of Technology Innovation" project. The objective was to create 10 sector-specific Circles of Knowledge, distributed in the Sicilian provinces
2008-present	Nominated Representative of the Region of Sicily, Italy by the Regional Health Council, for the establishment of the Italian REACH (a new integrated system for the Registration, Evaluation, Authorisation and Restriction of Chemical substances according to European Community Regulation n. 1907/2006 of the European Parliament and of the Council) network
2009-2011	Member of the Working Group of the Health Council of the Region of Sicily, Italy aimed at establishing a regional cell and tissue bank
2010	Member of the task force for Advanced Therapy Medicinal Products (ATMP) of the European Advanced Translational Research InfraStructure in Medicine (EATRIS). EATRIS aims at creating a distributed pan-European infrastructure consisting of a network of well-renowned biomedical translation research centers across Europe
2010	Member of the ISCD Research and Development Partnership Committee of the International and Commercial Services Division (ISCD) of the University of Pittsburgh Medical Center
2010	Member, representing ISMETT, of the Working Group of the Italian National Center for Biological Resources, aimed at establishing a national network for clinical research
2010	Member of the Technical Support Group of the Region of Sicily, Italy nominated by the Regional Health Council, for the Evaluation of clinical and translational biomedical research proposals funded by the Italian Ministry of Health
2011-present	Expert reviewer, on behalf of the US-Italy Fulbright Commission, of Fulbright competitions open to Italian candidates seeking to pursue a Master or Ph.D. program or to carry out a period of research in the US and to US candidates who plan to conduct research and/or teach in Italy
2011-present	Peer reviewer of the clinical and translational biomedical research proposals funded by the Italian Ministry of Health
2012	Peer reviewer of the ISSNAF Young Investigator Award

2012	Member of the Scientific Organizing Committee of the 8th Conference of Italian Researchers in the World held in Houston on December 1, 2012
2013	Member of the Scientific Organizing Committee and Moderator of Keynote Lecture Session of the 9th Conference of Italian Researchers in the World, held in Houston on December 14, 2013
2013-2014	Member of the International Scientific Committee and Chairman of the Tissue Engineering and Regenerative Medicine session of the XLI Annual Congress of the European Society for Artificial Organs (ESAO) held on September 17-20, 2014 in Rome, Italy
2014	Member of the working group defining the Memorial Hermann - Texas Medical Center Master Facility Plan. My role is to provide the specs for the stem cell production cleanroom laboratory and its equipment.
2014	Member of the Scientific Organizing Committee of the 10th Conference of Italian Researchers in the World, held in Houston on December 6, 2014
2015	Member of the International Scientific Committee of the XLII Annual Congress of the European Society for Artificial Organs (ESAO) held on September 2-5, 2015 in Leuven, Belgium

SERVICE ON THE UNIVERSITY OF TEXAS MEDICAL SCHOOL AT HOUSTON COMMITTEES:

2012-2018	Member of the Medical School Research Committee of the University of Texas Health Science Center at Houston (UTHealth)
2013	Peer reviewer, UTHealth Center for Clinical and Translational Sciences Pilot Project Awards Program
2013-2015	Faculty Senator, University of Texas Medical School at Houston
2013-2015	Member of the University of Texas Medical School at Houston Faculty Senate Promotions Subcommittee
2013-present	Interviewer for University of Texas Medical School at Houston Applicants
2013-present	Member of the UTHealth Skeletal Tissue Research Group
2015-present	Member of the UTHealth Core Lab and Service Center Council

2015-present Member of the UTHealth Stem Cell Research Oversight Committee

SPONSORSHIP OF CANDIDATES FOR GRADUATE DEGREE:

The University of Palermo (Italy) Management Engineering Degree Program

- Student name: Fabio Lopez
  Dissertation Title: Risk Management within the Quality Management System of a GMP Facility
  Period: July-November 2008
  Note: Mr. Lopez graduated summa cum laude with special mention of his dissertation and academic career. He continued collaborating with my unit until June 2010, becoming first author of a pioneering paper in the field of Risk Management applied to Cell Therapy Manufacturing.

SPONSORSHIP OF UNDERGRADUATE STUDENTS AT THE UNIVERSITY OF PALERMO, ITALY:

Biotechnology Degree Program

- Student name: Salvatore Mineo
  Dissertation Title: Isolation and Characterization of Human Fetal Liver Cells for Clinical Transplantation
  Period: January 2007 - July 2008

- Student name: Ignazio Romano
  Dissertation Title: Evaluation of the Physical and Microbiological Contamination in a Cellular Production Laboratory Compliant to Good Manufacturing Practice
  Period: October 2007 – October 2009

SPONSORSHIP OF POSTDOCTORAL FELLOWS:

- Name: Renato Mancuso
  Period: 2006-2008

- Name: Cinzia Chinnici
  Period: 2006-2010

- Name: Daniele Galvagno
  Period: 2006-2008

- Name: Giorgia Sisino
  Period: 2006-2009

- Name: Monica D’Amato
  Period: 2006-2009

- Name: Giandomenico Amico
  Period: 2007-2010

- Name: Claudia Coronnello
  Period: 2009

- Name: Cinzia Sausa
  Period: 2009

- Name: Suchit Sahai
  Period: 2013-2016

- Name: Kunjan Desai
  Period: 2016-present

TUTORSHIP OF SUMMER RESEARCH FELLOWS:

The University of Pittsburgh School of Medicine Summer Research Fellowship Program

- Student names: Jodie Bryk and Ian Yuan
  Project title: From Pre-neoplastic Lesions to Hepatocellular Carcinoma: a Comparative Gene Expression Study
  Period: Summer 2005

- Student Name: Srikanth Divi
  Project Title: 3D Perfusion Culture of Human Liver Fetal Progenitor Cells
  Period: Summer 2010

The University of Rochester School of Medicine and Dentistry Summer Research Fellowship Program

- Student name: Zachary Lill
  Project Title: Long-term Cultures of Human Bone Marrow Cells Using Bioreactors
  Period: Summer 2007

UT Medical School Summer Research Program

- Student name: Matteo Costantini (Rice University)
  Project Title: From Pre-Clinical Development of Wharton's Jelly-based Autologous Tissue Engineering Applications to Clinical Grade cGMP-compliant Manufacturing: a Translational Research Experience
  Period: Summer 2015

- Student name: Adam Saleh (UT Austin)
  Project Title:
  Period: Summer 2016

TUTORSHIP OF PROFESSIONAL TRAINEES:

- Trainee name: Marysuna Wilkerson (University of California, Berkeley)
  Project title: Long Term Preservation of Clinical-Grade Wharton's Jelly for Autologous Tissue Engineering Applications
  Period: August-December 2013

- Trainee name: Austin Sweat (UTHealth San Antonio)
  Project title:
  Period: May-July 2016

TUTORSHIP OF PRE-BACCALAUREATE TRAINEES:

- Trainee name: Chiara Sdringola (University of St. Thomas)
  Period: January-May 2016

TUTORSHIP OF INTERNATIONAL FACULTY EXCHANGE PROGRAM:

- Trainee name: José Julian Pérez Cordero
  Affiliation: El Bosque University, Medicine Faculty, Bogotá, D.C., Colombia
  Period: June-August 2015

CURRENT GRANT SUPPORT:

1. Source: National Institutes of Health, USA (R01)
   Duration: April 1, 2013 – February 28, 2018
   Title: Phase 2 Pediatric Autologous BMMNC for Severe Traumatic Brain Injury
   Role: Co-investigator (PI: Charles S. Cox, Jr., M.D.)
   Funding awarded: $2,529,546.00 (TC)
   Summary: This trial will examine the effects of using bone marrow derived cells to treat severe traumatic brain injury in children. The study will test if these cells preserve injured brain tissue after traumatic injury. Preservation of brain tissue is associated with improvement in functional and cognitive outcomes.

2. Source: National Institutes of Health, USA (SBIR)
   Duration: February 1, 2012 – July 31, 2017
   Role: Director, Cellular Therapy Core (PI: Charles Cox Jr., MD)
   Title: Cell-Based Therapy for Treatment of Traumatic Brain Injury (TBI)
   Funding awarded: $942,499.00 (TC)
   Summary: An initial GLP toxicity study in Phase 1, followed by sequential studies to address clinically relevant translational issues in progenitor cell therapy for neurological injury/disease.

3. Source: United States Department of Defense Joint Warfighter Medical Research Program
   Duration: August 16, 2016 – August 15, 2020

Role: Co-Investigator (PI: Charles S. Cox, Jr., M.D.)
Title: Treatment of Adult Severe TBI Injury Using Autologous Bone Marrow Mononuclear Cells
Funding awarded: $6,873,595.00 (TC)
Summary: The project plan will assess safety and functional outcomes following treatment of severe TBI in adults using autologous bone marrow mononuclear cells.

1. Source: Senator Lloyd and B.A. Bentsen Center for Stroke Research
   Duration: December 12, 2011 – December 31, 2017
   Role: Co-Investigator (PI: Charles Cox Jr., MD)
   Title: Amniotic Fluid Derived MSCs for Neurological Injury
   Funding: $486,654.00 (DC)
   Summary: This is a pre-clinical study aimed at developing a xeno-free method to isolate, expand and cryopreserve clinical-grade human amniotic fluid-derived mesenchymal stromal cells in compliance with cGMP and using them as a neuroprotective in the setting of HIE associated with cardiopulmonary bypass.

4. Source: Senator Lloyd and B.A. Bentsen Center for Stroke Research
   Duration: December 1, 2011 – December 31, 2017
   Role: Co-Investigator (PI: Charles Cox Jr., MD)
   Title: Imaging of Activated Microglia: Cell Therapy Targets for Neurological Injury
   Funding awarded: $300,000.00 (TC)
   Summary: The goal of this project is to develop novel imaging radioligand imaging techniques to study neuroinflammation after TBI.

5. Source: Cord Blood Registry, Inc./Mission Connect/TIRR Foundation/ Let's Cure CP
   Duration: October 1, 2013 – December 31, 2017
   Title: Autologous Cell Therapies for Cerebral Palsy (ACT for CP)
   Role: Director, Cellular Therapy Core (PI: Charles S. Cox, Jr., M.D.)
   Funding awarded: $1,300,000.00 (TC)
   Summary: This is a randomized, blinded, cross-over design Phase 2 clinical trial comparing cord blood vs. bone marrow mononuclear cells for cerebral palsy with specific imaging/clinical criteria.

6. Source: Cord Blood Registry, Inc.
   Duration: June 1, 2016 – May 31, 2018
   Title: Tools and Technologies for the Harvest/Storage/Deployment of Wharton's Jelly in Pediatric Craniofacial Surgery
   Role: Co-investigator (PI: Charles S. Cox, Jr., M.D.)
   Funding awarded: $307,359.00 (TC)
   Summary: This is a project aimed at developing the manufacturing and cryopreservation technology enabling Wharton's-jelly based Tissue Engineering applications in pediatric craniofacial surgery.

7. Source: Biostage
   Duration: September 1, 2016 – March 15, 2017
   Title: GLP Isolation, Expansion, Characterization and Release of Porcine Adipose-derived MSCs
   Role: Co-Investigator (PI: Olson, Scott, Ph.D.)
   Funding awarded: $237,534.00 (TC)
   Summary: The first phase of this project is aimed at preparing GLP-grade MSC-seeded scaffolds to support IND-enabling pre-clinical studies in which the combination product will be used to regenerate esophageal sections in the porcine model. The second phase is aimed at translating the technology into a cGMP-compliant clinical grade process to manufacture human MSC-seeded scaffolds to support a Phase I safety trial in humans.

OTHER SUPPORT:

1. Source: Evelyn H. Griffin
   Funds donated to date: $2,000,000.00
   Funds to be donated by Nov. 2019: $2,000,000.00
   Role: Director, Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory
   Summary: This is a philanthropic donation

2. Source: Immatics US, Inc.
   Duration: September 1, 2015 – December 31, 2018
   Funds: $946,125.90
   Role: Director, Cellular Therapy Core
   Summary: This is a service research agreement supporting manufacturing of therapeutic T cells

3. Source: UTHealth
   Duration: August 1, 2016 – December 1, 2016
   Funding: $40,000
   Summary: These funds were used to potentiate the Griffin laboratory processing capacity

PAST GRANT SUPPORT:

2. Source: UTMB Health
   Duration: July 1, 2015 – June 30, 2016
   Funds: $11,275.44
   Role: Director, Cellular Therapy Core
   Summary: This is an equipment lease to enable bone marrow processing at UTMB Health for our Phase 2 Pediatric Autologous BMMNC for Severe Traumatic Brain Injury trial

3. Source: UTHealth
   Duration: April – August 2015

Funds: $1,637,009.00
Role: Director, Cellular Therapy Core
Summary: These funds were used to purchase major equipment to potentiate the Cellular Therapy Core

4. Source: UTHealth Research Park Complex Behavioral and Biomedical Sciences cGMP Facility Park Complex
   Duration: March 2011 – November 2012
   Role: Director Griffin Stem Cell Laboratory
   Funding: $1,159,456.00

5. Source: Italian Ministry for Innovation and Technologies
   Duration: May 2005 – December 2010
   Role: Technical Director
   Title: Regenerative Medicine: From Research to Enterprise through Information and Communication Technology
   Funding awarded to ISMETT: € 5,300,000.00

6. Source: University of Pittsburgh Medical Center
   Duration: July 2006 – June 2007
   Role: Co-Investigator
   Title: Human Liver Progenitor Cell Transplantation (the MLS CellTxModule) using Bioreactor-Expanded Human Fetal Liver Cells
   Funding awarded to UPMC Italy: $530,415.00

7. Source: University of Pittsburgh Medical Center
   Duration: July 2007 – June 2008
   Role: Co-Investigator
   Title: Progenitor Cell Transplantation for Chronic Liver Disease, Diabetes Mellitus and Skin Injuries
   Funding awarded to UPMC Italy: $502,499.00

8. Source: National Institutes of Health (SBIR)
   Duration: February 2008 – January 2010
   Role: Project Director for UPMC Italy
   Title: Protection of Allogeneic Hepatocyte Transplants by Engineered Veto
   Funding awarded to UPMC Italy: $95,904.00

9. Source: Region of Sicily, Italy
   Duration: June 2008– March 2011
   Role: Technical Director
   Title: ICT-E2 STEP 2: The Cell Factory Collaboratory
   Funding awarded to ISMETT: € 1,164,661.60

10. Source: University of Pittsburgh Medical Center
    Duration: July 2008 – June 2009

Role: Co-Investigator
Title: Exploring Opportunities of Using Adult Stem Cells for the Development of Innovative Transplantation Therapies - for Liver Disease, Skin Injuries and Diabetes Mellitus
Funding awarded to UPMC Italy: $516,457.00

11. Source: Italian Ministry of Health
Duration: January 2009– January 2011
Role: PI for ISMETT
Title: Adult Mesenchymal Stem Cells: Differentiative Lineages and Applications in Autologous and Allogenic Implantation and Tissue Remodeling
Funding awarded to ISMETT: € 30,000.00

12. Source: University of Pittsburgh Medical Center
Duration: July 2009 – June 2010
Role: Co-Investigator
Title: Exploring Opportunities of Employing Fetal Adult Stem Cells for the Development of Innovative Transplantation Therapies
Funding awarded to UPMC Italy: $373,896.00

13. Source: University of Pittsburgh Medical Center
Duration: July 2010 – June 2011
Role: Co-Investigator (Resigned on January 24, 2011)
Title: Fetal Adult Stem Cells for the Development of Innovative Cell TransplantationTherapies
Funding awarded to UPMC Italy: $ 440,360.00

PUBLICATIONS:

A. Presented Abstracts

1) **Triolo, F.**, Caponetti, E., Mezzasalma, E., Salvato, B., Beltramini, M. and Heenan, R.K.: Investigation on the Quaternary Structure of Rapana thomasiana Hemocyanin. IX S.I.B.P.A. Congress - May 12-18, 1990, Marciana Marina, Italy

2) **Triolo, F.**, Triolo, R., Caponetti, E., and Floriano, M.A.. Chemical, Physical and Biological Applications of Neutron Scattering. 1st C.I.M.C.A. Meeting – February 24, 1995, Palermo, Italy

3) Caponetti, E., Floriano, M.A., **Triolo, F.** and Triolo, R.: SAXS and SANS Small Angle Scattering. VI Neutron Spectroscopy Annual Meeting - May 25-26, 1995, Rome, Italy

4) **Triolo, F.**, Heenan, R., Salvato, B., Beltramini, M., Caponetti, E., and Triolo, R.: Small Angle Neutron Scattering and Quaternary Structure of Hemocyanins.

ABCD-AGI-SIBBM-SIMGBM Joint Meeting - October 2-6, 1995, Montesilvano Lido (PE), Italy

5) Lencioni, S., Pellerito, A., Fiore, T., Maggio, F., **Triolo, F.**, and Pellerito, L.: Solid State and In Vivo Investigation on Organotin(IV) Orotates. XXIV National Congress of Inorganic Chemistry - June 25-29, 1996, Palermo, Italy

6) **Triolo, F.**, Triolo, A., Triolo, R., Betts, D., McClain, J., DeSimone, J., Wignall, G.D., Demé, B., Steytler, D.C., and Heenan, R.K.: Critical Micellisation Density: a SAS Structural Study of the Unimer-Aggregate Transition of block-copolymers in supercritical CO_2. SAS99: XI International Conference On Small Angle Scattering, May 17-20, 1999, Upton, NY

7) **Triolo, F.**, Triolo, A., Agamalian, M., Lin, J.S., Heenan, R.K., Lucido, G. and Triolo, R.: Fractal approach in petrography: Combining USANS, SANS and IANS. SAS99: XI International Conference On Small Angle Scattering, May 17-20, 1999, Upton, NY

8) Pellerito, C., Fiore, T., **Triolo, F.**, Mansueto, C., Nagy, L., and Pellerito, L.: Cytotoxic Activity of Organotin(IV) derivatives with m-tetra(4-sulfonatofenil) Porphinate Fe(III) cloride. Proc. XV National Congress of Analytic Chemistry, September 27 - October 1, 1999, Palermo, Italy

9) **Triolo, F.**, and Piñol-Roma, S.: Isolation and Characterization of Human Extranucleolar Pre-ribosomes. 39th American Society for Cell Biology Annual Meeting - December 11-15, 1999, Washington, DC
Mol. Biol. Cell 10:2538 Suppl., 1999

10) Triolo, A., **Triolo, F.**, Lo Celso, F., and Triolo, R.: From Unimer to Aggregates and Back: Exploring the Critical Micellization Density Concept. 8th Annual Meeting of the Italian Society of Synchrotron Light – June 29 – July 1, 2000, Palermo, Italy

11) **Triolo, F.**, and Piñol-Roma, S.: Nucleoplasmic pre-ribosomes from human cells: Isolation and Characterization. 5th International Conference on Ribosome Biogenesis and Nucleolar Function – August 17-21, 2000, Granlibakken, CA. See also T. Pederson (2001). Viewing the Ribosome and Visiting the Nucleolus at Lake Tahoe, RNA, 7, 1-4

12) Pellerito, C., Di Stefano, R., Scopelliti, M., Fiore, T., Duca, D., **Triolo, F.**, and Pellerito, L.: Interaction of Organotin(IV) moieties with MeCl[meso-tetra(4-sulfonatophenyl) porhine] (Me = Fe, Mn). Preliminary solid state investigation and antitumoral activity. 41st Mössbauer Discussion Group –September 3-4, 2000, University of Greenwich, London, UK

13) Lo Celso, F., Triolo, A., **Triolo, F.**, and Triolo, R.: Structural investigation on industrial appealing polymers dissolved in supercritical carbon dioxide. XXXI National Congress of Physical Chemistry – June 19-23, 2001, Padua, Italy

14) Triolo, A., **Triolo, F.**, Triolo, R., Lucido, G., Riso, A., and Lo Celso, F.: Fractal structure of geological materials. XXXI National Congress of Physical Chemistry – June 19-23, 2001, Padua, Italy

15) Lo Celso, F., Triolo, A., **Triolo, F.**, Thiyagarajan, P., Amenitsch, H., Steinhart, M., Kriechbaum, M., DeSimone, J.M., and Triolo, R.: A combined small-angle neutron and X-ray scattering study of block copolymers micellisation in supercritical carbon dioxide. XII International Conference on Small-Angle Scattering - August 25-29, 2002, Venice, Italy

16) Carruba, G., Granata, O.M., Campisi, I., **Triolo, F.**, Vizzini, G., Tamburo De Bella, M., Leonardi, V., Gridelli, B., and Agostara, B.: Biomolecular and genomic profiling of human liver tissues and cells. 7th World Congress on Gastrointestinal Cancer – June 15-18, 2005, Barcelona, Spain

17) Provenzani, A., Poma, P., Labbozzetta, M., Notarbartolo, M., Di Stefano, R., **Triolo, F.**, Vizzini, G., Gridelli, B., D'Alessandro, N.: Influence of CYP3A5 and MDR-1 Single Nucleotide Polimorphisms on the pharmacokinetics of tacrolimus in Caucasian liver transplant recipients. 33rd National Congress of the Italian Society of Pharmacology – June 6-9, 2007, Cagliari, Italy

18) Provenzani, A., Di Stefano, R., D'Alessandro, N., **Triolo, F.**, Vizzini, G.B., and Gridelli, B.: Possible pharmacological interaction between corticosteroids and a calcineurin inhibitor (tacrolimus) in liver transplant patients. 10th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology, September 9-14, 2007, Nice, France. Ther Drug Monit 29(4): 530-531, 2007

19) Marcenò, R., **Triolo, F.**, Cappuzzo, V., Amico, G., Gridelli, B., Vizzini, G., Gerlach, J., Miki, T., Chinnici, C., Ingrassia, F., Galvagno, D., Bavetta, R., Carcassi, C., Macchiarella, A., Mistretta, S., Merlo D., and Tortorici, G.: Immunological Advantages of Human Fetal Hepatocytes for the Treatment of Liver Disease? 22nd European Immunogenetics and Histocompatibility Conference, April 2-5, 2008, Toulouse, France
Tissue Antigens, 71(4):306-307, 2008

20) Provenzani, A., Notarbartolo, M., Biondi, F., Labbozzetta, M., Poma, P., Vizzini, G., Palazzo, U., Polidori, P., **Triolo, F.**, Gridelli, B., and D`Alessandro, N.: The Effect of CYP3A5 and ABCB1 Single Nucleotide Polymorphisms on Tacrolimus Dose Requirements in Caucasian Liver Transplant Patients. immunoTDM Warsaw 2008 International Conference Therapeutic Drug Monitoring in Optimizing the Immonosuppressive Therapy – September 26-27 2008, Varsavia, Poland

21) **Triolo, F.**, Di Bartolo, C., Lopez, F., Piazza, T., Gerlach, J.C., and Gridelli, B.: Application of a Quality Risk Management Model Approach for Cell Therapy Manufacturing to a Clinical Study on Human Fetal Liver Progenitor Cell Transplantation in Endstage Liver Disease. 2009 McGowan Institute Scientific Retreat – March 9-11, 2009, Farmington, PA

22) Piazza, T., Collura, F., Farinella, E., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: An Automated Information System for Human Fetal Liver Cell Manufacturing. 2009 McGowan Institute Scientific Retreat – March 9-11, 2009, Farmington, PA

23) Gridelli, B., Vizzini, G., Pietrosi, G., **Triolo, F.**, Luca, A., Cintorino, D., Conaldi, P.G., Amico, G., D'Amato, M., Chinnici, C., Sisino, G., Timoneri, F., Miki, T., and Gerlach, J.C.: Human liver progenitor cell transplantation (CTx) for endstage liver failure - initial report from a clinical feasibility study. 2009 McGowan Institute Scientific Retreat – March 9-11, 2009, Farmington, PA

24) Ring, A., Gerlach, J., Peters, G., Pazin, B.J., Minervini, C.F., **Triolo, F.**, Gridelli, B., and Miki, T.: Four-compartment 3D Perfusion Bioreactor Culture System Enhances Hepatic Maturation on Human Fetal Hepatocytes. 4th Annual Meeting of the European Association for the Study of the Liver. – April 22-26, 2009, Copenhagen, Denmark. J Hepatol, 50, S312-13, 2009

25) Amico, G., Chinnici, C., D'Amato, M., Sisino, G., Timoneri, F., Li Petri, S., Cintorino, D., Conaldi, P.G., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: Cytofluorimetric Characterization of Human Fetal Liver Cells. 6th National Congress of the Italian Society for Clinical and Experimental Cytometry – May 6-8, 2009, Catania, Italy **(best poster award)**

26) Giandalia, G., De Caro, V., Siragusa, M.G., Chiodo, F., Cordone, L., Gridelli, B., D'Amato, M., **Triolo, F.**, and Giannola, L.I.: Inclusion of Trehalose (TRH) into liposomes to regulate uptake of this cryoprotectant into human hepatocytes. Young Pharmaceutical Scientists Meet in Nice – June 7-8, 2009, Nice, France

27) **Triolo, F.**, Ring, A., Miki, T., Gridelli, B., and Gerlach, J.C.: Hepatic maturation of human fetal hepatocytes in four compartment 3D perfusion culture. Sicilian Biotechnology Day – June 20, 2009, Catania, Italy

28) Chinnici, C.M., Johnen, C., **Triolo, F.**, Gridelli, B., and Gerlach, J.C.: Development of a skin regeneration approach based on the combined use of human fetal keratinocytes and an innovative cell spray deposition system. Sicilian Biotechnology Day – June 20, 2009, Catania, Italy

29) Piazza, T., Collura, F., Farinella, E., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: Development of an automated information system for cell therapy manufacturing. Sicilian Biotechnology Day – June 20, 2009, Catania, Italy

30) Provenzani, A., Notarbartolo, M., Labbozzetta, M., Poma, P., Vizzini, G., Palazzo, U., Polidori, P., **Triolo, F.**, Salis, P., Caccamo, C., Bertani, T., Gridelli, B., and D'Alessandro, N.: Distribution of CYP3A5 and ABCB1 and their influence on tacrolimus kidney transplant patients. 11th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology – October 3-8, 2009, Montréal, Québec, Canada

31) Lopez, F., Di Bartolo, C., Piazza, T., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: A Quality Risk Management Model for Cell Therapy Manufacturing. 4th Healthcare Risk Management Forum. – November 24-27, 2009, Arezzo, Italy

32) Young, M., Chinnici, C., Plettig, J., Johnen, C., Bräutigam, K., **Triolo, F.**, Turner, M.E., Thompson, R.L., Over, P., Amico, G., and Gerlach, J.C.: Dermal Skin Stem Cells: Exploring Human Fetal Skin Progenitor Cells for Regenerative Medicine Cell-Based Therapy Development. 2010 McGowan Institute Scientific Retreat – March 7-10, 2010, Farmington, PA

33) Amico, G., Chinnici, C., Pasqua, S., Li Petri, S., Pietrosi, G., Vizzini, G., Conaldi, P.G., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: Cytofluorimetric Characterization of Human Fetal Liver Cells. 15th Leipziger Workshop, "Cytomics and Stem Cells" – April 22-24, 2010, Leipzig, Germany

34) Lopez, F., Di Bartolo, C., Piazza, T., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: A Quality Risk Management Model Approach for Cell Therapy Manufacturing. 19th Annual Meeting of the Society for Risk Analysis - Europe – June 21-23, 2010, London, UK

35) Pietrosi, G., Vizzini, G.B., Conaldi, P.G., D'Amato, M., Amico, G., **Triolo, F.**, Spada, M., Alio, L., Gerlach, J.C., and Gridelli, B.: Fetal Human Liver Progenitor Cells: A Potential Immune-Privileged Cellular Source from Non Heart Beating Donors. Proc. "From fetomaternal tolerance to immunomodulatory properties of placenta-derived cells in cell therapy" EMBO Workshop. October 3-6, 2010, Brescia, Italy **(best poster award)** - Placenta 32(Suppl. 4): S337, 2011

36) Gruttadauria, S., Seria, E., Pagano, D., Conaldi, P.G., Vizzini, G., Mangano, K., Liotta, R., Amico, G., Luca, A., **Triolo, F.**, Basile, F., and Gridelli, B.: Bone Marrow-Derived Mesenchymal Stem Cells To Augment Liver Regeneration in a Preclinical Model of Acute Liver Failure in Rats. The 2011 Joint International Congress of ILTS, ELITA, and LICAGE – June 22-25, 2011, Valencia, Spain. Liver Transplant, 17(6) Suppl. 1: S199-S200, 2011

37) Hetz, R.A., **Triolo, F.**, Olson, S.D., Smith, P., Day, M., Johnson, A., Moise, K.J. and Cox, C.S. Jr. Amniotic Fluid Derived Mesenchymal Stromal Cells: Characterization and Logistics of Clinical Grade Cell Production. Academic Surgical Congress – February 5-7, 2013 – New Orleans, LA – J Surg Res 179(2):189, 2013

38) Hetz R.A., **Triolo F.**, Olson S.D., Bedi S., Kota D.J., Roye J., Smith P., Day M.C., DiCarlo B., Cox Jr C.S. Amniotic fluid derived mesenchymal stem cells: potential hazardous in the treatment of traumatic brain injury. International Society of Stem Cell Research (ISSCR) 11th Annual Meeting – June 12-15 2013 – Boston, MA

39) Evans S., Kota D.J., Hetz H., Olson S.D., **Triolo F.**, Cox Jr C.S., Wenzel P. MSC licensing by biomechanical forces. International Society of Stem Cell Research (ISSCR) 11th Annual Meeting – June 12-15 2013 – Boston, MA

40) DiCarlo B., Kota D.J., **Triolo F.**, Adams B.D., Bailey V., Prabhakara K.S., Olson S.D. Looking for Biomarkers for Mesenchymal Stem Cell Potency. Mission Connect 2013 Annual Scientific Symposium – December 2013 – Houston, TX

41) Sahai, S., Wilkerson, M., Vitale, F., Tsentalovich, D., Kiran, S., Pasquali, M., Cox, C.S. Jr., and **Triolo, F.** Biophysical Characterization of Native Wharton's Jelly for Tissue Engineering Applications. 41st Annual Congress of the European Society for Artificial Organs – September 17-20, 2014 – Rome, Italy – Int J Artif Organs 37(8):595, 2014

42) Sahai, S., Wilkerson, M., Vitale, F., Tsentalovich, D., Kiran, S., Pasquali, M., Cox, C.S. Jr., and **Triolo, F**. Characterization of Native Wharton's Jelly: A Natural Tissue Engineering Construct. 2014 Annual Meeting of the Biomedical Engineering Society – October 22-25, 2014 – San Antonio, TX

43) Liao, G.P., Vojnits, K., Choi, Y., Aroom, K.R, Hetz, R.A., Xue, H., **Triolo, F.**, Li, Y., Lally, K.P., Cox, C.S. Jr. Human Amniotic Fluid derived Multipotent Stromal Cells Enhance Decellularized Diaphragm Scaffold Regeneration and Function in a Rodent Model of Congenital Diaphragmatic Hernia. American College of Surgeons Clinical Congress 2014 – October 26-30, 2014 – San Francisco, CA – J Am Coll Surg 219(3):S74-S75, 2014

44) Wilkerson, M., Sahai, S., Vitale, F., Tsentalovich, D., Kiran, S., Pasquali, M., Cox, C.S. Jr., and **Triolo, F**. Native Wharton's Jelly: a Natural Injectable Biomaterial for Autologous Tissue Engineering Applications. 10th Conference of Italian Researchers in the World – December 6, 2014, Houston, TX

45) Kota D.J., Liao G.P., Prabhakara K.S., DiCarlo B., Evans S., **Triolo F.**, Wenzel P., Cox C.S., Olson S.D. Predicting therapeutic efficacy of MSC in TBI through anti-inflammatory potency. Mission Connect 2014 Annual Scientific Symposium – December 2014 – Houston, TX

46) Liao, G.P., Hetz, R.A., Kota, D.J., Hughes, T.G., Corkins, C.J., Xue, H., Moise, K.J., Johnson, A., Olson, S.D., **Triolo, F.** and Cox, C.S. Jr. Donor Phenotype Influences Performance of Human Amniotic Fluid Derived Mesenchymal Stromal Cells as Therapy for Traumatic Brain Injury. APSA 2015, 46th Annual Meeting of the American Pediatric Surgical Association – April 30 – May 3, 2015, Fort Lauderdale, FL

B. Refereed Original Articles in Journals

1) **Triolo, F.**, Graziano, V., and Heenan, R.K.: Small Angle Neutron Scattering Study of the Quaternary Structure of Rapana thomasiana Haemocyanin. J Mol Struct, 383, 249, 1996

2) **Triolo, F.**, Pellerito, C., Stocco, G.C., Fiore, T., Maggio, F., Pellerito, L., and Triolo, R.: Organometallic Complexes with Biological Molecules. XIII. Organotin(IV)[meso-tetra(4-carboxyphenyl)porphinate]s and the cell-cycle: A Flow-cytometric Approach. Appl Organomet Chem, 13, 733, 1999

3) Fiore, T., Pellerito, C., Fontana, A., **Triolo, F.**, Maggio, F., Pellerito, L., Cestelli, A., and Di Liegro, L.: Organometallic Complexes with Biological Molecules. XII. Solid State and Solution Studies on Dialkyltin(IV) and Trialkyltin(IV)thiaminepyrophosphate derivatives. Appl Organomet Chem, 13, 705, 1999

4) **Triolo, F.**, Triolo, A., Agamalian, M., Lin, J.S., Heenan, R.K., Lucido, G., and Triolo, R.: Fractal approach in petrography: Combining ultra small angle, small angle and intermediate angle neutron scattering. J Appl Crystall, 33(3), 863, 2000

5) Triolo, A., **Triolo, F.**, Lo Celso, F., Betts, D.E., McClain, J.B., DeSimone, J.M., Wignall, G.D., and Triolo, R.: Critical Micellisation Density: a Small Angle Scattering Structural Study of the Monomer-Aggregate Transition of block-copolymers in supercritical CO_2. Phys. Rev. E, 62, 5839, 2000

6) **Triolo, F.**, Triolo, A., Triolo, R., Londono, J.D., Wignall, G.D., McClain, J.B., Betts, D.E., Wells, S., Samulski, E.T., and DeSimone, J.M.: Critical Micelle Density for the Self-assembly of Block Copolymer Surfactants in Supercritical Carbon Dioxide. Langmuir, 16, 416, 2000

7) **Triolo, F.**, Triolo, A., Triolo, R., Betts, D., McClain, J.B., DeSimone, J.M., Steytler, D.C., Wignall, G.D., Demé, B., and Heenan, R.K.: Critical micellisation density: a SAS structural study of the unimer-aggregate transition of block-copolymers in supercritical CO_2. J Appl Crystall, 33(3), 641, 2000

8) Triolo, R., Triolo, A., **Triolo, F.**, Steytler, D.C., Lewis, C.A., Heenan, R.K., Wignall, G.D., and DeSimone, J.M.: Structure of diblock copolymers in supercritical Carbon Dioxide and Critical Micellisation Pressure. Phys. Rev. E., 61, 4640, 2000

9) Mansueto, C., Puccia, E., Maggio, F., Di Stefano, R., Fiore, T., Pellerito, C., **Triolo, F.**, and Pellerito, L.: Organometallic complexes with biological molecules. XIV. Biological activity of dialkyl and trialkyltin(IV) - [meso-tetra(4-carboxy-phenil)porphinate] derivatives. Appl Organomet Chem, 14(5), 229, 2000

10) Puccia, E., Mansueto, C., Cangialosi, M.V., Fiore, T., Pellerito, C., **Triolo, F.**, and Pellerito, L.: Organometallic Complexes with Biological Molecules. XV. Effects of Tributyltin(IV)chloride on enzyme activity, Ca^{+2} and biomolecule content and synthesis in Ciona intestinalis (Urochordata) ovary. Appl Organomet Chem, 15, 213, 2001

11) Lo Celso, F., Triolo, A., **Triolo, F.**, McClain, J., DeSimone, J.M., Heenan, R.K., Amenitsch, H., and Triolo, R.: Industrial applications of the aggregation of block copolymers in supercritical CO_2: a SANS study. Applied Physics A- Materials Science & Processing 74(2): S1427-S1429, 2002

12) Triolo, A., Lo Celso, F., **Triolo, F.**, Amenitsch, H., Steinhart, M., Thiyagarajan, P., Wells-Kennedy, S., DeSimone, J.M., and Triolo, R.: Kinetics of block-copolymer aggregation in super critical CO_2. J Noncrystal Solids, 307, 725, 2002

13) Lo Celso, F., Triolo, A., **Triolo, F.**, Donato, D.I., Steinhart, M., Kriechbaum, M., Amenitsch, H., and Triolo, R.: SAXS investigation on aggregation phenomena in supercritical CO_2. Eur. Phys. J. E, 8, 311, 2002

14) **Triolo, F.**, Triolo, A., Lo Celso, F., Donato, D.I., and Triolo, R.: Dilute and semi dilute solutions of block copolymers in water, near-critical and super-critical CO_2: a small angle scattering study of the monomer-aggregate transition. Physica A, 304, 135, 2002

15) Lo Celso, F., Triolo, A., **Triolo, F.**, Thiyagarajan, P., Amenitsch, H., Steinhart, M., Kriechbaum, M., DeSimone, J.M., and Triolo, R.: A combined small-angle neutron and X-ray scattering study of block copolymers micellisation in supercritical carbon dioxide. J Appl Crystall, 36, 660, 2003

16) Provenzani, A., Notarbartolo, M., Labbozzetta, M., Poma, P., Biondi, F., Sanguedolce, R., Vizzini, G., Palazzo, U., Polidori, P., **Triolo, F.**, Gridelli, B. and D'Alessandro, N.: The effect of CYP3A5 and ABCB1 Single Nucleotide Polymorphisms on Tacrolimus Dose Requirements in Caucasian Liver Transplant Patients, Ann. Transplant., 14(1):23-31, 2009. See also Pharmacogenomics 10(5): 716, 2009

17) Frabetti, A., Vandini, A., Migliori, D., Balboni, P., **Triolo, F.**, and Mazzacane, S.: Experimental Evaluation of the Efficacy of Sanitation Procedures in Operating Rooms, Am J Infect Control, 37(8): 658-64, 2009

18) Schmelzer, E., **Triolo, F.**, Turner, M.E., Thompson, R.L., Zeilinger, K., Reid, L.M., Gridelli, B., and Gerlach, J.C.: Three-Dimensional Perfusion Bioreactor Culture Supports Differentiation of Human Fetal Liver Cells, Tissue Eng Part A, 16(6):2007-16, 2010

19) Ring, A., Gerlach, J., Peters, G., Pazin, B.J., Minervini, C.F., Turner, M.E., Thompson, R.L., **Triolo, F.**, Gridelli, B., and Miki, T.: Hepatic Maturation of Human Fetal Hepatocytes in Four-compartment 3D Perfusion Culture, Tissue Eng Part C Methods, 16(5):835-45, 2010

20) Lopez, F., Di Bartolo, C., Piazza, T., Passannanti, A., Gerlach, J.C., Gridelli, B., and **Triolo, F.**: A Quality Risk Management Model Approach for Cell Therapy Manufacturing, Risk Anal, 30(12):1857-71, 2010 (**January 2011 Publication of the month**, McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA)

21) Balmert, S.C., McKeel, D., **Triolo, F.,** Gridelli, B., Zeilinger, K., Bornemann, R., and Gerlach, J.C.: Perfusion Circuit Concepts for Hollow-Fiber Bioreactors Used as In Vitro Cell Production Systems or Ex Vivo Bioartificial Organs, Int J Artif Organs, 34(5):410-21, 2011

22) Pietrosi, G., Vizzini, G., Luca, A., Spada, M., Conaldi, P.G., **Triolo, F.,** Gerlach, J.C., and Gridelli, B.: Is Human Fetal Liver Stem Cell Transplantation a Panacea for Alcohol-Induced Liver Decompensation? Cell Transplant, 20(9):1497, 2011

23) Gridelli, B., Vizzini, G., Pietrosi, G., Luca, A., Spada, M., Gruttadauria, S., Cintorino, D., Amico, G., Chinnici, C., Miki, T., Schmelzer, E., Conaldi, P.G., **Triolo, F.**, and Gerlach, J.C.: Efficient human fetal liver cell isolation protocol based on vascular perfusion for liver cell-based therapy and a case report of fetal liver cell transplantation, Liver Transplant, 18:226-37, 2012

24) Johnen, C., Chinnici, C., **Triolo, F.**, Plettig, J., Bräutigam, K., Amico, G., Young, M., Over, P., Esteban-Vives, R., Schmelzer, E., Conaldi, P.G., Turner, M., Thompson, R., Zeilinger, K., Rubin, P., Vizzini, G., Gridelli, B. and Gerlach, J.C.: Phenotypical characterization of 6 - 21 week gestational age human dermis and epidermal cell Isolation methods for in vitro studies on epidermal progenitors, Burns, 39(2):300-10, 2013

25) Sahai, S., Wilkerson, M., Zaske, A.M., Olson, S.D., Cox, C.S. Jr. and **Triolo, F**. A Cost-Effective Method to Immobilize Hydrated Soft-Tissue Samples for Atomic Force Microscopy. BioTechniques, 61(4):206-09, 2016

26) Hetz, R.A., Liao, G.P., Aertker, B.M., Ewing-Cobbs, L., Juranek, J., Savitz, S.I., Jackson, M.L., Romanowska-Pawliczek, A.M., **Triolo, F.**, Dash, P.K., Pedroza, C., Lee, D. Worth, L., Aisiku, I., Choi, H.A., Holcomb, J.B., Kitagawa R.S. and Cox, C.S. Jr. Treatment of Severe Adult Traumatic Brain Injury Using Bone Marrow Mononuclear Cells, Stem Cells, in press

27) Liao, G.P., Choi, Y., Vojnits, K., Xue, H., Aroom, K., Meng, F., Pan, H.Y., Hetz, R.A., Corkins, C.J., Hughes, T.G., **Triolo, F.**, Lally, K.P., Cox, C.S. Jr. and Li, Y.

Tissue Engineering to Repair Diaphragmatic Defect in a Rodent Model. Submitted

C. Invited Lectures

1) **Triolo, F.**: The Mediterranean Cell Factory: A Joint Project of ISMETT and the University of Palermo. 12th Regional Congress of the Italian Diabetes Society – October 28-29, 2004, Messina, Italy

2) **Triolo, F.**, Piazza, T., and Romero Lauro, G.: Telescience and Virtual Collaboratories – Workshop on Image Analysis in Medicine and Biology – February 16, 2005, Palermo, Italy

3) **Triolo, F.**: Certified Cell Production for Clinics and Research through Good Manufacturing Practices. Biotecnologies: a transversal theme of the 7th European Research Framework Program – March 3, 2006, Messina, Italy

4) **Triolo, F.**, Gridelli, B., and Scardulla, C.: Congestive Heart Failure Treatment with Autologous Stem Cell Therapy. Adult Human Stem Cells: Signalling, Differentiation, Transplantation Workshop – May 19, 2006, Palermo, Italy

5) **Triolo, F.**: Fetal Progenitors for Cell-based Therapies. New Frontiers in Cell-based Research and Therapy at ISMETT Minisymposium. May 30, 2007, Palermo, Italy

6) **Triolo. F.**: Regeneration Biotechnology as a Source of Therapeutical and Economic Developments. Bioincubators of Ideas Meeting, sponsored by the National Committee for Biosafety, Biotechnology and Life Sciences of the Presidency of the Council of Ministers. October 3, 2007, Milan, Italy

7) **Triolo, F.**: Stem Cells and Regenerative Medicine: Possible Application in Dermatology. XII Oncoderm Meeting: Stem Cells and Dermatology – Physician-Patient Communication Techniques, December 15, 2007, Palermo, Italy

8) **Triolo, F.**: The New Frontiers of Regenerative Medicine. FIDAPA District Congress “Stem Cells: The Present and The Future” – October 19, 2008, Capo d’Orlando (ME), Italy

9) **Triolo, F.**: Development of a Cell Therapy Center: The ISMETT Experience in Palermo. Cell Therapy 2008: A European Perspective – October 27, 2008, Milan, Italy

10) **Triolo. F.**: Advanced Therapies and Regenerative Medicine. Introduction to Regenerative Medicine Workshop – November 10, 2008, Palermo, Italy

11) **Triolo, F.**: Regenerative Medicine and Neurodegenerative Diseases. Introduction to Regenerative Medicine Workshop – November 24, 2008, Palermo, Italy

12) **Triolo, F.**: Research Without Borders: Telescience and Virtual Collaboratories. Festival of Innovation – December 3, 2008, Bari, Italy

13) **Triolo, F.**: Cell Therapy Manufacturing and Advanced Therapies: The ISMETT experience in Palermo. Seminars of Medical Culture for the Population. Second Cycle – Year 2009 – February 11, 2009, Palermo, Italy

14) **Triolo, F.**, and Piazza, T.: Development of an Automated Information System for Cell Therapy Manufacturing. Bio-IT World Conference & Expo '09 Europe – October 6-8, 2009, Hannover, Germany

15) **Triolo. F.**: The Importance of Grantsmanship in Increasing Competitiveness. Info day – Funding Sources for Research – October 27, 2009, Palermo, Italy

16) **Triolo, F.**: Innovation in Cell Therapy. 9th National Pharmaceutical Conference – December 11, 2009, Catania, Italy

17) **Triolo, F.**: Developing a State-of-the-Art Cell Production Facility for the Clinical Translation of Innovative Cell-based Therapies, Roswell Park Cancer Institute – July 16, 2010, Buffalo, NY

18) **Triolo, F.**: Keep an eye on Regenerative Medicine: State of the Art and Future Perspectives in Ophthalmology. Grandangle 2010: Hot Topics in Ophthalmology – October 22-23, 2010, Milan, Italy

19) **Triolo, F.**: Innovations in Gene Therapy. Grandangle 2010: Hot Topics in Ophthalmology – October 22-23, 2010, Milan, Italy

20) **Triolo, F.**: Potentiating Translation of Innovative Cell-based Therapies in Regenerative Medicine at UTHealth. Center for Stem Cell and Regenerative Medicine (CSCRM) and Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM), University of Texas Medical School – June 4, 2012, Houston, TX

21) **Triolo, F.**: (Chair, Tissue Engineering and Imaging session): Implementing Clinical Trials in Regenerative Medicine: Challenges and Requirements. Nanocourse "Design and implementation for first-in-human oncology trials" – July 10, 2012, Houston, TX

22) **Triolo, F.**: Stem Cell and Regenerative Medicine Applications in Neurological Injury at UTHealth. 8th Conference of Italian Researchers in the World – December 1, 2012, Houston, TX

23) **Triolo, F.**: Achieving GMP Scalable Production for Study of Regenerative Medicine Products in Clinical Trials. REGEN 2013: Clinical Trials & Reimbursement. Improving Clinical Trial Design & Execution Strategies for Cell Based Regenerative Medicines – March 19-21, 2013, Boston, MA

24) **Triolo, F.**: Development of Cell-based Therapies and Regenerative Medicine Applications at UTHealth. Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research seminar series, University of Southern California – April 22, 2014, Los Angeles, CA

25) **Triolo, F.** Cell Banking to Enable Cell Therapy: Hurdles and Tricks to Make it Work. XLI Annual Congress of the European Society for Artificial Organs (ESAO) – September 17-20, 2014, Rome, Italy

26) **Triolo, F.** (Artificial vs. Biological Substitutes Crossfire Session) En Route to Regeneration: Keep Calm and Carry On. XLI Annual Congress of the European Society for Artificial Organs (ESAO) – September 17-20, 2014, Rome, Italy

27) **Triolo, F.** Establishment of a Clinical Grade Amniotic Fluid-derived Mesenchymal Stromal Cell Bank. 2014 Regenerative Medicine Symposium – Gulf Coast Cluster for Regenerative Medicine of the Gulf Coast Consortia for Quantitative Biomedical Sciences, October 3, 2014, Houston, TX

28) **Triolo, F.** "Is Your Idea a Good One if No One Knows About it: A Panel Discussion on Moving Your Research Ideas, Today, into Tomorrow's Cures". 2015 4th Tissue Engineering and Regenerative Medicine International Society (TERMIS) World Congress, September 8-11, 2015, Boston, MA

D. Invited Articles (Reviews, Editorials, etc.) in Journals

1) **Triolo, F.:** SAXS and SANS: A Biologist's Point of View. Notiziario Neutroni e Luce di Sincrotrone, 5 (2), 23, 1995

2) **Triolo, F.**, and Gridelli, B.: End Stage Organ Failure: Will Regenerative Medicine Keep its Promise? Cell Transplantation, 15(Suppl. 1):S3–S10, 2006

3) **Triolo, F.**, Pietrosi, G., Scardulla, C., and Gridelli, B.: Transplantation and Regeneration in the Heart of the Mediterranean. Mech. Ageing Dev., 128(1):5–8, 2007

4) **Triolo, F.** and Lo Celso, F.: Roberto Triolo - A 40 Year Legacy for Young Scientists. In M. Pagliaro (Ed.), FineCat 2015 Symposium on Heterogeneous Catalysis for Fine Chemicals Book of Abstracts, Simplicissimus Book Farm, New York, 2015. ISBN: 9788869094255

E. Chapters

1) Lo Celso, F., **Triolo, F.**, Triolo, A., Lin, J.S., Lucido, G., and Triolo, R.: Fractal Approach in Petrology: Combining Ultra Small Angle (USANS) and Small Angle Neutron Scattering (SANS). In: A. Messina (Ed.), Nuclear and Condensed Matter Physics, American Inst. of Physics, Melville (NY), pp. 138-141, 2000

2) **Triolo, F.**, Triolo, A., Lo Celso, F., Johnson Jr., J.S., Donato, D.I., and Triolo, R.: Modeling Small Angle Neutron Scattering Data from Polymers in Supercritical Fluids. In: A. Messina (Ed.), Nuclear and Condensed Matter Physics, American Inst. of Physics, Melville (NY), pp. 222-25, 2000

3) Pellerito, L., Barbieri, R., Di Stefano, R., Scopelliti, M., Pellerito, C., Fiore, T., and **Triolo, F.**: Toxic effects of organometallic compounds towards marine biota. In: A. Gianguzza, E. Pelizzetti and S. Sammartano (Eds.), Chemistry of Marine Water and Sediments, Springer-Verlag, New York, pp 337-382, 2002

4) Miki, T. and **Triolo F.** Functional Dualism of Perinatal Stem Cells. In: B. Arjmand (Ed.), Perinatal Tissue-Derived Stem Cells. Stem Cell Biology and Regenerative Medicine Series, Springer, New York, in press

F. Dissertations

1) **Triolo, F.**: Neutron Scattering from Hemocyanins. (pp. 1-119). Undergraduate Dissertation in Biological Sciences. University of Palermo, Palermo, Italy, 1994

2) **Triolo, F.**: Organometals and Biological Systems: Structural and Cytotoxic Investigations. (pp. 1-120). Ph.D. Dissertation in Chemical Sciences, University of Palermo, Palermo, Italy, http://www.opengrey.eu/item/display/10068/309481, 1999

3) **Triolo, F.**: Human Pre-Ribosomes: Isolation and Characterization. (pp. 1-109). Ph.D. Dissertation in Biomedical Sciences. Mount Sinai School of Medicine of New York University, New York, NY. ISBN: 0-493-48621-6. UMI Publication Nr. 3035739. ANN HARBOR, MI: ProQuest Information and Learning Co., 2002

TECHNOLOGY DEVELOPED:

1) **Triolo, F.**, and Piazza, T.: Biological Safety Cabinet with Integrated Telerobotic Video-monitoring System.

2) **Triolo, F.**, and Piazza, T.: cGMP-compliant Electronic Batch Records System for Cell Therapy Facilities.

3) **Triolo, F.**, and Piazza, T.: cGMP-compliant Environmental and Instrumental Monitoring System for cGMP Facilities.

4) Gerlach, J., Bold. T., and **Triolo, F.**: Automated cell infusion device with built-in shaker.

5) Piazza, T., Collura, F., Grosso, E., and **Triolo, F.**: Wireless Environmental and Cell Culture Monitoring System.

MEDIA APPEARANCES:

Television

1) News coverage of the opening of the State-of-the-Art Human Cell Processing cGMP Facility designed and directed by Dr. Triolo at the University of Pittsburgh Medical Center (UPMC)'s Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) in Palermo, Italy. Tg1 (Italy's national public TV channel RAI 1's newscast) – May 16, 2007

2) Special on the State-of-the-Art Stem Cell Production Facility designed and directed by Dr. Triolo at UPMC ISMETT in Palermo, Italy. Tg3 Sicilia (Regional broadcast of Italy's national public TV channel RAI 3's newscast) – May 17, 2008

3) Special on Regenerative Medicine, Bioreactors and Extracorporeal Support for Liver Failure. Elisir (Program on Health and Medicine related topics aired on Italy's national public broadcasting company RAI) – January 4, 2009

4) Special on the Tissue Engineering and Bone Regeneration Program at UPMC ISMETT in Palermo, Italy. Tg3 (Italy's national public TV channel RAI 3's newscast) Sicilia – June 11, 2009

5) Interview to Dr. Triolo with footing showing the Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory at UTHealth. RAI Italia (RAI Italia is the international television service of RAI Internazionale, a subsidiary of RAI, Italy's public national broadcasting company) – May 12, 2014

6) Interview to Dr. Triolo within the educational documentary "Texan Italian Innovation", which paints a comprehensive picture of the state of research and innovation in the Italian community in Texas. The piece highlights the work of diverse innovators from a wide range of fields, from biomedicine to modern languages to music. ITALchannel.tv – May 25, 2014

7) Interview to Dr. Triolo within the feature story "USA 2016. Verso il Super Tuesday, Viaggio tra gli Italiani in Texas" (USA 2016. Towards Super Tuesday. A Trip Among Italians in Texas). RAINews (RAI is Italy's public national broadcasting company) – February 26, 2016

Newspapers and Magazines

1) Dentro la Fabbrica delle Cellule (Inside the Cell Factory). Il Sole 24 Ore (one of Italy's major national newspapers) – May 31, 2007

2) Palermo, la Fabbrica degli Organi (Palermo, The Organ Factory). Corriere della Sera Magazine (one of Italy's major national newspapers) – December 13, 2007

3) Trapianti del Futuro. Nella "fabbrica di cellule" capaci di aggiustare gli organi (Transplants of the future. In the "factory of cells" able to repair organs). La Repubblica (one of Italy's major national newspapers) – May 4, 2010

4) Study Investigates Risk Management in Regenerative Medicine. UPMC International Extra! (also published on regenerativemedicine.net) – November 2010

5) Program Explores Use of Stem Cells to Treat Pediatric Birth Defects. Texas Medical Center News – August 1, 2012

6) Cervelli in fuga: Palermitano a Houston ai Vertici della Ricerca Scientifica (Brain Drain: a Native of Palermo in Houston at the Cutting-Edge of Scientific Research). SiciliaInformazioni.com (one of Sicily's major online newspapers) – May 28, 2014. The English version of the article, entitled "Brain Drain: a Native of Palermo in Houston", was published on NowItaly.com on May 30, 2014

7) Italian Doctors Honored in a Sold-out Casino Night that Leaves Everyone Feeling Lucky. CultureMap Houston and Houston City and Press – August 25, 2014

8) 2014 Italian Flame Awards Honor 21 Italians, Italian-Americans from the Medical Field. YourHoustonNews.com – August 26, 2014. Also published on EIN News World News Report – August 27, 2014

9) Houston, Ecco la Nuova Frontiera dei Cervelli "Made in Italy" (Houston, Here is the New Frontier of "Made in Italy" Brains). L'Espresso (one of Italy's major magazines) – September 2, 2014

10) It and US: 2014 Italian Flame Awards! We the Italians magazine – September 3, 2014

11) ICCC Gala and Casino Night. Houstonia Magazine – September 4, 2014

12) Italian Cultural Association Honors Faculty. UT Medical School at Houston News – September 4, 2014

13) Conservazione Cellule Staminali Cordone Ombelicale, Fabio Triolo (Umbilical Cord Stem Cell Storage, Fabio Triolo). Newsscienze.com – September 6, 2014

14) Premiazione degli Italian Flame Award per il 2014 (2014 Italian Flame Awards Ceremony) SiciliaInformazioni.com (one of Sicily's major online newspapers) – October 29, 2014

15) Lucio Luca Rende Onore ai Personaggi Siciliani "Dall'altra Parte della Luna" (Lucio Luca Honors Sicilian Personalities in "On the Other Side of the Moon"). ilsitodipalermo.it – November 24, 2014

16) "Dall'altra Parte della Luna": la Storia dei Siciliani d'America che ce l'Hanno Fatta. (On the Other Side of the Moon: the Story of Sicilians of the US who Have Succeeded). extraquotidiano.it – November 25, 2014.

17) Cento Anni tra i Banchi del CEI. Le Foto degli Studenti in Mostra. [100 Years Among the School Desks of the Ignatian Center for Education (CEI). An Exhibition of the Pictures of Distinguished Students]. LiveSicilia.it (one of Sicily's major online newspapers) – November 27, 2014.

18) Quali Sono i Siciliani che Hanno "Sfondato" negli USA? Lo Svela Lucio Luca nel suo Nuovo Libro (Who are the Sicilians who Have Made it Big in the USA? Lucio Luca Reveals it in his New Book). glittersicilia.it – December 1, 2014

19) Ellis Island, Addio. I Nuovi Siciliani Scoperti dall'America. (Farewell Ellis Island. The New Sicilians Discovered by America). La Repubblica (one of Italy's major national newspapers) – December 4, 2014

20) I Siciliani che mi Piacciono (The Sicilians I like). dipalermo.it (one of Palermo's major online newspapers) – December 6, 2014

21) Scienza, Palermo come Houston: 6 Dicembre Dedicato ai "Cervelli Made in Italy" (Science, Palermo like Houston: December 6 Dedicated to Made Italy Brains). meteoweb.eu – December 7, 2014

22) A Houston Cervelli Made in Italy nel Mondo (In Houston Made in Italy Brains of the World). SiciliaInformazioni.com (one of Sicily's major online newspapers) – December 8, 2014

23) La Biomedicina degli "Italian Brain" (The Biomedicine of Italian Brains). La Repubblica (one of Italy's major national newspapers) – December 9, 2014

24) Decima Conferenza dei Ricercatori Italiani nel Mondo, un Trionfo Italiano (10th Conference of Italian Researchers in the World: An Italian Triumph). Agenzia Internazionale Stampa Estero – December 15, 2014

25) Si è Svolta a Houston la X Conferenza dei Ricercatori Italiani nel Mondo (The 10th Conference of Italian Researchers in the World Took Place in Houston). Inform – December 15, 2014

26) La Decima Conferenza dei Ricercatori Italiani nel Mondo, un Trionfo del Tricolore (The 10th Conference of Italian Researchers in the World: A Triumph of the Tricolor). Corriere di Puglia e Lucania– December 14, 2014; We the Italians magazine – December 16, 2014

27) Terrasini: Dall'altra parte della Luna (Terrasini: On the other side of the moon). Ilvespro.it – December 21, 2014; a similar article was also published on terrasinioggi.it – December 23, 2014

28) IT and US: Dall'Altra Parte della Luna (On the Other Side of the Moon). We the Italians magazine – March 8, 2015

29) Quei Siciliani che ce l'Hanno Fatta, Storie di Emigrati di Successo negli USA (Those Sicilians who Have Succeeded, Stories of Successful Emigrants to the USA). La Repubblica (one of Italy's major national newspapers) – March 30, 2015

30) Texas all'Italiana. L'Eldorado è qui (Texas the Italian Way. The Eldorado is Here). La Repubblica Sera (nightly edition of La Repubblica, one of Italy's major national newspapers) – October 5, 2015

31) Italian President Meets with Faculty Members at UTHealth. University of Texas McGovern Medical School News – March 17, 2016

32) Houston, abbiamo… un palermitano di successo: dall'Ismett agli Usa, Fabio Triolo non si ferma più. [Houston, we have…a successful native of Palermo, from ISMETT (Mediterranean Institute for Transplantation and Advanced Specialized Therapies) to the USA, Fabio Triolo is unstoppable]. insanitas.it (One of Italy's leading healthcare portals) – July 25, 2016

Books

1) Goldstone, Bruce and Lascaro, Rita. Cells at Work: the Laboratory of Dr. Serafín Piñol-Roma. New York, NY: McGraw-Hill, 2002

2) Luca, Lucio. Dall'altra parte della luna: Siciliani d'America che ce l'hanno fatta (On the other side of the moon: Sicilians of the US who have succeeded). Palermo, IT: Pietro Vittorietti Edizioni, 2014

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

HUMAN RESEARCH CURRICULUM COMPLETION REPORT

Printed on 10/08/2014

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INSTITUTION University of Texas Health Science Center at Houston
EXPIRATION DATE 10/07/2017

GROUP 1 BIOMEDICAL RESEARCHER AND KEY PERSONNEL

COURSE/STAGE: Refresher Course/2
PASSED ON: 10/08/2014
REFERENCE ID: 11137207

REQUIRED MODULES	DATE COMPLETED
Biomed Refresher 2 - Instructions	10/08/14
Biomed Refresher 2 – History and Ethical Principles	10/08/14
Biomed Refresher 2 – Regulations and Process	10/08/14
Biomed Refresher 2 – Informed Consent	10/08/14
Biomed Refresher 2 – SBR Methodologies in Biomedical Research	10/08/14
Biomed Refresher 2 – Genetics Research	10/08/14
Biomed Refresher 2 – Records-Based Research	10/08/14
Biomed Refresher 2 - Populations in Research Requiring Additional Considerations and/or Protections	10/08/14
Biomed Refresher 2 – Vulnerable Subjects – Prisoners	10/08/14
Biomed Refresher 2 – Vulnerable Subjects – Children	10/08/14
Biomed Refresher 2 – Vulnerable Subjects – Pregnant Women, Human Fetuses, Neonates	10/08/14
Biomed Refresher 2 – FDA-Regulated Research	10/08/14
Biomed Refresher 2 – HIPAA and Human Subjects Research	10/08/14
Biomed Refresher 2 – Conflicts of Interest in Research Involving Human Subjects	10/08/14
How to Complete the CITI Refresher Course and Receive a Completion Report	10/08/14

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Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

CURRICULUM VITAE

DATE: October 1, 2016

NAME: Jenifer Juranek, Ph.D.

PRESENT TITLE: Associate Professor

ADDRESS: Department of Pediatrics
Division of Developmental Pediatrics
The University of Texas Medical School at Houston
6655 Travis, suite 1000
Houston, Texas 77030
(713) 500-8233
E-mail: Jenifer.Juranek@uth.tmc.edu

BIRTHDATE: December 27, 1969

CITIZENSHIP: United States

UNDERGRADUATE EDUCATION: B.S., Psychology Magna Cum Laude with Honors,1991
Valparaiso University
Valparaiso, Indiana

GRADUATE EDUCATION: Ph.D., Program in Neuroscience, Department of Psychology, 1997
University of California, Riverside
Riverside, California

POSTGRADUATE TRAINING: Postdoctoral Researcher, 1998-1999
Neurophysiology
Department of Neuroscience
University of California, Riverside
Riverside, California

Research Associate/Project Scientist, 1999-2005
Neuroimaging
Department of Pediatrics
University of California Irvine Medical Center
Orange, California

Postdoctoral Scholar as T32 trainee, 2005-2006
Neuroimaging
Department of Anatomy and Neurobiology
University of California, Irvine
Irvine, California

ACADEMIC APPOINTMENTS:

University of Texas – Medical School at Houston:
Department of Neurosurgery:
2006 Assistant Professor

Department of Pediatrics, The Children's Learning Institute:
2006 – 2013 Assistant Professor
2013 – current Associate Professor

PROFESSIONAL ORGANIZATIONS:

NATIONAL:
American Epilepsy Society, 2010
American Clinical Society for Magnetoencephalography, 2012

INTERNATIONAL:
International Neuropsychological Society, 2007
Society for Neuroscience, 1991
Organization for Human Brain Mapping, 2007

HONORS AND AWARDS:
1991 Magna cum laude, Valparaiso University, Indiana
1991 Graduate Opportunity Fellowship, University of California, Riverside
1996 Outstanding Teaching Assistant, University of California, Riverside

EDITORIAL POSITIONS:
Invited Reviewer**:**
2007 Annals of Dyslexia
2008 NeuroImage
2009 European Psychiatric Review
2010 The Cerebellum, NeuroImage
2011 The Cerebellum
2012 Neuropsychologia, European Journal of Pediatrics, Journal of Rehabilitative Medicine

SERVICE ON THE UNIVERSITY OF TEXAS MEDICAL SCHOOL AT HOUSTON COMMITTEES:
Member, Faculty Senate, 2011-2014
Member, CLI faculty search committee for expertise in pediatric fMRI, 2012-2013
Member, Steering Committee for the Scholarly Concentration in Neuroscience
2012: Roxanne Simmons (MS1 Student)

SERVICE TO THE COMMUNITY:

2004: Invited member, Evaluation Review Committee, Children's Hospital of Orange County/University of California Irvine Collaborative, Children and Families Commission of Orange County.

TEACHING EXPERIENCE:

1992-1997: University of California, Riverside, CA

1) For laboratory sections, my responsibilities included preparation of laboratory equipment and exercises, assistance of students with weekly 4 hour experiments, and grading of student lab reports.
2) For discussion sections, my responsibilities included preparation of weekly reviews of lecture material, preparation of weekly quizzes, and grading of exams.

1997		
Spring Quarter	Laboratory	Cellular Neuroscience
Winter Quarter	Laboratory	Cellular Neuroscience
1996		
Spring Quarter	Laboratory	Cellular Neuroscience
Winter Quarter	Laboratory	Cellular Neuroscience
1995		
Fall Quarter	Discussions	Neural Networks and Behavior
Spring Quarter	Laboratory	Cellular Neuroscience
Winter Quarter	Discussions	Cellular Neuroscience
1994		
Fall Quarter	Discussions	Brain and Behavior
Spring Quarter	Laboratory	Cellular Neuroscience
Winter Quarter	Discussions	Systems Neuroscience
1993		
Fall Quarter	Discussions	Cellular Neuroscience
Spring Quarter	Laboratory	Cellular Neuroscience
1992		
Fall Quarter	Discussions	Cellular Neuroscience

SPONSORSHIP OF CANDIDATES FOR POSTGRADUATE DEGREE:

Training/Mentoring graduate students from the University of Houston clinical neuropsychology program:

FY2011: Amery Treble, Tori Williams, Vindia Fernandez
1 Thesis committee: Lyla El-Messidi (March 25, 2011)
2 Thesis committee: Vindia Fernandez (July15, 2011)

FY2012: Emily Maxwell, Tori Williams
1 Thesis committee: Tori Williams (May 14, 2012)

2 Thesis committee: Emily Maxwell (June 26, 2012)
3 Dissertation Committee: Lyla El-Messidi (May 22, 2012)

FY2013: Amery Treble, Vindia Fernandez
1 Dissertation Committee: Amery Treble (October 18, 2012)
2 Dissertation Committee: Vindia Fernandez (June 12 , 2013)

FY2014: Kailyn Bradley, Tori Williams
1 Dissertation Defense Committee: Kailyn Bradley (August 15, 2014)
2 Dissertation Defense Committee: Vindia Fernandez (August 11, 2014)

FY2015: Tori Williams, Nikki Arrington, Lindsey Harrick
1 Dissertation Defense Committee Tori Williams (April 15, 2015)
2 Dissertation Defense Committee: Nikki Arrington (May 15, 2015)
3 Dissertation Defense Committee: Lindsey Harrick (July 24, 2015)

Training/Mentoring Graduate students from the University of Houston Developmental Cognitive Neuroscience program:

FY2008: Russ Jenkins, Alanna Gold, Lyla El-Messidi, Amery Treble
FY2009: Alanna Gold, Lyla El-Messidi, Amery Treble, Chad Johnson
FY2010: Amery Treble, Tori Williams, Vindia Fernandez.
FY2011: Amery Treble, Tori Williams, Vindia Fernandez, Emily Maxwell
FY2012: Kailyn Bradley as graduate research assistant (50% effort)
FY2012: Nikki Arrington as graduate research assistant (40% effort)
FY2013: Kailyn Bradley as graduate research assistant (50% effort)
FY2013: Aurora Ramos as graduate research assistant (50% effort)
FY2013: Nikki Arrington as graduate research assistant (40% effort)
FY2013: Ashley Ware as graduate research assistant (50% effort)
FY2014: Nikki Arrington as graduate research assistant (50% effort)
FY2014: Aurora Ramos as graduate research assistant (50% effort)
FY2015: Nikki Arrington as graduate research assistant (50% effort)
FY2015: Aurora Ramos as graduate research assistant (50% effort)

Training/Mentoring international students on scholarship:

FY2012: Tim Kunze from biomedical engineering at Ilmenau University in Germany. Title of Thesis: Probabilistic Connectivity Modeling to Maximize A Priori Information for MEG Source Localization.

SPONSORSHIP OF POSTDOCTORAL FELLOWS:

2009: Nikki Davis, PhD Vanderbilt University
Freesurfer/FSL full-time training for 1 week in neuroimaging analyses
2009-2011: Penny Dong, MD (Learning Disabilities and Spina Bifida)
2012-2014: Chad Johnson, PhD (Traumatic Brain Injury)
2012-2013: Maria Pilar Archila-Suerte, PhD (Learning Disabilities)
2013-2015: Anna Romanowska-Pawliczek, PhD (Autologous Bone Marrow)
2013-2015: Dana Demaster, PhD (Traumatic Brain Injury & Learning Disabilities)

CURRENT GRANT SUPPORT:

JW150014 PI: Charles S. Cox, Jr.
10/01/2016-09/01/2020 Co-I: Jenifer Juranek
Department of Defense/Joint Warfighter Medical Research Program $6,800,000 (TC)
Treatment of Adult Severe TBI Injury Using Autologous Bone Marrow Mononuclear Cells.
This project plan will assess structural and functional outcomes following treatment of severe TBI in adults using autologous bone marrow mononuclear cells.
Co-Investigator responsible for all MRI acquisitions and analyses

1 R01 NS077963-01A1 PI: Charles S. Cox, Jr.
04/01/2013 - 3/30/2018 Co-I: Jenifer Juranek
NIH/NINDS $ 488,363
Phase II trial of pediatric autologous bone marrow mononuclear cells (BMMNCs) for severe traumatic brain injury (TBI).
This project will determine the effect of intravenous infusion of autologous BMMNCs on brain structure and neurocognitive/functional outcomes after severe traumatic brain injury in young children.
Co-Investigator responsible for MRI acquisitions and analyses

BB-IND-12620, BB-IND-14214 Charles S. Cox. Jr. (PI)
9/1/2013 – 8/30/2017
Sponsors: TIRR Foundation, CBR, Inc., and Let's Cure CP Foundation
Autologous Cell Therapies for Cerebral Palsy
This clinical trial investigates autologous cell therapies in patients with cerebral palsy (CP). We aim to compare the effects of two specific autologous cell therapies - bone marrow derived mononuclear cells (BMMNCs) versus human umbilical cord blood cells (hUCBs).
Co-Investigator responsible for MRI acquisitions and analyses

2R01NS046308 PI: Linda Ewing-Cobbs
07/01/2011- 06/30/2017 (NCE) Co-I: Jenifer Juranek
NIH/NINDS $2,400,585 (DC)
Traumatic Stress After Pediatric Injury: Neurobiological Influences
This project will examine the impact of traumatic injury on the biomarkers of three stress-responsive neurobiological systems and their relation to cognitive and psychological health outcomes during the first year after TBI or extracranial injury.
Co-Investigator responsible for MRI acquisitions and analyses

P50HD052117-07 PD: Jack M. Fletcher (University of Houston)
12/01/2011- 11/30/2016 PI of Project 4: Jenifer Juranek
NIH/NICHD $1,250,000 (TC)
Learning Disabilities Research Center (LDRC).
The major goals of this project are to evaluate school-aged children and their response to reading intervention as part of a multidisciplinary center on learning disabilities involving a consortium of three Texas universities.
PI of LDRC Project 4: Neural Correlates of Reading Comprehension in Typical and Struggling Readers: A Multimodal Neuroimaging Study

Subcontract (NIH/NINDS) PI: Jenifer Juranek
8/1/2011 – 7/30/17 (NCE) $222,205 (DC)
A follow-up of children enrolled in the Management of Myelomeningocele Study (MOMS2).
The major goals of this project are to perform multimodal quantitative neuroimaging analyses on MRIs acquired at three study sites (e.g. UCSF, Children's Hospital of Philadelphia, and Vanderbilt University) where children with spina bifida either received prenatal surgery to correct the spinal defect before birth or received surgical treatment after birth. The total number of MRIs to be analyzed over the next five years is ~ 174.
PI responsible for MRI protocol development as well as all MRI acquisitions and analyses

PAST GRANT SUPPORT:

W81XWH-11-1-0460 PI: Charles S. Cox, Jr.
06/01/2011-11/30/2014 Co-I: Jenifer Juranek
USAMRRA $1,302,975 (DC)
Treatment of Adult Severe TBI Injury Using Autologous Bone Marrow Mononuclear Cells.
This project plan will assess safety and functional outcomes following treatment of severe TBI in adults using autologous bone marrow mononuclear cells.
Co-Investigator responsible for all MRI acquisitions and analyses

Subcontract PD: Leanne Tamm (UT Southwestern)
(University of Texas Southwestern) PI: Jenifer Juranek
1/1/11 – 12/31/2011 $6,750 (TC)
Attention Training Intervention Study in ADHD children
The major goal of this feasibility study is to evaluate pre- and post-changes in brain function following an intervention of attention training in children with ADHD.
Role: Co-investigator; PI of subcontract from UT Southwestern

P50 HD052117 PD: Jack M. Fletcher (University of Houston)
06/01/2006 – 11/29/2011 PI of Project 4: Andrew C. Papanicolaou
NIH/NICHD
Texas Center of Learning Disabilities
The major goals of this project are to establish a multidisciplinary center on learning disabilities involving a consortium of three Texas universities.
Role: Co-Investigator

P01 HD35946 PD: Jack M. Fletcher (University of Houston)
01/01/08 – 11/29/11 PI of subcontract to UT: Jenifer Juranek
NIH/NICHD $161,924 (TC)
Spina Bifida: Cognitive and Neurobiological Variability
The major goals of this project are to provide genetic, neuroimaging, and neurobehavioral studies of children with spina bifida.
Role: Co-investigator; PI of subcontract from University of Houston (01/01/08 – 11/29/11)

3P50HD052117-0351 PD: Jack M. Fletcher (University of Houston)
2/01/10 – 1/31/11 PI of subcontract to UT: Jenifer Juranek
NIH/NICHD $66,675 (TC)
ARRA - Texas Center of Learning Disabilities
The major goals of this project are to process neuroimaging data collected as part of the Texas Center for Learning Disabilities project.
Role: Co-Investigator; PI of subcontract from University of Houston (2/01/10 – 1/31/11)

R01-NS046308 PI: Linda Ewing-Cobbs
05/01/2004 –04/30/2010
NIH/NINDS
Academic Outcomes After Pediatric Traumatic Brain Injury
Major Goals: Investigate the effect of traumatic brain injury in pediatric subjects on academic performance using neuropsychological measures, MRI-based morphometry and diffusion tensor imaging.
Role: Co-Investigator (1/01/08 – 4/30/10)

P01 HD46261 PD: Jack M. Fletcher (University of Houston)
09/26/2003 – 07/31/2009 PI of subcontract to UT: Andrew C. Papanicolaou
NIH/NINDS
Cognitive, Instructional, and Neuroimaging Factors in Math
The major goal of this project is to provide studies of cognitive processes, response to instruction, and neuroimaging studies (magnetic source imaging, DTI, aMRI) of children with math difficulties.
Role: Co-Investigator (9/01/06 – 1/01/08)

P01 N537941 PD: Andrew C. Papanicolaou
12/01/2007 – 11/30/2009
NIH/NINDS
Functional Brain Reorganization in Stroke Recovery
The major goal is to characterize changes in spatiotemporal representation of language function in the brain after aphasia secondary to stroke. Dr.Breier is PI on Project 1: Functional Brain Reorganization in Recovery from Aphasia.
Role: Co-investigator (9/01/06 – 1/01/08)

T32 NS45540 PD: Tallie Z. Baram
2005 – 2006
NIH/NINDS
Epilepsy Research Training Program
The major goal is to provide training opportunities for postdoctoral fellows in epilepsy research.
Role: Postdoctoral Scholar

R01 NS035458 PI: Pauline A. Filipek
1999 – 2005
NIH/NINDS
Autism: A model of anomalous neural systems development
The major goals of the project are to investigate neurological (quantitative MRI image analyses), genetic, and behavioral markers of autism in children.
Role: Co-Investigator responsible for quantitative neuroimaging analyses.

PAST INTRAMURAL SUPPORT:

10/22/2003 Co-Investigator of a Pilot study intramurally funded by UCI's GCRC entitled, "*The Effects of Antenatal Betamethasone on Brain Development*". PI: Elysia Poggi-Davis, PhD

03/15/2005 Principal Investigator of a Pilot Study intramurally funded by UCI's GCRC entitled, *"Brain MRI & MRS Findings in ADHD Children".*

11/01/2005 Principal Investigator of Pilot study intramurally funded by UCI's GCRC entitled *"Effects of Seizures on White Matter Tracts and Behavior".*

07/01/2006 Lead Investigator of extramurally-funded research proposal sponsored by the Epilepsy Foundation, Special Research Grants Program, Targeted Research Initiative for Mood Disorders. Project entitled: *"Predictors of differential vulnerability to anxiety and depression in epilepsy: A diffusion tensor imaging study."*

03/01/2008 Principal Investigator of intramurally-funded research proposal sponsored by the Department of Pediatrics, University of Texas, Houston. Project entitled: *"Attention and Executive Systems in TBI children: A Multimodal and Quantitative Neuroimaging Study."*

03/01/2009 Co-Investigator of intramurally-funded research proposal sponsored by the Department of Pediatrics, University of Texas, Houston. Project entitled:" *Pre- and post-surgical changes in brain structure and function in patients with intractable epilepsy". PI: Gretchen Von Allmen, MD*

EXTRAMURAL GRANT SUBMISSIONS: (funded * and unfunded applications)
2009 Epilepsy Foundation: DTI correlates of anxiety & depression in pediatric epilepsy (PI: Juranek, J)
2009 1R01-NS-046308: Traumatic stress after pediatric injury: Neurobiological Influences
(PI: Ewing-Cobbs, L; Co-I: J Juranek)
2009 R01: Context and comprehension after right hemisphere brain damage
(PI: Blake, ML at UH; UTH site PI: Juranek, J)
2009 PPG renewal: Spina Bifida: Cognitive and neurobiological variability
(PD: Fletcher, JM at UH; Co-I Project 4: Juranek, J)
2010 1R01-HD-068422: Attention and movement in spina bifida and ADHD
(PD: Fletcher, JM at UH; UTH site PI: Juranek, J)
2010 1R01-NS-073658-01A1: Vulnerability of frontal lobe networks after brain injury in young children. (PI: Ewing-Cobbs, L; Co-I Juranek, J)
2010 NIH/NINDS: Stroke
(PI: Breier, J; Co-I Juranek, J)
2010 1R01-MH-094374-01: Neurophysiological markers of autism
(PI: Papanicolaou, AC; Co-I Juranek, J)
2010 1R01-HD-068468-01:Structural and functional correlates of motor skill learning in spina bifida (PI: Juranek, J)
***2010** 3P50-HD-052117-03S1: ARRA supplemental funding for LDRC
(PD: Fletcher, JM at UH; UTH site PI: Juranek, J)
***2010** 2R01-NS-046308: Traumatic stress after pediatric injury: Neurobiological Influences (PI: Ewing-Cobbs, L; Co-I Juranek, J)

***2010** DOD-W81XWH-11-1-0460: Treatment of adult severe TBI injury using autologous bone marrow mononuclear cells (PI: Cox, Charles, S; Co-I Juranek, J)
2011 1R01-HD-068468-01A1: Structural and functional correlates of motor skill learning in spina bifida (PI: Juranek, J)
2011 1R01-HD-068422-01: Attention and movement in spina bifida and ADHD (PI: Fletcher, JM at UH; UTH site PI: Juranek, J)

***2011** 1R01-NS-: Phase 2 pediatric autologous BMMNC for severe traumatic brain injury (PI: Cox, Charles S.; Co-I: Juranek, J)

***2011** 2 P50-HD-052116-06: Texas Center for Learning Disabilities (PD: Fletcher, JM at UH; PI of Project 4: Juranek, J)
2011 1R01-HD-046609B: Early and late predictors of risk and resiliency in adolescents with spina bifida (PI: Landry, S; Co-I Juranek, J)
2011 1R01-NS-079298-01: Imaging gray matter microstructure in vivo predicts histopathology in epilepsy (Co-PIs: Juranek, J and Von Allmen, G)

***2011 Subcontract**: A Follow-up study of children enrolled in the management of Myelomeningocele Study (MOMS2) (PI of Neuroimaging: Juranek, J)

***2013 BB-IND-12620, BB-IND-14214**
Sponsors: TIRR Foundation, CBR, Inc., and Let's Cure CP Foundation
Autologous Cell Therapies for Cerebral Palsy.This clinical trial investigates autologous cell therapies in patients with cerebral palsy (CP) (PI: Cox, Charles, S; Co-I Juranek, J).
2014 NIH: Concussive Trauma Encephalopathy (CTE) in professional football players (PI: Schultz, Paul; Co-I: Juranek, J.)
2014 NIH: Thrombelastography-guided Resuscitation of Pediatric Trauma and Associated Coagulopathy (PI: Cox, Charles, S.; Co-I Juranek, J.)
***2015** JW150014 DoD: Adult TBI Phase 2b: Treatment of adult severe TBI injury using autologous bone marrow mononuclear cells (PI: Cox, Charles, S; Co-I Juranek, J)

PUBLICATONS:

A. Abstracts (* presented)

1. Metzner, W.*, Juranek, J. Behavioral significance of multiple sensory maps in the electrosensory lateral line lobe (ELL) of the weakly electric fish, Eigenmannia. Society for Neuroscience 1996; 179.3

2. Juranek, J.*, Metzner, W. Cellular effects of different premotor circuitry on the pacemaker nucleus in two species of weakly electric fish. Society for Neuroscience 1996; 179.5

3. Metzner, W.*, Juranek, J. Visualization of pharmacological lesions made with biotinylated ibotenic acid. Society for Neuroscience 1997; 883.4

4. Juranek, J.*, Metzner, W. Changes in apparent input resistance in pacemaker cell types with different synaptic inputs, *in vivo*. Society for Neuroscience 1997; 101.14

5. Juranek, J.*, Currie, S.N. Fictive swimming elicited by electrical stimulation of the turtle spinal cord: Interactions with scratch reflex. Society for Neuroscience 1998; 654.22

6. Filipek, P.A.*, Juranek, J., Gargus, J.J, Smith, M., Ramos, E.R., Mays, L.Z., Bocian, M., Laulhere, T.M., Modahl, C., and Spence, M.A. Evidence of mitochondrial dysfunction in autistic patients with 15q inverted duplication. International Meeting for Autism Research 2001; 7.02

7. Filipek, P.A.*, Juranek, J., Nguyen, M., Cummings, C., and Gargus, J.J. Relative Carnitine Deficiency in Autism. Annals of Neurology 52(3S):S125-126; Child Neurologist Society, 2002.

8. Juranek, J.*, Filipek, P.A., Taylor, H.G., Bangert, B., Minich, N., and Hack, M. Anomalous Brain Development in Adolescent Survivors of Very Low Birthweight: A Structural Imaging Study. Child Neurologist Society, 2005.

9. Lin, J.*, Juranek, J., Franklin, D.L., Drescher, A., Maguire, G.A., and Cramer, S.C. Vulnerability of Frontal-Temporal Connections in Early Onset Focal Epilepsy. American Epilepsy Society, 2006.

10. Cramer, S.C.*, Parrish, T.B., Levy, R.M., Stebbins, G.T., Ruland, S.D., Lowry, D.W., Trouard, T.P., Squire, S.W., Weinand, M.E., Savage, C.R., Wilkinson, S.B., Juranek, J., Leu, S.Y., and Himes, D.M. An Assessment of Brain Function Predicts Functional Gains in a Clinical Stroke Trial. International Stroke Conference, 2007.

11. Hasan, K.M.*, Fletcher, J.M., Ewing-Cobbs, L., Sankar, A., Eluvathingal, T.J., Kramer, L.A., Ashtari, A., Juranek, J., Sarkari, S. and Papanicolaou, A.C. A Multi-Scale Whole-Brain Optimized Diffusion Tensor Imaging of Dyslexics at 3.0T. ISMRM, 2007.

12. Juranek, J.*, Castillo, E.M., Pazo-Alvarez, P., Ewing-Cobbs, L., Sarkari, S. and Papanicolaou, A.C. Anatomical and Functional Differences in Children with Spina Bifida: aMRI and MEG studies. International Neuropsychological Society, Bilbao, Spain, 2007.

13. Riley, J.*, Juranek, J., Drescher, A., Lin, J.J., and Cramer, S.C. Derangement of uncinate fasiculus myelin integrity as a function of age of seizure onset, as revealed by DTI tractography. Human Brain Mapping, Chicago, IL, 2007.

14. Kamali, A.*, Juranek, J., Hasan, K.M. Mapping the human brain fiber pathways using diffusion tensor imaging at high angular and spatial resolution. National Research Center at University of Texas Houston. University of Texas Houston, Medical School Building; 14th Annual Poster Session, 2007.

15. Hasan, K.M. *, Kamali, A., and Juranek, J. Mapping the Human Brain Fiber Tracts Relative to Deep and Cortical Gray Matter Using Diffusion Tensor Imaging at High Angular and Spatial Resolution. The 25th Annual Meeting of the Houston Society for Engineering in Medicine and Biology (HSEMB 08 Conference), Houston, TX, 2008.

16. Simos, P.G., Fletcher, J.M., Sarkari, S., Juranek, J., and Papanicolaou, A.C.* Aberrant spatiotemporal activation profiles associated with simple arithmetic operations in developmental math disability. The 36th Annual International Neuropsychological Society Meeting. Waikoloa, HI, 2008.

17. Dennis, M.*, Hopyan-Misakyan, T., Juranek, J., Cirino, P., Hasan, K., Fletcher, J. Strong and weak metric rhythm identification in spina bifida meningomyelocele in relation to parcellated anterior and posterior cerebellar volumes. Neuroscience & Music III: Disorders & Plasticity. Montreal, Canada, 2008.

18. Juranek, J.*, Prasad, M., Kramer, L., Ewing-Cobbs, L. Association between amygdala volume and self-reported measures of anxiety in children with TBI. The 37th Annual International Neuropsychological Society Meeting. Atlanta, GA, 2009.

19. Isenberg, A. L.*, Juranek, J., Filipek, P.A., Osann, K., Spence, M.A., Gage, N.M. An anatomical MRI investigation of asymmetries in frontal and temporal language association cortex in children with autism disorder. The 8th Annual International Meeting for Autism Research. Chicago, IL, 2009.

20. Juranek, J.* Increased cortical complexity in cortically-thin regions: an aMRI study in spina bifida. The 1st World Congress on Spina Bifida Research and Care. Orlando, FL, 2009.

21. Davis, N.*, Barquero, L., Juranek, J., Fan, Q., Zhang, W., Compton, D., Anderson, A. Is there a relationship between children's brain structure and their responsiveness to intervention? The 16th Annual Meeting of the Society of Scientific Study of Reading, Boston, Massachusetts, 2009.

22. Johnson, C. P.*, Juranek J., Kramer, L.A., Prasad, M.R., Swank, P.R., Blakeley, A., Kaplan, A.*, Ewing-Cobbs, L. Predicting attentional deficits following Traumatic Brain Injury through dissociated white matter pathways of attention: A diffusion tensor tractography study. The 38th Annual International Neuropsychological Society Meeting. Acapulco, Mexico, 2010.

23. Johnson, C.P.*, Juranek, J., Kramer, L.A., Prasad, M.R., Swank, P.R., Ewing-Cobbs, L. Predicting behavioral deficits following traumatic brain injury through damage to the uncinate fasciculus: A diffusion tractography study. The 38th Annual International Neuropsychological Society Meeting. Acapulco, Mexico, 2010.

24. Gold, A.*, Juranek, J., Prasad, M.R., Ewing-Cobbs, L. Attention networks in children with traumatic brain injury (TBI): Relations with regional brain volumetry. The 38th Annual International Neuropsychological Society Meeting. Acapulco, Mexico, 2010.

25. Blakely, A.*, Johnson, C.P., Juranek, J., Ewing-Cobbs, L., Kaplan, A., Prasad, M.R. Mean diffusivity of the orbitofrontal cortex is associated with inhibitory control in children with TBI. The 38th Annual International Neuropsychological Society Meeting. Acapulco, Mexico, 2010.

26. Treble, A.*, Juranek, J., Fletcher, J.M. Regions of increased and decreased cortical complexity in spina bifida: An aMRI study. The 38th Annual International Neuropsychological Society Meeting. Acapulco, Mexico, 2010.

27. Juranek, J.*, Treble, A., Law, N. Imaging the spina bifida brain for cross-disorder comparisons: Spatial patterns of cortical thickness and thinning, volumetrics of subcortical gray matter, and cerebellar parcellations. International Neuropsychological Society Annual Meeting, Boston, MA, 2011.

28. Bush, A.A.*, Juranek, J.,Tamm, L. Deficits in fluid reasoning associated with hypoactivation in ADHD: fMRI evidence. 22nd Eunethydis Meeting in Budapest, Hungary, 2011.

29. Juranek, J.*, Williams, V., Cirino, P.T., Dennis, M., Fletcher, J.M. aMRI and DTI of deep gray matter structures in children with and without spina bifida meningomyelocele. Annual Human Brain Mapping Meeting, Quebec City, Canada, 2011.

30. Juranek, J*. Neuroimaging in Spina Bifida: Findings from the SANDI Project. The Second International World Congress on Spina Bifida Research and Care, Las Vegas, NV, 2012.

31. Lankford, J.*, Juranek, J., Bhattacharjee, M., Von Allmen, G. White matter pathways in epileptic patients. First Annual Fellowship Research Symposium, Department of Pediatrics, UT-Health, Houston, TX, May 9-10, 2012.

32. Bradley, K.A., Juranek, J., Fletcher, J.M. Deterministic tractography of the corpus callosum in children with spina bifida myelomeningocele. Human Brain Mapping, Hamburg, Germany, 2014.

33. Roe, M.A.*, Martinez, J.E., Mumford, J.A., Juranek, J., Olmedo, L.A., Poldrack, R.A., Vaughn, S.R., Fletcher, J.M., Church, J.A. Neural correlations of reading comprehension pre- and post-intervention in struggling readers. Society for Neuroscience Conference, 2014.

34. Bradley, K.A.*, Juranek, J., Romanowska-Pawliczek, A., Hannay, H.J., Cirino, P.T., Dennis, M., Fletcher, J.M. Corpus callosum microstructure and auditory interhemispheric transfer in spina bifida myelomeningocele. International Neuropsychological Society Annual Meeting, Denver, CO, 2015.

35. Fernandez, V.G.*, Juranek, J., Romanowska-Pawliczek, A., Stuebing, K., Williams, V.J., Fletcher, J.M. Cortico-cerebellar connectivity in reading impaired children. International Neuropsychological Society Annual Meeting, Denver, CO, 2015.

36. Roe, M.A.*, Martinez, J.E., Mumford, J.A., Juranek, J., Olmedo, L.A., Poldrack, R.A., Vaughn, S.R., Fletcher, J.M., Church, J.A. Stop-signal inhibition in pre- and post-intervention struggling readers. Society for Research in Child Development Conference, 2015.

37. Roe, M.A.*, Martinez, J.E., Mumford, J.A., DeMaster, D.M., Juranek, J., Olmedo, L.A., Poldrack, R.A., Vaughn, S.R., Fletcher, J.M., Church, J.A. Neural correlations of reading comprehension and stop-signal inhibition in typical and struggling readers. Cognitive Development Society, 2015.

38. Romanowska-Pawliczek, A.*, Juranek, J., Cirino, P. T., Fletcher, J.M. A DTI study of structural connectivity within the reading network of young struggling readers. Human Brain Mapping, Honolulu, HI, 2015.

39. Cox, Charles, S., Dash, P., Juranek, J., Ewing-Cobbs, L. Progenitor cell therapy for adult TBI: preclinical findings and clinical outcomes. National Neurotrauma Society Sante Fe, New Mexico, June 2015.

40. Williams, V.J.*, Juranek, J., Stuebing, K., Cirino, P.T., Fletcher, J.M. Increased gyrification and thinner cortex in children with poor single word decoding skills. International Neuropsychological Society, Boston, MA, February 2016.

41. Roe, M. A.*, Deschner, L., Martinez, J. E., Mumford, J. A., DeMaster, D.M., Juranek, J., Church, J. A. Neural Correlates of Reading Comprehension in Struggling and Typical Readers. Cognitive Neuroscience Society, New York City, NY, April 2-5, 2016.

42. Roe, M. A.*, Martinez, J. E., Mumford, J. A., DeMaster, D.M., Juranek, J., Olmedo, L. A., Poldrack, R. A., Vaughn, S. R., Fletcher, J. M., Church, J. A. Neural correlates of reading comprehension and stop-signal inhibition in struggling and typical readers, Cognitive Development Society Columbus, OH, October 9-10, 2015.

43. Church, J.A.*, Roe, M. A., Juranek, J. J., DeMaster, D.M., Martinez, J. E., Vaughn, S. R., Fletcher, J. M. An fMRI study of sentence reading and response inhibition in pre- and post-intervention struggling readers. IDA Annual Reading, literacy, and learning Conference Grapevine, TX, October 28-31, 2015.

B. Refereed Original Articles in Journals

1. Metzner, W., Juranek, J. A sensory brain map for each behavior? Proceedings of the National Academy of Sciences, USA 94:14798-14803, 1997.

2. Metzner, W., Juranek, J. A method to biotinylate and histochemically visualize ibotenic acid for pharmacological inactivation studies. Journal of Neuroscience Methods 76:143-50, 1997.

3. Juranek. J., Metzner, W. Cellular characterization of synaptic modulations of a neuronal oscillator in electric fish. Journal of Comparative Physiology A 181:393-414, 1997.

4. Juranek. J., Metzner, W. Segregation of behavior-specific synaptic inputs to a vertebrate neuronal oscillator. Journal of Neuroscience 18(21):9010-9019, 1998.

5. Juranek, J, Currie, S.N. Electrically evoked fictive swimming in the low-spinal immobilized turtle. Journal of Neurophysiology 83:146-155, 2000.

6. Filipek, P.A., Juranek, J., Gargus, J.J., Smith, M., Ramos, E.R., Mays, L.Z., Bocian, M., Masser-Frye, D., Laulhere, T.M., Modahl, C.M., and Spence, M.A. Evidence of mitochondrial dysfunction in autistic patients with 15q inverted duplication. Annals of Neurology 53:801-804, 2003.

7. Filipek PA, Juranek J, Nguyen M, Cummings C, Gargus JJ. Relative carnitine deficiency in autism. Journal of Autism and Developmental Disorders 34(6):615-623, 2004.

8. Cramer, S.C., Shah, R., Juranek, J., Crafton, K.R., and Le, V. Activity in the Peri-infarct rim in relation to recovery from stroke. Stroke 37:111-115., 2006.

9. Juranek, J., Filipek, P.A., Berenji, G.R., Modahl, C., Osann, K., and Spence, M.A. Association between amygdala volume and anxiety level: A magnetic resonance imaging (MRI) study in autistic children. Journal of Child Neurology 21(12):1051-1058, 2006.

10. Cramer, S.C., Parrish, T.B., Levy, R.M., Stebbins, G.T., Ruland, S.D., Lowry, D.W., Trouard, T.P., Squire, S.W., Weinand, M.E., Savage, C.R., Wilkinson, S.B., Juranek, J., Leu, S.Z., and Himes, D.M. Predicting functional gains in a stroke trial. Stroke 38(7):2108-14, 2007.

11. Hasan, K.M., Sankar, A., Halphen, C., Kramer, L.A., Brandt, M.E., Juranek, J., Cirino, P.T., Fletcher, J.M., Papanicolaou, A.C., and Ewing-Cobbs, L.

Development and organization of human brain tissue compartments across lifespan using diffusion tensor imaging. Neuroreport 18(16):1735-1739, 2007.

12. Juranek, J., Fletcher, J.M., Hasan, K.M., Breier, J., Cirino, P., Pazo-Alvarez, P., Diaz, J., Ewing-Cobbs, L., Dennis, M., Papanicolaou, A. Neocortical reorganization in spina bifida. NeuroImage 40(4):1516-1522, 2008

13. Pazo-Alvarez, P., Simos, P.G., Castillo, E.M., Juranek, J., Passaro, A.D., Papanicolaou, A.C . MEG correlates of bimodal encoding of faces and persons' names. Brain Research 1230:192-201, 2008.

14. Simos, P.G., Kanatsouli, K., Fletcher, J.M., Sarkari, S., Juranek, J., Cirino, P., Papanicolaou, A.C. Aberrant spatiotemporal activation profiles associated with math difficulties in children: a magnetic source imaging study. Neuropsychology 22(5):571-584, 2008.

15. Lin, J.J., Riley, J.D., Juranek, J., Cramer, S.C. Vulnerability of the frontal-temporal connections in temporal lobe epilepsy. Epilepsy Research 82(2-3): 162-170, 2008.

16. Dennis M, Hopyan T, Juranek J, Cirino PT, Hasan KM, Fletcher JM. Strong-meter and weak-meter rhythm identification in spina bifida meningomyelocle and volumetric parcellation of rhythm-relevant cerebellum regions. Annals of the New York Academy of Sciences 1169:84-88, 2009.

17. Gage NM, Juranek J, Filipek PA, Osann K, Flodman P, Isenberg AL, Spence MA. Rightward hemispheric asymmetries in auditory language cortex in children with autistic disorder: An MRI investigation. Journal of Neurodevelopmental Disorders 1:205-214, 2009.

18. Breier JI, Juranek J, Schmadke S, Maher LM, Papanicolaou AC. Behavioral and neurophysiological response to therapy for chronic aphasia. Archives of Physical Medicine and Rehabilitation 90:2026-2033, 2009.

19. Juranek J, Dennis M, Cirino PT, El-Messidi L, Fletcher JM. The cerebellum in children with spina bifida and Chiari II malformation: Quantitative volumetrics by region. Cerebellum, 9(2):240-48, 2010.

20. Juranek J, Salman MS. Anomalous development of brain structure and function in spina bifida myelomeningocele. Developmental Disabilities and Research Reviews, 16(1), 23-30, 2010.

21. Dennis M, Salman MS, Juranek J, Fletcher JM. Cerebellar motor function in spina bifida meningomyelocele. Cerebellum, 9(4): 484-98, 2010.

22. Taylor HG, Filipek PA, Juranek J, Bangert B, Minich N, Hack M. Brain volume in adolescents with very low birthweight: effects of brain structure and associations with neuropsychological outcomes. Developmental Neuropsychology, 36(1):96-117, 2011.

23. Simos, P.G., Rezaie, R., Fletcher, J.M., Juranek, J., Passaro, A.D., Li, Z., Cirino, P.T., Papanicolaou, A.C. Functional disruption of the brain mechanism for reading: Effects of comorbidity and task difficulty among children with developmental learning problems. Neuropsychology, 25(4):520-534, 2011.

24. Simos, P.G., Papanicolaou, A.C., Castillo, E.M., Juranek, J., Cirino, P.T., Rezaie, R., Fletcher, J.M. Brain mechanisms for reading and language processing in spina bifida meningomyelocele: A combined magnetic source and structural magnetic resonance imaging study. Neuropsychology, 25(5):590-601, 2011.

25. Breier, J., Juranek, J., Papanicolaou, A.C. (2011). Changes in maps of language function and the integrity of the arcuate fasciculus after therapy for chronic aphasia. Neurocase 17(6): 506-517, 2011.

26. Rezaie R., Simos, P.G., Fletcher, J.M., Juranek, J., Cirino, P.T., Li, Z., Passaro, A.D., Papanicolaou, A.C. The timing and strength of regional brain activation associated with word recognition in children with reading difficulties. Frontiers in Human Neuroscience, 5(45):1-12, 2011

27. Simos, P.G., Rezaie, R., Fletcher, J.M., Juranek, J., Papanicolaou, A.C. Neural correlates of sentence reading in children with reading difficulties. Neuroreport 22:674-678, 2011.

28. Johnson CP, Juranek J, Kramer LA, Prasad MR, Swank PR, Ewing-Cobbs L. Predicting behavioral deficits in pediatric traumatic brain injury through uncinate fasciculus integrity. Journal of International Neuropsychology 15:1-11, 2011.

29. Juranek J, Johnson CP, Prasad M, Kramer LA, Filipek PA, Swank PR, Ewing-Cobbs L. Mean diffusivity in the amygdala correlates with anxiety in pediatric TBI. Brain Imaging and Behavior. Brain Imaging and Behavior 6(1):36-48, 2012.

30. Tamm, L, Juranek, J. Fluid reasoning deficits in children with ADHD: Evidence from fMRI. Brain Research 1465:48-56, 2012.

31. Treble A, Juranek, J, Stuebing, K.A., Dennis, M., Fletcher, J.M. Functional significance of atypical cortical organization in Spina Bifida Myelomeningocele: relations of cortical thickness and gyrification with IQ and fine motor dexterity. Cerebral Cortex 23: 2357-2369, 2013.

32. Landry, S.H., Taylor, H.B., Swank, P.R., Barnes, M., Juranek, J. Longitudinal mediators of social problem solving in spina bifida and typical development. Rehabilitation Psychology 27(3):364-377, 2013.

33. Clark, C., Fang, H., Espy, K., Filipek, P., Juranek, J., Bangert, B., Hack, M., Taylor, H.G. Relation of neural structure to persistently low academic achievement: A longitudinal study of children with differing birth weights. Neuropsychology 27(3):364-377, 2013.

34. Fernandez, V., Juranek, J., Stuebing, K., Fletcher, J.M., Volumetric analysis of regional variability in the cerebellum of children with dyslexia. The Cerebellum 12: 906-915, 2013.

35. Williams, V.J., Juranek, J., Stuebing, K., Cirino, P.T., Dennis, M., Fletcher, J.M. Examination of Frontal and Parietal tectocortical attention pathways in spina bifida meningomyleocele using probabilistic diffusion tractography. Brain Connectivity 3(5):512-522, 2013.

36. Dennis, M., Spiegler, B.J., Juranek, J., Bigler, E.D., Snead, C.O., Fletcher, J.M. Age, plasticity, and homeostasis in childhood brain disorders. Neuroscience and Biobehavioral Reviews 37: 2760-2773, 2013.

37. Ware, A. L., Juranek, J., Williams, V.J., Cirino, P.T., Dennis, M, Fletcher, J.M. Anatomical and diffusion MRI of deep gray matter in pediatric spina bifida. NeuroImage Clinical: 5:120-127, 2014.

38. Treble-Barna, A., Juranek, J., Stuebing, K., Cirino, P.T., Dennis, M., Fletcher, J.M. Prospective and episodic memory in relation to hippocampal volume in adults with spina bifida myelomeningocele. Neuropsychology 29(1):92-101, 2015.

39. Kulesz, P.A., Treble-Barna, A., Williams, V.J., Juranek, J., Cirino, P.T., Dennis, M., Fletcher, J.M. Attention in spina bifida myelomeningocele: relations with brain volume and integrity. NeuroImage Clinical 31(8): 72-78, 2015.

40. Kulesz, P.A., Tian, S., Juranek, J., Fletcher, J.M., Francis, D.J. Relations between volumetric measures of brain structure and attentional function in spina bifida: utilization of robust statistical approaches. Neuropsychology 29(2):212-225, 2015.

41. Williams, V.J., Juranek, J., Stuebing, K., Cirino, P.T., Dennis, M., Bowman, R.M., Blaser, S., Kramer, L.A., Fletcher, J.M. Postshunt lateral ventricular volume, white matter integrity, and intellectual outcomes in spina bifida and hydrocephalus. Journal of Neurosurgery, Pediatrics 15(4):410-419, 2015.

42. Dennis, M., Cirino, P.T., Simic, N., Juranek, J., Taylor, P., Fletcher, J.M. White and grey matter relations to simple choice and cognitive reaction time in spina bifida. Brain Imaging and Behavior 10(1):238-251, 2016.

43. Fernandez, V. G., Juranek, J., Romanowska-Pawliczek, A., Stuebing, K., Williams, V.J., Fletcher, J.M. White matter integrity of cerebellar-cortical tracts in reading impaired children: A probabilistic tractography study. Brain and Language (in press, 2015).

44. Johnson, C.P., Juranek, J., Swank, P.R., Kramer, L.A., Cox, C.S., Ewing-Cobbs,L. White matter and reading deficits after pediatric traumatic brain injury: A diffusion tensor imaging study. Neuroimage Clinical 9:668-677, 2015.

45. Bradley, K.A., Juranek, J., Romanowska-Pawliczek, A., Hannay, H.J., Cirino, P.T., Dennis, M., Fletcher, J.M. Plasticity of interhemispheric temporal lobe connections due to early disruption in CC development in spina bifida myelomeningocele. Brain Connectivity 6(3):238-248, 2016.

46. Ware, A.L., Kulesz, P.A., Williams, V.J., Juranek, J., Cirino, P.T., Fletcher, J.M. Gray matter integrity within regions of the dorsolateral prefrontal cortical-subcortical network predicts executive function and fine motor dexterity in spina bifida. Neuropsychology 30(4):492-501, 2016.

47. Ewing-Cobbs L., Johnson, CP, Juranek J, DeMaster D, Prasad M, Duque G, Kramer L, Cox CS, Swank PR. Longitudinal diffusion tensor imaging after pediatric traumatic brain injury: Impact of age at injury and time since injury on pathway integrity. Human Brain Mapping (in press, 2016).

48. Cox, CS, Hetz, RA, Liao, GP, Aertker, BM, Ewing-Cobbs, L, Juranek, J, Savitz, SI, Jackson, ML, Romanowska-Pawliczek, AM, Triolo, F, Dash, PK, Pedroza, C, Lee, D, Worth, L, Aisiku, I, Choi, HA, Holcombe, JB, Kitagawa, RS. Treatment of severe adult traumatic brain injury using bone marrow mononuclear cells. Stem Cells (in press, 2016).

C. Chapters

1. Juranek, J., Filipek, P.A. Neuroimaging in the Developmental Disorders. In Boller F, Grafman, J. (Eds). Handbook of Neuropsychology Second Edition. Rapin, I., Segalowitz, S. (Topic Eds). Volume 8, Part 1: Child Neuropsychology, Part 1, Chapter 7. Elsevier Science Publishers. Amsterdam, 2002. 175-194.
2. Spina bifida: brain and neurobehavioral outcomes. In Cognitive and behavioral neurology in developmental age. Riva, D and Bulgheroni, S (Eds). Editions John Libbey Eurotext, Montrouge, France, 2015.

D. Other Professional Communications

National Presentations:

1. Juranek, J. Pediatric Neuroimaging in ADHD Preschoolers. Invited speaker at Dopamine Network Meeting. New York, NY, 2003.

2. Juranek, J. Pediatric Neuroimaging. Invited participant to Trans-NIH Workshop. Bethesda, MD, 2004.

3. Juranek, J. Project 4: Brain Activation Profiles of Math Difficulties in Children: A Magnetic Source Imaging Study. Invited speaker at Fourth Annual PI Meeting: Mathematical Cognition and Specific Learning Disabilities Research Consortium. Bethesda, MD, 2007.

4. Juranek, J. Brain activation profiles of reading difficulties in children: A magnetic source imaging study. Invited speaker at First Annual PI Meeting: Learning Disabilities Research Center Consortium. Florida State University, FL, 2007.

International Oral Presentations:

1. Juranek, J. Advances in Pediatric Neuroimaging. Invited speaker (Research Symposium) at 15th Annual CHADD International Conference. Denver, CO, 2003.

2. Juranek, J. Anatomical and functional differences in children with spina bifida: aMRI and MEG studies. Symposium speaker at International Neuropsychological Society Annual Meeting. Bilbao, Spain, 2007.

3. Juranek, J. Increased cortical complexity in cortically-thin regions: an aMRI study in spina bifida. The 1st World Congress on Spina Bifida Research and Care. Orlando, FL, 2009.

4. Juranek, J., Treble, A., Law, N. Imaging the spina bifida brain for cross-disorder comparisons: Spatial patterns of cortical thickness and thinning, volumetrics of subcortical gray matter, and cerebellar parcellations. International Neuropsychological Society Annual Meeting, Boston, MA, 2011.

5. Juranek, J. Neuroimaging in Spina Bifida: Findings from the SANDI Project. The Second International World Congress on Spina Bifida Research and Care, Las Vegas, NV, 2012.

6. Romanowska-Pawliczek, A., Juranek, J., Cirino, P. T., Fletcher, J.M. A DTI study of structural connectivity within the reading network of young struggling readers. Human Brain Mapping, Honolulu, HI, June 2015.

7. Cox, Charles, S., Dash, P., Juranek, J., Ewing-Cobbs, L. Progenitor cell therapy for adult TBI: preclinical findings and clinical outcomes. National Neurotrauma Society Sante Fe, New Mexico, June 2015.

Local Presentations:

1. Juranek, J. Predictors of Differential Vulnerability to Anxiety and Depression in Epilepsy: A Diffusion Tensor Imaging Study. Invited speaker at UCI's Annual EpiCenter Symposium. Irvine, CA, 2006.

3. Juranek, J., Frye, R. Freesurfer: Automated cortical reconstruction and analysis. Invited lecture to teach background and methods for semi-automated morphometric analyses of brain MR images. Houston, TX, 2007.

4. Juranek, J., Fletcher, J.M. Invited speaker to briefly discuss with prospective graduate students current, cutting-edge approaches in multimodal and quantitative neuroimaging. Houston, TX, 2008.

5. Juranek, J. Invited speaker to present multi-modal neuroimaging methods as complementary information for integration with neuropsychological assessment data. Houston, TX, 2009.

6. Juranek, J. Surface-based analyses of aMRI in Spina bifida children. Children's Learning Institute Collaborative, The University of Texas Health Science Center at Houston, TX, 2009.

7. Juranek, J. Quantitative volumetric imaging in neurodevelopmental disorders. Brain, Behavior, & Imaging group Continuing Medical Education/Clinical Education series at Texas Children's Hospital, March 1, 2011. Invited by Dr. Eyal Muscal.

8. Juranek, J. Quantitative volumetric imaging in neurodevelopmental disorders. Pediatric Grand Rounds CME/CE series at UT-H, November 29, 2011. Invited by Dr. Ian Butler.

9. Juranek, J. Quantitative neuroimaging in spina bifida. Lonestar LEND program at UT-H, May 22, 2012. Invited by Dr. Pauline Filipek.

10. Juranek, J. Neuroimaging in struggling readers. Greater Houston Community Foundation at Cooley Center, September 10, 2016. Invited by Dr. Susan Landry.

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI)

HUMAN RESEARCH CURRICULUM COMPLETION REPORT

Printed on 09/23/2014

LEARNER Jenifer Juranek (ID: 1465450)
DEPARTMENT Pediatrics
PHONE 713.500.3813
EMAIL Jenifer.Juranek@uth.tmc.edu
INSTITUTION University of Texas Health Science Center at Houston
EXPIRATION DATE 09/22/2017

GROUP 1 BIOMEDICAL RESEARCHER AND KEY PERSONNEL

COURSE/STAGE: Refresher Course/2
PASSED ON: 09/23/2014
REFERENCE ID: 10753227

REQUIRED MODULES	DATE COMPLETED
Biomed Refresher 2 - Instructions	09/23/14
Biomed Refresher 2 – History and Ethical Principles	09/23/14
Biomed Refresher 2 – Regulations and Process	09/23/14
Biomed Refresher 2 – Informed Consent	09/23/14
Biomed Refresher 2 – SBR Methodologies in Biomedical Research	09/23/14
Biomed Refresher 2 – Genetics Research	09/23/14
Biomed Refresher 2 – Records-Based Research	09/23/14
Biomed Refresher 2 - Populations in Research Requiring Additional Considerations and/or Protections	09/23/14
Biomed Refresher 2 – Vulnerable Subjects – Prisoners	09/23/14
Biomed Refresher 2 – Vulnerable Subjects – Children	09/23/14
Biomed Refresher 2 – Vulnerable Subjects – Pregnant Women, Human Fetuses, Neonates	09/23/14
Biomed Refresher 2 – FDA-Regulated Research	09/23/14
Biomed Refresher 2 – HIPAA and Human Subjects Research	09/23/14
Biomed Refresher 2 – Conflicts of Interest in Research Involving Human Subjects	09/23/14
How to Complete the CITI Refresher Course and Receive a Completion Report	09/23/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid Independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Program Course Coordinator

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

DISCLOSURE: FINANCIAL INTERESTS AND ARRANGEMENTS OF CLINICAL INVESTIGATORS

Form Approved: OMB No. 0910-0396
Expiration Date: March 31, 2019

TO BE COMPLETED BY APPLICANT

The following information concerning Charles S. Cox, Jr., MD (*Name of clinical investigator*), who participated as a clinical investigator in the submitted study Autologous Wharton's Jelly for Augmented Repair of Cleft Palate (*Name of clinical study*) is submitted in accordance with 21 CFR part 54. The named individual has participated in financial arrangements or holds financial interests that are required to be disclosed as follows:

Please mark the applicable check boxes.

☐ any financial arrangement entered into between the sponsor of the covered study and the clinical investigator involved in the conduct of the covered study, whereby the value of the compensation to the clinical investigator for conducting the study could be influenced by the outcome of the study;

☐ any significant payments of other sorts made on or after February 2, 1999, from the sponsor of the covered study, such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation, or honoraria;

☒ any proprietary interest in the product tested in the covered study held by the clinical investigator;

☐ any significant equity interest, as defined in 21 CFR 54.2(b), held by the clinical investigator in the sponsor of the covered study.

Details of the individual's disclosable financial arrangements and interests are attached, along with a description of steps taken to minimize the potential bias of clinical study results by any of the disclosed arrangements or interests.

NAME	TITLE
Charles S. Cox, Jr.	M.D., Sponsor and Principle Investigator
FIRM/ORGANIZATION: UTHealth McGovern Medical School, 6431 Fannin Street, Dept of Pediatric Surgery, MSB 5.246, Houston, TX 77030	
SIGNATURE: Charles S. Cox	Date *(mm/dd/yyyy)*: 10/19/2016

This section applies only to the requirements of the Paperwork Reduction Act of 1995.

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Public reporting burden for this collection of information is estimated to average 5 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the necessary data, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information to the address to the right:

Do NOT send your completed form to the PRA Staff email address below.

Department of Health and Human Services
Food and Drug Administration
Office of Operations
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

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Dr. Cox has proprietary interest in a device for extracting Wharton's Jelly- but the device is not being used in this study. This study is being conducted under a UTHealth Research Conflict of Interest Management plan. The licensee of the device is not the sponsor of the trial.

RESEARCH CONFLICTS OF INTEREST MANAGEMENT PLAN
FOR CHARLES S. COX, JR., MD
Implemented August, 2014; Revised October, 2016

This Research Conflicts of Interest Management Plan (RCOI Management Plan) has been created by the Research Conflicts of Interest Committee (Committee) to assist in managing or reducing existing or potential financial conflicts of interest in research that have developed or may develop because Dr. Charles S. Cox, Jr. (Professor, Department of Pediatric Surgery), holds significant financial interests with **CBR SYSTEMS, INC. (CORD BLOOD REGISTRY®)** while simultaneously participating in research at The University of Texas Health Science Center at Houston (UTHealth) that is either sponsored by CBR Systems, Inc. and/or that might appear to affect or be affected by his financial interests in CBR Systems, Inc.

The UTHealth Research Conflicts of Interest Policy, HOOP Policy 94 (Policy), requires significant financial interests (*as defined in Attachment A*) that create or appear to create financial conflicts of interest in research to be managed, reduced, or eliminated. By accepting this RCOI Management Plan, the subject individual also agrees to abide by the university's Standards of Conduct (*Attachment C*).

DESCRIPTION OF FINANCIAL CONFLICT OF INTEREST

CBR Systems, Inc., also known as Cord Blood Registry®, is a private, for-profit company that operates a cord blood stem cell bank serving families in the U.S. and internationally. The company focuses on advancing the clinical application of cord blood stem cells by partnering with research institutions. It offers collecting, processing, and long-term cryopreservation of newborn cord blood and tissue stem cell units that are contained in the umbilical cord blood of newborns. The company also creates collection devices designed for cord blood stem cells, *e.g.*, CellAdvantage, a collection kit that is designed to preserve and safely deliver the blood and/or tissue. CBR is based in San Bruno, California and has a laboratory and storage facility in Tucson, Arizona.

Dr. Cox serves on the Scientific Advisory Board for CBR Systems, Inc. and receives significant personal compensation. He does not serve in any fiduciary position with CBR Systems, Inc. or hold any ownership interests. Dr. Cox stated that he is not involved in grant funding decisions in his Scientific Advisory Board position.

UTHealth related technology includes: 1) the umbilical cord processor/Wharton's Jelly extractor and 2) the Wharton's Jelly cryopreservation for autologous tissue engineering applications. UTHealth has optioned the patent application on the Wharton's Jelly extractor to CBR. There is also a Sponsored Research Agreement in place between UTHealth and CBR to develop the extractor, cryopreservation strategy and solutions, and pre-clinical testing, and the option agreement (which gives CBR the right to negotiate a license agreement) expires 1 year after the Sponsored Research Agreement ends.

SPONSORED RESEARCH

Dr. Cox participates in research at UTHealth that could appear to be related to his financial interest in CBR Systems, Inc. (*as listed in Attachment B*).

Should Dr. Cox propose to be involved in future related research, his participation in the research will mandate that it be reviewed by the Committee and this RCOI Management Plan will be updated accordingly.

RCOI MANAGEMENT PLAN

Dr. Cox, the Committee has reviewed your significant financial interests and your existing and proposed research. Your participation in the research creates the appearance of a potential conflict that will be

permitted insofar as you agree to this RCOI Management Plan to manage the identified potential financial conflicts of interest in the research.

In addition, the Committee will monitor this RCOI Management Plan on an annual basis to verify that you have complied with the terms of the Plan and to determine if additional strategies are required to effectively manage any potential financial conflicts of interest in research as needed.

Management of Potential Conflicts in the Conduct of Research:

1. The Committee has reviewed your significant financial interests with CBR Systems, Inc. and your existing and proposed research to determine whether safeguards will be required to promote objectivity in the research, *i.e*. to ensure, to the extent possible, that the design, conduct, and reporting will be free from bias resulting from your financial conflict of interest.

   Research that might appear to affect or be affected by your financial interests in CBR Systems, Inc. ("**conflicted research**") includes research that is sponsored by CBR Systems, Inc. and research funded by any external or internal source that uses, studies, or validates the technology or products licensed to CBR Systems, Inc., including but not limited to research that would result in bringing a product to market., and research funded by any external or internal source that harvests, stores, or uses umbilical cord tissue (a novel technology similar to the business model of CBR Systems, Inc.) that could potentially advance the need for the related technology and/or be used to improve upon the related technology.

   You have informed the Committee that for conflicted research you propose the following research methodologies (as applicable to each research): 1) if applicable, study subjects are randomized; 2) if applicable, clinical care providers and research team members are blinded to the treatment assignment; 3) objective study outcome measures are utilized; 4) you do not review or analyze the outcome measures (*e.g*., imaging, neurocognitive measures); 5) if applicable, subject monitoring procedures for the treatment and placebo groups during the first year of participation are identical; and 6) a Data Safety Monitoring Board is in place, in order to ensure, to the extent possible, that the design, conduct, and reporting will be free from bias resulting from your financial conflict of interest. The Committee has accepted these methodologies as appropriate for safeguarding the objectivity of the conflicted research.

   The Committee has determined that if you propose to participate in "**new conflicted research**" (i.e. initiation of a new protocol that is related to your financial interests with CBR Systems, Inc.), a re-evaluation of your methods for mitigating actual or perceived research bias will be performed. Depending upon the nature of the research, additional safeguards to those described above may be required. You may be asked to provide a description of the planned studies, including statements describing how the proposed studies constitute conflicted research, and the safeguards you will use to ensure objectivity in the design and conduct of the studies and the analysis and interpretation of data. Your participation in the planned studies must be reviewed and approved prior to the initiation of said research.

   The Committee will review your participation in future or existing projects according to the following guidelines:
   a. If there is a new protocol related to your significant financial interests, then it will need to be reviewed.
   b. If there is an existing protocol under the Plan and the proposed change to the protocol pertains to adding more study sites to increase enrollment, then it may not need re-review.
   c. If there is a change in the research that involves a change in the level of risk (ex. increased risk), then it will need re-review (to determine if additional safeguards are warranted).

d. If there is a change in your financial interests, then it will need to be re-reviewed by the Committee and the Plan updated accordingly.

The Committee has also determined that if you propose to participate in "**new conflicted clinical research**" (i.e. initiation of a new protocol that is related to your financial interests with CBR Systems, Inc. and involving human subjects), then the UTHealth guidance ("Research Conflict of Interest (RCOI) Review Process for Projects Involving Human Subjects") will be re-assessed. Your participation in the planned studies must be reviewed and approved prior to initiation of said research and a new RCOI Management Plan may be implemented for each new conflicted clinical research.

The Committee has determined that the continual and consistent implementation of these safeguards for all conflicted research conducted under this RCOI Management Plan will be required to maintain the objectivity of the research.

You are required to notify the Committee of changes in your financial interests or in your research related to CBR Systems, Inc. or its products prior to them occurring or when they occur so that any financial conflict of interest in the research may be re-evaluated and this RCOI Management Plan updated accordingly.

Reporting and Review of Related Significant Financial Interests:

2. You must submit a Research Conflict of Interest (RCOI) Disclosure Form: 1) when submitting all new research applications to the Sponsored Projects Administration (SPA), to the Committee for the Protection of Human Subjects (CPHS), to the Animal Welfare Committee (AWC) as requested by the AWC, or in association with proposed internal awards, department funds, or gifts; and 2) when new reportable financial interests are obtained by you and/or your covered family members during research award/contract periods.

   In addition, when proposing to participate in the design, conduct, or reporting of any other research involving the licensed technology or CBR Systems, Inc. products, regardless of the funding source or location of where the research is to be conducted, you must submit a Research COI Disclosure Form to the Office of the Executive Vice President/ Chief Academic Officer (EVP/CAO) for notification purposes.

3. You must submit a new Review and Approval Form when there are revisions in any sponsored research agreement or contract with CBR Systems, Inc. to ensure that a new Research COI Disclosure Form is submitted for the Committee to review.

4. You must provide additional information as requested within ten (10) business days to facilitate disclosure by UTHealth to sponsors regarding any identified potential financial conflict of interest in research, if required by sponsor guidelines (*e.g.*, federal agencies).

5. You must submit reports as requested by the President, his designee, and/or the Committee that include information about your research program, related significant financial interests, conflicts that may have developed and what steps were taken to minimize and/or eliminate actual or perceived bias in said research. The reports must be submitted annually unless the level of your financial interests or potential conflicts of interest in research requires more frequent monitoring.

Disclosure of Related Significant Financial Interests:

6. You must disclose your related significant financial interests and the institution's related significant financial interests in future publications and presentations that involve any conflicted research. The disclosure should be in the title footnotes, and recommended language is, "Dr. Cox and The University

of Texas Health Science Center at Houston (if applicable) have research-related financial interests in CBR Systems, Inc."

7. You must inform collaborators in writing that you have significant financial interests if they participate in your conflicted research. Notification must take place prior to the collaborator's participation in such research. Written confirmation of such discussions, signed and dated by each collaborator on the "Notification to Collaborators, Staff, and Trainees of Research Related Financial Interests" form, must be submitted to the Office of the EVP/CAO.

Management of Potential Conflicts in Trainee/Student Education:

8. You must inform trainees in writing of your significant financial interests (including but not limited to, pre- and post-doctoral students, collectively referred to as trainees) if they participate in your conflicted research, since your financial interests may potentially impact the trainees' research (*e.g.*, confidentiality issues, risks of publications delays, direction of research). Written confirmation of such discussions, signed and dated by each trainee on the "Notification to Collaborators, Staff, and Trainees of Research Related Financial Interests" form, must be submitted to the Office of the EVP/CAO prior to the trainee's participation in such research.

9. Thesis committees for trainees participating in your conflicted research must include at least one member who has no financial ties to CBR Systems, Inc. or to the research.

10. Trainee projects (*e.g.*, independent studies, course projects, theses) involving your conflicted research must be reviewed by a second faculty member for the potential impact on the trainee's research (*e.g.*, confidentiality issues, risks of publications delays, direction of research) upon initiation of the project and then annually thereafter. The second faculty member must not have financial ties to CBR Systems, Inc. or to the research.

11. UTHealth courses that involve your conflicted research must be co-directed by at least one faculty member who has no financial ties to CBR Systems, Inc. or to the research.

Management of Potential Conflicts in Human Subjects Research:

12. If you propose to participate in research involving human subjects at UTHealth, your participation in such research is subject to the Committee of the Protection of Human Subjects (CPHS) review and approval.

13. If you propose to participate in conflicted research involving human subjects at UTHealth, the Committee applies the guidance, Research Conflict of Interest (RCOI) Review Process for Projects Involving Human Subjects, modified from the American Association of Medical Colleges (AAMC) guidelines. If you sufficiently demonstrate that you have met these standards, the Committee will work with the CPHS to manage any identified potential financial conflicts of interest in research.

14. If you propose to participate in conflicted research involving human subjects at UTHealth, informed consent must be obtained by someone other than you and who has no financial ties to CBR Systems, Inc. or to the research.

15. If you participate in research conducted at another institution and the research is supported by CBR Systems, Inc. and/or the research might appear to affect or be affected by your financial interests, you must disclose the financial interests to that institution's Institutional Review Board.

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AGREEMENT

By signing below, I make the following representations to UTHealth:

I certify that the accuracy of the information in this RCOI Management Plan describing my outside activities and financial interests on which this Plan is based is true and accurate.

I have read, understand, and agree to comply with the requirements for managing potential financial conflicts of interest in research as set forth in this RCOI Management Plan.

I understand and acknowledge that a Standards of Conduct Attestation (Attachment C) will also be implemented to manage potential conflicts of commitment with my UTHealth responsibilities that may result from my outside activities with CBR Systems, Inc.

I understand and acknowledge that my failure to comply with applicable federal regulations, state laws, University of Texas Board of Regents *Rules and Regulations*, UTHealth policies, and the terms of this RCOI Management Plan may result in the total withdrawal of or limitations to UTHealth's approval of my activities with CBR Systems, Inc. and, further, may result in disciplinary action, up to and including termination of my employment at UTHealth.

I understand and acknowledge that it is my responsibility to inform the UTHealth Office of the Executive Vice President/ Chief Academic Officer in writing within thirty (30) days if any of the facts upon which this RCOI Management Plan is based should change. I understand and acknowledge that any material change in circumstances may require review by the Research Conflicts of Interest Committee and revision of this RCOI Management Plan.

Charles S. Cox, Jr., M.D. (Date)
Professor
Department of Pediatric Surgery

READ, ACKNOWLEDGED, AND AGREED TO:

Kevin P. Lally, M.D. (Date)
Professor and Chair
Department of Pediatric Surgery

APPROVED:
The University of Texas Health Science Center at Houston

George M. Stancel, Ph.D. (Date)
Senior Vice President for Academic and Research Affairs

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ATTACHMENT A
DEFINITION OF SIGNIFICANT FINANCIAL INTERESTS IN RESEARCH

UTHealth's definition of significant financial interests in research refers to anything of monetary value that would reasonably appear to affect or be affected by the outcome of the research, regardless of whether that entity is sponsoring the research.

Significant financial interests include not only the interests held by the individual but also the individual's covered family members (defined as spouse, dependent children/step-children, any other person financially dependent upon the employee regardless of legal or biological relationship, and any other person with whom the employee has joint financial interests such that the relationship or the interest could affect the employee's professional responsibilities at UTHealth).

Significant financial interests include, but are not limited to, the following:

- From a publicly-traded entity: the total amount of compensation for services or other payments (*e.g.*, consulting, advising, honoraria, paid authorship) received in the preceding twelve months and the value of stock, stock options, ownership interests, or rights to such interests held on the date of disclosure, that when aggregated exceeds $5,000.

- From a non-publicly traded entity: the total amount of compensation for services or other payments (*e.g.*, consulting, advising, honoraria, paid authorship) received in the preceding twelve months that when aggregated exceeds $5,000.

- From a non-publicly traded entity: any amount of stock, stock options, ownership interests, or rights to such interests held on the date of disclosure.

- Income from royalties, fees, and rights to such interests from an outside entity other than UTHealth.

- Service as an officer, director, or other fiduciary position for any entity from which the individual received remuneration or payment for expenses in the preceding twelve months.

- Gifts received from an outside entity in the preceding twelve months that exceed $250 in value, or multiple gifts from a single entity that in the aggregate exceed $250 in value.

- Reimbursed or sponsored travel in the preceding twelve months if the aggregated value of all payments from the sponsor/organizer (such as salary, consulting fees, honoraria, paid authorship, and travel) exceeds $5,000.

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ATTACHMENT B
RELATED RESEARCH

1-Title: "Autologous Cell Therapies for Cerebral Palsy-Chronic/ACT for CP"
Role: Principal Investigator
Sponsor: CBR Systems, Inc.
CPHS#: 12-0876
This Phase II clinical trial is a randomized, blinded, placebo-controlled, cross-over study designed to treat CP in pediatric patients with an IV infusion of autologous bone marrow mononuclear cells or autologous umbilical cord blood cells. The study aims to: 1) determine if autologous cells using either BMMNCs or hUCBs are safe to administer in children with CP; and 2) determine if late functional outcomes are improved following the administration of autologous cells compared with patients in the control group.

2-Title: "Assaying the potency of hUCB on BBB permeability after TBI"
Role: Principal Investigator
Sponsor: CBR Systems, Inc.
AWC#: 15-0027
This experiment uses cells harvested from umbilical cords and preserved using methods currently in use by CBR, Inc. The cells will then be used as an investigational treatment in animals that have undergone a well-established model of traumatic brain injury. Success of the treatment is determined by use of a dye that leaks into the brain at sites where the protective brain barrier is damaged. This animal model of TBI will then be compared to lab experiments that detect the ability of these cells to produce substances involved in the inflammatory response to injury.

3-Title: "Tools and Technologies for the harvest/ storage/ deployment of Wharton's Jelly in Pediatric Craniofacial Surgery"
Role: Co-investigator
Sponsor: CBR Systems, Inc.
Dr. Cox's involvement in this proposed study pertains to research on the Wharton's Jelly devices and not on the clinical uses of Wharton's Jelly. He will use a team based approach to iterative design of the related technology that will ultimately be milestone driven in terms of device function- either it works or doesn't based upon pre-determined go/ no go criteria once the project is funded. Dr. Cox/ CBR, Inc. will be "supplying" (harvesting, storing, providing) the Wharton's Jelly "material" for the surgery. Dr. Cox will not be involved in the consenting, data collection, or data reporting processes. It is anticipated that this project will migrate to a Phase I clinical trial.

4-Title: "Autologous Wharton's Jelly for Augmented Repair of Cleft Palate"
Role: Principal Investigator
Sponsor: Internal (UTHealth and Memorial Hermann Foundation)
CPHS#: 16-0738
The purpose of this study is to evaluate whether the harvesting, storage, reanimation, and surgical application of autologous Wharton's Jelly on alveolar cleft palate repair in children is safe and feasible. Secondary objectives will look at the effect of Wharton's Jelly on functional outcomes and bony healing post-repair through dental imaging (Xray and CT scan). Dr. Cox participated in the design of the research and as part of the team, he will participate in the writing of reports to the IRB and DSMB and in the publishing of research. He will not participate in the data collection or data analysis. For this study, Dr. Cox's team will manually extract the Wharton's Jelly and store the tissue locally. CBR Systems, Inc. is not sponsoring the study, not storing the tissue, and not supplying the materials, devices, or products used in this study.

CONFIDENTIAL

ATTACHMENT C
STANDARDS OF CONDUCT FOR CHARLES S. COX, JR., MD

(as per HOOP Policy 20- Conflict of Interest, Conflict of Commitment and Outside Activities)

By my signature below, I, Charles S. Cox, MD, acknowledge my understanding that:

A. My primary responsibility as a UTHealth employee is the accomplishment of the duties and responsibilities assigned to my position at the university. External consulting, outside employment or other activities that interfere with those duties and responsibilities are not acceptable.

B. Approval for me to conduct outside activities with CBR Systems, Inc. is contingent upon my agreement to and compliance with the following terms:

1. I will not use UTHealth time to conduct work for CBR Systems, Inc. unless conducted under a Sponsored Research Agreement (SRA) or other agreement, project, grant, or gift approved and administered by UTHealth. I will conduct work on behalf of or for the benefit of CBR Systems, Inc. either on my personal time away from UTHealth or on vacation leave from UTHealth.

2. I will not use any other UTHealth resources (*e.g.*, personnel, space, equipment, supplies, services) for the benefit of CBR Systems, Inc. unless conducted under an SRA or other agreement, project, grant, or gift approved and administered by UTHealth. Personnel hired by UTHealth and resources owned or purchased by UTHealth may only be used for the benefit of CBR Systems, Inc. if the use is referenced in an SRA, contract, or other document approved and administered by UTHealth.

3. I will not transact any business in my official UTHealth capacity with CBR Systems, Inc. In my work for which I am paid by UTHealth, I cannot participate in discussions, negotiations, or decision making about or with CBR Systems, Inc.

4. I will ensure that my work for CBR Systems, Inc. does not overlap with my work for UTHealth, unless conducted under an SRA or other agreement, project, grant, or gift approved and administered by UTHealth. I will not conduct work for which I am paid by UTHealth while simultaneously working for the benefit of CBR Systems, Inc. unless conducted under an SRA or other agreement, project, grant, or gift approved and administered by UTHealth.

5. I will work directly with my administrative supervisor to assure that I continue to meet my administrative, clinical, teaching, research, and service activities for UTHealth. During performance evaluations, I will discuss whether my activities with CBR Systems, Inc. are interfering with my work for which I am paid by UTHealth.

6. I will comply with UTHealth policies regarding participation in all outside activities, including the proportion of my professional efforts that may be devoted to such activities. I have read and understood the Decision Matrix for Faculty Outside Activities, referenced in HOOP 20. For those outside activities that require prior approval from my supervisor, I will use the online Outside Activities Request system.

7. I will conduct my activities so that UTHealth employees, colleagues, subordinates, trainees, fellows, and students understand when activities are being performed in my personal activity for CBR Systems, Inc. and when they are not. When I communicate with UTHealth colleagues,

subordinates, trainees, fellows, and students about CBR Systems, Inc., I will tell them that I am doing so in my capacity with CBR Systems, Inc. and not in the scope of the work for which I am paid by UTHealth.

8. I will not hire employees of CBR Systems, Inc. as an employee or contractor to UTHealth, unless otherwise specifically approved beforehand by my administrative supervisor and the Institutional Conflicts of Interest Committee.

9. I understand that if I provide administrative supervision of UTHealth employees or trainees who participate in research either sponsored by CBR Systems, Inc. or involving the related technology, personnel actions regarding those individuals (e.g., evaluations, appointments, reappointments, promotions, salary decisions) will be monitored on an annual basis by the Office of the EVP/CAO. I will work with the Office of the EVP/CAO and provide information about my supervisory activities upon request.

10. I have disclosed all of my financial interests, business relationships, advising, and consulting activities with CBR Systems, Inc. and any other outside entity on my Financial Disclosure Statement.

11. I will update my Financial Disclosure Statement as needed or at least annually, even if no changes have occurred.

12. I will disclose all material changes to my financial interests and activities with CBR Systems, Inc. to my administrative supervisor and on my Financial Disclosure Statement within thirty (30) days of the change.

13. I will ensure that my outside activities with CBR Systems, Inc., *i.e.*, those activities conducted outside the scope of an SRA or other agreement, project, grant, or gift approved and administered by UTHealth with CBR Systems, Inc., do not influence the way I perform my job at UTHealth or my independence of judgment in the performance of my duties at UTHealth. I will seek guidance from my administrative supervisor and the Office of the Executive Vice President/ Chief Academic Officer before taking actions in which both CBR Systems, Inc. and UTHealth are involved.

14. I will not disclose confidential information acquired through my employment at UTHealth to CBR Systems, Inc. This does not include information which is the subject of non-disclosure agreements or other appropriately executed documents between UTHealth and CBR Systems, Inc.

15. I will work with the Office of Technology Management regarding all intellectual property issues.

16. I understand that the HOOP Policy 20 and specific guidance documents regarding employee participation in outside activities and the management of conflicts of interest and commitment is available on the Office of the Executive Vice President/ Chief Academic Officer website.

CONFIDENTIAL

ATTESTATION

I attest to my understanding of the terms of these Standards of Conduct. I understand that failure to comply with applicable federal regulations, state laws, University of Texas Board of Regents *Rules and Regulations*, UTHealth policies, and these Standards of Conduct may result in the total withdrawal of or limitations to UTHealth's approval of my activities with CBR Systems, Inc. and, further, may result in disciplinary action, up to and including termination of my employment at UTHealth.

_____

Charles S. Cox, Jr., M.D. (Date)
Professor
Department of Pediatric Surgery

Form Approved: OMB No. 0910-0616. Expiration Date: 2/28/2018. See PRA Statement on page 2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Certification of Compliance

Under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. Name of Sponsor/Applicant/Submitter
Charles S. Cox, Jr., M.D.

2. Date of the Application/Submission Which This Certification Accompanies
10/19/2016

3. Address

Address 1 *(Street address, P.O. box, company name c/o)*
UTHealth McGovern Medical School, 6431 Fannin Street

Address 2 *(Apartment, suite, unit, building, floor, etc.)*
Department of Pediatric Surgery, MSB 5.246

City
Houston

State/Province/Region
Texas

Country
USA

ZIP or Postal Code
77030

4. Telephone and Fax Numbers *(Include country code if applicable and area code)*

(Tel): 713.500.7300

(Fax): 713.500.0714

PRODUCT INFORMATION

5. **For Drugs/Biologics:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s).
For Devices: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)

Human Native Wharton's Jelly

Continuation Page for #5

APPLICATION / SUBMISSION INFORMATION

6. Type of Application/Submission Which This Certification Accompanies

☐ IND ☐ NDA ☐ ANDA ☐ BLA ☐ PMA ☐ HDE ☐ 510(k) ☐ PDP ☒ Other

7. Include IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/ Other Number *(If number previously assigned)*

If BLA was selected in item 6, provide Supplement Number

8. Serial Number Assigned to Application/Submission Which This Certification Accompanies
00000

CERTIFICATION STATEMENT / INFORMATION

9. Check only one of the following boxes *(See instructions for additional information and explanation)*

☐ A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

☒ B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

☐ C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

Certification Statement / Information section continued on page 2

CERTIFICATION STATEMENT / INFORMATION *(Continued)*

10. If you checked box C, in number 9, provide the National Clinical Trial (NCT) Number(s) for any "applicable clinical trial(s)," under 42 U.S.C. § 282(J)(1)(a)(i), section 402(j)(1)(a)(i) of the Public Health Service Act, referenced in the application/ submission which this Certification accompanies. *(Add continuation page as necessary.)*

NCT Number(s): _____ _____ _____ _____ _____

Continuation Page for #10

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act.

Warning: A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. Name and Title of the Person who Signs Number 15

Name	Title
Charles S. Cox, Jr., M.D.	M.D., Sponsor and Principle Investigator

12. Address

Address 1 *(Street address, P.O. box, company name c/o)*: UTHealth McGovern Medical School, 6431 Fannin Street		
Address 2 *(Apartment, suite, unit, building, floor, etc.)*: Department of Pediatric Surgery, MSB 5.246		
City: Houston	State/Province/Region: Texas	
Country: USA		ZIP or Postal Code: 77030

13. Telephone and Fax Numbers *(Include country code if applicable and area code)*

(Tel): 713.500.7300

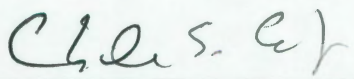
(Fax): 713.500.0714

14. Date of Certification

10/19/2016

15. Signature of Sponsor/Applicant/Submitter or an Authorized Representative *(Sign)*

Sign



This section applies only to requirements of the Paperwork Reduction Act of 1995.

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SECTION 1: INTRODUCTION

1.1 Introductory Statement:

This Phase 1 study will investigate the feasibility and safety of a single dose of autologous Wharton's Jelly to augment the surgical repair of Cleft Lip and Palate (CLP) in infants 1 to 2 years of age.

Despite nearly a century of experience repairing cleft palates, there is no consensus on the optimal strategy and timing of CLP repair, and the current gold standard surgery has a relatively high failure rate. Studies comparing various surgical protocols for CLP repair have primarily been small single center, retrospective reports lacking consistent outcome measures.

Wharton's jelly is a gelatinous connective tissue matrix made up of muco-polysaccharides and hyaluronic acid. It is the support structure of the umbilical cord/umbilical arteries and vein. The matrix is rich in primitive mesenchymal stromal cells (MSCs), various cytokines, nutrients and growth factors.

The use of autologous Wharton's Jelly (an otherwise biological waste product), instead of the gold standard cleft palate repair with bone graft represents a relatively low-cost/low-morbidity alternative.

1.1.1 Name of Biological-

Autologous human Wharton's Jelly with mesenchymal stem cells.

1.1.2 Pharmacological Class-

Not Applicable

1.1.3 Structural Formula-

Not Applicable

1.1.4 Formulation and Dosage for Proposed Study-

Autologous human Wharton's Jelly meeting release criteria with a volume $\geq$ 2mL. delivered in a sterile luer-lock syringe.

1.1.5 Route of Administration-

Instilled via injection into the tunnel created during Cleft Palate repair by Gingivoperiosteoplasty (GPP).

1.1.6 Objectives and Duration of the Proposed Clinical Investigation-

Primary Objective: To determine if the harvest/storage/reanimation/surgical

application of autologous Wharton's Jelly (WJ) to augment the surgical repair of alveolar cleft palate is safe and feasible.

Secondary Objectives: To evaluate the effect of WJ on functional outcomes post-repair as determined by dental x-ray and cone beam 3D CT examination of the bone bridge across the alveolus.

Duration: We have a sufficient clinical volume of potentially eligible patients to enroll all 25 subjects within the first year of study initiation. Subjects will be followed for approximately 2 years following consent. We anticipate completing the study within 3 to 4 years from initiation.

1.2 Summary of Previous Human Experience:

Published *in vivo* and *in vitro* research studies by Costello (2016) and others have described the potential role of WJ in regenerative medicine applications to act as a temporary scaffold for cell proliferation, bone regeneration and remodeling. (Gladysz, 2015) (Bongso, 2012) Human subjects clinical investigations using WJ have been underway since 2008. The Kalaszczynska, 2015 publication included in Section 8 of this application found 51 studies in the Clinical Trials Database (www.clinicaltrials.gov) using WJ for a wide range of therapeutic applications. To our knowledge, there have been no reports of adverse events directly related to autologous WJ administration.

1.3 Status in Other Countries:

We're unaware of any clinical investigations or marketing efforts that have been withdrawn in other countries due to concerns with the safety of autologous WJ.

1.4 References:

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Watson, N., Divers, R., Kedar, R., et al. Discarded Wharton's Jelly of the Human Umbilical Cord: A Viable Source for Mesenchymal Stem Cells. *Cytotherapy*. 2015;17(1): 18-24. DOI:10.1016/j.jcyt.2014.08.009

SECTION 2: GENERAL INVESTIGATIONAL PLAN

2.1 Rational:

The rationale for the study is that current surgical techniques for CLP repair have a relatively high failure rate and an autologous tissue engineering strategy may reduce complications and improve post-op. outcomes.

2.2 Indication to Be Studied:

This Phase 1 study will investigate the feasibility and safety of a single dose of autologous Wharton's Jelly to augment the surgical repair of Cleft Lip and Palate (CLP) in infants 1 to 2 years of age.

2.2.1 Background Information on CLP-

CLP is a congenital defect resulting from the failure of the median nasal process to fuse with the maxillary process and failure of the palatal shelves of the maxillary process to fuse to one another. CLP is the most common birth defect in the United States affecting more than 2,650 children born each year according to the Centers for Disease Control and Prevention. CLP treatment varies depending on severity. Historically, the cleft defect has been corrected between 8 to 12 years of age during the phase of mixed dentition with a secondary alveolar bone graft. In this procedure, cancellous bone is harvested from the patient's iliac crest and used to close the alveolar defect and any residual oro-nasal fistula. While successful in reconstructing the alveolus, the bone harvest equates to an additional surgery for the patient with associated donor site morbidities including intraoperative blood loss, pain, infection, and scarring.

Gingivoperiosteoplasty (GPP) is a surgical option for early repair of CLP in infants under 2 years of age. The procedure creates a gingivoperiosteal tunnel that facilitates bone ingrowth without the need of a bone graft. While patients benefit from a single surgery rather than multiple staged surgeries, long term follow-up of these patients has shown increased incidents of mid-facial hypoplasia, malocclusion, and persistent need for additional surgeries requiring bone grafts.

2.3 General Approach for Evaluation of Treatment:

Twenty-five subjects requiring surgical repair of the cleft palate will undergo an augmented repair using autologous Wharton's Jelly and will be assessed for safety, feasibility of study procedures, and functional outcome of the augmented surgical repair. Subjects will be monitored for safety throughout the hospital stay and at scheduled follow-up clinic visits at 14 days, 3, 6 and 12 mo. post-surgery, or more frequently if treatment related SAE's are suspected.

2.3.1 Inclusion Criteria-

1. Umbilical cord and placental tissue collected under the Protocol Tissue and Stem Cell Research Bank for Future Autologous Use, HSC-MS-15-0830, meeting QA release criteria at the time of collection and cryopreservation.
2. Presence of alveolar cleft defect in conjunction with cleft lip and palate based on birth assessment by the Cleft Palate team.
3. Planned cleft palate repair and follow-up with UTHealth Cleft Palate Team and Memorial Hermann Hospital-TMC.
4. Ability to obtain informed consent from the parent or legally authorized representative (LAR) within 1mo. of the scheduled GPP surgery.

2.3.2 Exclusion Criteria-

1. Birth assessment indicating: absence of alveolar cleft palate, cleft lip alone, cleft palate alone.
2. Presence of chorioamnionitis and/or neonatal sepsis.
3. Presence of significant cardiac comorbidities.
4. Cleft palate with an alveolar gap greater than 5mm.
5. Cleft palate unsuitable for pre-surgical nasoalveolar molding (NAM).
6. Unsuccessful NAM delaying and or preventing GPP repair.
7. Inability to return for follow-up evaluations.

2.3.3 Screening and Consent-

Following delivery and confirmation of alveolar cleft defect, the parents of potential subjects will be approached by one of the research team members. Information regarding study participation will be provided. Discussion on the alternatives to study participation will include the standard GPP without WJ or

other surgical options such as delayed CLP repair when the child is older. Parents will be given ample time to consider study participation and may take the consent home for review. For surgical repair using autologous WJ, consent must be obtained at least 1 month in advance. This will allow adequate time for WJ reanimation and QA procedures.

2.3.4 Study Treatment-

GPP is normally done when infants are between 12 to 18 months of age. The Stem Cell lab will be notified of the scheduled surgery date to allow time for WJ reanimation and QA tests. Parental consent will be reviewed at the time of admission to ensure willingness to continue with study procedures. The research and cleft palate teams will review the WJ release criteria and go/no go status immediately prior to surgery. The WJ cannot be returned to the stem cell lab and must be used within 6 hours after release.

Subjects will receive standard of care procedures for infants undergoing GPP repair with the addition of WJ.

The WJ will be delivered to the operating room in 3 mL. syringe within a sterile peel-pack. Standard hospital procedures will be followed to verify the ID's of the WJ and subject. The sterile peel-pack will then be opened and the WJ syringe presented to the surgeon. The surgeon will inject the WJ within the pocket of space created by elevation of the mucosal flaps. Finally, the lingual flaps will be closed. Following palatal closure, maxillary alveolar alginate impressions will be taken and poured into plaster cast impressions. These impressions will then undergo microCT scans and the size of the alveolar defect digitally registered to provide a baseline at the time of the WJ administration.

2.3.5 Follow-Up Period (Post Surgery to End of Study Visit)-

Subjects will be hospitalized overnight per standard of care for infants undergoing GPP. Outcome assessments will be made at standard of care post-op. visits: 14 Days (+/-7 Days), 3 Month (+/- 15 Days), 6 Month (+/- 21 Days), 1 Year (+/- 21 Days)

2.3.6 Evaluation of Safety (Primary Objective)-

Standard of care data will be collected at each follow-up visit including physical exam, medical history since discharge (unscheduled clinic or hospital visits for management of adverse events), and routine clinical lab and diagnostic test results. Any signs of infection (wound drainage, malodorous fluid, fever, increased pain or agitation with palpation of incision) will be treated with oral antibiotics for a course of 7 days. If this infection persists past this time, then operative drainage and irrigation of the pocket will be performed under general anesthesia.

2.3.7 Evaluation of Functional Outcome (Secondary Objective)-

Occlusal X-Ray: Occlusal x-ray images will be used for 2D evaluation of bone bridge presence or absence across the alveolus. Height and width of the alveolar bone will be compared to the non-cleft side for a percentage of fill in 2 dimensions.

Cone Beam CT: Cleft width, thickness, and vertical height of the bone bridge will be evaluated using CBCT images. Differences in permeability/Hounsfield units will be used to distinguish between existing and new bone. Digital volumetric analysis of the CBCT images will be used to calculate the volume of bone created within the cleft.

2.4 Research Plans for the First Year:

The activities detailed in the study protocol cover the entire 1st year of the study.

2.5 Risks:

The risks associated with autologous WJ administration are primarily limited to the remote chance of product contamination. There is always the potential risk of a rare and previously unknown allergic response to components associated with stem cell lab processing.

2.6 References:

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Hardwicke, J., Landini, G., Richard, B. Fistula Incidence after Primary Cleft Palate Repair: A Systemic Review of the Literature. *Plastic and Reconstructive Surgery*. 2014;134(4): 618-627. DOI:10.1097/PRS.0000000000000548

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Nguyen, C., Hernandez-Boussard, T., Davies, S., et al. Cleft Palate Surgery: An Evaluation of Length of Stay, Complications, and Costs by Hospital Type. *The Cleft Palate-Craniofacial Journal.* 2014; 51(4): 412-419. DOI: 10.1597/12-150

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SECTION 3: INVESTIGATIONAL BROCHURE

CIRCULAR OF INFORMATION

FOR THE USE OF CELLULAR THERAPY PRODUCTS

This circular was prepared jointly by the AABB, America's Blood Centers, the American Association of Tissue Banks, the American Red Cross, the American Society for Apheresis, the American Society for Blood and Marrow Transplantation, the College of American Pathologists, the Cord Blood Association, the Foundation for the Accreditation of Cellular Therapy, ICCBBA, the International Society for Cellular Therapy, the Joint Accreditation Committee of ISCT and EBMT, the National Marrow Donor Program, and Netcord. Federal law prohibits dispensing the cellular therapy products described in this circular without a prescription.

Contact Information

If you want to learn more about cellular therapy, contact any of the following co-sponsors of this publication.

AABB
www.aabbcct.org

American Association of Tissue Banks (AATB)
www.aatb.org

American Red Cross (ARC)
www.redcross.org

American Society for Blood and Marrow Transplantation (ASBMT)
www.asbmt.org

American Society for Apheresis (ASFA)
www.apheresis.org

America's Blood Centers (ABC)
www.americasblood.org

College of American Pathologists (CAP)
www.cap.org

Cord Blood Association (CBA)
www.cb-association.org/

Foundation for the Accreditation of Cellular Therapy (FACT)
www.factwebsite.org

ICCBBA
www.iccbba.org

International NetCord Foundation
www.netcord.org

International Society for Cellular Therapy (ISCT)
www.celltherapysociety.org

JACIE Accreditation Office
www.jacie.org

National Marrow Donor Program (NMDP)
www.nmdp.org

Table of Contents

Notice to All Users

The *Circular of Information for the Use of Cellular Therapy Products* (hereafter referred to as the *Circular*) is an extension of container labels, as the space on those labels is limited.[1] The focus of this *Circular* is restricted to unlicensed cellular therapy products that are minimally manipulated. These unlicensed products can be: hematopoietic progenitor cells (HPCs), leukocytes and other cells derived from bone marrow, umbilical cord blood, or cellular products collected by apheresis. This *Circular* does not apply to products that have already received a license as a cellular therapy product in the United States. Per requirements of other national competent authorities, cellular therapy products may be designated as licensed biological products, medical devices, or advanced therapy medicinal products. Principles expressed here may also be applied to other cellular therapy products. Cellular therapy products are biological products that contain living human cells and are intended for use in patient treatment. Professional judgment based on clinical evaluation determines the selection of products, dosage, rate of administration, and decisions in situations not covered in this general statement.

WARNING: Because cellular therapy products are derived from human blood or tissues, they may carry a risk of transmitting infectious agents, including bacteria, viruses, fungi, protozoa, and prions. Donor screening and testing procedures are in place to minimize the risk of transmitting such infections but cannot eliminate this risk. Transmission of malignant disease has been reported. Also, serious life-threatening septic and toxic reactions can result from administration of products containing bacterial toxins. In addition, cellular therapy products may contain certain immunizing substances other than those indicated on the label, such as red cells, mature white cells, platelets, and plasma proteins. Therefore, this *Circular*, in whole or in part, cannot be considered or interpreted as an expressed or implied warranty of the safety or fitness of the described products even when they are used for their intended purpose. Attention to the specific indications for cellular therapy products is needed to prevent inappropriate administration.

This *Circular* addresses some of the applicable regulations established by regulatory/competent authorities such as the Food and Drug Administration (FDA), the Health Resources and Services Administration (HRSA), and Directive 2004/23/EC (and other European Commission directives) of the European Parliament and the Council of the European Union (EU).[2-7] This *Circular* is not a comprehensive reference for applicable regulations.

The nomenclature used throughout this *Circular* is consistent with *ISBT 128* terminology and was current at the time of publication.[8-10] However, acronyms such as HPC(CB), MNC(A), and HPC(M) are used only as abbreviations and are not intended to be used on the full product labels. (Check ICCBBA's standard terminology[8] for appropriate usage of acronyms.) Users of this *Circular* should confirm that the terminology is still in effect before labeling and distributing a cellular therapy product for patient use.

General Information

This *Circular* was prepared by the Circular of Information for Cellular Therapy Products Task Force, consisting of representatives from the AABB, the American Association of Tissue Banks (AATB), the American Red Cross (ARC), the American Society for Blood and Marrow Transplantation (ASBMT), the American Society for Apheresis (ASFA), America's Blood Centers (ABC), the College of American Pathologists (CAP), the Foundation for the Accreditation of Cellular Therapy (FACT), ICCBBA, the International Society for Cellular Therapy (ISCT), the National Marrow Donor Program (NMDP), the Joint Accreditation Committee of ISCT and EBMT (JACIE), and NetCord. The text of this document has been approved by the board of directors of each of these organizations. Representatives from the FDA and HRSA participated in the deliberations of this task force.

This *Circular* is intended to provide general information to those who administer cellular therapy products, and serves as an extension and enhancement of the label found on the cellular therapy product. The task force has chosen to describe only those cellular therapy products that are most frequently used in clinical practice. **Not all cellular therapy products are described in this *Circular*.**

In order to address other cellular therapy products that are not listed in the *Circular*, this document is designed with a section of blank pages at the end to allow for inclusion of facility-specific information. It is important for users of this document to examine this section of the *Circular* for any additional information provided by the distributing facility and/or the manufacturer of the cellular therapy product. The portion preceding this section of the document cannot be changed.

This *Circular* is intended to be used by facilities based in different countries. The task force has made a concerted effort to accommodate both US and EU requirements in the document text. However, the regulatory approaches to cellular therapy products in the United States and the EU, as well as in other countries, differ in some aspects. Users should consult the appropriate regulatory authority for specific requirements related to their facility.

For investigational products manufactured and administered in the United States, an FDA-approved investigational new drug (IND) application or an investigational device exemption (IDE) is required. For investigational products manufactured and administered outside the United States, other local regulations apply. The relevant clinical protocol should be consulted for information regarding the indications for use, specific details for the administration of the product, and any expected toxicities. For corporate-sponsored or multicenter clinical trials, the indications and administration and toxicity information can also be found in the investigator's brochure.

Donors

Cellular therapy products described in this *Circular* have been collected from human donors for autologous or allogeneic administration. Autologous HPC collection usually occurs after mobilization of the donor's stem and progenitor cells with growth factors, chemotherapy, or both. Donors of other cellular therapy products may or may not require stimulation by growth factors, depending on the protocol employed. Allogeneic HPC collection usually occurs after mobilization with growth factors alone. Certain products such as HPCs from marrow [HPC, Marrow; or HPC(M)] and mononuclear cells from apheresis [MNC, Apheresis; or MNC(A)] are usually collected from donors who are not mobilized.

US federal regulations do not require testing of autologous donors for transmissible agents. However, the voluntary accrediting organizations (eg, AABB, FACT-JACIE) and some states or regions may require additional testing and/or testing of autologous donors. Allogeneic donors are screened through the use of questions designed to detect risk factors for infectious diseases transmissible by the cellular therapy product. Allogeneic donors are also tested for transmissible infectious diseases. (See Tables 1A and 1B.) The questions are based on donor screening requirements promulgated by regulatory agencies and criteria set forth by standard-setting organizations. A donor questionnaire and accompanying donor screening materials* have been developed for cellular therapy products and cord blood donors. The provision of truthful and accurate information by a donor during health/risk assessment is essential for the exclusion of donors whose cellular therapy products may transmit diseases to recipients.

Some allogeneic donors may not meet all the requirements; however, because of the patient's clinical circumstances, they may be approved for donation. In such situations, information regarding requirements that the donor has not met is included in the summary of records/information provided to the transplant center. The cellular therapy products from such donors are also labeled accordingly. (See Table 2.) Cellular therapy products from a donor with abnormal screening and/or test results may be administered to a recipient if the recipient has been advised of the risk, the recipient's physician has authorized the use of the product, and the product is appropriately labeled.

*An example of such a questionnaire, called the uniform donor questionnaire, has been prepared with input from the AABB, AATB, ASFA, and FACT, and can be accessed on the AABB website (www.aabb.org) under ''Programs & Services'' > ''Transfusion Medicine'' > ''Donor History Questionnaires.''

Cellular Therapy Product Sources

HPC, Marrow

HPC, Marrow [HPC(M)] preparations are collected as a source of HPCs. They are obtained by multiple needle aspirations from the posterior iliac crests of an autologous or allogeneic donor. The marrow is placed in a sterile container with an electrolyte solution and an appropriate anticoagulant. The cell suspension is passed through sterile filters to remove fat, bone particles, and cellular debris. The volume of HPC(M) products varies and may range from 100 mL to 2000 mL. Marrow contains mature red cells, white cells, platelets, committed progenitors of all lineages, mast cells, fat cells, plasma cells, and pluripotent hematopoietic cells. Some of these cells are capable of reconstituting the hematologic and lymphoid systems of an autologous or allogeneic recipient. These cells are usually processed before infusion but are sometimes infused in an unmodified state. The most common modifications of allogeneic HPC(M) products are reduction of the volume of ABO-incompatible red cells, removal of ABO-incompatible plasma, selection of CD34+ progenitor cells, and removal of donor T lymphocytes. The most common modification of autologous HPC(M) products is to reduce the volume by removing plasma and red cells before cryopreservation.

HPC, Apheresis

HPC, Apheresis [HPC(A)] preparations are collected as a source of HPCs obtained from the peripheral blood using an apheresis technology, usually after recombinant hematopoietic growth factor administration or other agents. Autologous donors may also have undergone chemotherapy mobilization. Allogeneic HPC(A) collections are frequently infused without further processing. Additional processing of allogeneic HPC(A) products includes reduction or removal of ABO-incompatible red cells, removal of ABO-incompatible plasma, selection of CD34+ progenitor cells, or removal of donor T lymphocytes. Common types of additional processing of autologous HPC(A) products are reduction of volume by removing plasma before cryopreservation and selection of CD34+ progenitor cells. HPC(A) products may be thawed and washed to remove dimethyl sulfoxide (DMSO).

HPC, Cord Blood

HPC, Cord Blood [HPC(CB)] preparations are collected as a source of HPCs obtained from the umbilical cord during the third stage of labor or after delivery of the placenta. After thorough cleansing of the cord, the blood is collected by gravity drainage into standard collection bags containing anticoagulant. Before cryopreservation, cord blood collections are usually processed by red cell and plasma reduction. HPC(CB) products are typically stored with final 10% DMSO cryoprotectant in bags with integral segments designed to be a source of material for identity and potency testing. Frozen cord blood products are transported to the transplant center before patient conditioning begins and are typically thawed using a wash or reconstitution method before infusion. HPC(CB) products that are not red cell reduced should be washed or diluted to lessen the potential effects of hemolysate.

Nucleated Cell Preparations

MNC, Apheresis

MNC, Apheresis [MNC(A)] preparations contain nucleated cells collected from the peripheral blood by an apheresis procedure and are intended for clinical use other than as HPCs. Autologous MNC(A) collections are generally further processed. Allogeneic MNC(A) collections are most commonly used as donor lymphocyte infusions (DLIs). The dose for MNC(A) is determined by institutional policies and is usually based on the number of T cells (eg, CD3+ cells), nucleated cells, or mononuclear cells.

NC, Cord Blood
NC, Cord Blood [NC(CB)] preparations are collected as a source of nucleated cells obtained from the umbilical cord during the third stage of labor or after delivery of the placenta and are intended for clinical use other than as HPCs.

NC, Whole Blood
NC, Whole Blood [NC(WB)] preparations contain nucleated cells collected as peripheral whole blood and are intended for clinical use other than as HPCs.

NC, Marrow
NC, Marrow [NC(M)] preparations contain nucleated cells collected from bone marrow and are intended for clinical use other than as HPCs.

Cellular Therapy Product Descriptions

Cellular therapy products consist of somatic-cell-based products [eg, HPC(A), HPC(M), HPC(CB), MNC(A), NC(WB)] that are collected or procured from the donor and intended for manipulation and/or administration to the patient.

HPC products contain hematopoietic stem and progenitor cells capable of providing hematopoietic and immune reconstitution after myeloablative or nonmyeloablative preparative regimens. The products contain pluripotent and lineage-committed hematopoietic progenitors as well as lymphocytes.

Actions
HPCs administered intravenously migrate to the marrow, where they divide and mature. The mature cells are released into the bloodstream, restoring blood counts and immunity.

The time from administration of HPCs to recovery of adequate or normal blood counts is variable. Allogeneic transplantation sometimes induces a graft-vs-tumor effect that is beneficial in recipients who receive a transplant for treatment of malignancies.

Allogeneic cellular therapy products may also be used to provide additional donor lymphocytes to enhance a graft-vs-leukemia effect. Other applications of cellular therapy products may have different potential mechanisms of action depending on the cell type and clinical setting.

Indications
Allogeneic HPC products are intended to provide hematopoietic reconstitution after myeloablative or nonmyeloablative preparative regimens for a wide range of disease states. For patients with certain malignancies, the product is also intended to provide immune reconstitution and immune-mediated therapy. Autologous HPCs are collected and used following myeloablative or myelotoxic therapy to enhance hematopoietic reconstiution. The therapy is intended to treat the patient's underlying malignancy, and autologous HPC products are administered to minimize morbidity and mortality caused by the myelotoxic effects of the therapy. Additional applications may be used as indicated in clinical trials and research protocols.

Contraindications
MNC(A) and NC(WB) are generally contraindicated for patients experiencing severe graft-vs-host disease (GVHD). Institutional policies and protocols and federal regulations dictate specific contraindications for cellular therapy product administration. Additional information regarding contraindications may be included at the end of this document, if provided by the distributing facility.

The following section provides common cellular therapy product descriptions in the product description format consistent with *ISBT 128* information and labeling standards.

HPCs contain self-renewing or multipotential stem cells capable of maturing into any hematopoietic lineage, lineage-restricted pluripotent progenitor cells, and committed progenitor cells. They may be collected from bone marrow [HPC(M)], peripheral blood with or without prior mobilization [HPC(A)], whole blood with mobilization (HPC, Whole Blood), or placental/umbilical cord blood [HPC(CB)]. They may then be subjected to volume reduction or further manipulations. (See below.)

HPC, (PLASMA REDUCED) PRODUCTS

HPC, APHERESIS
Plasma Reduced

HPC, CORD BLOOD
Plasma Reduced

HPC, MARROW
Plasma Reduced

These products contain the cellular elements of the starting HPC collection(s) that remain after the bulk of the plasma is removed by centrifugation.

HPC, (RBC REDUCED) PRODUCTS

HPC, CORD BLOOD
Red Cells Reduced

HPC, MARROW
Red Cells Reduced

These are the HPCs remaining after the mature red cells have been reduced by sedimentation, centrifugation, or lysis.

HPC, (BUFFY COAT ENRICHED) PRODUCTS

HPC, CORD BLOOD
Buffy Coat Enriched

HPC, MARROW
Buffy Coat Enriched

The buffy coat is the portion of an HPC product containing the nucleated cells after the bulk of the plasma and mature red cells has been removed by sedimentation or centrifugation techniques.

HPC, (MONONUCLEAR CELL ENRICHED) PRODUCTS

HPC, CORD BLOOD
Mononuclear Cell Enriched

HPC, MARROW
Mononuclear Cell Enriched

These are primarily mononuclear cells that remain after the depletion of mature red cells, polymorphonuclear leukocytes, and plasma by separation of the cells on the basis of their density. This is achieved using devices or density gradient solutions.

HPC, CRYOPRESERVED PRODUCTS

HPC, APHERESIS
CRYOPRESERVED

HPC, CORD BLOOD
CRYOPRESERVED

HPC, MARROW
CRYOPRESERVED

These are HPCs that have been frozen using cryoprotectant solutions and containers suitable for the purpose.

HPC, (CD34 ENRICHED) PRODUCTS

HPC, APHERESIS
CD34 Enriched

HPC, CORD BLOOD
CD34 Enriched

HPC, MARROW
CD34 Enriched

These products contain the cellular elements of HPCs that have been enriched by selection of CD34+ cells.

Other Cellular Products

These are nucleated cells from any source (marrow, peripheral blood, whole blood, or umbilical cord/placental blood) and intended for clinical use other than as HPCs. They may be further categorized according to the specific subpopulations.

MNC, APHERESIS; NC, WHOLE BLOOD; NC, MARROW

These products are most frequently used for DLIs. They are usually collected from the HPC donor and contain a mixture of mature nucleated cells (eg, T and B lymphocytes, granulocytes), red cells, and plasma.

Instructions for Storage and Administration of Cellular Therapy Products

The following instructions pertain to cellular therapy products described in this *Circular*:

- All products must be maintained in a controlled environment and stored under appropriate conditions as described in FDA regulations and applicable AABB, AATB, FACT-JACIE, NetCord-FACT, or NMDP standards.[12-16]

  Note: If the administration of a cellular therapy product is delayed, the distributing/issuing facility should be contacted for instructions on proper storage of the product during the delay.
- Before administration of the product, it is critical to coordinate patient and product preparation to support timely product infusion according to the facility standard operating procedure. Infusion coordination may include confirmation of the number of containers and type of product (fresh or cryopreserved), verification of product infusion order, verification of consent for infusion, and verification of patency of intravenous access for infusion of the product.
- The intended recipient and the product container must be properly identified according to facility standard operating procedure before the product is administered.
- The product must be inspected for changes in the integrity of the container and product condition before administration. Any questions about the product should be directed to the facility distributing or issuing the product.
- Aseptic technique must be employed when handling and administering the product.
- Products must *not* be administered through a filter designed to remove leukocytes.
- Products may be filtered through a 170- to 260-micron filter designed to remove clots.
- Products should be mixed thoroughly before use.
- Products must *not* be irradiated.
- No medications or solutions may be added to or infused through the same tubing as products, with the exception of 0.9% Sodium Chloride, Injection (USP) or facility-approved solutions, as directed by the distributing facility. Periodic observation of the patient is required during and after administration to detect adverse reactions. Vital signs must be recorded at a minimum before and after administration, or more often, if required, by facility standard operating procedure.

- Sequence and timing of multiple product infusions should be performed according to the administering facility's standard operating procedures. Adequate time between product infusions should be allowed to permit assessment for adverse reactions.

Dosage and Administration

The minimum number of HPCs necessary for engraftment in a myeloablated recipient has not been established for all HPC sources. However, eligibility criteria for some protocols may dictate a minimum number of cells to be collected and/or infused. Some examples of cell types measured to determine HPC dosage are CD34+ cells, nucleated or mononuclear cells, and colony-forming units–granulocyte-macrophage (CFU-GM). The dose for MNC(A) or NC(WB) is determined by institutional policies and is usually based on the number of T cells, nucleated cells, or mononuclear cells. For specific dosage and administration of other cellular therapy products, the investigator's brochure or special instructions should be followed. Such information may be found at the end of this document, if provided by the distributing facility.Administration of any cellular therapy product should begin only after identification of the product(s) and the intended recipient according to institutional policies. Manufacturers may recommend that products be filtered using a 170- to 260-micron filter to remove clumps or aggregates. Some institutions may have specific policies regarding the use of these filters for cellular therapy products. (See facility-specific section at the end of this document.) Cellular therapy product infusion should begin slowly and with sufficient observation to detect symptoms and/or signs suggestive of acute immunologic or infectious complications. Thereafter, the rate of infusion may be as rapid as tolerated. The administration time will be determined by the total volume to be infused and by whether the cells are fresh or previously cryopreserved. If the thawed products have not been washed to remove DMSO, care should be taken not to exceed 1 mL of DMSO per kilogram of recipient weight per day administration (eg, 100 mL of a 10% solution contains 10 mL of DMSO).

Storage

Cellular therapy products may be transported for administration in a fresh or cryopreserved state. They may require either long-term or short-term storage before administration. Institutional policies and protocols dictate specific storage requirements for cellular therapy products. The recommended storage duration and temperature may be included in the product labeling and should indicate the cell dose, storage temperature, and duration of storage to ensure acceptable cell viability and function. If an expiration date has been defined, it should be included on the product label. Before infusion, products received for the treatment of a patient should be stored according to the instructions on the label or those supplied in accompanying documentation. If there is an unexpected delay in administration and the product must be held for infusion after the expiration period indicated on the label, if applicable, the distributing/issuing and/or local cell processing facility should be contacted for further handling and storage instructions.

Noncryopreserved Cellular Therapy Products

Fresh products may be transported from distant collection facilities or undergo short-term local storage before administration.

Cryopreserved Cellular Therapy Products

Cryopreserved products may be received and stored long-term according to the manufacturer's directions or by a validated method. These products may be thawed at the local cell processing laboratory, with or without additional processing, or thawed at the bedside immediately before administration. These products should be infused as soon as possible after thawing occurs.

Cellular Therapy Product Labeling and Supporting Documents

At the time of issue, cellular therapy products will have the following information either on the affixed product label, on an attached label, or in accompanying documentation:

- Proper name of the product, including an indication of any qualification or modification.
- Unique identifier.
- Approximate volume.
- Name and volume of anticoagulant or other additives.
- Date and end time of collection.
- Expiration date and time (if applicable).
- Recommended storage temperature.
- Identity and address of collection facility or donor registry.
- Identity and address of processing/distributing facility.
- Statements regarding transmission of infectious diseases.
- Statement indicating "Do Not Irradiate."
- Biohazard or other warning label(s) (if applicable),
- Statements regarding recipient identification,
- Donor identifier and (if applicable) name.
- Recipient name and identifier.
- ABO group and Rh (D) type of donor or the ABO group and Rh (D) type of a cord blood product.
- Red cell compatibility testing results (if applicable).

Many products will be accompanied by additional records that are included to meet regulatory requirements. These accompanying records will include:

- A statement indicating whether the donor has been determined to be eligible or ineligible, or that the donor eligibility determination is incomplete.
- A summary of the records used to make the donor eligibility determination.
- Infectious disease testing results and supporting documents.
- For ineligible donors, a statement noting the reasons for ineligibility [21 CFR 1271.55(b)(4)].
- For products that are made available before the donor eligibility has been completed:
    - The results of any donor screening and testing that has been completed [21 CFR 1271.60(d)(2)(i) and (ii)].
    - A list of any screening and testing that has not yet been completed [21 CFR 1271.60(d)(2)(iii)].

International standards for nomenclature and labeling of cellular therapy products using *ISBT 128* have been determined by the International Cellular Therapy Coding and Labeling Advisory Group.[8-10]

Biohazard and Warning Labels

The application of biohazard and warning labels on the cellular therapy products summarized in Table 2 is defined by facility-specific policies and procedures. The FDA defines requirements for the use of biohazard and specific warning labels for products subject to the regulations as defined in 21 CFR 1271, implemented on May 25, 2005. As such, cellular therapy products subject to these FDA regulations require the use of these labels as specified by FDA for an "incomplete" or "ineligible" donor eligibility determination. Refer to 21 CFR 1271 for specific labeling guidance. Application of these labels extends outside the FDA-defined requirements, such as to HPC(M), based on voluntary adherence to professional industry standards and facility-specific guidance or other applicable laws. Questions about the interpretation of any label on a specific product should be directed to the facility distributing the product.

Side Effects and Hazards

Some side effects and complications may require reporting to a relevant national competent authority. See "Reporting of Adverse Reactions" later in this *Circular* for additional details.

The following side effects and hazards pertain to administration of cellular therapy products.

Immunologic Complications, Immediate

1. ***Acute Hemolytic Reactions*** can be a complication of cellular therapy product administration and can be caused by donor-recipient major or minor ABO or other blood group incompatibility. Acute hemolytic reactions may be immediate and occur up to 24 hours following infusion.

Signs and symptoms of acute hemolytic reactions may include one or more of the following:

- Chills.
- Fever.
- Headache.
- Burning sensation along the vein.
- Abnormal bleeding.
- Low back pain.
- Facial flushing.
- Chest pain; rapid, labored respirations.
- Tachycardia.
- Shock.
- Hemoglobinuria:
    - Development of a positive direct antiglobulin test (DAT).
    - Elevation of lactate dehydrogenase (LDH) or bilirubin.

Treatment:

- Measures to maintain or correct arterial blood pressure; correct coagulopathy, if present; and promote and maintain urine flow.

Prevention:

- Red cell reduction.
- Plasma reduction.

2. Febrile Nonhemolytic Reactions may reflect the action of cytokines, either present in the product or generated by recipient antibodies against infused white cells. These reactions occur more frequently in patients previously alloimmunized by transfusion or pregnancy.

Signs and symptoms of febrile nonhemolytic reactions include:

- Temperature elevation of 1 C (2 F) or more (shortly after or up to 2 hours following product administration and in the absence of another pyretic stimulus).
- Chills.

Treatment:

- Antipyretics.

Prevention:

- Antipyretics.

3. Allergic/Anaphylactoid/Anaphylactic Reactions are thought to be related to the presence of atopic substances capable of interacting with antibodies present in the donor or recipient plasma. In rare cases, anaphylaxis may occur. These reactions have typically been reported in IgA-deficient patients who have IgA-specific antibodies of the IgG and/or IgE class and who receive even small amounts of IgA-containing plasma. Allergic reactions to hydroxyethyl starch (HES) or DMSO used in cellular therapy product processing or cryopreservation may occur in sensitized patients.

Signs and symptoms of allergic reactions include:

- Urticaria (hives).
- Pruritus (itching).

- Bronchospasm and/or laryngospasm.
- Hypotension.
- Severe dyspnea.
- Facial, glottal, and/or laryngeal edema.
- Other symptoms such as facial burning and flushing, abdominal pain, nausea, vomiting, diaphoresis, diarrhea, and dizziness.

Treatment:

- Antihistamines.
- In severe cases, fluids, epinephrine, and/or steroids.

Prevention:

- Premedication with antihistamines is sometimes used to mitigate mild reactions.
- Washing of products can help prevent symptoms, but this procedure is usually reserved for patients with a history of severe/anaphylactic reactions.

4. ***Transfusion-Related Acute Lung Injury (TRALI)*** occurs when an acutely increased permeability of the pulmonary microcirculation allows massive leakage of fluids and protein into the alveolar spaces and interstitium. In many cases, the occurrence of TRALI is associated with the presence of leukocyte antibodies (eg, anti-HLA) in the donor or recipient. As such, these reactions are rare in recipients of HLA-matched products.

   In the absence of evidence for another cause of pulmonary compromise, signs and symptoms of TRALI include:

   - Acute respiratory distress within 6 hours of administration.
   - Hypoxemia.
   - Bilateral pulmonary infiltrates on frontal chest x-ray.

   Treatment:

   - Respiratory support.

   Prevention:

   - Plasma-reduction or washing can help reduce the risk of TRALI in the setting of a graft with known anti-HLA or antibody to human neutrophil antigen (HNA), but these procedures are rarely performed for this indication.

Immunologic Complications, Delayed

1. ***Alloimmunization to Antigens*** of red cells, white cells, platelets, or plasma proteins may occur after infusion of cellular products. Primary immunization does not become apparent until days or weeks after the immunizing event and does not usually cause symptoms or physiologic changes. However, in patients who have developed alloantibodies, if blood or cellular therapy products that express the relevant antigens are subsequently administered, there may be accelerated removal of cellular elements from the circulation and/or systemic symptoms that may contribute to graft failure, red cell aplasia, and transfusion refractoriness.

   Treatment:

   - Selective use of blood components for transfusion support that are negative for the antigen recognized by the alloantibody.

2. ***Delayed Hemolytic Reactions*** may occur in two different allogeneic settings. Clinically significant antibodies to red cell antigens in previously alloimmunized patients are usually detected by pretransfusion testing. Occasionally, however, levels may diminish to below the limits of detection. In these cases, antigens on transfused red cells can stimulate anamnestic production of antibody. The antibody levels may reach a significant circulating level while the transfused red cells are still present in the circulation, leading to hemolysis. The usual time frame for reappearance of antibody is 2 to 14 days after product administration. Delayed hemolysis may also occur in recipients who receive plasma-incompatible transplants, whether in regard to ABO antigens or to other red cell antigens. In

this setting, the infused donor's B lymphocytes may produce antibodies to red cell antigens, thus destroying the recipient's own remaining red cells in the 1 to 3 weeks after HPC product administration. This reaction may be sudden, severe, and life threatening, so at-risk recipients should be monitored for this occurrence.

Signs and symptoms of delayed hemolytic reactions may include:

- Unexplained fever.
- Unexplained decrease in hemoglobin/hematocrit.
- Mild jaundice.
- Development of a positive DAT.
- Elevation of LDH or bilirubin.
- Hemoglobinemia and hemoglobinuria (rare).
- Symptoms of acute intravascular hemolysis (rare).

Treatment:

- Use of antigen-negative red cells if transfusion is needed.
- More severe cases may require treatment similar to an acute hemolytic reaction and more rapid antigen-negative red cell replacement.

Prevention:

- Providing red cells after transplantation that are ABO compatible with the donor and recipient.

3. ***Graft-vs-Host Disease*** (GVHD) is an extremely serious condition that occurs frequently in recipients of allogeneic cellular therapy products.[17] GVHD occurs when viable T lymphocytes in the infused product engraft and react against tissue antigens in the recipient.

   Signs and symptoms:

   - Wide variety of immune-mediated tissue and organ damage.

   Treatment:

   - Posttransplant immunosuppression, according to institutional guidelines and policies.

   Prevention:

   - Use of optimally matched HLA-compatible donor grafts.
   - Incorporation of immune suppression into the transplant regimen.

Nonimmunologic Complications

1. ***DMSO Toxicity*** is the most common complication of cryopreserved product administration. DMSO is a cryoprotectant used to cryopreserve cellular therapy products. Side effects and symptoms are generally associated with histamine release.

   Signs and symptoms:

   - Coughing.
   - Flushing.
   - Rash.
   - Chest tightness and wheezing.
   - Nausea and vomiting.
   - Cardiovascular instability.

   Treatment:

   - Slowing the rate of infusion.
   - Medicating with antihistamines.
   - Treating symptoms.

   Prevention:

   - Prophylactic antihistamine therapy.
   - Decreased rate of administration.
   - Providing hard candy to prevent nausea caused by the odor and/or taste.

- Removing DMSO from the product by washing the cells before administration; while this may reduce the risk of symptoms, it is not generally required given typically mild reactions, and it may result in unintended cell loss.

2. ***Septic Infusion Reactions*** may result from bacterial contamination of cellular therapy products, but they rarely cause acute, severe, or life-threatening effects. Prompt recognition of a possible septic reaction is essential. The onset of high fever (>2 C rise in temperature) during or immediately after product administration should suggest the possibility of bacterial contamination and/or the presence of endotoxin in the product.
   Possible signs and symptoms of septic infusion reactions include:
   - Fever with chills.
   - Severe hypotension.
   - Dry, flushed skin.
   - Pain in abdomen and extremities.
   - Vomiting.
   - Bloody diarrhea.

   Treatment:
   - Prompt and appropriate use of antimicrobial agents with modification based on evaluation of blood culture results from the patient and the product when available.

   Prevention:
   - Appropriate aseptic technique during all aspects of product collection, manufacturing, and infusion.
   - Appropriate antibiotic prophylaxis should be considered when using nonconforming products with positive culture results according to institutional protocol and relevant national competent authorities.

3. ***Fat Emboli***, small fat droplets in marrow products, may block capillary perfusion and cause respiratory distress.
   Signs and symptoms of fat emboli syndrome (FES) include:
   - Dyspnea.
   - Hypoxia.
   - Tightness of the chest.
   - Coughing.
   - Petechiae.
   - Confusion (mental status change).

   Treatment:
   - Supplemental oxygen therapy.
   - Ventilation as needed.
   - Corticosteroids, including methylprednisolone, which have reduced posttraumatic hypoxemia believed to be due to FES.

   Prevention:
   - Routine filtering of bone marrow products with 170- to 260-micron filters before infusion.

4. ***Transmission of Infectious Disease and/or Disease Agents*** may occur because cellular therapy products are collected from human blood and/or tissues. Disease may be caused by known or unknown agents. Donor selection criteria, screening, and testing are designed to minimize the potential risk of disease transmission. These procedures aim to identify potential donors with increased risk of infection with human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis, as well as other agents. (See “Donors” section.) These measures do not totally eliminate the risk of transmitting these agents. Cytomegalovirus (CMV) may, unpredictably, be present in white-cell-containing products from donors previously infected with this virus, which can persist lifelong despite the presence of serum antibodies. Up to 70% of donors

may be CMV-seropositive. Transmission of CMV may be of concern in immunocompromised transplant recipients if they are CMV-seronegative. Administering CMV-seronegative cellular therapy products reduces the risk of CMV transmission. For some infectious agents, there are no routine tests to predict or prevent disease transmission.

Treatment:

- Based on implicated infectious agent.

Prevention:

- Minimize by robust screening procedures, identification of infectious donors, and proper labeling.

5. ***Bleeding Due to Excessive Anticoagulation*** can occur if heparin or other anticoagulants were added to the product during collection and/or processing and remain in the cellular therapy product when administered.

   Treatment:

   - Anticoagulant specific.
   - A reversal agent can be considered.

   Prevention:

   - Product/anticoagulant specific.
   - Infusion rates may be adjusted depending on the clinical conditions; products may be washed when cell loss is not a concern.

6. ***Circulatory Overload*** leading to pulmonary edema can occur after infusion of excessive volumes or at excessively rapid rates. Pulmonary edema should be promptly and aggressively treated. In at-risk patients, the infusion of colloid preparations (including plasma products and the suspending plasma in cellular therapy products) should be reduced to a minimum.

   Signs and symptoms of circulatory overload may include:

   - Dyspnea.
   - Peripheral edema.
   - Rapid increase of blood pressure.

   Treatment:

   - Diuresis.

   Prevention:

   - Minimize the volume of colloidal preparations and, if appropriate, split or volume-reduce the product for infusion.
   - Reduce the rate of administration.

7. ***Hypothermia*** is related to the temperature of the cellular therapy product and the rate of infusion and can be caused by rapid infusion of large volumes of cold products. Hypothermia carries a risk of cardiac arrhythmia or cardiac arrest. A blood warming device should not be used unless approved by the manufacturer of the cellular therapy product.

   Treatment:

   - Warm the patient.

   Prevention:

   - Decrease infusion rate when clinically appropriate.

8. ***Nonimmunologic Hemolysis*** can result from lysis of red cells in the product, which may occur at any time during processing, cryopreservation, thawing, and administration. This lysis may be caused by osmotic stress, mechanical injury, shear stress, coadministration with incompatible fluids, or intrinsic red cell abnormalities such as hemoglobinopathies or enzyme deficiencies. Some hemoglobinuria can be seen even with products containing only small amounts of free hemoglobin and does not necessarily indicate a reaction.

   Signs and symptoms:

   - May be the same as hemolytic reactions, either delayed or immediate.

Treatment:

- Same as treatment of hemolytic reactions.

Prevention:

- High levels of free hemoglobin can be removed by washing the product when clinically appropriate and using isotonic solutions during product preparation.
- Prevention relates to proper product handling during all steps of product collection, manufacturing, and administration.

Reporting of Adverse Reactions

Any adverse reaction that is defined as a suspected or proven unfavorable response to administration of cellular therapy products and is manifested by signs and symptoms (including microbial contamination of a product or suspected disease transmission during or after product administration) must be documented and reported in accordance with the facility's policies and/or applicable laws and regulations. At a minimum, any such event must be reported to the patient's physician and to the medical director of the facility that issued the product.

The reporting requirements vary based on the regulatory oversight required by the type of product and manufacturing process. The user must contact the manufacturing/distributing facility for specific requirements.

Entities involved in the manufacture of the product must be contacted in the investigation/reporting of an adverse reaction, as applicable.

Table 1A. US Minimal Requirements for Testing for Transmissible Agents in Cellular Therapy Products*†

Testing for Infectious Agents	Donors of HPC(M), HPC(A), MNC(A), and NC(M)	Donors of HPC(CB) and NC(CB)	Donors of NC(WB)
Timing of specimen collection	Up to 30 days before or 7 days after collection	Up to 7 days before or after collection	Up to 7 days before or after collection
Human immunodeficiency virus, types 1 and 2 (HIV-1, HIV-2)‡	X	X (MS)	X
Hepatitis B virus (HBV)	X	X (MS)	X
Hepatitis C virus (HCV)	X	X (MS)	X
Human T-cell lymphotropic virus, types I and II (HTLV-I, HTLV-II)	X	X (MS)	X
Cytomegalovirus (CMV) (if allogeneic)	X	X (MS)	X
Treponema pallidum (syphilis)	X	X (MS)	X

*Testing is performed according to manufacturers' instructions using donor screening tests that have been licensed, approved, or cleared by the US Food and Drug Administration (FDA) for transmissible agents as defined by the FDA.[11] More than one test may need to be conducted to adequately and appropriately test for a single communicable disease agent or disease. Refer to the Center for Biologics Evaluation and Research website for donor eligibility guidance and a list of approved tests. FDA-licensed, -approved, or -cleared donor screening tests are available for West Nile Virus (WNV) and HBV nucleic acid testing (NAT) and *Trypanosoma cruzi*; testing may be implemented per facility-specific guidance prior to an FDA testing requirement. Additional tests for infectious transmissible agents may be required or performed.

†US federal regulations do not require testing of autologous donors for transmissible agents. However, the voluntary accrediting organizations (eg, AABB, FACT-JACIE) and some states or regions may require additional testing for allogeneic or autologous donors. See references for a list of selected publications containing testing requirements and standards. Required testing must be performed by a laboratory that is either certified to perform such testing on human specimens under the Clinical Laboratory Improvement Amendments of 1988 (42 USC §263a and 42 CFR 493) or has met equivalent requirements as determined by the Centers for Medicare and Medicaid Services.

‡Establishments not using FDA-licensed screening tests for HIV-1 group O antibodies must evaluate donors for risk associated with HIV-1 group O infection.

HPC(M) = hematopoietic progenitor cells from marrow; HPC(A) = HPCs from apheresis; MNC(A) = mononuclear cells from apheresis; NC(M) = nucleated cells from marrow; HPC(CB) = HPCs from cord blood; NC(CB) = NCs from cord blood; NC(WB) = NCs from whole blood; MS = maternal sample.

Table 1B. EU Minimal Requirements for Testing for Transmissible Agents in Cellular Therapy Products*†‡

	Donors of HPC(M), HPC(A), MNC(A), NC(M) And NC(WB)	Donors of HPC(CB)[§] and NC(CB)
Timing of specimen collection	Up to 30 days before collection	Day of or up to 7 days after delivery
Human immunodeficiency virus, type 1 and type 2 (HIV-1, HIV-2)	X	X (MS/CBU)
Hepatitis B virus (HBV)	X	X (MS/CBU)
Hepatitis C virus (HCV)	X	X (MS/CBU)
Treponema pallidum (syphilis)	X	X (MS/CBU)
Human T-cell lymphotropic virus, type I (HTLV-I)	X[‖]	X (MS)

*The tests must be carried out by a qualified laboratory authorized as a testing center by the competent authority in the Member State, using CE-marked testing kits where appropriate. The type of test used must be validated for the purpose in accordance with current scientific knowledge. In certain circumstances, additional testing may be required depending on the donor's history and the characteristics of the tissue or cells donated (eg, malaria, toxoplasma, cytomegalovirus, Epstein-Barr virus, *Trypanosoma cruzi*).

†Member countries of the European Union may amend and/or introduce additional requirements. In some settings, testing by more than one method may be required for some infectious agents. This table is not intended to reflect all national variations but rather to present general requirements within the EU directives.

‡The governmental regulations require testing of autologous donors for transmissible agents only if removed cells are to be stored or cultured. However, the voluntary accrediting organizations (eg, AABB, FACT-JACIE) may require testing of autologous donors.

§The testing is repeated on the CBU if the CBU is stored for a long period of time; alternatively, nucleic acid testing (NAT) technology is used.

‖Performed on donors living in or originating from high-prevalence areas, or with sexual partners originating from those areas, or whose parents originate from those areas.

HPC(M) = hematopoietic progenitor cells from marrow; HPC(A) = HPCs from apheresis; MNC(A) = mononuclear cells from apheresis; NC(M) = nucleated cells from marrow; NC(WB) = NCs from whole blood; HPC(CB) = HPCs from cord blood; NC(CB) = NCs from cord blood; MS = maternal sample; CBU = cord blood unit.

See references for a list of selected publications containing testing requirements and standards.

Table 2. Biohazard and Warning Labels on Cellular Therapy Products Collected, Processed, and/or Administered in the United States

	Status				Product Labels*			
	All Donor Screening and Testing Performed per FDA Criteria	**Abnormal Results of Donor Screening†**	**Abnormal Results of Donor Testing†**	**Urgent Medical Need‡**	**Biohazard Legend [21 CFR 1271.3(h)]**	**Not Evaluated for Infectious Substances**	**WARNING: Advise Patient of Communicable Disease Risks**	**WARNING: Reactive Test Results for (name of disease agent or disease)**
Donor Eligibility Determination Required [21 CFR 1271.45(b)]								
1. Allogeneic donors with incomplete donor eligibility determination§	No	No	No	Yes	NR (see footnote§)	R	R	NA
Allogeneic donors with incomplete donor eligibility determination§	No	No/Yes	Yes	Yes	NR (see footnote§)	R	R	NR (see footnote§)
Allogeneic donors with incomplete donor eligibility determination§	No	Yes	No	Yes	NR (see footnote§)	R	R	NA
2. Allogeneic donors found ineligible:								
A first-degree or second-degree blood relative	Yes	No/Yes	Yes	Yes	R	NA	R	R
A first-degree or second-degree blood relative	Yes	Yes	No	Yes	R	NA	R	NA
Unrelated donor	Yes	No/Yes	Yes	Yes	R	NA	R	R
Unrelated donor	Yes	Yes	No	Yes	R	NA	R	NA
Donor Eligibility Determination Not Required [21 CFR 1271.90(a)]								
3. Autologous donors‖								

*Application of biohazard and warning labels extends outside the HCT/Ps described in 21 CFR 1271 based on voluntary adherence to professional standards and applies to all products defined in this *Circular*, including HPC(M), which is not regulated under 21 CFR 1271. FDA eligibility processes and associated biohazard and warning labels were required on and after May 25, 2005 and may or may not be implemented for units collected before this date per facility-specific policy.
†When abnormal results of any donor screening or testing are identified in the donor, the transplant physician is notified of those results.
‡Urgent medical need must be documented when a donation is used for transplantation from a related or an unrelated donor with an "incomplete" eligibility status, or when a donation is used for transplantation from an unrelated donor with an "ineligible" eligibility status. When an HCT/P is made available for use before the donor eligibility determination is completed under the urgent medical need provision, the physician using the HCT/P must be notified that the testing and screening were not complete, and the notification must be documented [21 CFR 1271.60(d)(3)].
§Donor eligibility status of "incomplete" means donor eligibility determination was not completed per US requirements. The donor eligibility determination must be completed for donor screening and testing per FDA requirements during or after the use of the cellular therapy product. When not feasible to complete screening and/or testing per FDA criteria, documentation should be on file to justify. The biohazard label may be applied per facility-specific guidance for "incomplete" eligibility status.
‖FDA does not require donor testing or screening for autologous donors; FDA requires all autologous donations to be labeled "For Autologous Use Only." When all donor screening and testing is not completed per FDA requirements, the label "Not Evaluated for Infectious Substances" is also required. Any abnormal donor screening or testing results (even though neither screening nor testing is mandated for this group of donors) require appropriate labeling [21 CFR 1271.90(a)(b)].
HCT/Ps = human cells, tissues, and cellular and tissue-based products; CFR = Code of Federal Regulations; HPC(M) = hematopoietic progenitor cells from marrow; FDA = Food and Drug Administration; NR = not required; R = required; NA = not applicable.

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SECTION 4.1

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate

ClinicalTrials.gov Identifier: NCT__ __ __ __ __

BB IND #__ __ __ __

UTHealth IRB# HSC-MS-16-0738

IND Sponsor and Principal Investigator: Charles S. Cox, Jr., M.D.
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Title:	Autologous Wharton's Jelly for Augmented Repair of Cleft Palate
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ABBREVIATIONS

AE- Adverse Event

aMRI- Anatomical Magnetic Resonance Imaging

BA- Bone Area

BMP- Bone Morphogenic Protein

CBCT- Cone Beam Computed Tomography

CBER- Center for Biologics Evaluation and Research

CBVI- Cone Beam Volumetric Imaging

CCI- Controlled Cortical Impact

CFU- Colony Forming Unit

CLA- Cleft Lip Adhesion

CLP- Cleft Lip and Palate

CP- Cleft Palate

cGMP- Current Good Manufacturing Practices

CFR- Code of Federal Register

CMHH- Children's Memorial Hermann Hospital

CRF- Case Report Form

CT- Computer Tomography

CTCAE- Common Terminology Criteria for Adverse Events

DSMB- Data Safety and Monitoring Board

EMR- Electronic Medical Record

FDA- Food and Drug Administration

GCP- Good Clinical Practice

GPP- Gingivoperiosteoplasty

hUCB- Human Umbilical Cord Blood Cells

HIPPA- Health Insurance Protection and Portability Act

IMM- Independent Medical Monitor

IND- Investigational New Drug

IRB- Institutional Review Board

LAR- Legally Authorized Representative

MHH- Memorial Hermann Hospital

MNC- Mononuclear Cells

MSC- Mesenchymal Stromal Cell

MSM- Medical Safety Monitor

MRI- Magnetic Resonance Imaging

NAM- Nasoalveolar Molding

NCI-CTCAE- National Cancer Institute-Common Terminology Criteria for Adverse Events

NSC- Neural Stem Cells

nWJ- Native Wharton's Jelly

PI- Principal Investigator

PSC- Perinatal Stem Cells

QA- Quality Assurance

QC- Quality Control

ROI- Region of Interest

SAE- Serious Adverse Events

SMC- Safety monitoring Committee

TA- Tissue Area

TEM- Transmission Electron Microscopy

WBC- White Blood Count

UC- Umbilical Cord

Protocol Synopsis

Protocol Title	Autologous Wharton's Jelly for Augmented Repair of Cleft Palate
IND #	BBXXXXX
NCT#	NCTXXXXXXXX
UTHealth IRB #	HSC-MS-16-0738
Study Design	Prospective Phase 1 Study
Study Population	Infants with cleft palate and planned gingivoperiosteoplasty (GPP) who have previously enrolled in the protocol *Tissue and Stem Cell Research Bank for Future Autologous Use*, HSC-MS-15-0830.
Primary Objectives	To determine if the harvest/storage/reanimation/surgical application of autologous Wharton's Jelly (WJ) to augment the surgical repair of alveolar cleft palate is safe and feasible.
Secondary Objectives	To evaluate the effect of WJ on functional outcomes post-repair as determined by dental x-ray and cone beam 3D CT examination of the bone bridge across the alveolus.
Sample Size	25 Subjects
Study Intervention	Standard GPP surgical repair for cleft palate augmented with Autologous WJ.
Inclusion Criteria	**1.** Umbilical cord and placental tissue collected under the Protocol *Tissue and Stem Cell Research Bank for Future Autologous Use*, HSC-MS-15-0830, meeting QA release criteria at the time of collection and cryopreservation. **2.** Presence of alveolar cleft defect in conjunction with cleft lip and palate based on birth assessment by the Cleft Palate team. (*Refer to Appendix A for ICD Codes*) **3.** Planned cleft palate repair and follow-up with UTHealth Cleft Palate Team and Memorial Hermann Hospital-TMC. **4.** Ability to obtain informed consent from the parent or legally authorized representative (LAR) within 1mo. of the scheduled GPP surgery.
Exclusion Criteria	**1.** Birth assessment indicating: a. Absence of alveolar cleft palate, b. Cleft lip alone, c. Cleft palate alone. **2.** Presence of chorioamnionitis and/or neonatal sepsis. **3.** Presence of significant cardiac comorbidities. **4.** Cleft palate with an alveolar gap greater than 5mm. **5.** Cleft palate unsuitable for pre-surgical nasoalveolar molding (NAM). **6.** Unsuccessful NAM delaying and or preventing GPP repair. **7.** Inability to return for follow-up evaluations.
Safety Follow-Up	Subjects will be monitored for safety throughout the hospital stay and at scheduled follow-up clinic visits at 14 days, 3, 6 and 12 mo. post-surgery, or more frequently if treatment related SAE's are suspected.
Safety Monitoring	**1.** A medical safety monitor (MSM) will review treatment summaries for each subject in real time to ensure good clinical practice and to quickly identify safety concerns. **2.** The Data Safety Monitoring Board (DSMB) will review safety data. Members will be clinical experts (Oral and Maxillofacial Surgery, Cleft Palate Repair, etc.) with no conflict of interests. **3.** An independent Contract Research Organization (CRO) will audit study records for protocol and GCP compliance.

1. STUDY OBJECTIVES

1.1 Primary Objective:
To determine if the harvest/storage/reanimation/surgical application of autologous Wharton's Jelly to augment the surgical repair of alveolar cleft palate is safe and feasible.

1.2 Secondary Objectives
Determine the effect of Wharton's Jelly on functional outcomes post-repair as determined by dental x-ray and cone beam 3D CT examination and quantification of bone bridge formation and volumetrics.

1.3 Primary Endpoints:
Safety and feasibility of using autologous Wharton's Jelly as measured by: (1) volume recovered relative to need for repair, (2) Go/No Go on release criteria post-harvest and reanimation, (3) presence of ectopic/overgrowth of palatal tissue, (4) other AEs.

1.4 Secondary Endpoints:
Palatal bone density at 12 months post repair as determined by volumetric bone density imaging.

1.5 Efficacy Outcome Measures:
Alveolar bone formation (presence/density) as determined by dental x-rays and digital volumetric dental imaging. Absence of alveolar fistula's lessening the potential need for secondary procedures including bone grafting.

2. INTRODUCTION

2.1 Cleft Lip & Palate (CLP) Background
CLP is a congenital defect resulting from the failure of the median nasal process to fuse with the maxillary process and failure of the palatal shelves of the maxillary process to fuse to one another. (Ruppel, 2012) CLP defects are the most common congenital facial anomaly affecting 1 in 500 to 750 live births in the United States per year. (Shaye, 2014 - Ahmed, 2015) Infants with CLP lack not only the soft tissue of skin and muscle in their lip and palate, but also the bony support of their alveolus. Abnormal dental development is common and includes congenitally missing teeth, supernumerary teeth, malformed teeth, and ectopic teeth. (Long, 2000) There are additional challenges in managing CLP associated with a clinical syndrome. Estimates range from 30 to 50% and include Van der Woude syndrome, 22Q deletion syndrome, and Down syndrome. (Mitchell, 2009 - Cohen, 2002)

CLP Treatment varies depending on severity. Historically, the cleft defect has been corrected between 8 to 12 years of age during the phase of mixed dentition with a secondary alveolar bone graft. In this procedure, cancellous bone is harvested from the iliac crest and used to graft the alveolar defect along with closure of any residual oro-nasal fistula. While successful in reconstructing the alveolus, this equates to an additional surgery for the patient with associated donor site morbidities including intraoperative blood loss, pain, infection, and scarring. Bone graft reabsorption is common and may be as high as 50%. (Feichtinger, 2007) A higher rate of post-op. fistulas has also been reported. (Matic, 2008)

2.2 Gingivoperiosteoplasty (GPP) for CLP Repair

GPP is usually performed on infants 9 to 12 months of age. At the time of the primary palate repair, the alveolar flaps are closed across the bony defect. While patients benefit from a single surgery rather than multiple staged surgeries, long term follow up of these patients has shown that primary bone graft (prior to age 2) results in increased mid-facial hypoplasia, malocclusion, and persistent need for additional bone requiring a secondary bone graft. (Hseih, 2010, Power, 2008) Also, due to size, donor sites for infant bone grafts are limited and carry a higher morbidity than older children or adults undergoing the same procedure. To mitigate this donor site effect, bone morphogenic protein (BMP), a potent bone generating cytokine, has been used to facilitate osteogenesis across the alveolar defect. While robust bone formation resulted, subsequent fusion of facial sutures significantly reduced facial growth. In perhaps the most successful report, 63% of patients with unilateral clefts and 50% of patients with bilateral clefts had adequate bone formation following GPP. While "success" was demonstrated by the presence of bone within the alveolar cleft, many orthodontists and surgeons would argue that more bone is needed to stabilize both dental eruptions and/or future lefort surgeries to advance the maxilla. (Matic, 2008, Wang, 2013)

2.3 Potential Role of Native Wharton's Jelly (nWJ) in Regenerative Medicine:

Regenerative biomaterials present exciting strategies for repair of damaged or diseased tissues. A variety of tissue engineering applications based on a combination of extracellular matrix or polymer scaffolds and various cell types have shown promising clinical results. A particularly interesting, yet largely unexplored natural biomaterial is native Wharton's Jelly (nWJ), the connective tissue matrix of the umbilical cord (UC). A better understanding of the biomechanical characteristics of nWJ will help exploit its potential to repair tissues and develop innovative WJ-based regenerative therapies.

2.3.1 Biophysical Characterization of nWJ:

Wharton's jelly is a gelatinous connective tissue matrix made up of mucopolysaccharides and hyaluronic acid. It is the support structure of the umbilical cord/umbilical arteries and vein. Primitive mesenchymal stromal cells (MSCs) are lodged in the matrix. These cells meet the criteria for MSCs in that they have a similar immunophenotype, self-renew, and can be induced to differentiate into various cell types *in vitro*. Wharton's jelly represents an ideal autologous hydrogel scaffold for soft-tissue reconstruction—if it can be stored and re-animated while retaining the desirable biophysical characteristics with or without MSC viability. When placed in an osteogenic niche such as healing bone, these cells tend to differentiate into bone AND provide the stromal support function for ingrowth of native tissue.

2.4 Study Rationale

Despite nearly a century of experience repairing cleft palates, there is no consensus on the optimal strategy and timing of CLP repair. Studies comparing various surgical protocols for CLP repair have primarily been small single center, retrospective reports lacking consistent outcome measures.

The rationale for the study is that current surgical techniques for CLP repair have a relatively high failure rate and an autologous tissue engineering strategy may reduce complications and improve post-op. outcomes. The use of Wharton's Jelly (an otherwise biological waste product), instead of harvest of bone graft represents a relatively low-cost/low-morbidity alternative for efficacious repair of a bony defect. The use of the —de-gel" approach is a

classic tissue engineering strategy. The reason that this approach is particularly appealing for cleft palate is related to the ability to diagnose the problem in utero, so that the preparations for harvest and storage of Wharton's Jelly are made, and that the cells embedded in the matrix are mesenchymal stromal cells (MSCs). The key component of this strategy is that there are both MSCs AND an autologous hydrogel that will allow the matrix to stay in place once applied to the defect. Further, the volume of Wharton's Jelly is biologically feasible to impact the repair of this type of defect in infants/children.

The proposed protocol is designed to test the feasibility of harvesting and preserving Wharton's jelly to be used later as an autologous hydrogel scaffold that supports bony in-growth across cleft palate reconstructions. To determine the feasibility of this proposed approach, we plan to use autologous human umbilical cords to harvest the Wharton's jelly. Under cGMP/cGTP conditions, we have examined the effects of cryopreservation and re-animation on the biological properties/biophysical characteristics of Wharton's jelly. This protocol seeks to test the surgical feasibility, safety, and influence on bony healing after repair of alveolar cleft palate in children.

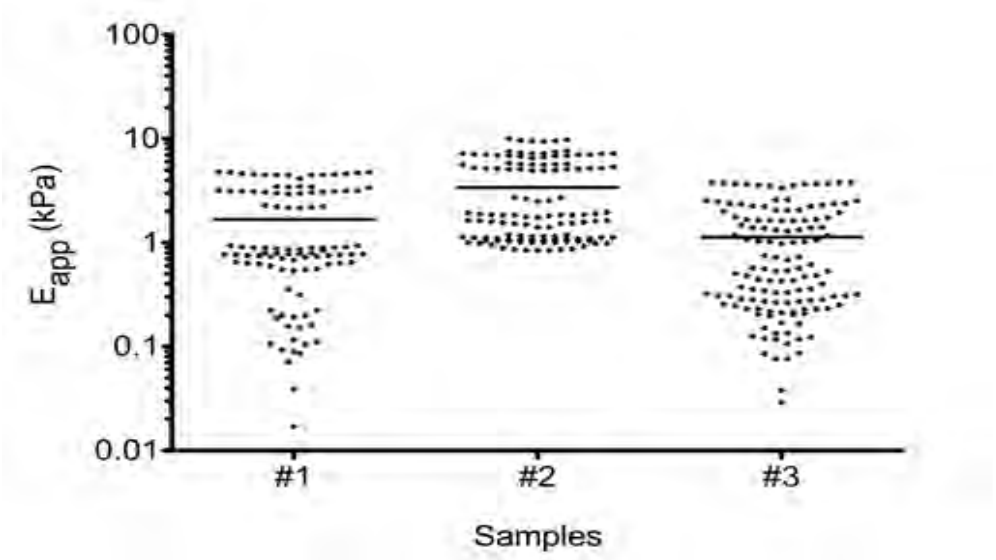
3. WHARTON's JELLY PRECLINICAL DATA

As our goal is to implant nWJ at defect sites that will create mechanical stresses on the implant, it is necessary to determine how nWJ will respond to various mechanical stresses of physiological relevance. In this context we analyzed nWJ tissue using rheology, a technique that allows us to understand the behavior of nWJ under different conditions of mechanical deformation, revealing the macroscopic tendencies of the nWJ to flow or resist conformational change, and also gives us great insight into the microstructural mechanisms of this behavior.

3.1 Determination of Elasticity and Differentiation Potential

Atomic Force Microscopy measurements of native Wharton's Jelly (nWJ) yielded a Young's modulus of 2.06 ± 1.18 kPa, consistent with that of some of the body's softest tissues, such as brain and liver (Fig. 1).

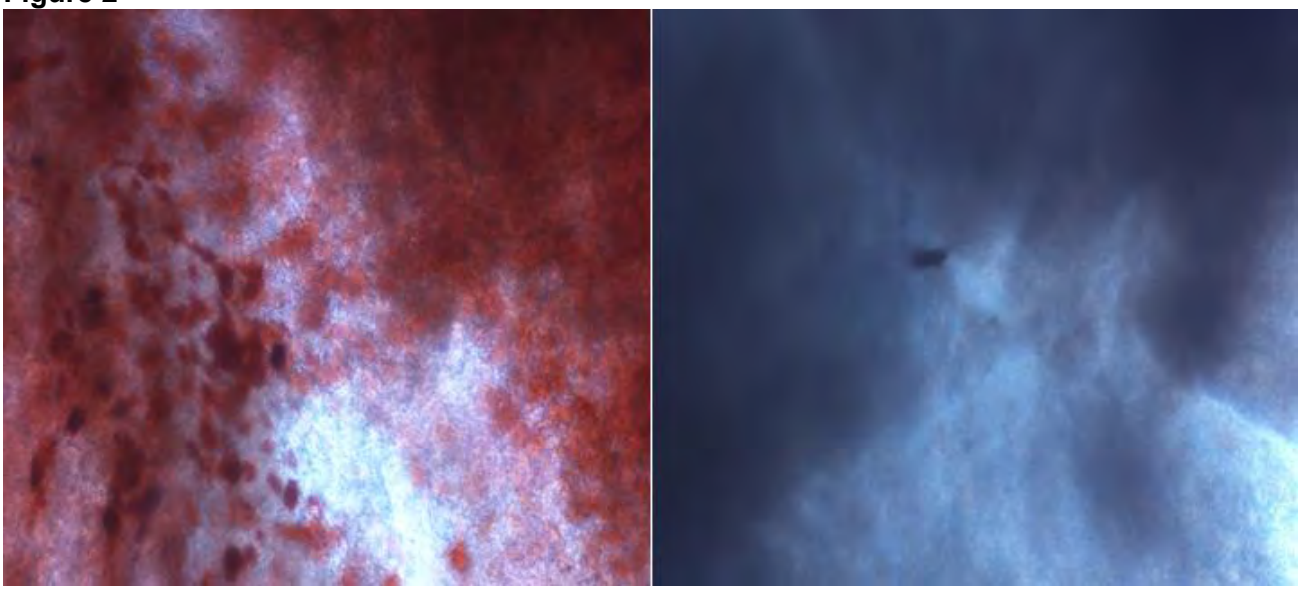
Figure 1



nWJ elasticity falls within the range of the body's softest tissues

Moreover, when cultured in osteogenic differentiation medium (MEMα supplemented with 10% FBS, 50 μg/mL gentamycin, 1x l-glutamine, 0.2 mM ascorbic acid, 0.1 μM dexamethasone, and 10 mM β-glycerophosphate), nWJ showed significant in situ osteogenic differentiation of nWJ cells and mineral deposition as evidenced by strong Alizarin Red S staining (Fig. 2, left panel). By contrast, no staining was observed when nWJ was maintained in control medium (MEMα supplemented with 10% FBS and 50 μg/mL gentamycin) (Fig. 2, right panel). Taken together, these data suggest its potential use for regenerative applications in bone tissue repair.

Figure 2



nWJ-embedded perinatal stem cells can differentiate into bone. Alizarin Red S Staining of nWJ cultured in osteogenic medium (left panel) or control medium (right panel) for 14 days.

3.2 Viability and Functionality after Cryopreservation

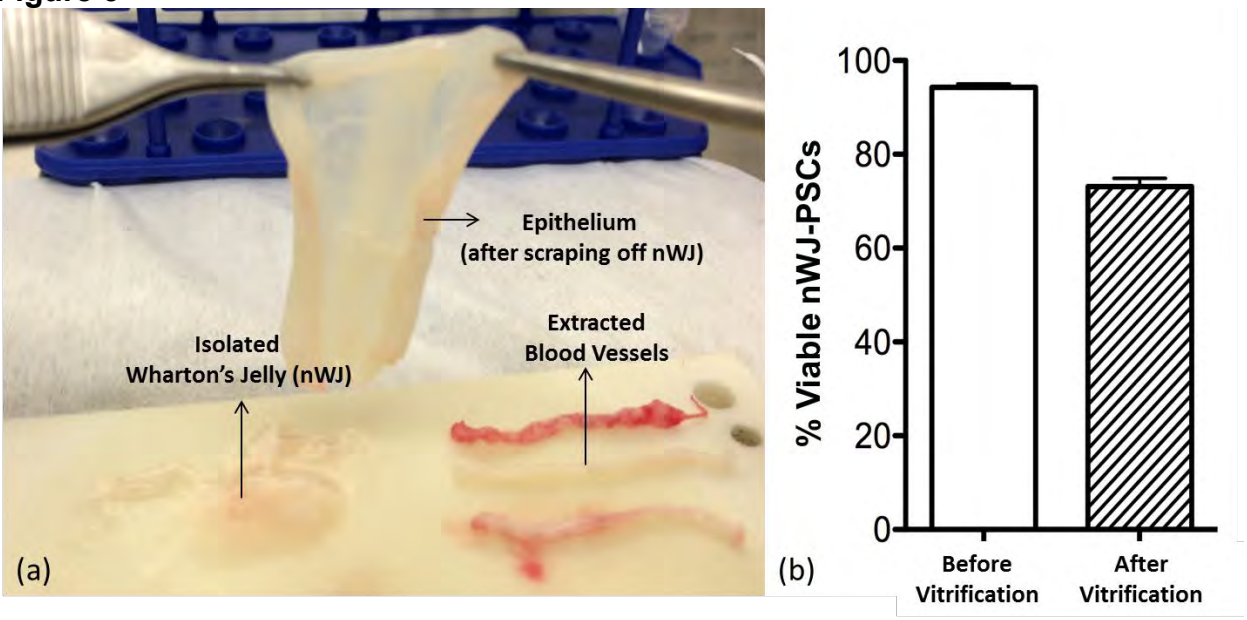
To examine the effect of the vitrification/thaw processes on the viability of perinatal stem cells (PSC) contained in nWJ, PSC from fresh nWJ and from vitrified/thawed nWJ were extracted by digesting the matrix for 30 minutes at 37°C, using an enzymatic solution containing Collagenase (1mg/ml), Hyaluronidase (1mg/ml), DNaseII (200μg/ml), Trypsin inhibitor (0.1mg/ml) and BSA (1mg/ml). Enzymatic digestion was followed by filtration through a 100μm mesh and viability was determined using the trypan blue exclusion method. The vitrification and thaw processes were carried out according to the methods described in the DP:2.1 Human Native Wharton's Jelly Harvest, Vitrification and Thaw SOP.

Isolation and assessment of post-vitrification survival of nWJ cells

After removing blood vessels from the UC segments, nWJ was scraped off the amniotic epithelium (Fig. 3a) and vitrified using a two-step exposure to equilibrium solution (ES) and vitrification solution (VS) provided in the clinical grade Vit Kit®-Freeze kit (Irvine Scientific, CA). To assess post-vitrification survival of nWJ cells, the vitrified sample was warmed rapidly at 37°C, and nWJ was sequentially transferred in serial dilutions of sucrose in DPBS 1X.

Fig. 3b shows viability data obtained from cells extracted from vitrified nWJ compared to that of cells extracted from fresh nWJ. Mean viability of nWJ-PSCs from freshly harvested nWJ was 94.25±1.24% compared to 73.11±3.08% after vitrification (Fig. 3b). Drastically lower viabilities were obtained when testing other vitrification solutions as well as after conventional DMSO-based slow-freezing methods (data not shown).

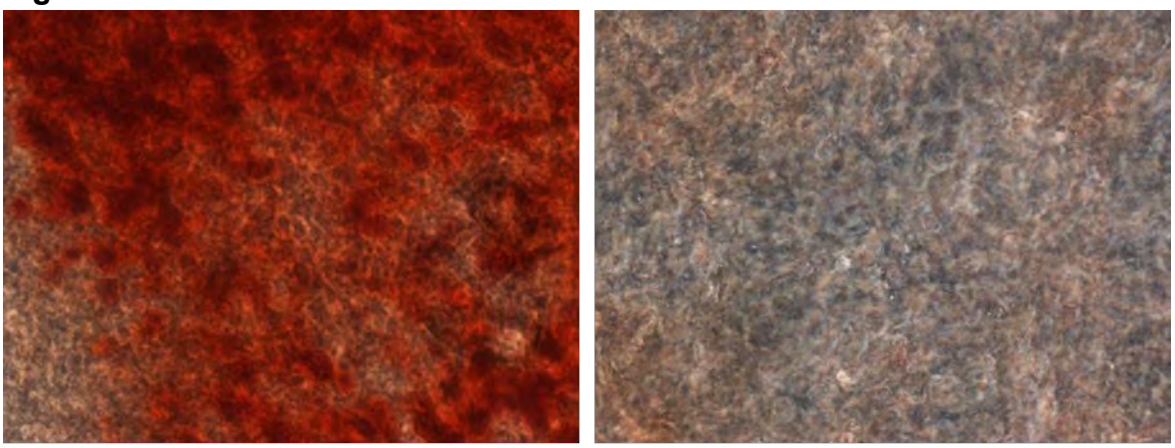
Figure 3



nWJ isolation and effect on nWJ-PSCs viability after vitrification. (a) The three blood vessels are first removed and nWJ is then scraped off the amniotic epithelium. (b) Viability of nWJ-PSCs before and after vitrification (p-values <0.05).

The effect of the vitrification/warming process on nWJ-embedded PSC function was also investigated by culturing thawed nWJ in osteogenic differentiation medium. After 14 days of culture, nWJ vitrified in and thawed, retained in situ osteogenic differentiation and mineral deposition capacity of embedded cells as evidenced by Alizarin Red S staining (Fig. 4a). By contrast, no staining was observed when nWJ was maintained in control medium (Fig. 4b).

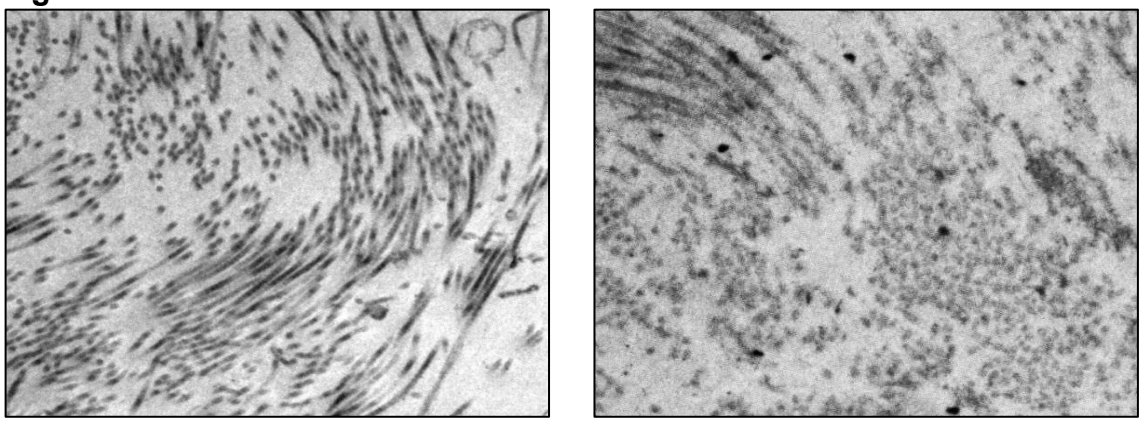
Figure 4



Vitrified nWJ-embedded perinatal stem cells can differentiate into bone after 2 weeks in situ. Alizarin Red S Staining of nWJ cultured in (a) osteogenic medium and (b) control medium.

Furthermore, transmission electron microscopy (TEM) of warmed nWJ vitrified using the Vit Kit®-Freeze kit showed good preservation of matrix structure (Fig. 5, left panel) when compared to fresh nWJ (Fig. 5, right panel).

Figure 5

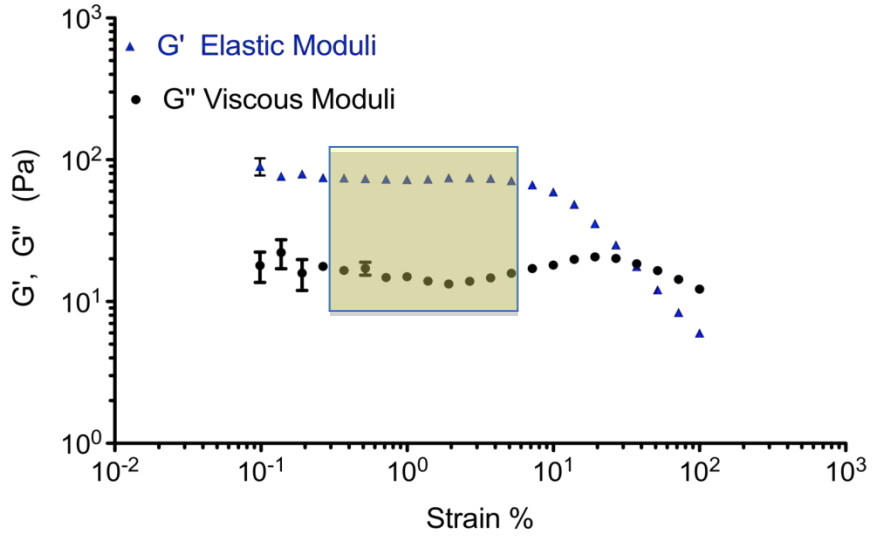


TEM of nWJ vitrified with the Vit Kit®-Freeze kit and warmed (left panel) and of fresh nWJ (right panel)

3.3 Determination of Linear Viscoelastic Region

nWJ has both fluid and solid characteristics. In rheology, the solid behavior of a material is represented by the Elastic Modulus (G′), while fluid behavior is represented by the Viscous Modulus (G″). The strain sweep test was used to obtain information on the range of strains (amount of lateral deformation) over which the structure of nWJ is stable. As seen in Fig. 6, G′ is dominant in nWJ, indicating that elastic behavior predominates in this semi-solid gel. It is crucial to determine the region of linear viscoelasticity in the strain sweep test as G′ and G″ are influenced by both the strain percentage and the frequency of oscillation (stress) on the material, so in order to gain reliable data on their behavior, one of these variables must be kept constant. Choosing a strain-percentage within the linear viscoelastic area ensured that further readings reflect material properties. Such a plot is extremely useful because it represents a signature of the microstructure of the material.

Figure 6

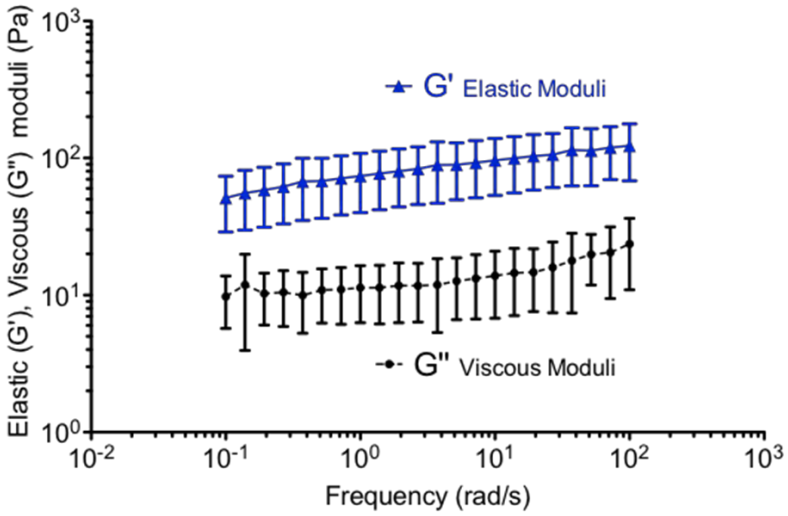


Strain Sweep Test showing the range of strains at which nWJ structure is stable. It also shows elastic behavior is predominant in nWJ.

3.4 Structural Response to Deformations

We used the Dynamic Frequency Sweep (DFS) test to investigate the unique interplay of viscous and elastic behavior in nWJ. The data pattern in Fig. 7 is consistent with that of glasslike colloids/hydrogels, which are designed after human tissues to begin with. The data show that nWJ is relatively stable over an oscillation frequency of 0.1 to 100 rad/sec, which is the physiologically relevant range. Furthermore, elastic and viscous moduli (G′, G″) increased with increasing frequency, indicating that the tissue stiffens in response to mechanical stress.

Figure 7

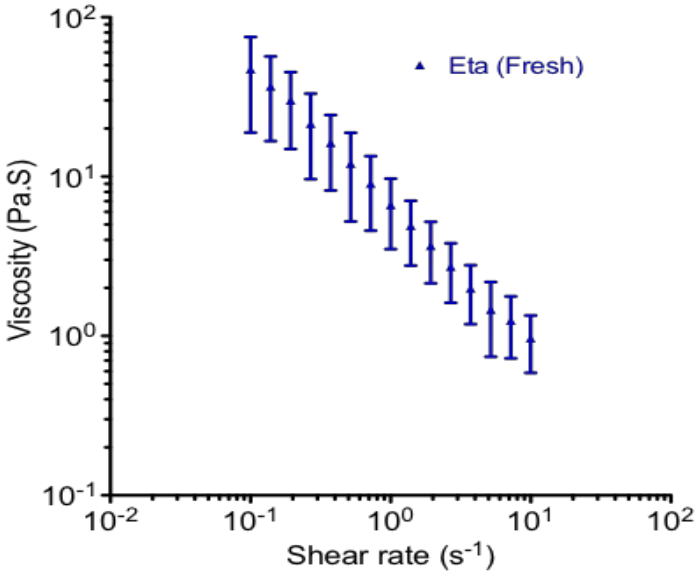


Dynamic Frequency Sweep test showing that the elastic modulus is dominant over the viscous modulus at physiologically relevant frequencies. As these increase, the nWJ does not have time to relax and thus stiffens.

3.5 Viscosity Profiling

A viscoelastic material can either become more or less viscous under stress. For nWJ, the shear viscosity shows an inverse dependence on shear rate, which indicates a shear thinning behavior (Fig.8).

Figure 8



nWJ displays shear-thinning, a phenomenon in which viscosity decreases with increasing applied shear rate.

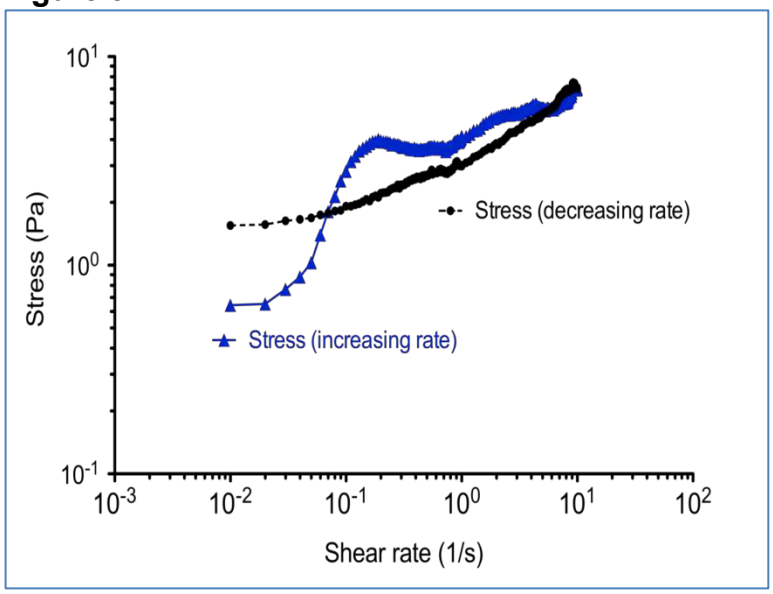
3.6 Thixotropic Behavior

An interesting feature of shear thinning materials is that thinning can be time dependent and reversible. If a material responds differently to shear stress depending on the previous shear history, it will have a time dependent response to shear stress. It is possible to test this attribute by applying a linear acceleration to a set shear rate, followed by a deceleration (thixotropic loop test). A thixotropic material will behave differently on the way up (loading curve) and on the way down (unloading curve), thereby revealing a gap in the middle, known as the hysteresis. The area of the hysteresis corresponds to the thixotropic nature of the material and its level of microstructural changes in response to shear stress.

As increasing shear rate is applied to nWJ, there is an increase in the stress response of the material, indicating that the nWJ is resisting flow and building up pressure. As it reaches its yield stress, the tissue transitions from a linear relationship between shear rate and stress to a more fluid flow. After the yield stress, the data in the graph reflect fluid behavior until it reaches the peak shear rate. During the linear deceleration phase, the nWJ follows a different path, with a lower stress response at each shear rate. This indicates that it is storing less energy, and is instead flowing more like a fluid due to the aligning of the microstructure at the high shear rate.

The data in Fig. 9 show that nWJ has a high degree of thixotropy, as seen in the loop between the loading (blue) and unloading (black) curves. Thixotropy is one of the most desirable properties of injectable scaffolds. Being thixotropic in nature and potentially able to deform and fit any complex anatomical defect makes nWJ a very promising natural injectable scaffold.

Figure 9

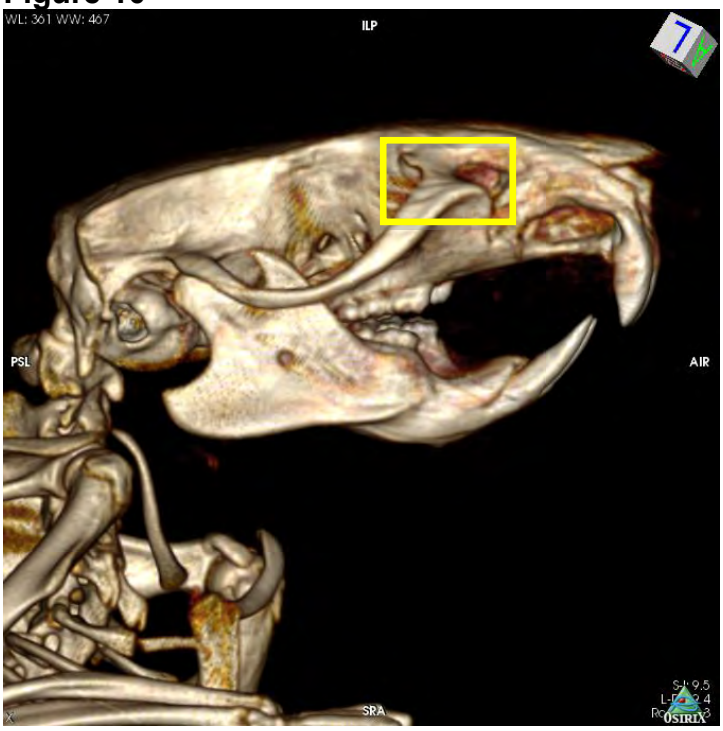


Thixotropic Loop Test showing that nWJ is a natural thixotropic material

3.7 In Vivo Pre-Clinical Data

The first set of experiments were designed to determine the effect of vitrified/warmed nWJ implant on bone regrowth and healing over 8 weeks in an alveolar defect model representative of cleft palate surgery. A 7x4x3 mm critical-size alveolar bone defect model in the rat was used. The defect site was analyzed using flat panel CT scanning on day 0 and 2, 4 and 8 weeks post-surgery. Animals were sacrificed only after the last CT scan for histological analysis.

Figure 10



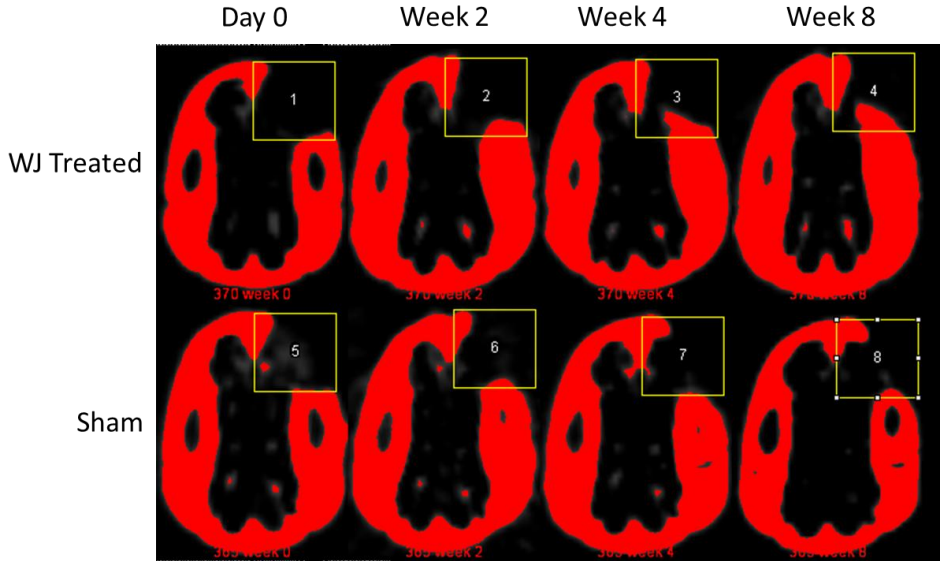
Flat panel CT: The eXplore Locus Ultra Pre-Clinical cone-beam CT (GE Healthcare, London, ON) was used to examine bone formation over time in individual rats. The images were reconstructed using MicroView software (Parallax Innovations, Ilderton, ON, CA) and analyzed with ImageJ software (NIH, Bethesda, MD, USA).

3D flat panel CT image showing the defect site (outlined in yellow).

3.7.1 Quantification of bone density

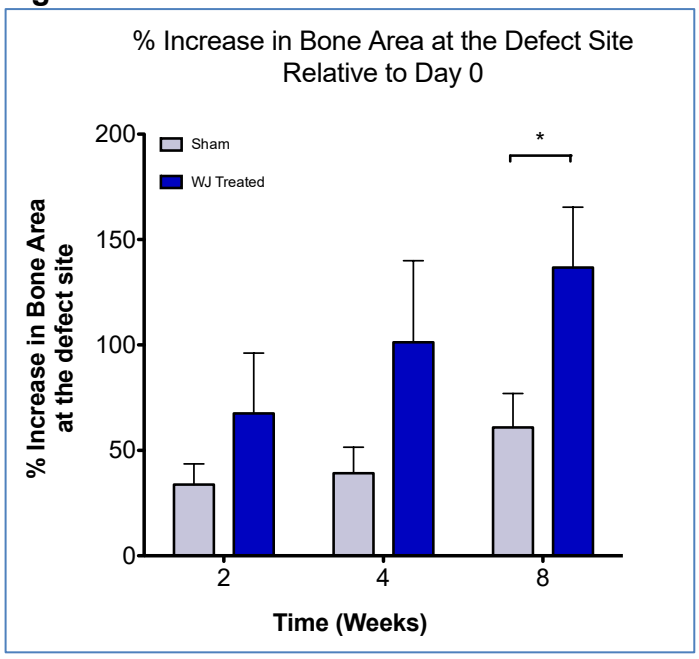
Quantification of bone was carried out using ImageJ to isolate relevant data in the flat panel CT images with a threshold on pixel intensity, which separates the bone from other soft tissues and the background. A region of interest (ROI) was selected based on the anatomical location of the defect site. The size and location of the ROIs were consistent for all images. The area of the bone (defined by threshold) in the ROI was obtained from the software at each point. The data were analyzed relative to day 0 to obtain the percent increase in bone area at week 2, 4 and 8 post-surgery.

Figure 11



Analysis of Bone Area within selected flat panel CT image ROI at Day 0 and 2,4 and 8 weeks post-surgery

Figure 12

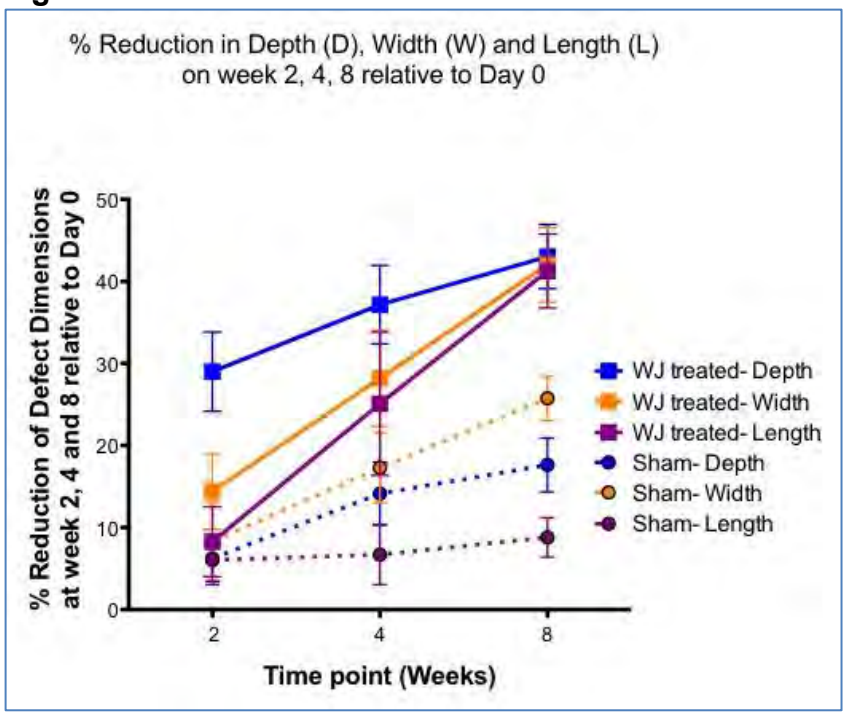


*Percent Increase in Bone Area at 2, 4 and 8 weeks post-surgery compared to day 0. * indicates statistical significance (p-values <0.05)*

A higher increase in bone area in rats treated with nWJ compared to the sham rats was observed. In nWJ treated rats, an increase in bone area of 67.49 ± 28.62%, 101.23 ± 38.76% and 136 ± 17.91% was observed at week 2, 4 and 8 post-surgery, respectively (Fig 12). In sham rats, an increase in bone area of 33.80 ± 9.74%, 39.16 ± 12.34% and 60.83 ± 16.14% was observed at week 2, 4 and 8 post-surgery, respectively (Fig 12).

To analyze the defect dimensions, the flat panel CT images were analyzed using MicroView. The percent change of the average width, depth, and length of the defect were measured at weeks 2, 4 and 8 post-surgery. The data show greater reduction of all three dimensions in rats treated with nWJ compared to sham rats. In nWJ treated rats at weeks 2, 4, and 8 post surgery, respectively, the depth was reduced by 29.01 ± 4.82%, 37.16 ± 4.77%, and 43.03 ± 3.91%, the width by 14.41 ± 4.62%, 28.17 ± 5.84%, and 42.05 ± 4.54%, and the length by 8.27 ± 4.24%, 25.09 ± 8.73%, and 41.27 ± 6.39% (Fig 13). In sham rats at weeks 2, 4, and 8 post surgery, respectively, the depth was reduced by 6.32 ± 2.84%, 14.14 ± 3.84%, and 17.63 ± 3.28%, the width by 8.33 ± 4.69%, 17.28 ± 4.28%, and 25.76 ± 2.67%, and the length by 6.04 ± 3.05%, 6.71 ± 3.67%, and 8.81 ± 2.39 (Fig 13).

Figure 13



Percent reduction in defect dimensions at 2, 4 and 8 weeks post-surgery compared to day 0. Statistical significance (p-values <0.05) between nWJ treated and sham rats was observed for all measured dimensions at weeks 4 and 8 post-surgery.

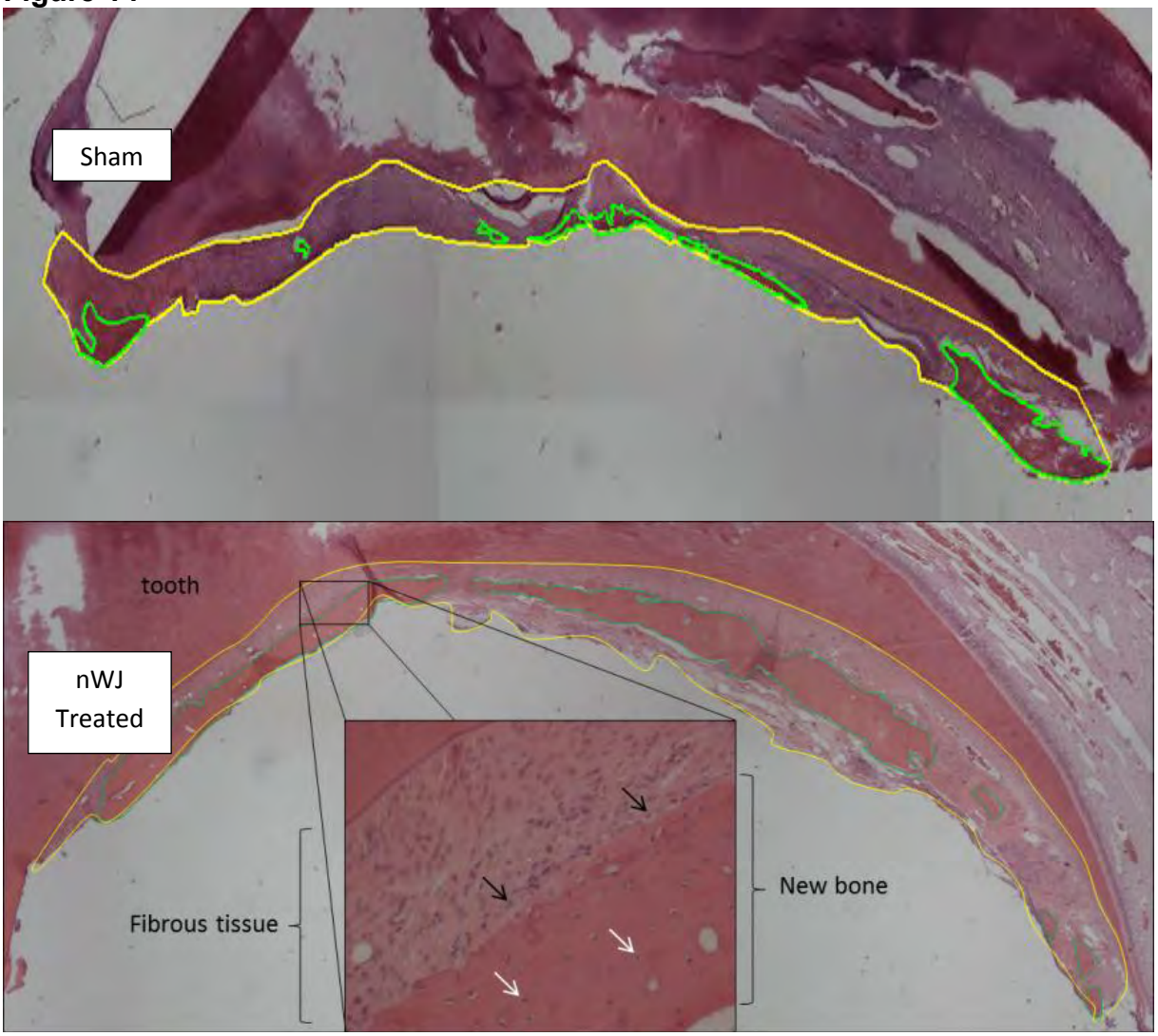
3.7.2 Histology

After the last flat panel CT scan (week 8 post-surgery), the animals were sacrificed and the defect site was harvested for histological analysis. The sample was fixed in 10% formalin for 24 hours, demineralized, paraffin embedded, and 5μm thick slices were cut in the sagittal plane and H&E stained.

3.7.3 Quantification of bony ingrowth

The slices were viewed and imaged at 4X magnification using a Nikon Eclipse Ti-E microscope. NIS-Elements software was used to stitch together images and obtain composite images of the whole defect area, and to analyze ROIs in that area. Newly formed bone and dense fibrous scar tissue could be distinguished with the H&E staining. Newly formed bone was selected in a ROI and defined as new Bone Area (BA) (circumscribed by the green line in Fig 14). The perimeter of the entire defect was selected in a ROI and defined as total Tissue Area (TA) (circumscribed by the yellow line in Fig 14). The results were expressed as new Bone Area/total Tissue Area (BA/TA).

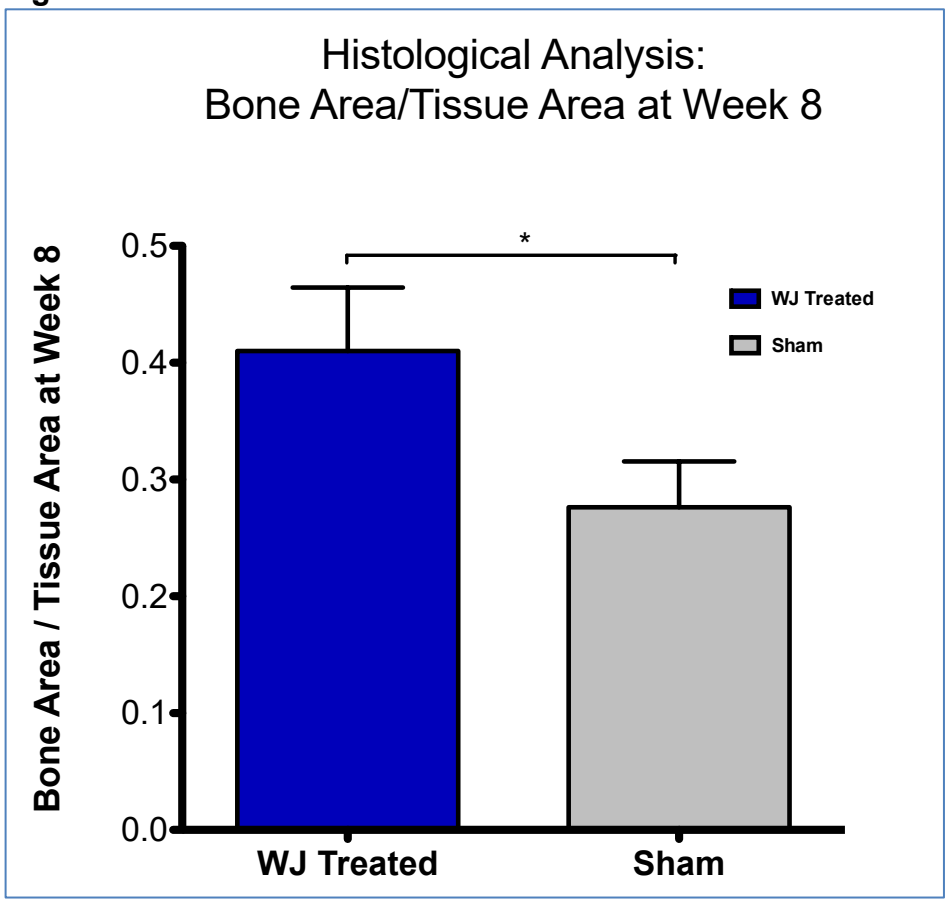
Figure 14



H&E stained sections of defect site in sham and nWJ treated rats. Total Tissue Area (TA) outlined in yellow, New Bone Area (BA) outlined in green, black and white arrowheads respectively indicate representative osteoblasts and osteocytes.

A greater BA/TA ratio was observed in nWJ treated rats (0.41 ± 0.05) compared to sham rats (0.26 ± 0.04), consistent with significantly greater percentage of newly formed bone in the total defect site area of the nWJ treated rats (Fig 15).

Figure 15



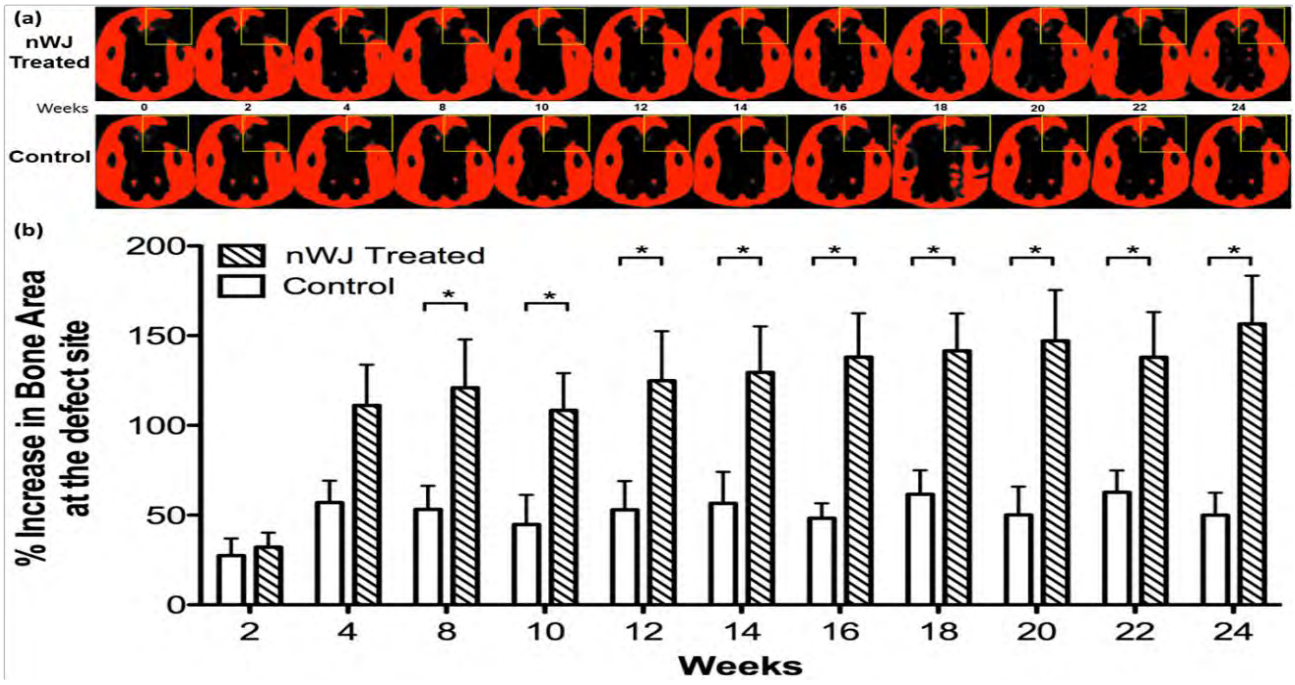
*Ratio of newly formed bone area to total newly formed tissue area in the defect site. * indicates statistical significance (p-values <0.05)*

3.7.4 Repeated, longer-term in vivo experiments

The same model system was used to repeat the initial studies, extending the follow-up to 24 weeks total. Fig.16a represents the montage of flat panel CT images of nWJ treated and control animal, demonstrating increasing bone formation over a twenty four week time period for animals treated with nWJ, whereas control animals showed minimal bone formation. Twenty four weeks postoperatively, the percent increase in new bone formation in the nWJ treated group (156.57±26.85%) was markedly higher than that in the control group (49.97±12.51%) ($p<0.05$) (Fig.16b) and the evaluation demonstrated significant increase in new bone formation in animals treated with nWJ as compared to control group throughout the study (Fig. 16b, Table 1). Furthermore,the histological results obtained from H&E staining of decalcified specimens supported the flat panel CT findings (Fig. 17). In the nWJ treated animals, light microscopy revealed the presence of osteoblastic activity, osteocytes inside lacunae's, fibrous tissue, blood vessel ingrowth, and the presence of new bone growth (BA/TA) of 0.48 ± 0.18 after twenty four weeks. Whereas, for the control group, there was presence of osteoblastic activity, osteocytes inside lacunae's, fibrous tissue, but absence of blood vessel ingrowth and significantly lower new bone growth (BA/TA) of 0.32 ± 0.15.

Altogether, these data indicate that implantation of nWJ tissue promotes bone regeneration in the alveolar defect of animals.

Figure 16



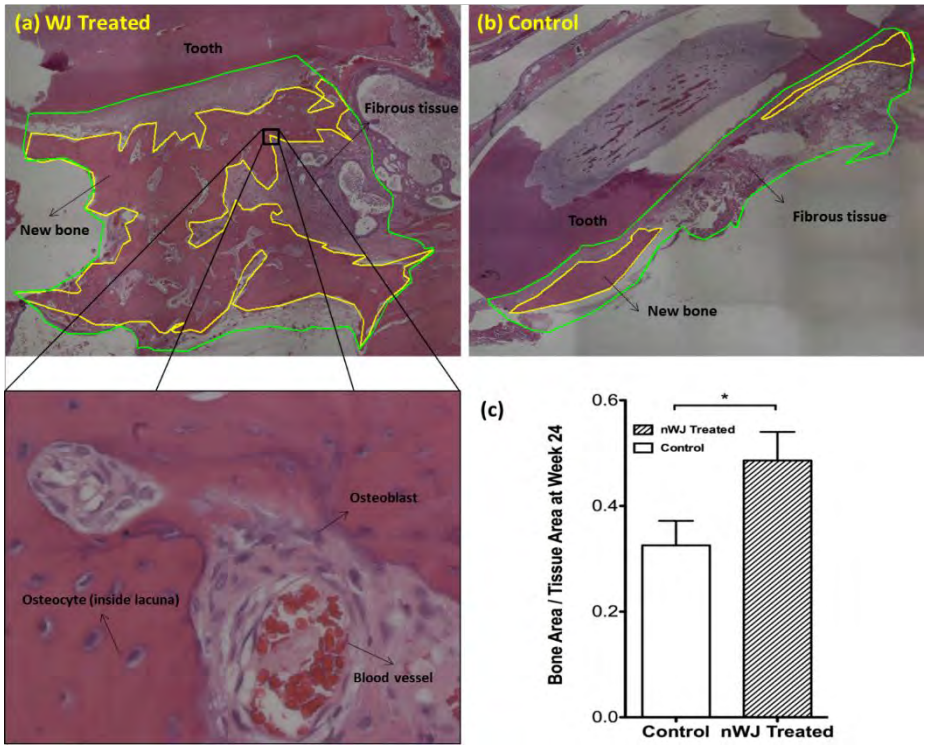
*Montage of flat panel CT and quantification of new bone area regenerated at the defect site. (a) Representative montages of the nWJ treated and the control rat CT scan over 24 weeks. (b) Quantification of the percent increase in bone area at 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24 weeks post-surgery compared to day 0. nWJ treated group regenerated significantly (*p-values <0.05) more new bone than control group.*

Table 1

Groups / Weeks	nWJ Treated (n=12)	Control (n=12)
2	32.10±8.16%	27.43±9.57%
4	111.13±22.69%	56.99±12.21%
8	120.96±27.01%	53.15±13.14%
10	108.38±20.68%	44.74±16.51%
12	124.91±27.58%	52.96±15.93%
14	129.49±25.70%	56.57±17.52%
16	138.11±24.41%	48.20±8.37%
18	141.59±20.72%	61.66±13.30%
20	147.11±28.31%	50.14±15.72%
22	137.97±25.14%	62.70±12.17%
24	156.57±26.85%	49.97±12.51%

Quantification of new bone area regenerated at the defect site in control and nWJ treated animals.

Figure 17



4 STUDY DESIGN

4.1 Study Design

Twenty-five subjects requiring surgical repair of the cleft palate will undergo an augmented repair using autologous Wharton's Jelly and will be assessed for safety, feasibility of study procedures, and functional outcome of the augmented surgical repair.

Subjects will be monitored for safety throughout the hospital stay and at scheduled follow-up clinic visits at 14 days, 3, 6 and 12 mo. post-surgery, or more frequently if treatment related SAE's are suspected.

4.2 Rational for Study Outcome Measures

4.2.1 Outcome Assessment Time Point

Several longitudinal studies have been conducted assessing bone graft outcomes and the rate of bone reabsorption at various time points up to 11 years post CLP repair. (Enemark, 1987- Feichtinger, 2007 – Ruppel, 2012) Minimal bone graft changes were observed after one year post surgery and support the predictability of a one year post-op. assessment of alveolar bone graft success or failure. The presence of an intact bony bridge across the alveolus at the one year post-op time point is the best surrogate for identifying —success" of this project in the short term. The current protocol is somewhat different in that we plan to not use a true —bone graft", and the procedure will be performed in conjunction with an early GPP at 12 to 18 mo. of age. Thus, the classic scales for bone graft assessment have not been validated for this approach.

4.2.2 Bone Graft Evaluation Scale

Several standardized scales have been developed to assess cleft palate repair including the Bergland, Kindelan, Chelsea, and Americleft SWAG Scales. (Ruppel, 2012) The scales were

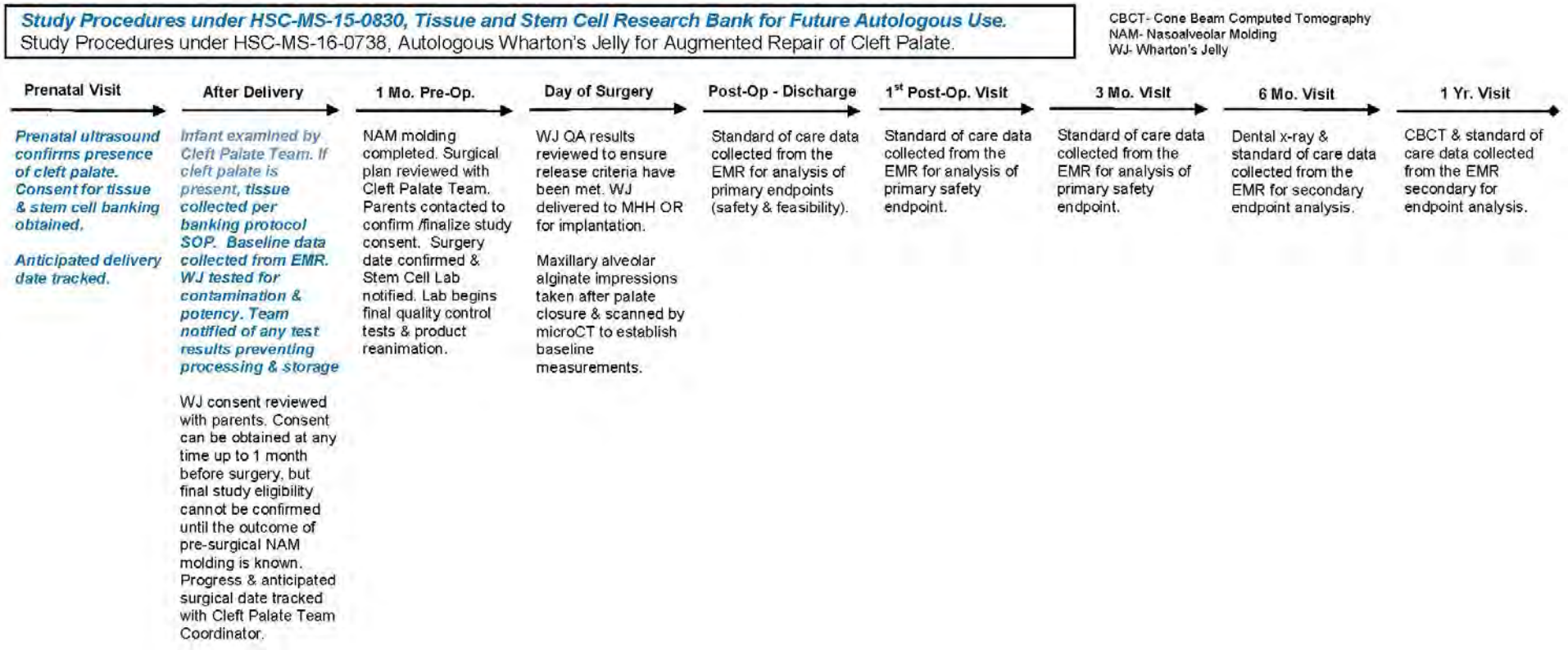
designed to measure alveolar repair with bone grafting in in older children and adults. Therefore, the use of these scales is not applicable in this protocol. We plan to assess: (1) presence/absence of the bone bridge across the alveolus as a binary outcome, (2) occlusal radiographs to assess volumetric bone formation on treated vs. non-cleft alveolar ridge to obtain a 2-dimensional percentage of bone fill, and (3) CBCT

4.2.3 Imaging Outcome Measures

Frequent dental radiographs and Cone Beam Computed Tomography (CBCT) scans are standard of care procedures for infant's pre and post CLP repair. CBCT is routinely used by cleft surgeons to visualize the 3-D morphology of the bone bridge and measure bucco-palatal width. Recent advances in CBCT imaging software have established the reliability and validity of bone density measures. (Shirota, 2010 - Molteni 2013 - Marques de Moura, 2016).

5. SELECTION AND ENROLLMENT OF SUBJECTS

Figure18: Study Flow



5.1 Pre-Consent Screening

Parents who have received the diagnosis of a cleft lip during fetal ultrasound will be invited to participate in the research protocol *Tissue and Stem Cell Research Bank for Future Autologous Use*, HSC-MS-15-0830. The invitation to participate in the study will occur after the initial prenatal consultation with the Cleft Palate team. At present, ultrasounds can only detect the presence of a CLP defect. It is currently impossible to determine the severity of the CLP defect and the potential need for surgical repair. Therefore, parents will be given the opportunity to participate in HSC-MS-15-0830 after ultrasound diagnosis of CLP. At birth, the infant will be examined and screened. The presence of a cleft at the level of the alveolus will be noted and is a critical component of enrollment eligibility for this study. Infants who have evidence of an intact, non-clefted alveolus will be excluded from the study.

5.2 Consent

Following delivery and confirmation of alveolar cleft defect, the parents of potential subjects will be approached by one of the research team members. Information regarding study participation will be provided. Discussion on the alternatives to study participation will include the standard GPP without WJ or other surgical options such as delayed CLP repair when the child is older. Parents will be given ample time to consider study participation and may take the consent home for review. For surgical repair using autologous WJ, consent must be obtained at least 1 month in advance. This will allow adequate time for WJ reanimation and QA procedures.

5.3 Eligibility Requirements

5.3.1 Inclusion Criteria

1. Umbilical cord and placental tissue collected under the Protocol Tissue and Stem Cell Research Bank for Future Autologous Use, HSC-MS-15-0830, meeting QA release criteria at the time of collection and cryopreservation.
2. Presence of alveolar cleft defect in conjunction with cleft lip and palate based on birth assessment by the Cleft Palate team. (Refer to Appendix A for ICD Codes)
3. Planned cleft palate repair and follow-up with UTHealth Cleft Palate Team and Memorial Hermann Hospital-TMC.
4. Ability to obtain informed consent from the parent or legally authorized representative (LAR) within 1mo. of the scheduled GPP surgery.

5.3.2 Exclusion Criteria

1. Birth assessment indicating:
   a. Absence of alveolar cleft palate,
   b. Cleft lip alone,
   c. Cleft palate alone.
2. Presence of chorioamnionitis and/or neonatal sepsis.
3. Presence of significant cardiac comorbidities.
4. Cleft palate with an alveolar gap greater than 5mm.
5. Cleft palate unsuitable for pre-surgical nasoalveolar molding (NAM).
6. Unsuccessful NAM delaying and or preventing GPP repair.
7. Inability to return for follow-up evaluations.

6 STUDY PROCEDURES

Table 2

	Prenatal	Delivery	Pre-Surgical Clinic Visits	1 Mo. Before Surgery	Day of Surgery	Post-op. to Discharge	14 Day Post-Op Visit (+/- 7 Days)	3 Month Visit (+/- 15 Days)	6 Month Visit (+/- 21 Days)	1 Year Visit (+/- 21 Days)
HSC-MS-15-0830 Consent	X [1]									
Cord Blood/Placental Tissue Collection		X [1]								
Stem Cell Lab Product QC Tests		X [1]								
Cleft Palate / Research Team Consultation, Exam	X [1]	X[2]	X[2]		X	X	X	X	X	X
HSC-MS-16-xxxx Consent		X[3]	X	X						
WJ Reanimation & Final QA Testing				X						
Cleft Palate Repair with Wharton's Jelly					X					
Maxillary Aveolar Impression					X					
Standard of Care Data Collected from EMR		X[4]			X[4]	X[4]	X[4]	X[4]	X[4]	X[4]
Occlusal X-Ray									X	
3D CT/CBCT										X
Evaluate for AE/SAEs		X			X	X	X	X	X	X

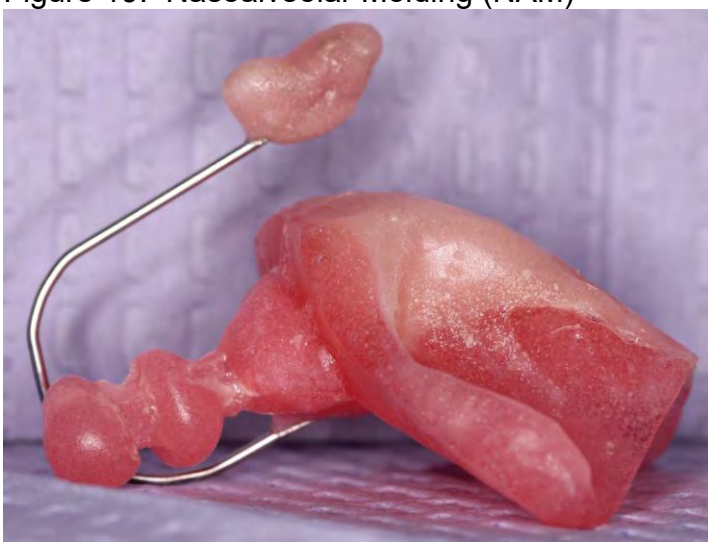
1. Protocol: *Tissue and Stem Cell Research Bank for Future Autologous Use*. **2.** To confirm presence of cleft palate defect requiring surgical repair and outcome of NAM. **3.** Consent for cleft palate surgical repair with banked WJ can be obtained at any time up to 1 month before the planned surgery. **4.** Including vital signs, weight, facial photography (if done), operative data, LOS, and other routine lab and diagnostic test results.

6.1 Screening Period (Birth up to CLP Repair)

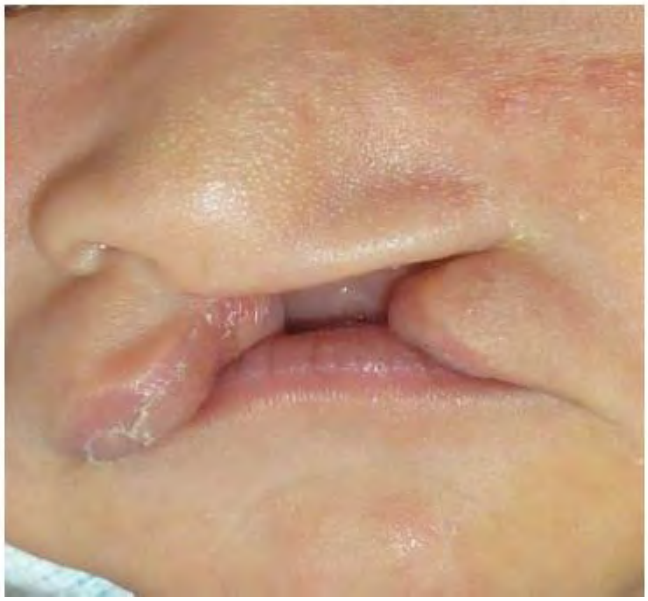
Full physical examinations will be performed and assessment of type and extent of cleft documented. Standardized patient photographs will be taken. The usual standard of care for newborn CLP infants will be provided. CLP infants are discharged home when they are tolerating adequate feeds and showing appropriate weight gain.

The cleft team will meet with the parents at routinely scheduled clinic visits after discharge to monitor the infant's progress and finalize the plan for surgical treatment. Infants with a complete cleft of their lip (unilateral or bilateral) will initiate nasoalveolar molding (NAM) therapy at one month of age. The goal of presurgical NAM is to align and approximate the alveolar cleft segments and correct soft tissue and nasal cartilage deformities. Briefly, dental impressions will be taken of the maxillary arches and used to create a custom dental appliance. Over the course of the next two to three months, the appliance will be molded and shaped to approximate the two edges of the maxillary arches together to facilitate the needed gingivoperiosteoplasty (GPP) at the time of the palate repair. This molding is essential as alveolar gaps of more than 5mm of distance across the alveolus are not candidates for GPP. This short distance allows for both adequate soft tissue closure of the gingival mucosa at the time of the GPP as well as an appropriate distance for osteogenesis to occur.

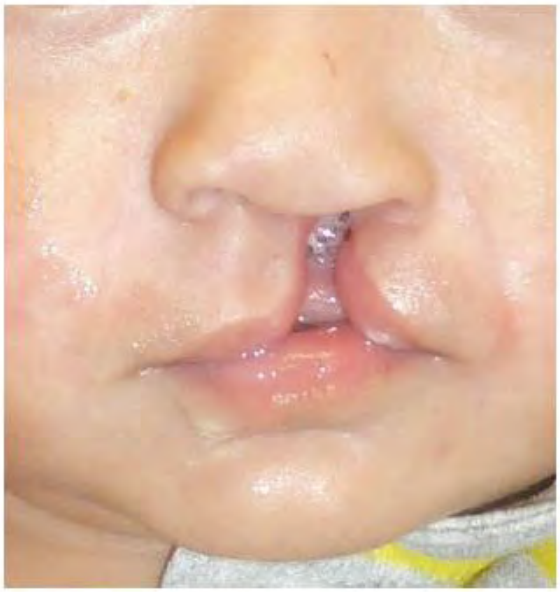
Figure 19: Nasoalveolar Molding (NAM)



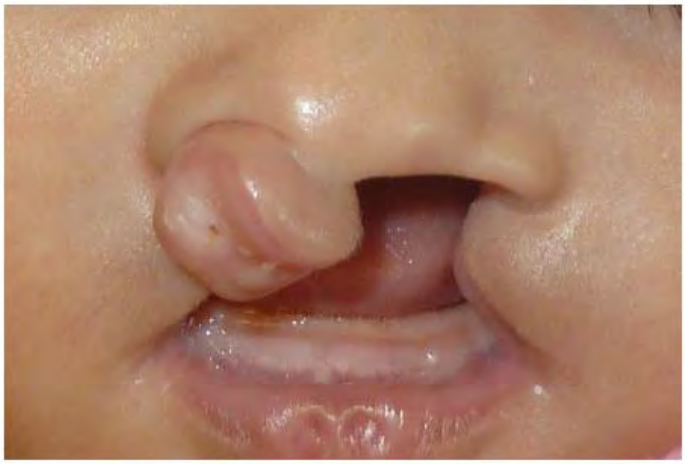
Before NAM



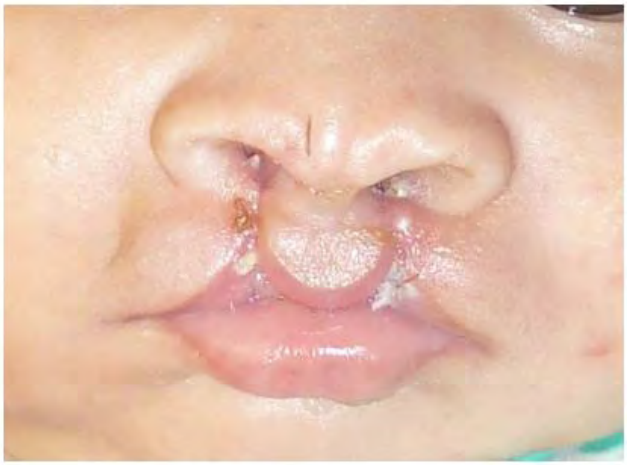
After NAM



Before NAM



After NAM



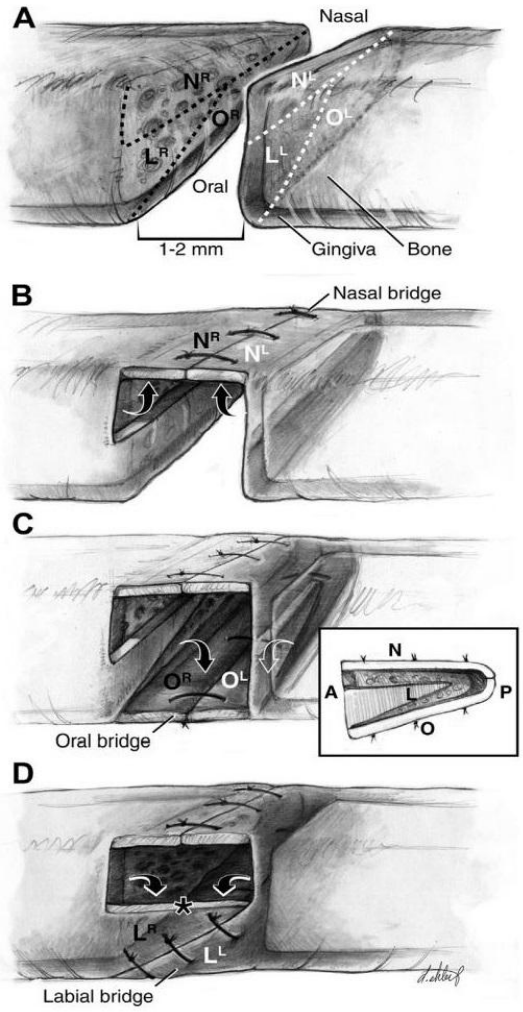
NAM isn't required for infants with incomplete clefts of the lip or isolated cleft palates that involve only the alveolus. For infants unable to tolerate or complete NAM therapy, a cleft lip adhesion surgery (CLA) may be performed at 2-3 months of age, prior to their definitive cleft lip repair.

6.2 Study Treatment

When NAM molding has been completed and the surgical plan finalized, the research team will contact the infant's parents and confirm their desire to augment the cleft palate repair with autologous WJ. GPP is normally done when infants are between 12 to 18 months of age. The Stem Cell lab will be notified of the scheduled surgery date to allow time for WJ reanimation and QA tests. Parental consent will be reviewed at the time of admission to ensure willingness to continue with study procedures. The research and cleft palate teams will review the WJ release criteria and go/no go status immediately prior to surgery. The WJ cannot be returned to the stem cell lab and must be used within 6 hours after release.

Subjects will receive standard of care procedures for infants undergoing GPP repair with the addition of WJ. Briefly, muscosal flaps are created along the anterior margin of the alveolus and closed primarily, including the incisive surface. The palate is repaired using a posterior Furlow palatoplasty with bilateral hard palate mucosal flap elevation, (figure 20 below).

Figure 20:



Gingivoperiosteal flap design and elevation for the Millard GPP. The dissection is limited to the tissues within the cleft. The flaps are named by the part of the periosteal tunnel they construct. A, anterior; LL, left labial flap; LR, right labial flap; NL, left nasal flap; NR, right nasal flap; OL, left oral flap; OR, right oral flap; P, posterior; *, Marks the mucosal edge repaired to the lip mucosa. (From Losee J, Kirschner R. Comprehensive cleft care. New York: McGraw-Hill; 2008. p. 833; with permission.)

The nasal floor is repaired up to the level of the incisive foramen. Acelluar dermal matrix may be used to patch any defects in the nasal closure to prevent fistula formation. Gingival mucosal flaps are elevated off of the lingual surface of the alveolar cleft.

The WJ will be delivered to the operating room in 3 mL. syringe within a sterile peel-pack. Standard hospital procedures will be followed to verify the ID's of the WJ and subject. The sterile peel-pack will then be opened and the WJ syringe presented to the surgeon. The surgeon will inject the WJ within the pocket of space created by elevation of the mucosal flaps. Finally these lingual flaps are closed primarily to seal off the alveolar cleft defect.

Following palatal closure, maxillary alveolar alginate impressions will be taken and poured into plaster cast impressions. These impressions will then undergo microCT scans and the size of the alveolar defect digitally registered to provide a baseline at the time of the WJ administration.

6.3 Follow-Up Period (Post-Surgery to End of Study Visit)

6.3.1 Safety Assessments
Subjects will be hospitalized overnight per standard of care for infants undergoing GPP. Outcome assessments will be made at standard of care post-op. visits:
14 Days (+/-7 Days), 3 Month (+/- 15 Days), 6 Month (+/- 21 Days), 1 Year (+/- 21 Days)

Standard of care data will be collected at each follow-up visit including physical exam, medical history since discharge (unscheduled clinic or hospital visits for management of adverse events), and routine clinical lab and diagnostic test results.

Any signs of infection (wound drainage, malodorous fluid, fever, increased pain or agitation with palpation of incision) will be treated with oral antibiotics for a course of 7 days. If this infection persists past this time, then operative drainage and irrigation of the pocket will be performed under general anesthesia.

6.3.2 Alveolar Cleft Assessments
Occlusal X-Ray: Occlusal x-ray images will be used for 2D evaluation of bone bridge presence or absence across the alveolus. Height and width of the alveolar bone will be compared to the non-cleft side for a percentage of fill in 2 dimensions.

Cone Beam CT: Cleft width, thickness, and vertical height of the bone bridge will be evaluated using CBCT images. Differences in permeability/Hounsfield units will be used to distinguish between existing and new bone. Digital volumetric analysis of the CBCT images will be used to calculate the volume of bone created within the cleft.

7. SAFTEY MONITORING AND REPORTING

7.1 Safety Monitoring Plan
The study will be conducted according to ICH Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and applicable State and Federal Regulations. Although this study has a relatively small sample size, we plan to use a DSMB instead of a SMC- monitoring approach. We have chosen this plan due to the target vulnerable study population of injured children, and the specialized safety review procedures required for autologous stem cell

research. A Medical Safety Monitor (MSM), an Independent Medical Monitor (IMM) and a Data Safety Monitoring Board (DSMB) will provide study oversight as outlined below.

7.2 Medical Safety Monitor (MSM)

Michael Lypka, MD., DMD, will serve as the Medical Safety Monitor. Dr. Lypka is an Assistant Professor of Pediatric Surgery at the Univ. of Missouri-Kansas City School of Medicine and is a board certified oral and maxillofacial surgeon. Dr. Lypka has extensive experienced with cleft lip and palate surgery and potential postoperative complications. Dr. Lypka's primary responsibility will be to review SAEs in real time to ensure good clinical practice and to quickly identify safety concerns. He may suggest protocol modifications to prevent the occurrence of particular adverse events. Dr. Lypka is not affiliated with UTHealth in any manner, nor does he practice within the Memorial Hermann Hospital System. The MSM will work in conjunction with the IMM and the DSMB to ensure the safety of the intervention in this trial.

7.3 Independent Medical Monitor (IMM)

Study monitors from Juno Research Inc. will serve as the IMM. Juno Research has provided this service for previous TBI trials at UT Health and Memorial Hermann Hospital. The IMM's primary responsibility will be to review documentation of consent and verify study eligibility from source documents, verify source documents to CRF's for errors and protocol deviations, and review subject records for treatment related AEs/SAEs. The IMM will make recommendations to the PI regarding continuation, modification or conclusion of the trial, while protecting the confidentiality of the trial data, and will provide monitoring reports for the MSM and DSMB review. In the unlikely event of unexpected or unduly high rate of SAEs, the IMM will notify the PI, MSM, and the DSMB of these findings. The IMM will review the medical record and study data collected following post-infusion Day 30 for the first enrolled subject. Thereafter, monitoring visits will be scheduled based on study enrollment, or at least every 6 months, or as recommended by the study sponsor, the MSM, DSMB, FDA, or IRB.

7.4 Data Safety Monitoring Board

The DSMB will assess the trial performance in terms of patient recruitment and retention, protocol adherence and data quality/completeness. This will be done in concert with the IMM reports and the MSM reports. The DSMB will monitor interim data with regard to safety, but since this is not an efficacy/Phase 3 study, no efficacy/interim analysis will take place. Potential conflicts can be adjudicated by consultation with the Internal Advisory Committee (Wade, Tyson, Willerson), composed of senior faculty members of various Departments (none involved in the trial). The DSMB will review any protocol modifications and how these could potentially adversely impact on the trial/and the potential impact on scientific integrity of the study and subject safety.

7.4.1 DSMB Membership

Dr. Russell Reid (University of Chicago), Dr. Justine Lee (UCLA), and Dr. Joseph Losee (Pittsburgh) will serve as DSMB members. None of the DSMB members have a conflict of interest or financial interest in the study, nor do they have a vested interest in the study outcomes.

7.4.2 DSMB Meetings

Initial Meetings-Prior to study initiation, the DSMB will meet in conjunction with the study coordinator, PI, MSM, and statistical consultant. The protocol will be reviewed and clarity on

AE reporting, monitoring and analysis plan will be reviewed. Any ambiguities in the protocol will be addressed. The board will formulate its operating procedures and frequency of meetings, typically every 6 months or more frequently if needed. The meetings are usually conducted via teleconference.

7.4.3 DSMB Reports

The format and reporting requirements will be established in conjunction with the DSMB at the initial meeting. The UTHealth Program Manager and the study statistician will prepare study reports for submission to the DSMB members 14 days prior to the meeting. DSMB reports will focus on accrual and demographics, data completeness and study performance measures. The report will include summary statistics of adverse events, a review of baseline characteristics, drop-out rates, and potential efficacy measures. Any new information that would impact the conduct of the trial will be reviewed.

7.4.4 Communication of DSMB Recommendations

The Board will provide verbal reports to the PI regarding performance and safety of the study, including any concerns that need immediate action. Thereafter, the DSMB will issue a written summary of the Board's recommendations, documenting: date of review, all data reviewed, recommendations, date of next review.

Early termination: As this is not a Phase 3 efficacy study, futility or early stopping due to efficacy will not be cause for early termination. However, if the DSMB believes that the study is not being conducted according to high scientific or ethical standards or poses an unreasonable risk to study participants, then the DSMB will recommend early termination or suspension of the protocol. Prior to this, a plan will be developed for notifying study participants, the UTHealth IRB, and FDA, and all other participants in the study.

The UTHealth Program Manager will be responsible for MedDRA coding of FDA MedWatch 3500 forms and assembling cumulative reports of all SAEs to meet requirements as mandated by the FDA, the DSMB, and the UTHealth IRB.

7.5 Reporting of Serious Adverse Events (SAEs)

The study population is not expected to have a large number of serious adverse events. The following events will trigger UTHealth IRB notification and Medical Safety Monitor/DSMB review.

- Any subject death, regardless of relationship to the GPP/WJ surgical intervention.
- SAEs with a suspected or known causal relationship to the GPP/WJ surgical intervention , as determined by the site PI.
- Any communication from the FDA or other State or Federal Agencies concerning the trial.

7.6 Grading of Adverse Events

Adverse events will be recorded from the time of consent until the subject completes the final study visit or until the subject (or LAR) prematurely withdraws from participation. The severity of the adverse event will be graded according to the criteria set forth in the National Cancer Institute's Common Toxicity Criteria for Adverse Events Version 4.0 (CTCAE). The CTCAE provides a common language to describe levels of severity facilitating interpretation and analysis, and clinical significance of the adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in

the NCI- CTCAE:

- **Grade 1** = mild adverse event
- **Grade 2** = moderate adverse event
- **Grade 3** = severe and undesirable adverse event
- **Grade 4** = life-threatening or disabling adverse event
- **Grade 5** = death

7.7 Relationship of Adverse Event to Stem Cell Infusion

The causal relationship of the AE to the study intervention will be determined by the principal investigator. The determination will be recorded on the appropriate CRF and reporting forms (if applicable).

7.8 Study Stopping Rules

The stopping rules listed below will trigger cessation of enrollment and potential study closure following a comprehensive DSMB safety review. The IRB, the FDA, and the study sponsor will be promptly notified.

- Death.
- Any Grade 4-5 Adverse Event as defined in the NCI CTCAE v4.0 and determined to be temporally-related by the Medical Safety Monitor and/or DSMB.

8. SUBJECT WITHDRAWAL

8.1 Description of Subject Completion

Subjects will be considered to have completed the study if they underwent the cleft palate repair with autologous WJ completed the 1 year follow-up visit.

8.2 Withdrawal of Individual Subjects

There may be circumstances that prevent a subject from receiving the cleft palate repair with autologous WJ. Circumstances include postponement of surgery and/or change in the surgical plan due to the subject's clinical condition, or failure to meet the final WJ release criteria on the day of surgery.

If consent is withdrawn before the 1 year visit, the reason for withdrawal will be documented in the study records. Study data collected up to the point of withdrawal will remain in the study database for analysis.

9. STATISTICAL CONSIDERATIONS

Currently the success rate with GPP with full naso-alveolar molding pre-operatively is approximately 60% in selected patients. While the rodent data are compelling and convincing in terms of augmented bone growth, we do not have any strong data to determine the potential treatment effect size in this protocol. Therefore, a true statistical analysis regarding efficacy is not valid. This protocol should yield those data on treatment effect size such that a true sample size determination can be made for future studies.

9.1 Primary and Secondary Outcome Analysis

The long term objective of this study is to reduce the need for secondary bone graft in the cleft lip and palate patient population. By performing the GPP with addition of WJ at the time of the primary palatal repair, we hope to show:

1. Formation of bone within the GPP zone.
2. Absence of an alveolar fistula decreasing the potential need for a secondary bone graft following GPP.
3. No change on the incidence of orthognathic surgery due to maxillary hypoplasia.

Descriptive statistics for baseline characteristics, known or suspected to be associated with outcomes, will be prepared. The variables considered in such a description can be categorized as: 1) demographic characteristics; 2) medical history/co-morbidities; 3) physical examination; 4) laboratory data; and 5) quality of life and psychosocial data.

Table 3:

Mortality (Current mortality rate is ≤ 0.01%)
Airway Compromise/Reintubation (Most common complication, 2% overall)
Transfusions- Intraoperative or within 24hr. of surgery (Current rate is 0.3%)
Duration of Surgery
Duration of Anesthesia
Duration of PACU Stay
Duration of IV fluid Therapy
Unanticipated Post-OP Admission to PICU
Hemorrhage/Hematoma
Wound Infection 3-4% (Schonmeyr, 2014)
Wound Dehiscence 3-4% (Schonmeyr, 2014)
Wound Complication 4.5% (Schonmeyr, 2014)
LOS (National average is 1 day without Co-Morbidities, >2 days with Co-Morbidities)
Return to Surgery within 24hr. (Estimate 2.4%, Deshpande, 2014)
Hospital Readmission within 1 month of surgery
Unscheduled Clinic or Emergency Room Visit(s) within 1 month of surgery
Co-Morbidities (Congenital Heart Defects, Low Birth Weight, Chromosomal Abnormalities)
Alveolar Fistula Formation (5 to 8% for Furlow Repair, Shaye, 2015)
Hanging Palate
Partial or Total Flap Necrosis

9.2 Sample Size and Accrual

As this is primarily a safety study, we should be able to ascertain serious AEs with 25 patients. We have already identified clinical volumes that support approximately 40-45 patients/year that meet screening criteria for enrollment, thus accrual should not be problematic.

10. DATA MANAGEMENT

10.1 Record Retention

Data will be obtained from a variety of sources including laboratory results and notebooks, electronic medical records, medical charts, neuropsychological and cognitive test data, and imaging reports and files. Data from these source materials will be entered onto a standardized set of paper Case Report Forms (CRFs) and entered into an encrypted database by the research coordinator. The period of paper and electronic record retention will be consistent with the record retention policies of UTHealth, the study sponsor, and the FDA.

11. HUMAN SUBJECTS

11.1 Institutional Review Board (IRB) Review and Informed Consent
This protocol, the informed consent document, and any subsequent modifications will be reviewed and approved by the IRB, the DSMB, and the FDA (if applicable). A signed consent form will be obtained from the parent(s) or Legally Authorized Representative (LAR) after a thorough explanation of the purpose of the study, the study procedures, follow-up visits, and potential risks/benefits. Adequate time to ask questions and make a decision about participation has been allowed. A copy of the signed consent form will be given to the parent(s) or LAR. In addition, a written note will be placed in the patient's medical chart documenting the communication between investigator and the parent(s) or LAR about the research. This note will include what was discussed; the fact that any questions were answered, and that a copy of the consent form was given to take home.

11.2 Risks

11.2.1 Autologous Wharton's Jelly and MSC's:
Cell Processing: Processing of the Wharton's Jelly is done under strict sterile conditions; however, there is a rare chance the tissue could be contaminated during processing as rigorous quality control testing is carried out to minimize the risk of issuing a contaminated final product. In brief, an aliquot of the product to be implanted will be thawed two months prior to the implantation date for quality control testing, including mycoplasma and long term sterility (14 days aerobic and anaerobic bacterial cultures). In addition, on the day of implantation, additional quality control testing of the final product, including Gram stain and Endotoxin, will be required for product release. 14 day sterility cultures of the released product will also be inoculated and if contamination is detected (usually within 3-7 days), the microbial organisms will be identified and results will be sent to the facility and the PI, so that appropriate antibiotic prophylaxis may be considered for the patient. There is always the potential risk of an allergic response to components associated with stem cell lab processing.

11.2.2 Potential Loss of Confidentiality: Despite strict measures to ensure the confidentiality of subject study records, there is always the remote risk of loss of confidentiality.

11.3 Risk Mitigation Plan:
Tissue processing will be conducted at the FDA-registered and FACT (Foundation for the Accreditation of Cellular Therapy) accredited UTHealth-Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory, an ISO 7 facility fully compliant to current Good Manufacturing Practice (cGMP) of the FDA.

Study procedures will be conducted by highly trained experts in conjunction with close monitoring to minimize potential risks.

To protect against loss of confidentiality, subject names will be replaced by a study identification number (ID) on case report forms and on information entered into the study database. Study records will be stored in locked file cabinets located in the study coordinator's office. Strict IT security measures will be followed including database storage on Zone 100, firewall protected and encrypted network servers within UTHealth. No identifying information will be mentioned in any presentations or publications.

11.4 Potential Benefits

This study has the potential to improve functional outcomes for infants undergoing alveolar cleft palate repair and also eliminate the need for secondary surgery.

11.5 Study Modification/Discontinuation

The study may be modified or discontinued at any time by the DSMB, IRB, FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

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Appendix A: Diagnostic Codes Associated with Cleft Palate

Diagnosis	ICD-9 Code	ICD-10 Code
Cleft lip unspecified	749.20	Q37.9
Unilateral Cleft Palate with Cleft Lip, Complete	749.21	*Q37.1, Q37.3, Q37.5, Q37.9*
Unilateral Cleft Palate with Cleft Lip, Incomplete	749.22	*Q37.1, Q37.3, Q37.5, Q37.9*
Bilateral Cleft Palate with Cleft Lip, Complete	749.23	*Q37.0, Q37.2, Q37.4, Q37.8*
Bilateral Cleft Palate with Cleft Lip, Incomplete	749.24	*Q37.0, Q37.2, Q37.4, Q37.8*
Cleft Palate with Cleft Lip, other Combinations	749.25	

SECTION 4.2

INFORMED CONSENT FORM TO TAKE PART IN RESEARCH

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate

HSC-MS-16-0738

INVITATION TO TAKE PART

You are invited to take part in a research project called, "Autologous Wharton's Jelly for Augmented Repair of Cleft Palate", conducted by Charles S. Cox, MD, of the University of Texas Health Science Center. For this research project, he will be called the Principal Investigator or PI.

You're being invited to participate in the program because you are participating in the protocol "*Tissue and Stem Cell Banking for Future Autologous Use*", HSC- MS-15-0830, and your child was born with a cleft palate requiring surgical repair. The word "*autologous*" means "*from self*".

Your decision to allow your child to take part is voluntary. You may refuse to allow your child to take part or choose to stop your child from taking part, at any time. This will not change the services available to your child from healthcare providers with the University of Texas Health Science Center at Houston (UTHealth) and Memorial Hermann Healthcare System.

You may refuse to answer any questions asked or written on any forms. This research project has been reviewed by the Committee for the Protection of Human Subjects (CPHS) of the University of Texas Health Science Center at Houston as HSC-MS-16-0738.

PURPOSE

In the tissue and stem cell banking protocol mentioned above, you stored your child's umbilical cord and placental tissue for potential future use at the time of cleft palate repair. This study consent describes how Wharton's Jelly (WJ), a fluid extracted from your child's umbilical cord and placental tissue will be used to enhance the standard surgical repair for cleft palate. Your child's cleft palate surgeon will review a standard surgical consent form with you before the actual surgery.

The purpose of this study is to demonstrate the safety and feasibility of using autologous WJ along with standard cleft palate surgical repair, and to determine if surgery with WJ is better than standard surgery alone.

There are several different ways to repair a cleft palate depending on severity. The cleft palate can be repaired in multiple, staged surgeries as a child grows or from a single surgical procedure during infancy called Gingivoperiosteoplasty or GPP. The goal of GPP is to close the cleft defect and provide a stable dental arch and adequate bone support for tooth eruption without limiting facial growth. The GPP surgical procedure uses nearby tissue and bone to close the cleft and creates a tunnel of space to guide future bone growth. Some cleft palate surgeons use bone grafts or synthetic bone-like material to fill in the space created by GPP, but results are unpredictable and often unsuccessful. Performing a bone graft with the GPP carries additional risks for infants. Additional surgery may be needed following GPP if bone growth into the cleft space is inadequate. Estimates on the need for additional surgery after GPP vary based on the severity of the cleft palate defect, but can be as high as 40 to 50%.

Autologous WJ is ideally suited to fill in the space created by GPP as the stem cells are able to mature into bone and support ingrowth of the surrounding tissue.

Repair of the cleft palate with autologous WJ is an investigational procedure and not yet approved by the U.S. Food and Drug Administration (FDA). The research study is being conducted under the FDA IND # __ __ __ __. The study is being conducted at Children's Memorial Hermann Hospital, TMC and is expected to enroll 25 children.

PROCEDURES

If you decide to enroll your child in this study, the information collected under the tissue and stem cell banking protocol will be used for eligibility screening. You will be immediately notified of test results preventing tissue and stem cell banking and participation in this study protocol. Your child will receive routine, pre-surgical care during clinic visits with the cleft palate team. Screening and data collection from routine clinic visits will continue until a surgical plan has been finalized by your child's cleft palate surgeon. It's important to remember that every child is unique. Depending on the severity of the cleft, some children may not be eligible for GPP. The timing of surgery will vary based on the individual needs of the child and their response to pre-surgical treatment.

The research study coordinator may contact you by telephone, e-mail, or in person at scheduled clinic visits to monitor your child's pre-surgical progress and planned date of surgery. About 1 month before your child's scheduled surgery, the UTHealth Stem Cell Lab will begin preparing the WJ. It's important for you to understand that once this process begins, the WJ cannot be re-frozen. Please notify the research team as soon as possible if the date of surgery changes.

In collaboration with the cleft palate surgeon, the research team will meet with you and your child briefly on the morning of surgery to verify the surgical plan and your desire to continue with study procedures. Final plans will be made to deliver the WJ directly to the operating room for immediate use. Except for the use of autologous WJ during surgery, your child will receive the same standard of care tests and procedures routinely provided to all infants having a GPP.

Standard of care test results and medical record information will be collected throughout the hospital stay and at follow-up clinic visits 7 to 14 days after surgery, then at 3mo., 6mo. and 1 year post surgery. Dental x-rays and facial CT imaging are routinely performed after cleft palate repair to monitor healing and bone growth. Information from dental x-rays and CT images taken at the 6mo. and 1yr. visits will be used to evaluate the benefit of GPP surgery with autologous WJ.

TIME COMMITMENT

The time commitment for parents is limited to discussion of this consent and then occasional contact with the research team by telephone, e-mail, letter, or in person during routine pre-op clinic visits. The research team will briefly meet with parents on the morning of surgery (30min.), and then at each scheduled follow-up clinic visit (30min.) for a total time commitment of about 2.5 to 3 hours.

BENEFITS

If you decide to have your child take part in this research study, your child may or may not directly benefit. However, the knowledge we gain from this study may give us information about how to better repair cleft palate defects in the future.

The University of Texas Health Science Center at Houston may benefit from your child's participation and/or what is learned in this study.

RISKS AND/OR DISCOMFORTS

Over 50 clinical research studies have been conducted since 2008 exploring the benefit of WJ for a variety of diseases and conditions. To date, no adverse events or chronic side effects associated with WJ have been reported, however, there may be unknown risks not yet discovered.

There is a remote chance of a surgical site infection from WJ that became contaminated during processing. Strict quality control measures are in place to prevent accidental contamination and to ensure the integrity of the WJ product at the time of delivery to the operating room.

There is always the potential risk of a rare allergic response to materials and/or agents used with processing and storage of WJ.

Every effort will be made to protect the confidentiality of information we collect on you and your child, as required by law for stem cell banking and release of stored cellular products. Despite our best efforts, there is always the potential risk of a breach of confidentiality.

ALTERNATIVES

You may choose to have your child's cleft palate surgery done without autologous WJ or, rather than GPP, a staged cleft palate repair at a later time. Your cleft palate surgeon will discuss other surgical alternatives.

STUDY WITHDRAWAL

Your decision to take part is voluntary. You may decide to stop your child's participation any time. A decision to withdrawal from the study will not change the routine healthcare services available to you and your child. If you decide to withdraw your child from the study, the data collected up to the point of withdrawal may still be used.

If for any reason your child becomes ineligible for GPP with autologous WJ, data collection and study procedures for this protocol will cease. Options for disposal of your child's banked WJ will be presented and are described in the tissue and stem cell banking consent.

The study PI, Charles Cox, M.D., can stop your child's study participation at any time for reasons including loss of funding for the stem cell research bank, new information supporting other more effective or safer treatments, or at the request of the FDA or other State or Federal Agencies.

IN CASE OF INJURY

If your child suffers an injury as a result of taking part in this research study please understand that nothing has been arranged to provide free treatment of the injury or any other type of payment. However, necessary facilities, emergency treatment and professional services will be available to your child, just as they are to the general community. You should report any such injury to Charles Cox, MD, at 713-500-7329 and to the Committee for the Protection of Human Subjects at 713-500-7943. You will not give up any of your child's legal rights by signing this consent form.

COSTS, REIMBURSEMENT AND COMPENSATION

You and your child will not be paid for taking part in this study.

You and your child will not incur any additional costs for surgery to repair the cleft palate using autologous WJ. You or your child's insurance provider will be responsible for the cost of all routine, standard of care hospital and/or clinic visits, diagnostic tests, procedures, and surgery.

The University of Texas Health Science Center at Houston owns any data collected and the use of the data, results, treatments or inventions that can be made from the research. The University's ownership includes the right to license or transfer the use or ownership to other parties, including without limitation, commercial entities contracting with the UTHealth. There are no plans to compensate you or your child for any patents or discoveries that may result from participation in this research study. You and your child will not be paid for any use of study data, samples or results.

CONFIDENTIALITY

Please understand that representatives of the Food and Drug Administration (FDA), Juno Research, Inc., the Data Safety Monitoring Board, and the Committee for the Protection of Human Subjects at the University of Texas Health Science Center at Houston may review your child's research and/or medical records for the purposes of verifying research data, and will see personal identifiers. Your child will not be personally identified in any reports or publications that may result from this study. Personal information about you and your child that is gathered during this study will remain confidential to the extent permitted by law. There is a separate section in this consent form that you will be asked to sign which details the use and disclosure of protected health information.

CONFLICT OF INTEREST

The PI, Charles S. Cox, M.D., is an inventor of the device used to process WJ, for which a patent may be filed by the institution. If the patent is pursued, based on data from this and other research, royalties and other compensation may be received by the institution and the investigator. Thus the University of Texas Health Science Center at Houston and the PI have a potential financial interest in the outcome of this study.

CLINICAL TRIALS.GOV REGISTRATION

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify your child. At most, the Web site will include a summary of the results. You can search this Web site at any time.

NEW INFORMATION

While taking part in this study, the study team will notify you of new information that may become available and could affect your willingness to stay in the study. They will notify you of this information by either telephone call, e-mail, or letter.

QUESTIONS

If you have questions at any time about this research study, please feel free to contact Dr. Cox or a study coordinator at 713-500-7329, as they will be glad to answer your questions. You can contact the study team to discuss problems, voice concerns, obtain information, and offer input in addition to asking questions about the research.

AUTHORIZATION TO USE AND DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

Patient Name: _____ Date of birth:_____

Protocol Number and Title: HSC-MS-16-0738
Autologous Wharton's Jelly for Augmented Repair of Cleft Palate

Principal Investigator: Charles S. Cox, MD

If you sign this document, you give permission to The University of Texas Health Science Center at Houston AND/OR Memorial Hermann Healthcare System to use or disclose (release) your child's health information that identifies you for the research study named above.

If you sign this document, you give permission to the researchers to obtain health information from the following health care providers:

Name of Provider	Address of Provider	Fax Number of Provider

The health information that we may use or disclose (release) for this research includes all information in a medical record including results of physical examinations, medical history, and lab tests. Information disclosed or released is de-identified.

The health information listed above may be used by and/or disclosed (released) to researchers and their staff. The researchers may disclose information to employees at The University of Texas Health Science Center at Houston AND/OR Memorial Hermann Healthcare System for the purposes of verifying research records. The researchers may also disclose information to the following entities:

- Juno Research, Inc. (A contract research organization monitoring the conduct and progress of the study)
- Data Safety Monitoring Board (Independent Physicians monitoring subject safety)
- Food and Drug Administration
- Companies engaged with The University of Texas Health Science Center at Houston for the commercialization of the results of the research study.

The University of Texas Health Science Center at Houston AND/OR Memorial Hermann Healthcare System is required by law to protect your health information. By signing this document, you authorize The University of Texas Health Science Center at Houston AND/OR Memorial Hermann Healthcare System to use and/or disclose (release) you and your child's health information for this research. Those persons who receive the health information may not be required by Federal privacy laws (such as the Privacy Rule) to protect it and may share information with others without your permission, if permitted by laws governing them.

If all information that does or can identify your child is removed from the health information, the remaining information will no longer be subject to this authorization and may be used or disclosed for other purposes. No publication or public presentation about the research described above will reveal your child's identity without another authorization from you.

Please note that health information used and disclosed may include information relating to HIV infection; treatment for or history of drug or alcohol abuse; or mental or behavioral health or psychiatric care. In case of an adverse event related to or resulting from taking part in this study, you give permission to the researchers involved in this research to access test, treatment and outcome information related to the adverse event from the treating facility.

Please note that you do not have to sign this Authorization, but if you do not, your child may not participate in this research study. University of Texas Health Science Center AND/OR Memorial Hermann Healthcare System may not withhold treatment or refuse treating you if you do not sign this

Authorization.

You may change your mind and revoke (take back) this Authorization at any time. Even if you revoke this Authorization, researchers may still use or disclose health information they already have obtained about you and your child as necessary to maintain the integrity or reliability of the current research. To revoke this Authorization, you must write to:

Charles S. Cox, MD
UTHealth McGovern Medical School,
6431 Fannin Street, MSB 5.246
Houston, Texas 77030
Fax: 713-500-0647

Privacy Officer
Memorial Hermann Healthcare System
909 Frostwood
Houston, Texas 77024
Fax: 713-338-4542

This Authorization will expire fifteen (15) years after the end of the study.

SIGNATURES

Sign below only if you understand the information given to you about the research and you choose to take part. Make sure that any questions have been answered and that you understand the study. If you have any questions or concerns about your rights as a research subject, call the Committee for the Protection of Human Subjects at (713) 500-7943. You may also call the Committee if you wish to discuss problems, concerns, and questions; obtain information about the research; and offer input about current or past participation in a research study. If you decide to take part in this research study, a copy of this signed consent form will be given to you.

Printed Name of Subject		
Print Name of Legally Authorized Representative	Signature of Legally Authorized Representative	Date / Time
Print Name of Legally Authorized Representative	Signature of Legally Authorized Representative	Date / Time
Printed Name of Person Obtaining Consent	Signature of Person Obtaining Consent	Date / Time

CPHS STATEMENT: This study (HSC-MS-16-0738) has been reviewed by the Committee for the Protection of Human Subjects (CPHS) of the University of Texas Health Science Center at Houston. For any questions about research subject's rights, or to report a research-related injury, call the CPHS at (713) 500-7943.

SECTION 4.3

UTHealth
The University of Texas
Health Science Center at Houston

Committee for the Protection of Human Subjects
6410 Fannin Street, Suite 1100
Houston, Texas 77030

Dr. Charles Cox
UT-H - MS - Dept of Pediatric Surgery

NOTICE OF APPROVAL TO BEGIN RESEARCH **October 17, 2016**

HSC-MS-16-0738 - Autologous Wharton's Jelly for Augmented Repair of Cleft Palate

Number of Subjects Approved: Target: 25 **/Screen:** 35

PROVISIONS: This approval relates to the research to be conducted under the above referenced title and/or to any associated materials considered at this meeting, e.g. study documents, informed consent, etc.

APPROVED: At a Convened Meeting on 09/09/2016

EXPIRATION DATE: 08/31/2017

CHAIRPERSON: Charles C. Miller, III, PhD

Subject to any provisions noted above, you may now begin this research.

CHANGES: The principal investigator (PI) must receive approval from the CPHS before initiating any changes, including those required by the sponsor, which would affect human subjects, e.g. changes in methods or procedures, numbers or kinds of human subjects, or revisions to the informed consent document or procedures. The addition of co-investigators must also receive approval from the CPHS. ALL PROTOCOL REVISIONS MUST BE SUBMITTED TO THE SPONSOR OF THE RESEARCH.

INFORMED CONSENT DETERMINATION:
Signed Parental Consent/Two Parent Signature

INFORMED CONSENT: Informed consent must be obtained by the PI or designee(s), using the format and procedures approved by the CPHS. The PI is responsible to instruct the designee in the methods approved by the CPHS for the consent process. The individual obtaining informed consent must also sign the consent document. Please note that only copies of the stamped approved informed consent form can be used when obtaining consent.

HEALTH INSURANCE PORTABILTY AND ACCOUNTABILITY ACT (HIPAA):

HIPAA Authorization required:
HIPAA Authorization within consent form

Waiver for Screening and Recruitment granted:
Information to be accessed:
Name, Street address, City, Birth date, Treatment/Service Dates, Telephone numbers, Medical Record Number

Information to be retained:
Name, Street address, City, Zip Code, Birth date, Treatment/Service Dates, Date of death, Telephone numbers, Email Address, Medical Record Number, Biometric identifiers, Full-face photographic images

UNANTICIPATED RISK OR HARM, OR ADVERSE DRUG REACTIONS: The PI will immediately inform the CPHS of any unanticipated problems involving risks to subjects or others, of any serious harm to subjects, and of any adverse drug reactions.

RECORDS: The PI will maintain adequate records, including signed consent documents if required, in a manner that ensures subject confidentiality.

SECTION 4.4 CRF # 1: Inclusion and Exclusion Criteria

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

Inclusion Criteria: *(To be eligible, all questions must be answered "**YES**")*

1. Umbilical cord and placental tissue collected under the protocol *Tissue and Stem Cell Research Bank for Future Autologous Use*, HSC-MS-15-0830, meeting QA release criteria at the time of collection and cryopreservation.	☐ Yes	☐ No
2. Presence of alveolar cleft defect in conjunction with cleft lip and palate based on birth assessment by the Cleft Palate team.	☐ Yes	☐ No
3. Planned GPP cleft palate repair and follow-up with UTHealth Cleft Palate Team and Memorial Hermann Hospital-TMC.	☐ Yes	☐ No
4. Ability to obtain informed consent from the parents or legally authorized representative (LAR) within 1mo. of the scheduled GPP surgery.	☐ Yes	☐ No

Exclusion Criteria: *(To be eligible, all questions must be answered "**NO**")*

1. Birth assessment indicating absence of alveolar cleft palate, Cleft lip alone, Cleft palate alone.	☐ Yes	☐ No
2. Presence of chorioamnionitis and/or neonatal sepsis.	☐ Yes	☐ No
3. Presence of significant cardiac comorbidities.	☐ Yes	☐ No
4. Cleft palate with alveolar gap greater than 5mm.	☐ Yes	☐ No
5. Cleft palate unsuitable for pre-surgical nasoalveolar molding (NAM).	☐ Yes	☐ No
6. Unsuccessful NAM delaying and or preventing GPP repair.	☐ Yes	☐ No
7. Inability to return for follow-up evaluations.	☐ Yes	☐ No

Patient Eligibility Verified By:

Printed Name	Signature	Date	Time
_____	_____	_____	_____

CRF #2: Demographics

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Date of Birth: ___ ___ / ___ ___ / ___ ___ ___ ___
   mm *dd* *yyyy*

2. Gender: ☐ Female ☐ Male

3. Ethnicity: *(check one selection as most closely identified)*

   ☐ Hispanic or Latino ☐ Not Hispanic or Latino ☐ Unknown ☐ Not Reported

4. Race: *(check one selection as most closely identified)*

   ☐ American Indian or Alaskan Native ☐ Asian ☐ Black or African American
   ☐ Native Hawaiian or Other Pacific Islander ☐ White ☐ Unknown
   ☐ Not Reported

5. Maternal Ethnicity: *(check one selection as most closely identified)*

   ☐ Hispanic or Latino ☐ Not Hispanic or Latino ☐ Unknown ☐ Not Reported

6. Maternal Race: *(check one selection as most closely identified)*

   ☐ American Indian or Alaskan Native ☐ Asian ☐ Black or African American
   ☐ Native Hawaiian or Other Pacific Islander ☐ White ☐ Unknown
   ☐ Not Reported

7. Paternal Ethnicity: *(check one selection as most closely identified)*

   ☐ Hispanic or Latino ☐ Not Hispanic or Latino ☐ Unknown ☐ Not Reported

8. Paternal Race: *(check one selection as most closely identified)*

   ☐ American Indian or Alaskan Native ☐ Asian ☐ Black or African American
   ☐ Native Hawaiian or Other Pacific Islander ☐ White ☐ Unknown
   ☐ Not Reported

CRF #3: Baseline Medical History

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Date of assessment: ___ ___ / ___ ___ / 2 0 ___ ___
mm dd yy

Enter all significant medical history items. Use only one line per description. Print additional pages if needed.

Body System Codes		
(1) Constitutional symptoms (e.g., fever, weight loss)	**(6)** Gastrointestinal	**(11)** Psychiatric
(2) Eyes	**(7)** Genitourinary	**(12)** Endocrine
(3) Ears, Nose, Mouth, Throat	**(8)** Musculoskeletal	**(13)** Hematologic/Lymphatic
(4) Cardiovascular	**(9)** Integumentary (skin and/or breast)	**(14)** Allergic/Immunologic
(5) Respiratory	**(10)** Neurological	

Body System Code	Medical History Term * (one item per line)	Start Date * (mm/dd/yyyy)	Ongoing?	End Date (mm/dd/yyyy)
(Example) **4**	(Example) Hypertension	03/99/2009	☒ Yes ☐ No	____ / ____ /20____
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown
		____ / ____ / _____ ☐ Unknown	☐ Yes ☐ No	____ / ____ / _____ ☐ Unknown

Signature: ______________________________ Date: ___ ___ / ___ ___ / 2 0 ___ ___ Page __ of __
mm dd yy

CRF #4: Wharton's Jelly Release Criteria

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

Complete this CRF on the day of surgery before the subject enters the operating room.

The following criteria must be **Positive or Yes** for nWJ release and implantation:

1. Potency test (reaction to Alizarin Red staining): ☐ Positive ☐ Negative

2. Volume greater than 2mL.: ☐ Yes ☐ No

The following criteria must be **Negative** for nWJ release and implantation:

1. Bacterial and fungal long term sterility test results: ☐ Positive ☐ Negative

2. Mycoplasma test result: ☐ Positive ☐ Negative

3. Gram Stain: ☐ Positive ☐ Negative

4. Endotoxin test result: ☐ Positive ☐ Negative

CRF #5: Hospital Admission - Discharge

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Hospital Admission:
   Date: ___ ___ / ___ ___ / 2 0 ___ ___ (mm / dd / yy) Time: ___ ___ / ___ ___ (hh / mm)

2. Was WJ used to augment the GPP? ☐ Yes ☐ No *(record early termination on end of study CRF)*

3. WJ Dose/Volume: __ __ mL.

4. Duration of Surgery: ___ ___ / ___ ___ (hh / mm)

5. Duration of Anesthesia: ___ ___ / ___ ___ (hh / mm)

6. Duration of PACU Stay: ___ ___ / ___ ___ (hh / mm)

7. Duration of IV Fluids: ___ ___ / ___ ___ (hh / mm)

8. Did the subject require reintubation for compromised airway? ☐ Yes ☐ No

9. Did the subject require an unanticipated post-op. admission to PICU? ☐ Yes ☐ No

10. Did the subject return to surgery within 24hr? ☐ Yes *(record reason below)* ☐ No
    - ☐ Post-Op. Hematoma
    - ☐ Post-Op Hemorrhage
    - ☐ Wound Dehiscence
    - ☐ Other:_____

11. Hospital Discharge:
    Date: ___ ___ / ___ ___ / 2 0 ___ ___ (mm / dd / yy) Time: ___ ___ / ___ ___ (hh / mm)

12. Hospital LOS: ___ ___ / ___ ___ (hh / mm)

CRF #6: Post-Operative Visit

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Visit Time Point:

☐ 2 Week Post-Op Visit ☐ 3 Month Post-Op Visit ☐ 6 Month Post-Op Visit

☐ 1 Year Post-Op Visit ☐ Extra Post-Op Visit: _____
(Reason for Visit)

2. Visit Date: ___ ___ / ___ ___ / 2 0 ___ ___
mm dd yy

3. Heart Rate: ___ ___ ___
(beats per minute)

4. O_2 Saturation: ___ ___ ___ (%)

5. Blood Pressure: ___ ___ ___ / ___ ___ ___ mmHg **Check here** ☐ if not done.
(Systolic Diastolic)

6. Temperature: __ __ __ ^{0}F ☐ Temporal ☐ Tympanic ☐ Other *(specify)*: _____

7. Weight: ___ ___ ___ ☐ Pounds ☐ Kilograms **Check here** ☐ if not done.

8. Height: __ __ __ ☐ Inches ☐ Centimeters **Check here** ☐ if not done.

9. Did the visit include dental x-rays? ☐ Yes ☐ No

10. Did the visit include a CBCT? ☐ Yes ☐ No

11. Have there been any unscheduled clinic/emergency room visits or hospital admissions since surgery? ☐ Yes ☐ No

12. Oral Exam: ☐ Normal Post-Op Exam ☐ Alveolar Fistula ☐ Flap Necrosis
☐ Hanging Palate ☐ Wound Infection
☐ Other Wound Complication:_____

Signature:_____ Date: ___ ___ / ___ ___ / 2 0 ___ ___
mm dd yy

CRF #7: End of Study

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Off study date: ___ ___ / ___ ___ / 2 0 ___ ___
   mm dd yy

2. Did the subject undergo an augmented GPP using autologous WJ and complete the follow-up post-op. visits?

   ☐ **Yes** *(**Stop here** and proceed to PI signature.)* ☐ **No** *(Answer next question.)*

3. Reason for early withdrawal: *(Select One)*

   ☐ Unable to complete the cleft palate repair with WJ.

   ☐ Subject/LAR refused further participation *(withdrew consent).*

   ☐ Based on other clinical considerations, it was in the subject's best interest to withdrawal.

   ☐ Lost to follow-up.

   ☐ Died. **Check here** ☐ if cause of death is unknown.

Cause of Death *(List primary cause first)*	ICD-10-CM Codes *(xxx.xx format)*
1	__ __ __ . __ __
2	__ __ __ . __ __
3	__ __ __ . __ __
4	__ __ __ . __ __
5	__ __ __ . __ __

   ☐ Other, *(Specify)*: _____

INVESTIGATOR'S SIGNATURE

I have reviewed this subject's record including all case report forms and pertinent attachments, and have found them to be true, legible, accurate and a complete account of the data generated during this study.

Investigator's Signature: _____ Date: ___ / ___ / 20___

Investigator's Name: *(print)* _____

CRF #8: Adverse Events / Serious Adverse Events *(print additional pages as needed)*

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Did the subject experience any adverse events during the study? ☐ Yes ☐ No

‡Adverse Event	Start Date *(mm/dd/yyyy)*	End Date *(mm/dd/yyyy)*	*CTCAE Grade	± Suspected?	Expected? *(Given Trauma Injuries)*	SAE?	Outcome	MedDRA System Organ Class (SOC) Preferred Term (PT)	PI/Co-PI Initials
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	
	/ /20	/ /20 ☐ Ongoing ☐ Unknown	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5	☐ YES ☐ NO	☐ YES ☐ NO	☐ YES ☐ NO	☐ Recovered/Resolved ☐ Ongoing ☐ Unknown ☐ Fatal ☐ Recovered/Resolved with Sequelae	SOC PT	

Complete at end of study.

PI Name:______________________ PI Signature:______________________

‡ As defined in the TRDB or AABB Circular. Refer to section 7.5 of protocol.
* Common Terminology Criteria for Adverse Events (CTCAE V4.0) Grade
± FDA Definition of Suspected AE/SAE: **Yes =** Causal Relationship to Study Intervention is Reasonably Possible. **No =** Unrelated to Study Intervention.

Page __ of __

SERIAL # 0000

CRF #9: Protocol Deviations *(Print Additional Pages as Needed)*

Autologous Wharton's Jelly for Augmented Repair of Cleft Palate. HSC-MS-16-0738
Subject ID: ___ / ___ / ___ Initials of Individual Completing Form: ___ / ___ / ___

1. Where there any deviations from the protocol for this subject? ☐ Yes ☐ No

Description of Protocol Deviation	Date of Discovery	PI/Co-PI Initials
	/ /20	
	/ /20	
	/ /20	
	/ /20	
	/ /20	
	/ /20	

Complete at end of study.

PI Name:_____ PI Signature:_____

SECTION 5.1: Product Manufacturing and Characterization

I. Product Manufacturing – Components

A. **Tissue-based product:**
Potential mothers who meet study criteria will be enrolled into the study. After the birth of the child with cleft –craniofacial anomaly, the umbilical cord attached to the placenta will be collected and placed in a sterile container with transport medium. The umbilical cord will be transported in a validated transport cooler at ambient temperature to the UTHealth - Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory for harvesting and cryopreservation of the Wharton's Jelly.

B. **Maternal Screening:**
Donors will be screened according to current blood donor regulations by Gulf Coast Regional Blood Center using FDA-approved tests where available. Testing will be performed according to applicable processing facility SOPs.

C. **Maternal Testing:**
Chagas disease, Hepatitis B core, Hepatitis B surface antigen, Hepatitis C, HTLV 1 and 2, Syphilis, HIV 1 and 2, Nucleic acid testing (NAT) using non-pooled samples will test for HIV, HBV, HCV (Procleix Ultrio plus) and West Nile virus.

Collection of samples for infectious disease marker (IDM) testing will be carried out according to 21 CRF 1271.80(b1).

Products with positive results or with pending eligibility results will be quarantined and labeled according to 21CFR 1271.60 and applicable facility SOPs.

D. **Reagents:** The following reagents and materials will be used for manufacturing:

1. Transport medium (sterile PBS without Ca^{++} and Mg^{++} supplemented with 50U/mL penicillin G, 50µg/mL gentamycin, 5µg/mL amphotericin B and 500 IU heparin)
2. Wash buffer (sterile PBS without Ca^{++} and Mg^{++} supplemented with 50µg/mL gentamycin)
3. Sterile Gauze pads
4. Sterile 50 mL conical tubes
5. Sterile 9 x 9 cm O-wrap Cryogenic Overwrap Bags (Origen Product code OW0909)
6. Vitrification Freeze Kit (Irvine Scientific Catalog # 90133-DSOC)
7. 10% Povidone-Iodine (Medline)
8. Sterile deionized (DI) water
9. Sterile 2, 5, 10 and 25 mL cell culture pipettes

E. **Reagent Qualification**
All reagents have the manufacturer's certificate of analysis on file. These are maintained according to applicable regulation.

F. **Combination Products**
Not applicable

G. **Drug or Device Components**
Not applicable

II. Product Manufacturing – Preparation of Autologous tissue

Human Umbilical Cord (UC) will be collected at Memorial Hermann Hospital. The UC will be submerged into the transport medium in a sterile container and will be placed in an insulated cooler validated for short distance transportation of tissue-and cell-based therapy products. The cooler will be hand carried to the processing facility: UTHealth - Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory.

UTHealth-Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory

1. **Facility Demographics**

Location:
Behavioral and Biomedical Science Building (BBSB)
1941 East Road, 6th Floor
Houston, TX 77054

FDA Establishment Identifier (FEI):
3009561521

Reporting Official:
Fabio Triolo, PhD - Director
713-486-2542

Accreditations:
Foundation for the Accreditation of Cellular Therapy (FACT) for Cellular Therapy Product Processing with more than minimal manipulation

a. Location and General Description

UTHealth-Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory (Griffin Facility hereafter) is part of the Cellular Therapy Core (CTC) at UTHealth and is a 1,850 sq. ft. state-of-the-art multiple ISO class Human Cell Processing cGMP Facility. It has three ISO 7 clean-room laboratories engineered to comply with current Good Manufacturing Practice (cGMP) and Good Tissue Practice (GTP) requirements. The core cell processing suites are used to independently perform cGMP-grade cell and tissue processing. Each core laboratory has its own entrance and exit to ensure unidirectional personnel flow. Facility function is managed by a computerized control system interfaced with an environmental monitoring system that allows continuous monitoring of the critical equipment and environmental performance. An integrated door interlock system interfaces with an institutional access control and management system to prevent simultaneous door openings that would affect differential pressures designed to maintain rooms to ISO Standards.

The Griffin Facility supports the University of Texas Health Science Center at Houston (UTHealth) School of Medicine, Program in Regenerative Medicine and its clinical tissue engineering and cell transplantation activity by producing clinical grade human tissue-based products as well as human somatic cellular products, including, but not limited to, those derived from bone marrow, peripheral and umbilical cord blood. The Facility is exclusively used for human cell and tissue processing and its mission is to provide innovative clinical-grade cell- and tissue-based products and advanced therapies with quality suitable for intended use in humans.

Table 1.0 Room Listing and ISO Classification

ROOM NAME	ROOM NUMBER	ISO CLASSIFICATION
Quarantine Room	6302	Not Classified
Released Supply Room	6304	Not Classified
Main Entry/ Exit Room	6318	ISO 9
Change Room	6318A	ISO 9
Janitorial Room	6J03	ISO 9
Bulk Material Pass Through	6306	ISO 8
Sterile Gowning Room	6308	ISO 8
Clean Storage Room	6308A	ISO 8
De-gowning Room	6318B	ISO 8
Exit Hallway	6318C	ISO 8
Entry Hallway	6306A	ISO 8
Production Suite No. 1	6310	ISO 7
Production Suite No. 2	6312	ISO 7
Production Suite No. 3	6314	ISO 7

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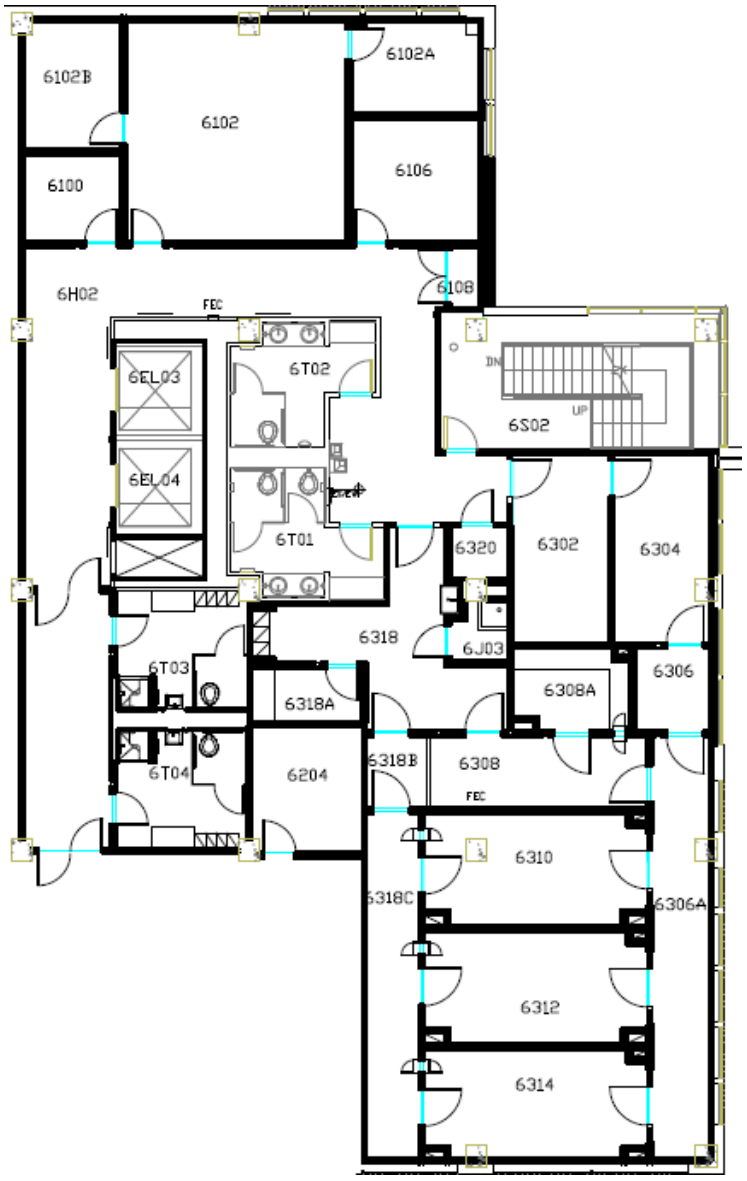
1) Floor Plan – General



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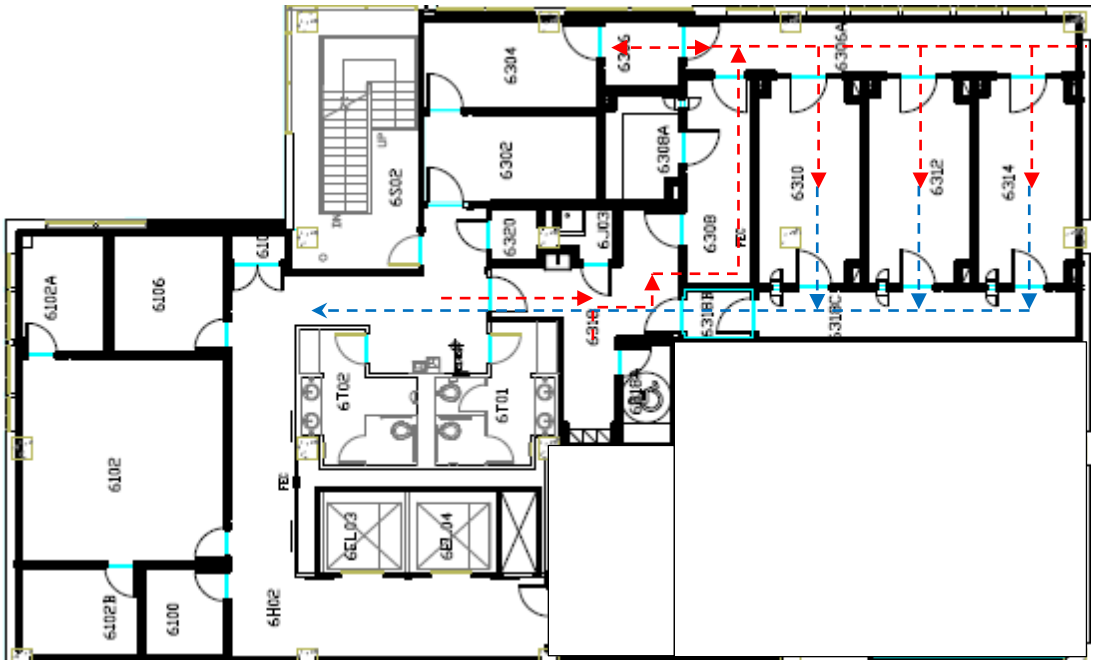
2) Personnel flow



i. Staff trained and released to perform production activities enter through the Facility's main Entry/Exit (6318) after hand washing and ensuring all prohibited materials are removed (jewelry, cell phones, etc.). Street clothes are changed to clean room (non-sterile) low particulate release undergarments in the Changing Room (6318A) and non-sterile gloves, face mask, hair and beard covering and autoclavable clean room clogs are donned.

Staff then proceed immediately to Sterile Gowning (6308) where they don disposable sterile coveralls, boots and gloves. Clean supply items needed are taken from 6308A and staff then proceed to the selected and prepared suite for production. They may pick up newly released items from the Materials Pass-Through (6306) as needed.

All activity is strictly outlined in facility SOPs (GP: 1-2), which include detail on aseptic behavior and processing activity while inside the facility. Once staff are finished with their tasks, they leave via 6318C, through sterile degowning (6318B) and then change back into street clothes into changing room 6318A, before leaving the facility.

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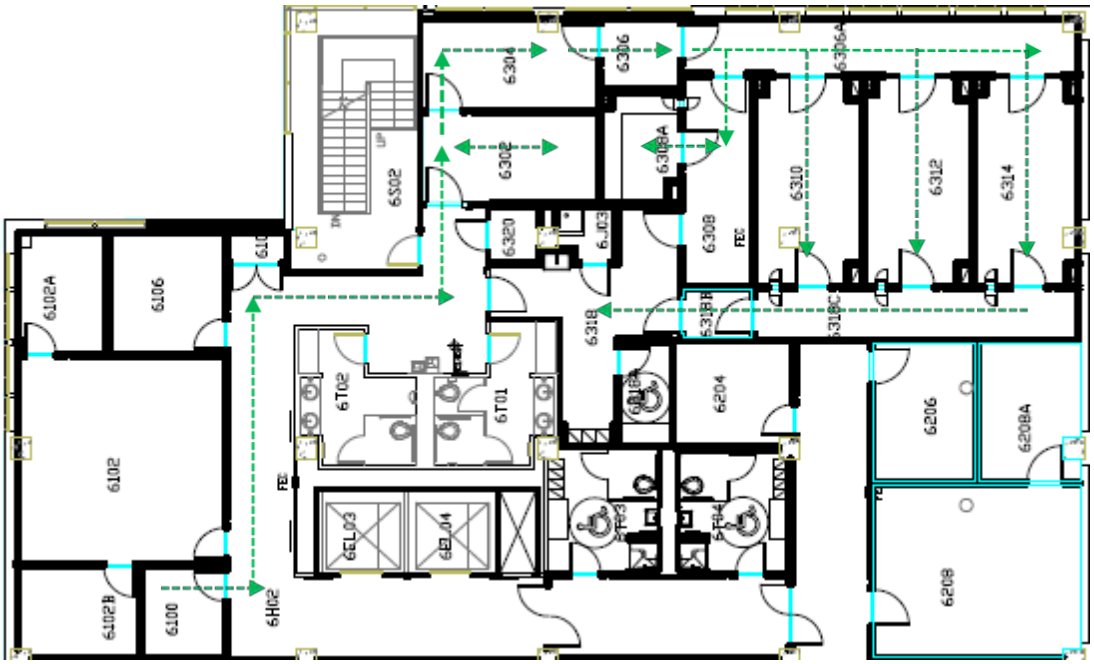
3) Materials flow (See SOPs GP:9 and 10)



ii. Most materials are received outside the administrative offices. Items are inspected, unpacked from shipping containers and organized on shelves in the storage room 6100 and room 1408 (the latter is on the ground floor of the building). All storage areas are continuously monitored for temperature and humidity. Sterile items are inspected for expiration and a Certificate of Analysis (COA), which is kept on file in the QA Manager's office. Reagents or supplies deemed critical cannot be used without a COA or other qualification.

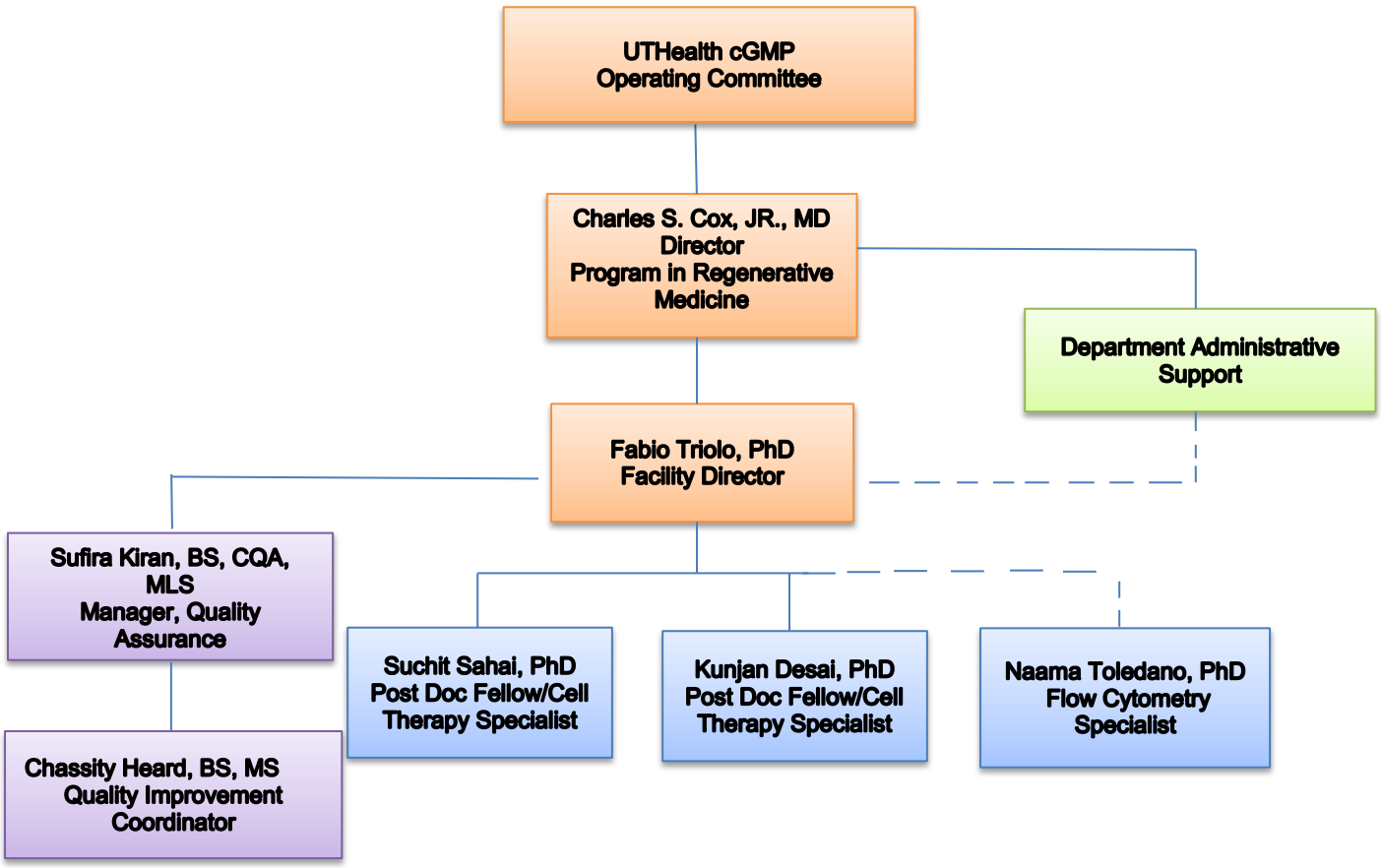
Reagents are moved into the Quarantine room (6302) where they are stored at the appropriate temperature, if COAs, validation or qualification is needed. If released for operations, they are moved directly into the Released Supply room (6304, duplicate cold storage equipment exists in both areas). When ready for production activities, staff removes these items, cleans/disinfects them thoroughly using 70% isopropanol or quaternary ammonium and places them on a cart in the Materials Pass-Through (6306).

Personnel who have donned sterile garments retrieve items from the pass-through either for production or storage in the Clean Storage room (6308A). Any non-temperature-sensitive items not used during production must be placed back into the Clean Storage room. All trash is removed from the facility through the exit route. Biohazardous trash is autoclaved and discarded according to facility and institutional policy.

All biological raw materials entering the facility are inspected, verified by two people against accompanying documentation and stored according to requirements. Stored products are segregated and clearly labeled. During processing, only one product is processed in a production suite at a time. Two

different products, even of the same type for the same trial, cannot be processed in the same suite at the same time. If consecutive processing sessions are required, SOP GP: 3 dictates cleaning as well as other steps similar to a manufacturing line change.

2. **Facility Personnel**
   a. The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory Organizational Chart



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b. Key Personnel

1) Director, Program of Regenerative Medicine

Charles S. Cox, Jr., M.D. is the George and Cynthia Mitchell Distinguished Chair in Neurosciences and directs the Pediatric Surgical Translational Laboratories and Pediatric Program in Regenerative Medicine at the UTHealth Medical School. He also directs the Pediatric Trauma Program at the UTHealth Medical School/Children's Memorial Hermann Hospital in the Texas Medical Center. The Pediatric Translational Laboratories and Pediatric Program in Regenerative Medicine are a multi-disciplinary effort that addresses problems that originate with traumatic injury and the consequences of resuscitation and critical care. It includes a multidisciplinary clinical and translational laboratory team based on complementary research functional working groups focused on development of cellular therapies for neurological injury. In addition to the ISO 7 Griffin cellular production facility, the Program also includes the Judith R. Hoffberger translational laboratory, a contamination-controlled facility for process development, optimization and scale-up. The Program focuses on progenitor cell based therapy (stem cells) for traumatic brain injury, and related neurological injuries (hypoxic-ischemic encephalopathy, stroke, spinal cord injury), and completed the first acute, autologous cell therapy treatment Phase I study for traumatic brain injury in children. It also develops novel bio-hybrid organs using cell-based and tissue engineering approaches to trauma and injury related problems.

2) Facility Director

Fabio Triolo, D.d.R., M.Phil., Ph.D., graduated summa cum laude in Biological Sciences from the University of Palermo, where he also completed a Research Doctorate (D.d.R.) in Chemical Sciences in 1999 and obtained Italian Board of Biology certification in 2001. From 1996 to 2001 he was a Fulbright Fellow at Mount Sinai School of Medicine of New York University, where he was conferred a Master of Philosophy (M.Phil.) and a Doctor of Philosophy (Ph.D.) in Biomedical Sciences in 2000 and 2002, respectively. Immediately after, he was recruited by the University of Pittsburgh Medical Center at the Mediterranean Institute for Transplantation and Advanced Specialized Therapies (ISMETT) in Italy, where he designed a state-of-the-art Human Cell Processing Facility compliant to FDA and EU cGMPs, made it operational, founded and co-directed the Regenerative Medicine and Cell Therapy until being recruited by the University of Texas Health Science Center at Houston in January 2011.

He has a broad background in aseptic methods of harvesting, purification, processing, culture, storage and characterization of human somatic cells and extensive experience in Good Manufacturing Practice-compliant stem cell production for regenerative medicine applications. For example, he was the first to publish specific risk analysis approaches and procedures applicable to cell therapy manufacturing and to provide a specific model for guidance of cell transplantation centers and cell processing facilities, especially if approaching risk management for the first time (Lopez et al., Risk Analysis, 30(12):1857-71, 2010). He was also the first person in the Region of Sicily to ever be authorized by the Italian Drug Agency (AIFA) and the Italian Ministry of Education, University and Research, to act as "Qualified Person" (according to European directive 2001/83/EC) of cGMP facilities authorized to produce cell therapy products. The only other person ever authorized

in the Italian Region of Sicily was trained in the cGMP facility directed by Dr. Triolo. Since 2008 he has been a member of the National Reference Pole for the Coordination of Biological Resource Centers and Biobanks, nominated by the National Committee for Biosafety, Biotechnology and Life Sciences of the Italian Presidency of the Council of Ministers. He actively participated to the drafting and review of several national guidelines including the Italian Presidency of the Council of Ministers guidelines for biological banks for infectious diseases, the Italian Presidency of the Council of Ministers guidelines for biobanks and biological resource centers for storage of human samples for research purposes, the Italian Ministry of Health guidelines for procurement, processing, storage and distribution of cells and tissues for clinical use, and the National Transplant Center guidelines for procurement, processing, preservation, storage and distribution of pancreatic islets and hepatocytes.

He also served on the task force for Advanced Therapy Medicinal Products (somatic cell therapy, gene therapy and tissue engineering products) of the European Advanced Translational Research InfraStructure in Medicine (EATRIS), aimed at creating a distributed pan-European infrastructure consisting of a network of well-renowned biomedical translation research centers across Europe.

In January 2011 he was recruited by the University of Texas Health Science Center at Houston (UTHealth) where he set-up, validated and directs the Evelyn H. Griffin cellular production facility. He also directs the Judith R. Hoffberger translational laboratory under the Program of Regenerative Medicine. Dr. Triolo supervises and actively participates in the cell and tissue manufacturing activities and is involved in several research endeavors of the Program. These include the development of human adult and fetal cell-based therapies to improve neurological conditions, such as anoxic brain injury at birth, cerebral palsy, traumatic brain injury and stroke, all of which are still unmet medical needs that have not been able to be satisfied by conventional healthcare therapies. Dr. Triolo’s research interests also include the development of innovative autologous tissue engineering applications based on extra-embryonic tissues (e.g., Amniotic Fluid, Wharton’s Jelly). He is currently Associate Professor in the Department of Pediatric Surgery, Associate Professor of Clinical and Translational Sciences and Director of the Cellular Therapy Core. Today, thanks to Dr. Triolo, UTHealth has an active and growing FDA-compliant biomanufacturing program that supports multiple cell therapy trials, with a focus on neurological injury.

3) Quality Assurance Manager
Sufira Kiran, BS, CQA (ASQ), MLS (ASCP) is the Quality Assurance (QA) Manager of the Cellular Therapy Core (Griffin and Hoffberger facilities) at UTHealth. She is responsible for managing the quality assurance program to ensure that all products manufactured possess the required quality, safety, purity and potency characteristics according to current FDA and FACT standards. She completed her Clinical Laboratory Science degree at UTMB Health, Galveston and was immediately recruited at UT MD Anderson Cancer Center Stem Cell Therapy Lab where she acquired ten years of manufacturing experience in cord blood banking where she was in-charge of developing and validating new procedures. She visited cord blood programs across America to learn cord blood processing and cryopreservation techniques. The program was able to acquire FACT accreditation within a few years of operation. Her background broadly covers standard operating procedure development, reviews and maintenance. She has extensive internal auditing

experience along with tracking and trending events/deviations/complaints for quality improvement purposes. She was responsible for creating an elaborate training program for new employee via video presentations and hands-on training methods. She is also an active member of the Lab Practices Committee of the International Society for Cellular Therapy.

4) Quality Improvement Coordinator
Chassity Heard BS, MS is Quality Improvement Coordinator of the Cellular Therapy Core at UTHealth. She assists the Facility Director and the QA Manager in keeping the Griffin facility in compliance with local, state, and federal regulations and also assists staff in consistently manufacturing products that are safe, pure, and effective. She conducts internal/external routine audits and proficiency testing. She also reviews, analyzes, and summarizes data and provides recommendations for corrective action and preventative action (CAPA). She helps in writing, reviewing, and revising technical SOPs and policies, ensuring they are updated and in compliance with regulatory bodies. She assists the QA manager in gap analysis and in preparing any missing documentation noted by accreditation organizations in a timely manner. She plays a significant role in preparation for FACT accreditation by working closely with accreditation counselors/coordinators to complete required documentation. She has a Bachelor's degree in Clinical Laboratory Science, a Master's degree in Health Administration, and five years of experience in a laboratory setting.

5) Cell Manufacturing Technologist
This position works with and under the supervision of the Facility Director on projects related to cell therapy and regenerative medicine. In accordance with cGMP guidelines, he/she performs a wide variety of complex biologic manufacturing processes, including, but not limited to tissue digestion, volume reduction, mononuclear cell separation, dose allocation, cryopreservation, thawing, cell culture and expansion, and related procedures (e.g., determination of cell count, viability, identity and potency) for autologous and allogeneic cell products. He/she is trained in accurate documentation according to cGMP and assists in performing facility and equipment duties (e.g., environmental monitoring of the facility, equipment, room clearance/cleaning, trouble shooting).

Suchit Sahai received his Ph.D. in Biomedical Engineering from the University of South Carolina in 2013 and has since been working at UThealth as a postdoctoral research fellow spearheading the efforts leading to the development of clinical-grade processes to harvest, vitrify and thaw Wharton's Jelly, as well as its characterization and preclinical development of innovative Wharton's jelly-based tissue engineering approaches for the treatment of congenital defects. He is actively involved in clinical-grade cell manufacturing activities supporting multiple clinical trials. His key responsibilities are to help in the translation, scale-up and validation of promising new therapeutic technologies developed at a preclinical level, into clinical-grade processes that can be used to manufacture cell-based and/or tissue engineered products for human clinical applications.

Kunjan Desai received his PhD in Cryobiology and molecular biology from the University of Bedfordshire (UK) in 2012. He then carried out postdoctoral research

aimed at developing innovative xeno-free vitrification protocols and molecular assays for various cells and tissues in Sweden at Linköping University and in the USA at Georgia Regents University. He was recruited at UTHealth in February 2016 to develop and/or optimize clinical-grade cryopreservation/thaw conditions for human tissues to be used in clinical applications of regenerative medicine. He is also currently being trained in cGMP-compliant cell and tissue manufacturing activities.

c. Training (GP:4)

Training is overseen by cGMP Facility management and must be performed by a qualified and fully trained staff member on the task being trained. The Facility Director (FD) is ultimately responsible to ensure training is complete and personnel are qualified to work inside the facility. This responsibility may be delegated. The cGMP Facility training is primarily performed by the QA Manager where it involves general or non-technical policies and procedures. Technical training is determined on a project by project basis, overseen by the FD and documented in that individual's training file.

cGMP Facility staff must possess the necessary education, training and experience to ensure competent performance of the assigned functions. Training is a crucial staff development measure intended to maintain the employee's skill features and adapt them to changed conditions. Training requirements extend to all employees whose activity affects product quality, safety and quality-related aspects of facility operations. Training is pertinent to the particular operations that an employee performs and to cGMP regulations as they relate to employee's functions and responsibilities.

Employee training is conducted by qualified individuals on a continuing basis, and employee competency is assessed with sufficient frequency to assure that employees remain familiar with applicable GMP requirements. Training is documented and retained for all essential functions. The QA Manager maintains current personnel files on each individual employee, documenting education, current job description, training/retraining records, as well as competency assessments.

Training courses are executed when the employee first starts their job and then continuously thereafter.

1. All new UTHealth employees attend new employee orientation. This includes general university overview and history, reviewing human resource policy/procedure, Vision, Mission, Organization Charts, IT, etc.
2. UTHealth also requires on line classes including but not limited to conduct, safety, patient information confidentiality and ethics standards.
3. The cGMP Facility also promotes participation in relevant conferences, courses and web seminars which are considered appropriate to increase the staff qualification. Participation is based on time and budget availability and requires FD approval.
4. Quality Assurance (QA) specific training sessions are organized by the QA Manager. QA training includes:
    a. QA principles and the cGMP Facility Quality Management System (QMS), including the quality management software (Q-Pulse)
    b. Regulations
    c. Non-Technical SOPs (theoretical and practical training)

5. Tests are given when applicable to assess training comprehension after each training session, accompany SOP revision or whenever considered appropriate by the QA Manager or FD.

Staff never perform a technical procedure independently until fully trained and released by the QA Manager or FD to do so. This is documented on the competency assessment, which shows the dates the procedure was performed and is signed by the employee and trainer.

Revised SOPs usually require a revision training exam based on the technical or procedural changes in the revision. Whether an exam is required or not, a Revision Training Roster must be initialed and dated by all trained staff indicating the revision draft has been reviewed and/or the revision exam has been completed successfully. This occurs prior to the new revision being activated (SOP QA: 2).

3. **Quality Management System** (SOP QA:1)

a. Program Overview

The Griffin Facility performs various steps in the manufacture of human cells, tissue and cellular and tissue-based products (HCT/Ps) for Phase I and II clinical trials and maintains a detailed Quality Management System (QMS hereafter). The QMS incorporates the organizational elements from FACT-JACIE International Standards for Cellular Therapy Product Collection, Processing and Administration and the Federal regulations found in 21 CFR Parts 1271 (cGTP), 210/211 and 820. The overall goal is to prevent the introduction, transmission, or spread of communicable diseases through the manufacturing and use of HCT/Ps, and to assure HCT/Ps have the required safety, purity, potency and effectiveness.

The QMS identifies and monitors specific operational systems in cGMP operations. The QA Manager and FD monitor implemented systems to ensure the goals and objectives are consistent with Good Laboratory and Manufacturing Practices (GLP/GMP).

Specific aspects incorporated into the QMS include but are not limited to:

- Appropriate organizational structure with staff properly qualified and trained
- Detailed protocol development, review and revision
- Detailed document control
- Written Agreements
- Outcome analysis
- Quality audits
- Deviations, complaints and related quality improvement initiatives
- Product tracking and labeling
- Validation
- Environmental Control
- Vendor, supply and reagent qualification
- Equipment management, including calibration, validation and maintenance
- Inventory control
- Process change control
- Product receipt, storage and distribution

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b. QMS Software

The cGMP Facility uses Q-Pulse Quality Management Software produced by Gael Ltd. (www.gaelquality.com) which is 21 CFR part 11 compliant and validated. Q-Pulse is a software application that helps organizations manage Quality, Safety and Risk effectively. Eliminating effort duplication and the time spent searching for information in a plethora of systems, spreadsheets, databases, reports and filing cabinets, Q-Pulse provides all employees with a central, focal point for all compliance data, materials and activities. In addition, Q-Pulse makes management aware of areas in need of attention if their compliance status is to remain current. Q-Pulse provides a suite of integrated modules to manage business functions effectively and efficiently including:

- Document Control
- Audits
- Corrective / Preventative Action (CAPA)
- Incident Reporting and Investigations
- Analysis of Findings, CAPAs, Incidents, Customer Complaints, Returns, etc.
- Assets
- Employee Competence & Development
- Customer Feedback
- Supplier Performance

Q-Pulse is highly configurable in terms of structure, data security, workflows, alerts and escalation.

Q-Pulse offers version tracking, edit security, active version control and many other aspects required for documents in a regulated industry. Q-pulse allows us to demonstrate compliance, reduce risk exposure and present opportunities to continually improve the organization.

Additionally, Q-Pulse offers mobile applications for use with an iPad. Using Q-Pulse's iPad applications allows us access to required documents in the sterile environment without introducing paper in the facility.

c. Document Control (SOPs QA:1, QA:2, GP:10)

This is handled almost solely using Q-Pulse at the Griffin Facility. All regulated documents are securely housed on the server database, with controlled access granted by the QA Manager. Staff are given access and permissions based on their responsibilities and training. All staff has access to active documents, which are viewed as "read only" to prevent accidental editing. Using Q-Pulse, staff is always guaranteed to have the most recent version available from any PC or iPad used in the facility.

Q-Pulse also manages the review and revision process. Once in draft, a document can only be "checked out" by one person at a time who has the correct permissions. Approvals are also managed within the system.

In order to minimize the impact when computer systems are down, all documents also have a printed master. Standard Operating Procedures are physically signed by the QA Manager and FD and are available as needed. Obsolete versions are archived according to compliance guidelines. This allows the facility to have a paper trail to back up the electronic system.

d. Deviation Management (SOP QA:3)

This process is also managed in Q-Pulse. The CAPA module is highly customizable allowing the facility to create several different types of non-conformance templates (including deviations and customer complaints). Stages within a Record allow staff to document the investigation, root cause analysis, corrective and preventive actions and follow up. Once complete, the Record is then reviewed by the QA Manager and the FD and is signed electronically. The document is then printed and archived for availability if the computer system is ever down.

Q-Pulse also incorporates an Analysis Module in which deviations can be tracked and trended based on various input parameters. This facilitates continuous quality improvement over time.

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4. Processing

a. Critical Equipment List

	Equipment	Model Number	Serial Number	Install Date
6302	Thermo Forma -80 Freezer	8692	825945-55	10/14/2011
	Thermo Revco Refrigerator	REL-3004A	0117981201110509	10/14/2011
	Thermo Revco -20 Freezer	UGL2320A	0117970601110505	10/14/2011
	MVE LN_2 Vapor Freezer	819P-190F-GB	CAB2111140013	01/20/2012
6304	Thermo Forma -80 Freezer	8690	824383-218	10/14/2011
	Thermo Revco Refrigerator	REL-5004A	*0117963801110430	10/14/2011
	Thermo Revco -20 Freezer	UGL2320A	*0116086601110624	10/14/2011
	MVE LN_2 Vapor Freezer	819P-190F-GB	CAB2111160001	01/20/2012
	Thermo CryoMed Controlled Rate Freezer	TF7452	511263-313	10/14/2011
6310	Thermo Revco Undercounter Refrigerator	REL404A	0117982201110510	10/14/2011
	Thermo Revco Undercounter -30 Freezer	ULT430A	0117982001110510	10/14/2011
	Thermo Forma Incubator	3140	357354-440	10/14/2011
	Thermo Forma Incubator	3140	3205553-438	10/14/2011
	Thermo Sorvall Centrifuge	RC3BP+	41192130	10/14/2011
	NuAire Labgard Class II A2 BSC	425-400	134523121009	12/22/2009
	NuAire Labgard Class II A2 BSC	425-400	134529121109	12/22/2009
	Olympus Inverted Microscope	CKX41SF	1A41768	07/13/2011
6312	Thermo Revco Undercounter Refrigerator	REL404A	0117973801110510	10/14/2011
	Thermo Revco Undercounter -30 Freezer	ULT430A	0117973701110510	10/14/2011
	Thermo Forma Incubator	3140	357354-441	10/14/2011
	Thermo Forma Incubator	3140	357354-439	10/14/2011
	Thermo Sorvall Centrifuge	RC-4	41200171	10/14/2011
	Biosafe Cell Processing Unit	Sepax 2 RM	6019.0151	11/11/2011
	Biosafe Cell Processing Unit	Sepax 2 RM	6020.0151 (temporarily off-site)	11/11/2011
	NuAire Labgard Class II A2 BSC	425-400	134521121009	12/22/2009
	NuAire Labgard Class II A2 BSC	425-400	134530121109	12/22/2009
	Mettler Toledo Balance	XS2002S	B146463080	04/04/2012
	Olympus Inverted Microscope	CKX41SF	1A41778	07/13/2011
6314	Thermo Revco Undercounter Refrigerator	REL404A	0117982101110510	10/14/2011
	Thermo Revco Undercounter -30 Freezer	ULT430A	0117973601110509	10/14/2011
	Thermo Forma Incubator	3140	357354-443	10/14/2011
	Thermo Forma Incubator	3140	357354-442	10/14/2011
	Thermo Sorvall Centrifuge	RC-4	41203492	10/14/2011
	NuAire Labgard Class II A2 BSC	425-400	134567121109	12/22/2009
	NuAire Labgard Class II A2 BSC	425-400	134567121109	12/22/2009
	Biosafe Cell Processing Unit	SEPAX 2 RM	6273.0133	07/10/2013
	Biosafe Cell Processing Unit	SEPAX 2 RM	6274.0133	07/10/2013
	Olympus Inverted Microscope	CKX41SF	1A41931	07/13/2011
	Mettler Toledo Balance	XS2002S	B350095388	04/30/2014
	Genesis TCD	B40	G100104	01/05/2012

	Genesis TCD	B40	G100576	01/03/2016
	Genesis TCD	B40	G100590	01/03/2016
	Endosafe PTS	PTS KINETIC READER	5197	03/14/2012
	Endosafe PTS	PTS KINETIC READER	8590	01/02/2016
	Genesis Rapid Seal II Tube Sealer	SE640	643236	01/05/2012
	Genesis Rapid Seal II Tube Sealer	SE640	643531	01/03/2014
	Genesis Rapid Seal II Tube Sealer	SE640	643943	01/03/2016
	Sysmex Hematology Analyzer	KX-21N	F3345	02/27/2013

All critical processing equipment underwent extensive validation (IQ, OQ and PQ). All validation protocols, execution documentation and raw data is on file with the QA Manager on site.

b. Validation, Calibration and Certification (SOP QA:4)

Equipment, reagents and supplies deemed critical to product manufacturing are validated prior to use in HCT/P manufacturing. New or significant alteration in a procedure vital to product safety, purity and potency is also validated so expected consistency is maintained. A validation plan tests an item/process under normal operational parameters and worst case conditions as applicable. The extent or detail of a validation plan is dependent on its potential to affect HCT/P safety, purity and potency. Validation is mandatory for any reagent that is not FDA-approved for human use or is not accompanied by a COA. The QA Manager or FD determines when to validate other reagents, supplies, equipment and procedures.

Validation plans are drafted, reviewed, revised and approved prior to execution. Plan execution entails data collection and analysis, followed by conclusions and recommendations based on the data. Plan approval by the QA Manager and FD is required for all validation plans. All Plan/Execution approval signatures are required before putting the item into active use.

Validation of equipment, reagents, supplies or procedures/processes is done according to established protocols. All validation activities and results, including the approver signature and date are documented and kept on file according to regulations. When a validated process changes or equipment needs repair, the QA Manager and FD review the details to determine if revalidation is required.

All personnel performing validation activities possess training appropriate to the validation tasks undertaken. The training must be complete, understood and documented as part of the critical skills necessary to conduct a validation activity. It provides the guidance for personnel involved in validation activities on cGMP awareness training, safety, and specific job skills training. Training records are maintained for each individual.

All instruments that are directly associated with a process or used to monitor a process are maintained in a calibrated state. Calibration activities are performed on a planned basis or when a new instrument is placed in use.

c. Quality Control and Preventive Maintenance (SOP EQ:1 and individual equipment SOPs)

The Griffin Facility frequently and consistently performs quality control (QC) monitoring and preventive maintenance (PM) of critical laboratory operations (facility mechanical systems and maintenance; equipment and instrument maintenance). Careful attention to regularly scheduled cleaning, maintenance, and calibration tasks is paramount. Performance and system problems are tracked and observed for trends. The goal is to ensure the highest level of equipment and vital system performance and minimize faulty equipment/systems downtime.

Procedures outline tasks to be completed when equipment is found not performing according to established parameters. A strict process is followed when any item critical to product safety, purity or potency is found out of specification. This includes a complete look back and if needed, additional product testing to ensure stored or released product still meets acceptance criteria.

Equipment and facility cleaning is thoroughly covered in applicable SOPs. Strict guidelines are followed for sanitization procedures, which are verified periodically by active air sampling and contact plating. The policy indicates how the facility should be cleaned room by room, and the process to clean from top to bottom, entry to exit, in the most aseptic manner possible. It also outlines the cleaning agents used at the Facility, which are rotated to prevent microbial resistance. Equipment specific SOPs indicate the cleaning procedure and frequency though this information is referenced in more general facility SOPs.

d. Instrument and Environmental Monitoring (SOP QA:5, QA:11)

The Griffin Facility has installed the REES Scientific E-Centron Environmental Monitoring System. REES continuously monitors all critical systems in real time and can provide daily reports that show periodic readings when conditions are normal. When in alarm, the system tracks the alarm time and severity, who was notified and who responded. Comments are input regarding alarm correction, corrective actions, deviations, etc. Alarm notification is by audible alarm and an auto-dialer telephone system with a voice synthesized response. The auto dialer progresses through a programmed list of telephone numbers until a response is received. Alarms may be temporarily inhibited until the problem can be evaluated and resolved. Personnel can reactivate an inhibited alarm when the problem is resolved or probe will automatically reset when the inhibit time period expires. This robust system monitors all critical equipment and facility functions to include:

- Cold Storage
- Liquid Nitrogen Storage (including supply manifold)
- Incubators
- Room temperature and humidity for critical areas
- Differential pressure
- Particle counts

This system was installed, calibrated and validated by trained REES technicians. Validation demonstrates the computer system, probes, transmitters and data retention all perform according to manufacturer specifications and 21 CFR Part 11. Validation and calibration is performed on all systems annually.

Routine viable environmental monitoring program is in place to ensure manufacturing environment is within defined limits for viable airborne and surface microbial contamination.

5. **Production Controls**

a. Facility and Environmental

This facility is utilized exclusively for human cell and tissue processing and its mission is to provide innovative clinical-grade cell- and tissue-based products and advanced therapies with the adequate quality for the intended use in humans, hence the facility is a barrier, and not a containment, facility. From a barrier perspective, spaces leading from the primary entry point toward production suites are progressively more positive with 3 pressure differentials above the neutral zone at the entry.

In addition to positive pressurization to maintain a clean environment, several pairs of doors are interlocked to prevent airborne contaminants from passing between spaces. The Griffin Facility maintains documentation showing compliance with sanitation protocols via testing for viable and non-viable particle counts inside the cleanroom areas.
To eliminate potential product contamination, access to Production Suites No.1, 2, and 3, and associated support facilities provide segregation and logical personnel, equipment and material flow through the use of interlocked doors and gowning protocols.

The facility and HVAC systems underwent extensive validation (IQ, OQ and PQ). All validation protocols, execution documentation and raw data is on file with the QA Manager on site.

The Facility was designed with an integrated non-viable particle counting system with return air duct probes in the most critical spaces (all ISO 7 certified rooms). These are monitored constantly by the REES system. Facility staff perform periodic environmental monitoring using volumetric air sampling techniques to measure viable airborne particles. These results are compared against facility validation data in addition to tracking and trending over time. Facility surfaces are also tested in like fashion to qualify and certify cleaning agents used in addition to staff cleaning tasks. All excursions outside set facility parameters require investigation and appropriate CA/PA.

1) Facility Design
   i. HVAC Design
   - Environments or areas are defined as classified and non-classified. Classified areas are required for the production of human derived cellular based products. Classified areas design guidelines are established by the International Standards, ISO 14644.
   - Design of HVAC systems servicing clinical operations is intended as a barrier and not a containment facility, designed to protect the product. Applicable procedures are in place to minimize risk of contamination and cross-contamination.
   - Design temperature ranges provide the basis for appropriate temperature control performance. The design specification is 20°C (68°F) year round. cGMP Facility operational temperature range, including ISO7, ISO8, ISO9, and non-classified areas are 20°C +/- 3°C (63°F to 73°F).

- Relative humidity (RH) ranges are established to prevent corrosion, condensation, and product contamination, to limit microbial impact on the area, to protect hygroscopic materials or products, and to provide comfort for personnel. The design specification for all areas in the facility is 25% to 60% RH.
- Directional air flow is designed to provide airflow from clean to less clean areas and to prevent flow reversal. It is used to establish a critical barrier in cross-contamination prevention.
- Door interlocks are used to transition from one classified area to an area of lower classification. They are designed to meet the requirements of the area of higher classification when tested under at-rest conditions. Directional airflow between areas of different classification are designed to maintain ≥ 0.02” wc across the door. All interlocks are designed to prevent both doors from being opened simultaneously.
- There is no direct access from the space of highest classification without a transitional classified area in between.
- Directional air flow is monitored from an automated monitoring system, with local alarming to support the operation. Differential pressure is the first barrier in preventing contamination of the production areas and product.
- Single-pass air is supplied through terminal HEPA filtration of at least 99.97% in support of the designated area classifications.
- Air changes per hour (ACH) are defined based on the size of the room, operations to be carried out, heat load, calculated moisture load, and the number of people to be present in the room. Design ACH for ISO 7 areas is 60 ACH. The design criteria were selected to guarantee that area requirements for control of cross contamination and cleanliness are met.

ii. Air Handling Unit (AHU) Design

- Two dedicated air handlers are installed to support the cGMP space.
- AHUs have 30% carbon, 30% and 85% pre-filters, hydronic heating coil, clean steam humidifier, two cooling coils and HEPA filters.
- The units are 100% outside air and the temperature is maintained through Phoenix control valves with hydronic reheat coils.
- Production suites are served with N+1 HEPA fan/filter units which bring in outside air and return air to maintain no less than 60 air changes to the specific spaces.
- The supply is high with hemispherical diffusers to maintain low velocities to limit turbulence. Air is exhausted and returned in low return grilles to maintain constant piston of clean air through the space per ISO and ASHRAE recommendations.
- The AHU is located in an enclosed structure with consideration given to maintainability.
- Access panels are provided to for each component section as required to facilitate preventive maintenance activities.

iii. Chilled Water/Heating Water System

- The chilled and heating water is supplied to the cGMP area from the campus central plant.
- The sole purpose of the chiller and boiler system is to provide water to the HVAC system, hence it is considered a secondary utility.

iv. Vacuum

- Vacuum for the cGMP facility is supplied from the campus central plant.

v. Compressed Gases
- Compressed gas cylinders and piping to the point of use are installed in the cGMP area to provide CO_2 and N_2. The cylinders contain medical grade gas. These are all outside the classified processing areas.

vi. Steam Systems
- There is no steam required in the cGMP area. The clean steam that serves the humidifiers in the cGMP AHUs is made by RO water through steam to steam generators in the building penthouse delivered to the AHUs through stainless steel welded piping.

b) Product

The Griffin Facility employs several product processing controls. This begins with thorough inspection and document review at product receipt, which is then documented in the batch record. Additionally, harvest sterility and viability provide information on the condition the material was received in. Depending on the protocol, in process testing will take place at critical steps to ensure accurate production. Additionally, all final products are tested for safety, quality, purity, potency and identity to ensure the product meets release and final acceptance criteria.

c) Personnel (SOP GP:1)

The Griffin Facility has several SOPs governing staff activity and behavior. The training noted above is required for all staff entering the facility to ensure they have the skills to operate in controlled environments. Staff are observed performing activities using sterile technique to prevent contamination and cross contamination.

Periodic microbial plating is performed on staff as well, to ensure they are operating properly. Significant growth results will prompt investigation and retraining as needed.

6. **Product Quality Control**

a. Final Product Testing – Analysis and Methods

All products manufactured at the Griffin Facility are subject to substantial testing prior to release. As final product requires immediate implantation due to protocol and patient need, long term sterility results on final product will be received after implantation. All results are documented in batch records specific to each clinical trial and patient.

The following are performed by the Griffin Facility or by qualified testing vendors:

Sterility

Sterility testing for product release is performed by STAT Gram stain at the Memorial Hermann Hospital, Laboratory Services, a CAP accredited and CLIA certified lab (see attached certificates). This provides results within ~1 hour and is a required release criteria.

Long term sterility testing is also performed in the same laboratory on all manufactured products. Samples for aerobic, anaerobic and fungal growth are taken from the starting material and on the final product, inoculated into the BacT/ALERT 3D system by

bioMerieux (see bioMerieux Master File BB-MF #13225, Sections III, IV and Appendices A-C). The samples are incubated for 14 (bacterial) or 28 (fungal) days after which final reports are issued. If contamination is detected before that (usually within 3-7 days), the bottle is removed, microbial organisms are plated and identified at that time, and results are sent to the facility.

Mycoplasma
Mycoplasma testing is performed on the final product by Clongen Laboratories in compliance with the "Points To Consider" document published by the FDA in 1993 (http://www.clongen.com) or using a PCR-based rapid detection method with equivalent sensitivity and specificity.

Endotoxin
Endotoxin levels in the final product for release testing is measured using the Endosafe PTS system by Charles River Laboratories. This test is a miniaturized version of the standard LAL assay and the cartridges employed have received FDA approval. As with all other equipment and critical processes, the Endosafe and the procedure has been fully validated.

Potency
Potency is assessed by placing Wharton's Jelly tissue in osteogenic differentiation and control media for 14 days. At the 14th day the samples are stained with Alizarin Red stain. Bright red or orange staining determines mineral deposition which indicates a positive result.

- **IND Specific Product Manufacturing at The Griffin Facility** (SOP GP:22)
  When collected at the hospital facility, the Umbilical Cord (UC) is placed in sterile container containing transport medium and stored at 2-8°C until processing. Upon arrival at the cell processing facility, the UC is carefully rinsed in wash buffer until complete blood and blood clots are removed. UC is then dipped in 10% providone-iodine bath and dubbed dry with sterile gauze.

  The UC is then transferred to the dissecting stage with clamps attached at both ends. The cord is measured and length/weight parameters are documented.

  The UC is cut into small segments and rinsed in wash buffer to remove any remaining blood clots. On each segment a small incision is made parallel along the axis of the UC which is deep enough to cut through the epithelium using a scalpel with blade nr. 22. The UC is unraveled by pulling epithelium on each side of the incision until the end of the segment is reached. The cord segment is laid flat, pinned on the dissecting stage with native Wharton's Jelly (nWJ) side up. All three blood vessels are removed and only translucent yellow connective tissue which consists of nWJ is left. The nWJ is scrapped off by using a scalpel with blade nr. 18 and is collected into a 50 mL conical tube containing 25-30 mL of wash buffer. The nWJ is centrifuged for 5 minutes at 400g at RT and the supernatant is aspirated carefully. The nWJ at the bottom of the conical tube is collected and added into another 50 mL conical tube containing 2 mL of Equilibrium solution (Irvine Scentific) and left for 3 minutes at room temperature. Centrifugation for 1 minute follows, after which the supernatant is aspirated and 2 mL of Vitrification Solution (Irvine Scientific) is added and sample is left for 3 minutes at room temperature. Then the nWJ is carefully collected, measured and transferred into three 5x9 cm O-wrap bags:

- Bag 1: Implantation product (2-3 mL)
- Bag 2: QC samples (0.3 mL)
- Bag 3: Reference Sample (0.1 mL)

All bags are heat sealed to 5x5 cm size ensuring air and bubbles are removed. All three bags are snap frozen (vitrified) by completely dipping the bags into liquid nitrogen. The 3 vitrified samples are transferred into the liquid nitrogen vapor freezer.

The QC sample will be thawed two months prior to the implantation date. The sample is thawed at 38-39°C for 30 seconds. The bag is opened and the nWJ is transferred to a 50 mL conical tube. The nWJ is transferred sequentially for 5 minutes at RT in 1M, 0.75M, 0.5M, and 0.25M sucrose solutions. Then the nWJ is centrifuged for 5 minutes at 400g at RT and the supernatant and/or tissue is utilized for quality control testing:

- Mycoplasma
- Long term sterility (14 days aerobic and anaerobic bacterial culture and 28 day fungal culture)
- Potency osteogenic differentiation assay

On the day of implantation, the following additional quality control assays will be performed after thawing of the final product:

- Gram stain
- Endotoxin
- Long term sterility (14 days aerobic and anaerobic bacterial culture and 28 day fungal culture)
- Target Dose: $\geq$ 2 mL of nWJ

Release Criteria:

- QC testing on QC sample aliquots of nWJ (2 months prior to transplantation)
  - Long Term Sterility (bacterial and fungal) (R): Negative
  - Potency (R): Positive to Alizarin Red Staining
  - Mycoplasma (R): Negative
- QC testing on Implantation product (On the day of transplantation)
  - Gram stain (R): Negative
  - Endotoxin (R): 0.5 EU/mL
- Target dose
  - $\geq$2 mL of nWJ

Final Implantation Product (FP) preparation: Sterile luer-lock cap is attached to a 3 mL syringe. The plunger of the syringe is removed and stored aseptically. 2-3 mL of nWJ is poured into the syringe and plunger is reinserted. Syringe is inverted and the nWJ is collected at the base of the plunger. The leur-cap is loosened and plunger is depressed to remove any air from the syringe. Syringe is re-capped and final volume is documented.

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Product Testing

a. ***Testing and Criteria***

Test	Method	Criteria	Sensitivity or Tolerance	Specificity	Results Available Prior to Release
Sterility (R)	Gram Stain	Negative	N/A	Gram-positive microbes	Yes
Purity (R) Endotoxin	Endosafe PTS (LAL)	<0.5 EU/mL	≤0.1 EU/mL	N/A	Yes
Dose (R)	Volume	≥2 mL	N/A	N/A	Yes
Long Term Sterility (bacterial and fungal)- Thawed nWJ tissue (R)	BacT/ALERT Culture	Negative	N/A	N/A	Yes
Long Term Sterility (bacterial and fungal) – Post-thaw sucrose solution	BacT/ALERT Culture	Negative	N/A	N/A	No
Mycoplasma (R)	Points to Consider or equivalent	Negative	<100 CFU	Most common species	Yes
Potency (R)	Alizarin Red Staining	Positive	N/A	N/A	Yes

- **Description of Test Methods**
  a. Products specific to this IND are tested using the methods described in section 6 above.
- **Product Tracking**
  a. Each incoming product is identified using a unique component/Donor Information Number (DIN) combination. This can be linked directly to the patient's medical record number and to any other donor identifiers. The product retains the DIN throughout manufacturing and release. This system provides complete product, donor and recipient traceability from donation through administration and follow-up.

  b. If a positive sterility test result is received following product administration, the recipient's physician is contacted immediately. The organism is identified and submitted for sensitivity testing, the results of which are also communicated to the physician immediately upon receipt.

- **Product Labeling**
  a. The Griffin Facility uses following local code:
  A0001, Umbilical Cord/For Further Processing (Local Code)
  A0002, Wharton Jelly Tissue/ Cryopreserved/For Further Processing (Local Code)
  A0003, Wharton Jelly Tissue/ For Therapeutic Use (Local Code)

Biohazard and Warning Labels for Autologous Donor

		Status			Product Label				
		All Donor Screening and Testing Completed	Abnormal Results of Donor Screening	Abnormal Results of Donor Testing	Biohazard Legend	For Autologous Use Only	Not Evaluated for Infectious Substances	WARNING: Advise patient of communicable disease risks	WARNING: Reactive test results for (name of disease agent or disease)
Donor Eligibility Determined Not Required [21 CFR 1271.90(a)]									
Autologous Donors	1271.90 (a)(b)								
Autologous Donors	1271.90 (a)(1)(2)	**No**	**No**	**No**		**X**	**X**		
Autologous Donors	1271.90(b)(1)(3)	**Yes**	**No/Yes**	**Yes**	**X**	**X**			**X**
Autologous Donors	1271.90(b)(1)(3)	**Yes**	**Yes**	**No**	**X**	**X**			

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- **APPENDIX (Griffin Facility)**

1) Process Validation Summary:

		Acceptance Criteria Met?	Comments
Run 1	Unit Identifier: **WJ-VAL-1**		
Quality Control Assays on thawed sections vitrified for quality testing			
Long Term Sterility (Bacterial and fungal): Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 11/25/2015	N/A
Potency: 14 days Osteogenic Differentiation Medium Culture: Positive to Alizarin Red Staining Actual Result: Positive		☒ Y ☐ N Initials/Date: SK 11/04/2015	N/A
Mycoplasma Testing: Negative Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 01/20/2016	N/A
Quality Control Assays on Thawed Infusion Product			
Dose: 2-3 mL Actual Dose: 2.1 mL		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Sterility-Gram Stain: Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Endotoxin: < 0.5EU/mL Actual Result: < 0.1EU/mL		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Long Term Sterility (Bacterial and fungal): Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 11/25/2015	N/A

		Acceptance Criteria Met?	Comments
Run 2	Unit Identifier: **WJ-VAL-2**		
Quality Control Assays on thawed sections vitrified for quality testing			
Long Term Sterility (Bacterial and fungal): Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 11/25/2015	N/A
Potency: 14 days Osteogenic Differentiation Medium Culture: Positive to Alizarin Red Staining Actual Result: Positive		☒ Y ☐ N Initials/Date: SK 11/04/2015	N/A
Mycoplasma Testing: Negative Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 01/20/2016	N/A
Quality Control Assays on Thawed Infusion Product			
Dose: 2-3 mL Actual Dose: 3.1 mL		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Sterility-Gram Stain: Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Endotoxin: < 0.5EU/mL Actual Result: < 0.1EU/mL		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Long Term Sterility (Bacterial and fungal): Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 11/25/2015	N/A

		Acceptance Criteria Met?	Comments
Run 3	Unit Identifier: **WJ-VAL-3**		
Quality Control Assays on thawed sections vitrified for quality testing			
Long Term Sterility (Bacterial and fungal): Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 11/25/2015	N/A
Potency: 14 days Osteogenic Differentiation Medium Culture: Positive to Alizarin Red Staining Actual Result: Positive		☒ Y ☐ N Initials/Date: SK 11/04/2015	N/A
Mycoplasma Testing: Negative Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 01/20/2016	N/A
Quality Control Assays on Thawed Infusion Product			
Dose: 2-3 mL Actual Dose: 2.7 mL		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Sterility-Gram Stain: Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Endotoxin: < 0.5EU/mL Actual Result: < 0.1EU/mL		☒ Y ☐ N Initials/Date: SK 10/21/2015	N/A
Long Term Sterility (Bacterial and fungal): Negative (No organisms found) Actual Result: Negative		☒ Y ☐ N Initials/Date: SK 11/25/2015	N/A

1) Griffin cGMP Facility – FACT Accreditation Certificate

Accredited

Foundation for the Accreditation of Cellular Therapy

certifies that

UTHealth- Medical School

The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory

Houston, Texas

Having Met the Requirements of FACT
is Granted Accreditation for

Cellular Therapy Product Processing with More than Minimal Manipulation

From: January 29, 2016 To: January 29, 2019

UTHealth- Medical School, The Evelyn H. Griffin Stem Cell Therapeutics Research Laboratory
Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Dennis Gastineau, MD
FACT President

Phyllis I. Warkentin, MD
Chair, Accreditation Committee

FACT FOUNDATION FOR THE ACCREDITATION OF CELLULAR THERAPY
AT THE UNIVERSITY OF NEBRASKA MEDICAL CENTER

2) Memorial Hermann Hospital Laboratory Services – Accreditation Certificates

Feb. 5. 2015 1:23PM

CENTERS FOR MEDICARE & MEDICAID SERVICES

CLINICAL LABORATORY IMPROVEMENT AMENDMENTS

CERTIFICATE OF ACCREDITATION

LABORATORY NAME AND ADDRESS	CLIA ID NUMBER
MEMORIAL HERMANN LABORATORY SERVICES 6411 FANNIN SUITE JG405 HOUSTON, TX 77030-1501	45D0053104
	EFFECTIVE DATE
	02/09/2015
LABORATORY DIRECTOR	EXPIRATION DATE
ROBERT HUNTER MD	02/08/2017

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C. 263a) as revised by the Clinical Laboratory Improvement Amendments (CLIA), the above named laboratory located at the address shown hereon (and other approved locations) may accept human specimens for the purposes of performing laboratory examinations or procedures.

This certificate shall be valid until the expiration date above, but is subject to revocation, suspension, limitation, or other sanctions for violation of the Act or the regulations promulgated thereunder.

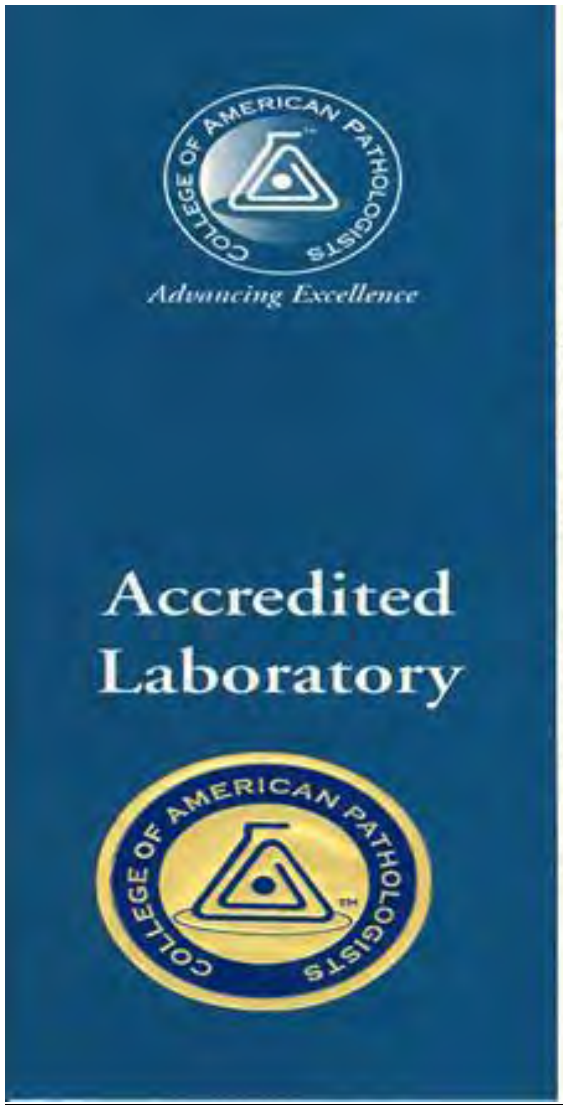
CMS

Karen W. Dyer, Acting Director
Division of Laboratory Services
Survey and Certification Group
Center for Clinical Standards and Quality

If you currently hold a Certificate of Compliance or Certificate of Accreditation, below is a list of the laboratory specialties/subspecialties you are certified to perform and their effective date:

LAB CERTIFICATION (CODE)	EFFECTIVE DATE	LAB CERTIFICATION (CODE)	EFFECTIVE DATE
BACTERIOLOGY (110)	07/28/1995	ANTIBODY IDENTIFICATION (540)	07/28/1995
MYCOLOGY (120)	07/28/1995	COMPATIBILITY TESTING (550)	07/28/1995
PARASITOLOGY (130)	07/28/1995	HISTOPATHOLOGY (610)	07/28/1995
VIROLOGY (140)	07/28/1995	CYTOLOGY (630)	07/01/2003
GENERAL IMMUNOLOGY (220)	06/30/2003		
ROUTINE CHEMISTRY (310)	07/28/1995		
URINALYSIS (320)	07/28/1995		
ENDOCRINOLOGY (330)	07/28/1995		
TOXICOLOGY (340)	06/30/2003		
HEMATOLOGY (400)	07/28/1995		
ABO & RH GROUP (510)	07/28/1995		
ANTIBODY TRANSFUSION (520)	07/28/1995		
ANTIBODY NON-TRANSFUSION (530)	07/28/1995		

FOR MORE INFORMATION ABOUT CLIA, VISIT OUR WEBSITE AT WWW.CMS.GOV/CLIA
OR CONTACT YOUR LOCAL STATE AGENCY. PLEASE SEE THE REVERSE FOR
YOUR STATE AGENCY'S ADDRESS AND PHONE NUMBER.



The College of American Pathologists

certifies that the laboratory named below

Memorial Hermann Texas Medical Center
Laboratory Services
Houston, Texas
Robert L. Hunter, MD,PhD

CAP Number: 2108501
AU-ID: 1186020
CLIA Number: 45D0053104

has met all applicable standards for accreditation and is hereby accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur prior to March 18, 2017 to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Chair, Commission on Laboratory Accreditation

President, College of American Pathologists

AABB Accreditation

Memorial Hermann The Medical Center Hospital

having been assessed by AABB, has been found to meet the requirements of applicable Standards of this organization and therefore is granted this

CERTIFICATE OF ACCREDITATION

for the following activities:

Transfusion Activities

In Witness whereof the undersigned, being duly authorized, have caused this Certificate to be issued and the AABB Corporate Seal to be affixed.

Effective Dates

April 01, 2015 - March 31, 2017

President, AABB

Chair, Accreditation Program Committee

ineScientific

PRODUCT: Vitrification Freeze Kit (Vit Kit-Freeze)
Equilibration Solution-ES (90131)
Vitrification Solution-VS (90132)

LOT #90133-DSOC150702

CATALOG #90133-DSOC

MFR DATE: 07/27/15

EXPIRES: 05/31/16

STORAGE: 2°- 8°C

PREPARED BY: JN 07/30/15

CERTIFICATE OF ANALYSIS

Products manufactured by Irvine Scientific are produced in accordance with the Guideline for Manufacture of In Vitro Diagnostic Products and the Good Manufacturing Practices (GMP's) for Medical Devices. Irvine Scientific is licensed by both Federal and State agencies and is inspected regularly for compliance.

Chemicals used in Irvine Scientific products meet ACS, USP or NF standards. Incoming chemicals are released in accordance with approved specifications.

All donors used to obtain the human albumin were tested and found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), antibodies to Human Immunodeficiency Virus (HIV), and Hepatitis C virus (HCV) by approved testing methods.

		Vitrification Freeze Media	
Assay	**Specification**	**Lot #90131150502: Result**	**Lot #90132150502: Result**
Sterility[1]	Pass	Pass	Pass
pH	7.1 - 7.5	7.3	7.4
Osmolality:			
of 90131 1:1 dilution	1055 – 1445	1294 mOsm/Kg H_2O	
of 90132 1:3 dilution	1100 – 1588		1375 mOsm/Kg H_2O
Endotoxin[2]	≤0.6 EU/mL	<0.06 EU/mL	<0.12 EU/mL
Albumin Recovery[3]	≥85%	141%	144%

	Kit:
Mouse Embryo Test (One-Cell)[4]	
-Control[5]:	
% of embryos developing ≥ 80%	90% (28/31)
-This lot:	
% of embryos developing≥ 80%	90% (28/31)

[1]In accordance with the Current USP <71>; 21 CFR, Part 610.12.
[2]Utilizes a gel clot assay with a sensitivity of 0.03 EU/mL.
[3]Albumin concentration is determined by the BCG method.
[4]Fresh one-cell mouse embryos (n=31) were exposed to each medium for a limited time then washed and cultured in growth medium (HTF+0.4%BSA). Test results indicate the percentage of mouse embryos developing to fully expanded blastocysts after 96 hours in culture.
[5]Control embryos were cultured in growth medium only.

Rev 2

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3) Applicable Griffin Facility SOPs and Documents

QA: 1 – Quality Management System

QA: 2 – SOP Preparation, Approval and Review

QA: 3 – Managing Deviations and Complaints

QA: 4 – Validation Procedures

QA: 5 -- Environmental Monitoring with REES Scientific E-Centron

QA: 6 – Audit

QA: 7 – Reagent and Supply Receipt & inspection

QA: 8 – Vendor Qualification

QA: 10 – Change Control

QA: 11 – Routine Environmental Monitoring

EQ: 1 – Equipment Installation, Calibration and Preventive Maintenance

EQ: 4 – Biological Safety Cabinet Operation

EQ: 5 -- Endotoxin Testing Using the Endosafe PTS

EQ: 6 – Mettler Toledo Precision Electronic Balance Operation and Maintenance

EQ: 9 – Genesis RAPID Seal II Tube Sealer Operation and Maintenance

EQ: 10 – Polyscience Digital water bath Operation and Maintenance

EQ: 11 – Operation and Maintenance of Thermo Scientific Incubator

F: 48 – Human Native Wharton’s Jelly Harvest, Vitrification and Thaw Batch Record

GP: 1 – cGMP Facility – Lab Flow and Personnel Conduct

GP: 2 – cGMP Facility – Gowning

GP: 3 – cGMP Facility – Cleaning

GP: 4 – cGMP Facility – Personnel Competency and Training

GP: 8 – Product Release, Transport and Recall

GP: 10 – Labeling

GP: 17 – Cell Product Storage and Disposal

GP: 18 – Cell Product Quarantine

GP: 19 – Record Retention and Storage

GP: 22 – Human Native Wharton's Jelly Harvest, Vitrification and Thaw

P: 1 – Emergency and Disaster Plan

P: 2 – Safety Program

VAL: 4.1 – Human Native Wharton's Jelly Harvest, Vitrification and Thaw Validation Protocol

SECTION 6: PHARMACOLOGY AND TOXICOLOGY INFORMATION

6.1 Pharmacology and Drug Distribution:

Not Applicable.

SECTION 7: PREVIOUS HUMAN EXPERIENCE

7.1 Clinical Research Experience with WJ:

As mentioned in Section 1 of the application, numerous studies are investigating the therapeutic potential of WJ for a wide variety of illnesses and conditions. In preparation for submitting this IND application, the clinical trails registration site, www.clinicaltrials.gov was queried using the search terms “Wharton’s jelly” or “Wharton’s jelly mesenchymal stem cells” or” umbilical cord mesenchymal stem cells”. Studies with a status of “unknown” were excluded. The search yielded 84 interventional research studies, with 13 indicating a status of “completed”. To date, no study results have been reported. Most of the research protocols listed an allogenic source for the WJ product reflecting the immune-privileged status of WJ MSC’s. We are unaware of any clinical research protocols or marketing applications that have been withdrawn due to safety concerns with WJ.

The following table provides summary information of the registered studies at www.clinicaltrials.gov

SECTION 7.1 TABLE A

Study 1:	
NCT Number:	NCT02834858
Title:	Umbilical Cord Mesenchymal Stem Cells Infusion for Diabetes Related Vascular Complications
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Peripheral Vascular Disease|Ischemia|Diabetic Foot
Interventions:	Biological: Umbilical Cord Mesenchymal Stem Cells
Sponsor/Collaborators:	Jie Shen|Nanfang Hospital of Southern Medical University|Academy Military Medical Science, China |The Fifth Affiliated Hospital of Southern Medical University|Southern Medical University, China|The Third Affiliated Hospital of Southern Medical University
Gender:	Both
Age:	18 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	240
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study|Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
First Received:	9-Jul-16
Start Date:	Jan-16
Completion Date:	Dec-19
Last Updated:	14-Jul-16
Last Verified:	Jul-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-18
Outcome Measures:	Area of diabetic foot ulcers|Improvement of transcutaneous oxygen partial pressure (TcPO2)|Improvement of microvascular cutaneous reactivity by laser Doppler perfusion monitoring (LDPM)|Pain (Visual-Analog Scale)|Walking distance (treadmill) if possible
URL:	https://ClinicalTrials.gov/show/NCT02834858
Study 2:	
NCT Number:	NCT02763423
Title:	Allogeneic Umbilical Cord Mesenchymal Stem Cell Transplantation for Type 1 Diabetes With Diabetic Ketoacidosis
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Ketoacidosis, Diabetic
Interventions:	Procedure: umbilical cord mesenchymal stem cell
Sponsor/Collaborators:	The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Both
Age:	12 Years to 35 Years Â (Child, Adult)
Phases:	Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Endpoint Classification: Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	NJGYNFM-SC-02
First Received:	19-Apr-16
Start Date:	Jan-09
Completion Date:	Dec-19
Last Updated:	4-May-16
Last Verified:	May-16
Acronym:	UCMSCDKA
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-19

Outcome Measures:	Changes from baseline exogenous insulin dose at different time points post treatment|C-peptide level|HbA1c level|titres of islet antigen antibodies
URL:	https://ClinicalTrials.gov/show/NCT02763423
Study 3:	
NCT Number:	NCT02790762
Title:	Human Umbilical Cord-Mesenchymal Stem Cells for Pneumoconiosis
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Pneumoconiosis
Interventions:	Biological: Human umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Shenzhen Hornetcorn Bio-technology Company, Second Affiliated Hospital of University of South China
Gender:	Both
Age:	18 Years to 75 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	10
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	HYK-pneumoconiosis
First Received:	20-May-16
Start Date:	Jun-16
Completion Date:	Oct-17
Last Updated:	31-May-16
Last Verified:	May-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-17
Outcome Measures:	Severity of adverse events|Immunological Indicator in serum|The level of ceruloplasmin in serum|The level of procollagen peptide in serum|Lung function as assessed by spirometry|Chest high kilovolt X-ray examination
URL:	https://ClinicalTrials.gov/show/NCT02790762
Study 4:	
NCT Number:	NCT02285673
Title:	Efficacy of Umbilical Cord Mesenchymal Stem Cells in Duchenne Muscular Dystrophy
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Duchenne Muscular Dystrophy
Interventions:	Biological: Umbilical Cord Mesenchymal Stem Cell
Sponsor/Collaborators:	Acibadem University
Gender:	Male
Age:	7 Years to 20 Years Â (Child, Adult)
Phases:	Phase 1|Phase 2
Enrollment:	10
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	DMD-UC-MSC-1
First Received:	5-Nov-14
Start Date:	Nov-13
Completion Date:	Nov-15
Last Updated:	6-Nov-14
Last Verified:	Nov-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-15
Outcome Measures:	Duchenne muscular dystrophy gene expression
URL:	https://ClinicalTrials.gov/show/NCT02285673

Study 5:	
NCT Number:	NCT02283879
Title:	Human Umbilical Cord Mesenchymal Stem Cell in Cerebral Hemorrhage Sequela
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Cerebral Hemorrhage
Interventions:	Biological: Human umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Shenzhen Hornetcorn Bio-technology Company, Fifth Affiliated Hospital of Guangzhou Medical University
Gender:	Both
Age:	40 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	HYK-Cerebral hemorrhage
First Received:	3-Nov-14
Start Date:	Mar-15
Completion Date:	Apr-17
Last Updated:	23-May-16
Last Verified:	Jul-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Safety evaluation through vital signs, the results of clinical lab tests and adverse events (AEs) Improvement of infarct size measured by brain MRI|Modified Barthel index, National Institutes of Health stroke scale(NIHSS) score
URL:	https://ClinicalTrials.gov/show/NCT02283879
Study 6:	
NCT Number:	NCT02652351
Title:	Human Umbilical Cord-Mesenchymal Stem Cells for Hepatic Cirrhosis
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Hepatic Cirrhosis
Interventions:	Biological: Human umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Shenzhen Hornetcorn Bio-technology Company,Second Affiliated Hospital of University of South China
Gender:	Both
Age:	18 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	HYK-hepatic cirrhosis
First Received:	7-Jan-16
Start Date:	Mar-16
Completion Date:	Oct-16
Last Updated:	20-May-16
Last Verified:	Jan-16
Results First Received:	No Study Results Posted
Primary Completion Date:	May-16
Outcome Measures:	Severity of adverse events|Hepatic function Liver fibrosis index
URL:	https://ClinicalTrials.gov/show/NCT02652351
Study 7:	

NCT Number:	NCT02291926
Title:	Human Umbilical Cord Mesenchymal Stem Cell Transplantation in Articular Cartilage Defect
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Cartilage Diseases|Osteoarthritis
Interventions:	Biological: Human umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Shenzhen Hornetcorn Bio-technology Company, LTD The Fifth Affiliated Hospital of Guangzhou Medical University
Gender:	Both
Age:	18 Years to 75 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	HYK-Articular Cartilage Defect
First Received:	7-Nov-14
Start Date:	Dec-14
Completion Date:	Dec-16
Last Updated:	23-May-16
Last Verified:	Jul-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-16
Outcome Measures:	Severity of adverse events|Magnetic resonance imaging (MRI) of the knee|Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)
URL:	https://ClinicalTrials.gov/show/NCT02291926
Study 8:	
NCT Number:	NCT02481440
Title:	Umbilical Cord Mesenchymal Stem Cells Transplantation to Patients With Spinal Cord Injury
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Nerve and Spinal Cord Injuries
Interventions:	Biological: Umbilical Cord Mesenchymal Stem Cells
Sponsor/Collaborators:	Limin Rong|Third Affiliated Hospital, Sun Yat-Sen University
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 3
Enrollment:	44
Study Types:	Interventional
Study Designs:	Allocation: Non-Randomized|Endpoint Classification:Safety/Efficacy Study|Intervention Model: Factorial Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	UC-MSC-SCI
First Received:	15-Jun-15
Start Date:	Jan-14
Completion Date:	Dec-18
Last Updated:	22-Jun-15
Last Verified:	Jun-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	Changes in motor and sensory assessment by the ASIA score|Number of participants with adverse events|Changes in electromyogram and electroneurophysiologic test
URL:	https://ClinicalTrials.gov/show/NCT02481440
Study 9:	

NCT Number:	NCT02287831
Title:	Umbilical Cord Mesenchymal Stem Cells Injection for Diabetes Secondary Peripheral Arterial Disease
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Diabetes|Peripheral Arterial Disease
Interventions:	Biological: umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Institute of Hematology & Blood Diseases Hospital
Gender:	Both
Age:	18 Years to 75 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	13ZCZDSY02200
First Received:	2-Nov-14
Start Date:	Feb-14
Completion Date:	null
Last Updated:	24-Feb-16
Last Verified:	Feb-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-16
Outcome Measures:	Angiographic evaluation of angiogenesis at ischemic limb Ankle-Brachial pressure index|Walking distance|Pain|Laser Doppler evaluation of blood perfusion at ischemic limb
URL:	https://ClinicalTrials.gov/show/NCT02287831
Study 10:	
NCT Number:	NCT02235844
Title:	Allogeneic Human Umbilical Cord Mesenchymal Stem Cells for a Single Male Patient With Duchenne Muscular Dystrophy (DMD)
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Duchenne's Muscular Dystrophy
Interventions:	Biological: Umbilical Cord Mesenchymal Stem Cells
Sponsor/Collaborators:	Allergy and Asthma Consultants, Wichita, Kansas Aidan Foundation|Neil H. Riordan PhD
Gender:	Male
Age:	28 Years to 31 Years Â (Adult)
Phases:	Phase 1
Enrollment:	1
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	IND 16026 DMD Single Patient
First Received:	8-Sep-14
Start Date:	Sep-14
Completion Date:	Sep-18
Last Updated:	8-Sep-14
Last Verified:	Sep-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Sep-17

Outcome Measures:	Adverse Events|Change from baseline of weight Change of muscle diameter (circumferential measurements) from baseline|Change from baseline of Pulmonary Maximum Expiratory Pressure|Change from baseline of Pulmonary Forced Vital Capacity|Maximum Change from baseline of Predicted Inspiratory Pressure %|Change from baseline of Predicted Maximum Expiratory Pressure %|Change from baseline of Predicted Forced Vital Capacity %
URL:	https://ClinicalTrials.gov/show/NCT02235844
Study 11:	
NCT Number:	NCT02643823
Title:	Human Umbilical Cord-Mesenchymal Stem Cells for Rheumatoid Arthritis
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Rheumatoid Arthritis
Interventions:	Biological: hUC-MSC + DMARDs|Drug: DMARDs
Sponsor/Collaborators:	Shenzhen Hornetcorn Bio-technology Company, LTD Futian People's Hospital
Gender:	Both
Age:	18 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety Efficacy Study|Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	HYK-Rheumatoid Arthritis
First Received:	23-Dec-15
Start Date:	Jan-16
Completion Date:	Jun-17
Last Updated:	23-May-16
Last Verified:	Dec-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-17
Outcome Measures:	Severity of adverse events|RA Serology Disease Activity Score (DAS 28) Index
URL:	https://ClinicalTrials.gov/show/NCT02643823
Study 12:	
NCT Number:	NCT02277145
Title:	A Study on Radiation-induced Pulmonary Fibrosis Treated With Clinical Grade Umbilical Cord Mesenchymal Stem Cells
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Post-radiotherapy Pulmonary Fibrosis
Interventions:	Biological: clinical grade umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Jianwu Dai|Southwest Hospital, China, Chinese Academy of Sciences
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	14
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CAS-XDA001-SH/IGDB
First Received:	20-Oct-14
Start Date:	Oct-14
Completion Date:	Dec-16
Last Updated:	9-Oct-16
Last Verified:	Oct-16

Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Composite indicators, including quantitative analysis of CT density histograms, self-evaluation and changes of TGF-Î²1 content|Safety Evaluation|Clinical Indicator 1: change in blood gas analysis|Clinical Indicator 2: change in pulmonary function analysis|Clinical Indicator 3: 6-minute walk test distance|Clinical Indicator 4: change in MRC chronic dyspnea scale|Clinical Indicator 5: change in St. George's Respiratory Questionnaire (SGRQ) scale|Immunological Indicator in serum 1: T lymphocyte counts in peripheral blood|Immunological Indicator in serum 2: response level of CD4+ T lymphocyte subsets (such as Th1 / Th2)|Immunological Indicator in serum 3: concent of immunoglobulin|Immunological Indicator in serum 4: expression levels of various cytokines (IL-1, IL-3, IL-6, IL-8, TNF-Î±, GM-CSF, etc)|Immunological Indicator in serum 5: subtype analysis and phagocytic activity analysis of macrophage|Immunological Indicator in serum 6: expression levels of IL-12, IL-10|Immunological Indicator in lavage fluid 1: T lymphocyte counts in peripheral blood|Immunological Indicator in lavage fluid 2: response level of CD4+ T lymphocyte subsets (such as Th1 / Th2)|Immunological Indicator in lavage fluid 3: concent of immunoglobulin|Immunological Indicator in lavage fluid 4: expression levels of various cytokines (IL-1, IL-3, IL-6, IL-8, TNF-Î±, GM-CSF, etc)|Immunological Indicator in lavage fluid 5: subtype analysis and phagocytic activity analysis of macrophage|Immunological Indicator in lavage fluid 6: expression levels of IL-12, IL-10|Inflammatory Indicators: measured by routine blood test including C-reactive protein (CRP)|Fibrosis Indicators in serum 1: content of transforming growth factor -Î±/Î² (TGF-Î±/TGF-Î²)|Fibrosis Indicators in serum 2: content of hydroxyproline|Fibrosis Indicators in serum 3: content of matrix metalloproteinase 1/7(MMP1/MMP7)|Fibrosis Indicators in lavage fluid 1: content of transforming growth factor -Î±/Î² (TGF-Î±/TGF-Î²)|Fibrosis Indicators in lavage fluid 2: content of hydroxyproline|Fibrosis Indicators in lavage fluid 3: content of matrix metalloproteinase 1/7(MMP1/MMP7)
URL:	https://ClinicalTrials.gov/show/NCT02277145

Study 13:	
NCT Number:	NCT01739777
Title:	Randomized Clinical Trial of Intravenous Infusion Umbilical Cord Mesenchymal Stem Cells on Cardiopathy
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Dilated Cardiomyopathy
Interventions:	Biological: ucMSC|Other: Controls
Sponsor/Collaborators:	Universidad Los Andes, Chile
Gender:	Both
Age:	18 Years to 75 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety Efficacy Study|Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	UANDES-C4C|CORFO-11IEI-9766
First Received:	29-Nov-12
Start Date:	Dec-12
Completion Date:	Jun-15
Last Updated:	2-Jun-15
Last Verified:	Jun-15
Acronym:	RIMECARD
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-15

Outcome Measures:	Change in global left ventricular ejection fraction, Change in functional capacity measured in O2 consumption, Occurrence of major adverse cardiac event, Change in high sensitivity C-reactive protein (hs CRP), Reduction in level of B-type natriuretic peptide (BNP)
URL:	https://ClinicalTrials.gov/show/NCT01739777
Study 14:	
NCT Number:	NCT02192749
Title:	Allogeneic Umbilical Cord Mesenchymal Stem Cell Therapy for Autism
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Autism
Interventions:	Biological: Umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	6 Years to 16 Years Â (Child)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	TBS-UCMSC-ASD001
First Received:	12-Jul-14
Start Date:	Jul-14
Completion Date:	May-18
Last Updated:	17-Oct-16
Last Verified:	Oct-16
Acronym:	null
Results First Received:	No Study Results Posted
Primary Completion Date:	Sep-17
Outcome Measures:	Number of participants with adverse events|Number of participants with a change in disability as measured by the Autism Treatment Evaluation Checklist (ATEC)|Number of participants with a change in disability as measured by the Childhood Autism Rating Scale (CARS)|Change from baseline macrophage-derived chemokine (MDC)|Change from baseline thymus and activation-regulated chemokine (TARC)
URL:	https://ClinicalTrials.gov/show/NCT02192749
Study 15:	
NCT Number:	NCT02580019
Title:	Umbilical Cord Derived Mesenchymal Stem Cells Treatment in Ischemic Stroke
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Stroke
Interventions:	Biological: Human umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 2
Enrollment:	2
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	Hospital307
First Received:	2-Jul-15
Start Date:	Feb-16
Completion Date:	Dec-17

Last Updated:	30-Nov-15
Last Verified:	Jul-15
Acronym:	Recruiting
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Number of treatment related-adverse events during the study period. Comparison of National Institutes of Health stroke scale (NIHSS).|Comparison of modified Rankin scale (mRS).|Distinguish of EuroQol 5d (EQ-5D) between pre- and post-treatment 180 days.|Comparison of infarct size measured by brain MRI.
URL:	https://ClinicalTrials.gov/show/NCT02580019
Study 16:	
NCT Number:	NCT02776943
Title:	UCMSC Transplantation in the Treatment of Cartilage Damage
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Cartilage Damage|Degenerative Osteoarthritis
Interventions:	Biological: umbilical cord mesenchymal stem cells|Device: Hyaluronic acid
Sponsor/Collaborators:	South China Research Center for Stem Cell and Regenerative Medicine
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study|Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Investigator)|Primary Purpose: Treatment
Other IDs:	UCMSC-4
First Received:	14-Mar-16
Start Date:	Jun-16
Completion Date:	Jun-19
Last Updated:	16-May-16
Last Verified:	Jan-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-18
Outcome Measures:	Knee Function Change and Improvement|Severity of adverse events
URL:	https://ClinicalTrials.gov/show/NCT02776943
Study 17:	
NCT Number:	NCT01342250
Title:	Human Umbilical Cord Mesenchymal Stem Cells Transplantation for Patients With Decompensated Liver Cirrhosis
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Liver Cirrhosis
Interventions:	Biological: conventional therapy plus low dose hUC-MSCs treatment Biological: conventional therapy plus medium dose hUC-MSCs treatment|Biological: conventional therapy plus high dose hUC-MSCs treatment
Sponsor/Collaborators:	Shenzhen Beike Bio-Technology Co., Ltd. No.85 Hospital, Changning, Shanghai, China
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	BKCR-LD-1.0(2010)

First Received:	21-Apr-11
Start Date:	Oct-10
Completion Date:	Oct-11
Last Updated:	13-Oct-11
Last Verified:	Oct-11
Results First Received:	No Study Results Posted
Primary Completion Date:	Jul-11
Outcome Measures:	Overall Survival (OS)|Liver function improvement|The size of liver and the width of portal venous|Incidence of hepatocellular carcinoma within 1 year|Child-Pugh score, MELD score,SF36-quality of life (SF36-QOL)|The clinical symptom improvement(including appetite, debilitation, abdominal distension, edema of lower limbs, et al)
URL:	https://ClinicalTrials.gov/show/NCT01342250
Study 18:	
NCT Number:	NCT02698813
Title:	Safety Study of Filler Agent Composed of Umbilical Cord Mesenchymal Stem Cells and Hyaluronic Acid
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Senescence Wrinkles, Acne, Pitting Scar
Interventions:	Biological: umbilical cord mesenchymal stem cells and hyaluronic acid|Drug: hyaluronic acid
Sponsor/Collaborators:	South China Research Center for Stem Cell and Regenerative Medicine
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 1
Enrollment:	30
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver)|Primary Purpose: Treatment
Other IDs:	UCMSC-HA-1
First Received:	4-Feb-16
Start Date:	Dec-16
Completion Date:	null
Last Updated:	3-Mar-16
Last Verified:	Feb-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-18
Outcome Measures:	Proportion of patients with non-serious and serious adverse events Wrinkle Severity Rating Scale (WSRS) Evaluation|Global Aesthetic Improvement Scale (GAIS)
URL:	https://ClinicalTrials.gov/show/NCT02698813
Study 19:	
NCT Number:	NCT02302599
Title:	Mesenchymal Stem Cells to Treat Type II Diabetes
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Type II Diabetes
Interventions:	Biological: Umbilical cord mesenchymal stem cells|Biological: Controlled suspension liquid
Sponsor/Collaborators:	Chinese PLA General Hospital
Gender:	Both
Age:	20 Years to 60 Years Â (Adult)
Phases:	Phase 1
Enrollment:	200

Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study|Intervention Model: Parallel Assignment|Masking: Double Blind (Investigator, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	CHIN-PLAGH-ST-003
First Received:	12-Nov-14
Start Date:	Jan-13
Completion Date:	Dec-15
Last Updated:	26-Nov-14
Last Verified:	Nov-14
Acronym:	UC-MSCs
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-15
Outcome Measures:	Change in MSCs Level From Baseline to Week 48 From Baseline Change From Baseline in Fasting Glucose Over Time
URL:	https://ClinicalTrials.gov/show/NCT02302599
Study 20:	
NCT Number:	NCT01494480
Title:	The Clinical Trial on the Use of Umbilical Cord Mesenchymal Stem Cells in Amyotrophic Lateral Sclerosis
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Amyotrophic Lateral Sclerosis
Interventions:	Procedure: stem cell transplantation
Sponsor/Collaborators:	General Hospital of Chinese Armed Police Forces
Gender:	Both
Age:	20 Years to 65 Years Â (Adult)
Phases:	Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Allocation: Non-Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	20111207ALS
First Received:	7-Dec-11
Start Date:	Mar-12
Completion Date:	Apr-15
Last Updated:	18-Jun-12
Last Verified:	Jun-12
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-13
Outcome Measures:	Nerve functional evaluation|Forced vital capacity|Blood test|Urinal test Electrophysiology examination
URL:	https://ClinicalTrials.gov/show/NCT01494480
Study 21:	
NCT Number:	NCT01489267
Title:	A New Method to Treat Hereditary Cerebellar Ataxia -Umbilical Cord Mesenchymal Stem Cells Transplantation
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Hereditary Cerebellar Ataxia.
Interventions:	Other: stem cell transplantation
Sponsor/Collaborators:	General Hospital of Chinese Armed Police Forces
Gender:	Both
Age:	18 Years to 65 Years Â (Adult)

Phases:	Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Allocation: Non-Randomized|Endpoint Classification: Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	20111031SCA|20111031limin
First Received:	30-Oct-11
Start Date:	Dec-11
Completion Date:	Jul-14
Last Updated:	18-Jun-12
Last Verified:	Jun-12
Acronym:	SCA
Results First Received:	No Study Results Posted
Primary Completion Date:	Jul-13
Outcome Measures:	blood test|Nerve functional evaluation|Urinal test Electrophysiology examination
URL:	https://ClinicalTrials.gov/show/NCT01489267
Study 22:	
NCT Number:	NCT02668068
Title:	A Study on Pneumoconiosis Treated With Whole-lung Lavage Combined With Mesenchymal Stem Cells
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Pneumoconiosis
Interventions:	Procedure: large volume whole-lung lavage (WLL)|Biological: clinical grade umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Jianwu Dai|Southwest Hospital, China|Nanjing Chest Hospital Chinese Academy of Sciences
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	80
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Treatment
Other IDs:	CAS-XDA-SH/NCH/IGDB
First Received:	20-Jan-16
Start Date:	Jan-16
Completion Date:	Dec-17
Last Updated:	26-Jan-16
Last Verified:	Jan-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-17
Outcome Measures:	Incidence of Treatment-Emergent Adverse Events (Safety Evaluation) Imaging indicator: Quantitative analysis of CT density histograms|Clinical Indicator 1: change in blood gas analysis|Clinical Indicator 2: change in MRC chronic dyspnea scale|Clinical Indicator 3: change in St. George's Respiratory Questionnaire (SGRQ) scale|Immunological Indicator in serum : response level of CD4+ T lymphocyte subsets (Th1/Th2/Th17)|Immunological Indicator in lavage fluid : response level of CD4+ T lymphocyte subsetsTh1/Th2/Th17)|Immunological Indicator in serum : expression levels of various cytokines including TNF-Î±, IL1-Î², MIP-1Î±, TIMP1, PDGF|Immunological Indicator in lavage fluid: expression levels of various cytokines including TNF-Î±, IL1-Î², MIP-1Î±, TIMP1, PDGF|Fibrosis Indicators in serum: expression levels of TGF-Î²1, hydroxyproline, MMP2, MMP9|Fibrosis Indicators in lavage fluid: expression levels of TGF-Î²1, hydroxyproline, MMP2, MMP9|self-evaluation
URL:	https://ClinicalTrials.gov/show/NCT02668068

Study 23:	
NCT Number:	NCT01985464
Title:	Umbilical Cord Tissue-derived Mesenchymal Stem Cells for Rheumatoid Arthritis
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Rheumatoid Arthritis
Interventions:	Biological: Umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	18 Years and older Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	TBS-UCMSCRA-001
First Received:	31-Oct-13
Start Date:	Oct-13
Completion Date:	Dec-18
Last Updated:	17-Oct-16
Last Verified:	Oct-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Apr-18
Outcome Measures:	Number of participants with adverse events|Number of participants with a change in disease activity index as measured by 28-DAS Score|Number of participants with a change in current disease activity as measured by EULAR Response Criteria|Change from baseline quality of life measure (based on Stanford HAQ)|Change from baseline C-reactive protein|Change from baseline erythrocyte sedimentation rate (ESR)|Change from baseline anti-citrulline antibody measure|Change from baseline rheumatoid factor (RF)
URL:	https://ClinicalTrials.gov/show/NCT01985464
Study 24:	
NCT Number:	NCT02034188
Title:	Feasibility Study of Human Umbilical Cord Tissue-Derived Mesenchymal Stem Cells in Patients With Multiple Sclerosis
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Multiple Sclerosis
Interventions:	Biological: Umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	18 Years to 55 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	TBS-UCMSC-001
First Received:	9-Jan-14
Start Date:	Jan-14
Completion Date:	Mar-16
Last Updated:	26-Aug-16
Last Verified:	Feb-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Mar-16

Outcome Measures:	Number of participants with adverse events|Number of participants with a change in disability as measured by Expanded Disability Status Scale (EDSS)|Number of participants with a change in neurological impairment as measured by Scripps Neurological Rating Scale|Number of participants with a change in cognitive function as measured by the â€¢ Paced Auditory Serial Addition Test (PASAT)|Number of participants with a change in upper extremity function as measured by the Nine Hole Peg Test|Number of participants with a change in mobility and leg function as measured by the 25 foot walking test|Number of participants with a change in quality of life as measured by the Short form 36 (SF-36) quality of life questionnaire|Number of participants experiencing pulmonary edema as measured by 12-lead electrocardiogram (ECG)|Number of participants with a change in brain or spinal cord lesions as measured by gadolinium-enhanced magnetic resonance imaging (MRI)
URL:	https://ClinicalTrials.gov/show/NCT02034188
Study 25:	
NCT Number:	NCT01343511
Title:	Safety and Efficacy of Stem Cell Therapy in Patients With Autism
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Autism
Interventions:	Biological: human cord blood mononuclear cells|Biological: human cord blood mononuclear cells and human umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Shenzhen Beike Bio-Technology Co., Ltd. Shandong Jiaotong Hospital|Association for the Handicapped Of Jinan
Gender:	Both
Age:	3 Years to 12 Years Â (Child)
Phases:	Phase 1|Phase 2
Enrollment:	37
Study Types:	Interventional
Study Designs:	Allocation: Non-Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	BKCR-AUTISM-1.0(2009)
First Received:	26-Apr-11
Start Date:	Mar-09
Completion Date:	May-11
Last Updated:	13-Oct-11
Last Verified:	Oct-11
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-10
Outcome Measures:	Childhood Autism Rating Scale, Clinical Global Impression Scale, Aberrant Behavior Checklist, Adverse Event and Serious Adverse Event
URL:	https://ClinicalTrials.gov/show/NCT01343511
Study 26:	
NCT Number:	NCT02666391
Title:	Safety and Exploratory Efficacy Study of UCMSCs in Patients With Ischemic Heart Disease (SEESUPIHD)
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Acute Myocardial Infarction|Myocardial Infarction|Ischemic Cardiomyopathy
Interventions:	Biological: umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	South China Research Center for Stem Cell and Regenerative Medicine Sun Yat-sen University
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2

Enrollment:	64
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	UCMSC-2
First Received:	21-Jan-16
Start Date:	May-16
Completion Date:	Dec-17
Last Updated:	24-Jan-16
Last Verified:	Jan-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	Change in global left ventricular ejection fraction (LVEF)measured by echocardiography.|Change in infarct size and myocardial viability within the infarcted region measured by emission computed tomography (ECT).|Pump failure Killip classification|New York Heart Association(NYHA) classification|Occurrence of major adverse event
URL:	https://ClinicalTrials.gov/show/NCT02666391
Study 27:	
NCT Number:	NCT02223897
Title:	Mesenchymal Stem Cells Transplantation for Ischemic-type Biliary Lesions
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Ischemic-type Biliary Lesions
Interventions:	Drug: huc-MSCs|Drug: Placebo
Sponsor/Collaborators:	Yang Yang|Third Affiliated Hospital, Sun Yat-Sen University
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 2|Phase 3
Enrollment:	66
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Prevention
Other IDs:	2014006
First Received:	21-Aug-14
Start Date:	Jul-14
Completion Date:	Jun-18
Last Updated:	21-Aug-14
Last Verified:	Aug-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-17
Outcome Measures:	The incidence of ITBLs|Changes in biliary enzymology|Biliary blood supply
URL:	https://ClinicalTrials.gov/show/NCT02223897
Study 28:	
NCT Number:	NCT02003131
Title:	Safety and Feasibility Study of Mesenchymal Trophic Factor (MTF) for Treatment of Osteoarthritis
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Osteoarthritis of the Knee
Interventions:	Biological: Trophic factors from umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	18 Years to 80 Years (Adult, Senior)

Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Non-Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	TBS-MTFOA-001
First Received:	2-Dec-13
Start Date:	Dec-13
Completion Date:	Jun-17
Last Updated:	4-Feb-16
Last Verified:	Feb-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Number of participants with adverse events|Number of participants with a change in joint function from baseline WOMAC assessment at 12 months|Number of participants with a change in radiogrpahic evidence of knee OA from baseline Kellegren-Lawrence grading system at 12 months
URL:	https://ClinicalTrials.gov/show/NCT02003131
Study 29:	
NCT Number:	NCT02192736
Title:	Safety and Feasibility Study of Intranasal Mesenchymal Trophic Factor (MTF) for Treatment of Asthma
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Asthma
Interventions:	Biological: Trophic factors from umbilical cord mesenchymal stem cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	21 Years to 60 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	TBS-MTFAS-001
First Received:	14-Jul-14
Start Date:	Jul-14
Completion Date:	Oct-17
Last Updated:	17-Oct-16
Last Verified:	Oct-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-17
Outcome Measures:	Number of patients with adverse events|Number of patients with a change in pulmonary function from baseline as measured by Forced Expiratory Volume (FEV1) following American Thoracic Society (ATS) guidelines|Number of patients with a change in pulmonary function from baseline as measured by Forced Vital Capacity (FVC) following American Thoracic Society (ATS) guidelines|Number of patients with a change in quality of life from baseline as measured by the University of Pittsburgh Medical Center (UPMC) Asthma Questionnaire
URL:	https://ClinicalTrials.gov/show/NCT02192736
Study 30:	
NCT Number:	NCT02669199
Title:	MSCs Source of Sweat Gland Cells of Large Area Skin Injury Patients Transplant of the Wound

Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	MSCs
Interventions:	Biological: MSCs
Sponsor/Collaborators:	Chinese PLA General Hospital
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 1
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CHIN-PLAGH-ST-007
First Received:	21-Jan-16
Start Date:	Jan-12
Completion Date:	Dec-15
Last Updated:	27-Jan-16
Last Verified:	Jan-16
Acronym:	MSCs
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-15
Outcome Measures:	Adverse Events|Relative Wound Area Regression of 40% or More at 12 Week
URL:	https://ClinicalTrials.gov/show/NCT02669199
Study 31:	
NCT Number:	NCT02815423
Title:	Safety and Exploratory Efficacy Study of UCMSCs in Patients With Fracture and Bone Nonunion
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Fracture|Bone Nonunion
Interventions:	Biological: UCMSCs|Biological: Percutaneous
Sponsor/Collaborators:	South China Research Center for Stem Cell and Regenerative Medicine Guangzhou Panyu Central Hospital
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	UCMSC-6
First Received:	20-Apr-16
Start Date:	Jan-17
Completion Date:	Jan-20
Last Updated:	23-Jun-16
Last Verified:	Mar-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-19
Outcome Measures:	Radiological progression of bone fusion|Comparison of the rate of complications between the 2 groups|Incidence of increased temperature sensitivity by questionnaire|Incidence and severity of infections at grafting sites by questionnaire
URL:	https://ClinicalTrials.gov/show/NCT02815423
Study 32:	
NCT Number:	NCT02444858

Title:	Human Umbilical-Cord-Derived Mesenchymal Stem Cell Therapy in Paraquat Poisoning Induced Lung Injury
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Paraquat Poisoning|Lung Injury
Interventions:	Biological: UCMSC group|Other: Control group(Normal saline)
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences Ivy Institute of Stem Cells Co. Ltd
Gender:	Both
Age:	15 Years to 60 Years Â (Child, Adult)
Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Treatment
Other IDs:	307-IVY-SC-004
First Received:	12-May-15
Start Date:	May-15
Completion Date:	Dec-17
Last Updated:	12-May-15
Last Verified:	Apr-15
Acronym:	UCMSC-PQLI
Results First Received:	No Study Results Posted
Primary Completion Date:	May-17
Outcome Measures:	Safety will be determined by the assessment of major adverse events. The efficacy of UC-MSC treatment was measured clinical evaluation.|The efficacy of UC-MSC treatment was measured by chest computerized tomography.|The efficacy of UC-MSC treatment was monitored by pulmonary function.|The efficacy of UC-MSC treatment was measured by lab Indicators.
URL:	https://ClinicalTrials.gov/show/NCT02444858

Study 33:	
NCT Number:	NCT02444455
Title:	Human Umbilical-Cord-Derived Mesenchymal Stem Cell Therapy in Acute Lung Injury
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Acute Lung Injury|Acute Respiratory Distress Syndrome
Interventions:	Biological: UCMSC group
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences Ivy Institute of Stem Cells Co. Ltd
Gender:	Both
Age:	35 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	307-IVY-SC-003
First Received:	8-May-15
Start Date:	May-15
Completion Date:	Dec-17
Last Updated:	11-May-15
Last Verified:	May-15
Acronym:	UCMSC-ALI
Results First Received:	No Study Results Posted
Primary Completion Date:	Mar-17

Outcome Measures:	Safety will be determined by the assessment of major adverse events, Quantify pulmonary respiratory function measured by chest computerized tomography|The efficacy of UC-MSC treatment was measured by arterial blood gas analysis|The efficacy of UC-MSC treatment was measured by biological markers,including markers of inflammationï¼ŒIL-6|The efficacy of UC-MSC treatment was measured by biological markers,including markers of inflammationï¼ŒIL-8
URL:	https://ClinicalTrials.gov/show/NCT02444455
Study 34:	
NCT Number:	NCT02439541
Title:	Human Umbilical-Cord-Derived Mesenchymal Stem Cell Therapy in Ischemic Cardiomyopathy
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Chronic Ischemic Heart Disease|Heart Failure|Angina
Interventions:	Biological: UCMSC group
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences|Ivy Institute of Stem Cells Co. Ltd
Gender:	Both
Age:	18 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Non-Randomized|Endpoint Classification:Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	307-IVY-SC-001
First Received:	5-May-15
Start Date:	May-15
Completion Date:	Dec-18
Last Updated:	14-Mar-16
Last Verified:	Mar-16
Acronym:	UCMSC-Heart
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	Number and nature of adverse events|Incidence of major adverse coronary events (MACE)|Exercise Time and Level|Quantify myocardium perfusion measured by SPECT|Assessment of heart function by left ventricular ejection fraction|Clinical Improvement in NYHA Classification
URL:	https://ClinicalTrials.gov/show/NCT02439541
Study 35:	
NCT Number:	NCT02442037
Title:	Human Umbilical-Cord-Derived Mesenchymal Stem Cell Therapy in Active Ulcerative Colitis
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Ulcerative Colitis
Interventions:	Biological: UCMSC group|Other: Control group(Normal saline)
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences, Ivy Institute of Stem Cells Co. Ltd
Gender:	Both
Age:	18 Years to 65 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional

Study Designs:	Allocation: Randomized|Endpoint Classification:Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Treatment
Other IDs:	307-IVY-SC-002
First Received:	6-May-15
Start Date:	May-15
Completion Date:	Dec-17
Last Updated:	14-May-15
Last Verified:	May-15
Acronym:	UCMSC-UC
Results First Received:	No Study Results Posted
Primary Completion Date:	May-17
Outcome Measures:	Safety will be determined by the assessment of major adverse events. Clinical response (CDAI points)|Endoscopic improvement is assessed by UCEIS.|Level of C-reactive protein
URL:	https://ClinicalTrials.gov/show/NCT02442037
Study 36:	
NCT Number:	NCT02947191
Title:	Collagen Membrane Combined With HUC-MSCs Transplantation in Patients With Nasal Septum Perforation
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Chronic Nasal Septum Perforation
Interventions:	Biological: Collagen membrane + HUC-MSCs
Sponsor/Collaborators:	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Both
Age:	18 Years to 45 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	12
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CAS-XDA-NSP/IGDB
First Received:	25-Oct-16
Start Date:	Nov-16
Completion Date:	Dec-19
Last Updated:	25-Oct-16
Last Verified:	Oct-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-19
Outcome Measures:	The change of integrity of nasal septum assessed by endoscopic examination|The change of nasal mucosal physiology assessed by olfactory test|The change of nasal mucosal physiology assessed by nasal mucociliary clearance time (MCT)|The change of nasal airway resistance assessed by rhinomanometry|The change of nasal obstruction assessed by acoustic rhinometry|The change of nasal obstructive symptom assessed by visual analogue scale (VAS)|The change of nasal symptom severity assessed by total nasal symptom score (TNSS)|Change from baseline in computed tomography (CT)
URL:	https://ClinicalTrials.gov/show/NCT02947191
Study 37:	
NCT Number:	NCT02635464
Title:	Human Umbilical Cord-derived Mesenchymal Stem Cells With Injectable Collagen Scaffold Transplantation for Chronic Ischemic Cardiomyopathy
Recruitment:	Recruiting
Study Results:	No Results Available

Conditions:	Chronic Ischemic Cardiomyopathy
Interventions:	Biological: hUC-MSCs+Injectable collagen scaffold+CABG Biological: hUC-MSCs+CABG|Procedure: CABG
Sponsor/Collaborators:	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Both
Age:	35 Years to 65 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	45
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	CAS-XDA-CIC/IGDB
First Received:	13-Dec-15
Start Date:	Oct-15
Completion Date:	Mar-18
Last Updated:	19-Dec-15
Last Verified:	Dec-15
Acronym:	null
Results First Received:	No Study Results Posted
Primary Completion Date:	Mar-18
Outcome Measures:	Incidence of treatment-emergent adverse events|Myocardial blood flow|Left ventricle ejection fraction (LVEF)|Infarct size|New York Heart Association (NYHA) Functional Classification|Canadian Cardiovascular Society (CCS) Angina Grading Scale
URL:	https://ClinicalTrials.gov/show/NCT02635464
Study 38:	
NCT Number:	NCT02323477
Title:	Human Umbilical Cord Stroma MSC in Myocardial Infarction
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Chronic Ischemic Cardiomyopathy|Coronary Artery Bypass Surgery
Interventions:	Biological: stem cell transplantation
Sponsor/Collaborators:	Ankara University|Hacettepe University|Turkiye Yuksek Ihtisas Education and Research Hospital|ATIGEN-CELL|Dr. Sami Ulus Children's Hospital|Ankara Yildirim BeyazÄ±t University
Gender:	Male
Age:	30 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	79
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification:Safety/Efficacy Study Intervention Model:Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose:Treatment
Other IDs:	741.STZ.2014
First Received:	18-Dec-14
Start Date:	Feb-15
Completion Date:	Sep-17
Last Updated:	28-Dec-15
Last Verified:	Dec-15
Acronym:	HUC-HEART
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-17
Outcome Measures:	ventricular remodeling
URL:	https://ClinicalTrials.gov/show/NCT02323477
Study 39:	

NCT Number:	NCT02648386
Title:	Stem Cell Therapy Combined With NeuroRegen Scaffold in Patients With Erectile Dysfunction After Rectal Cancer Surgery
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Rectal Cancer|Erectile Dysfunction
Interventions:	Procedure: Laparoscopic surgery|Device: NeuroRegen scaffold transplantation|Biological: NeuroRegen scaffold/BMMCs transplantation|Biological: NeuroRegen scaffold/HUC-MSCs transplantation
Sponsor/Collaborators:	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Male
Age:	20 Years to 65 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	34
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	CAS-XDA-ED-IGDB
First Received:	5-Jan-16
Start Date:	Jan-16
Completion Date:	Dec-18
Last Updated:	14-Apr-16
Last Verified:	Dec-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-18
Outcome Measures:	Safety and Tolerability assessed by Adverse Events|IIEF-5 (International Index of Erectile Function)|Mean scores of the Sexual Encounter Profile (SEP) Question 2, 3|Penile cavernosal artery peak systolic velocity (PSV)|Nocturnal penile tumescence (NPT)|The change of results of Nerve electrophysiological examination|Maximum Flow Rate (Qmax)
URL:	https://ClinicalTrials.gov/show/NCT02648386
Study 40:	
NCT Number:	NCT01844063
Title:	Safety and Efficacy of Diverse Mesenchymal Stem Cells Transplantation for Liver
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Liver Failure
Interventions:	Genetic: Conventional plus BM-MSC treatment|Genetic: Conventional plus UC-MSC treatment|Drug: Conventional treatment
Sponsor/Collaborators:	Third Affiliated Hospital, Sun Yat-Sen University
Gender:	Both
Age:	18 Years to 65 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	210
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	The Third Affiliated Hospital
First Received:	20-Apr-13
Start Date:	Jul-13
Completion Date:	Jan-19
Last Updated:	4-Jul-13
Last Verified:	Jul-13
Results First Received:	No Study Results Posted

Primary Completion Date:	Jan-16
Outcome Measures:	survival rate|Liver function|Marker of liver cancer|degree of hepatic necrosis|improvement of symptoms|score for Model for End-Stage Liver Disease|improvement of immune function|complications|incidence of hepatocellular
URL:	https://ClinicalTrials.gov/show/NCT01844063
Study 41:	
NCT Number:	NCT02685722
Title:	UC-MSCs Gel Treatment Difficult Healing of Skin Ulcers
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Difficult to Healing of Skin Ulcers
Interventions:	Biological: UC-MSCs Gel group|Other: Gel group
Sponsor/Collaborators:	Chinese PLA General Hospital
Gender:	Both
Age:	17 Years to 78 Years Â (Child, Adult, Senior)
Phases:	Phase 1
Enrollment:	20
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CHIN-PLAGH-ST-008
First Received:	21-Jan-16
Start Date:	Jan-12
Completion Date:	Dec-15
Last Updated:	18-Feb-16
Last Verified:	Feb-16
Acronym:	UC-MSCs
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-15
Outcome Measures:	Adverse Events|Relative Wound Area Regression of 40% or More at 6 Week
URL:	https://ClinicalTrials.gov/show/NCT02685722
Study 42:	
NCT Number:	NCT02237846
Title:	Clinical Study of Umbilical Cord Tissue Mesenchymal Stem Cells (UC-MSC) for Treatment of Osteoarthritis
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Osteoarthritis of the Knee
Interventions:	Biological: Human umbilical cord tissue-derived mesenchymal stem cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	18 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CNEI-2014-TBS-UCMSCOA-001
First Received:	9-Sep-14
Start Date:	Sep-14
Completion Date:	Aug-19
Last Updated:	17-Oct-16
Last Verified:	Oct-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Nov-18

Outcome Measures:	Number of participants with adverse events|Number of participants with a change in joint function from baseline WOMAC assessment|Number of participants with a change in radiogrpahic evidence of knee OA from baseline Kellegren-Lawrence grading system
URL:	https://ClinicalTrials.gov/show/NCT02237846
Study 43:	
NCT Number:	NCT00823316
Title:	Safety and Efficacy Study of Umbilical Cord Blood-Drived Mesenchymal Stem Cells to Promote Engraftment of Unrelated Hematopoietic Stem Cell Transplantation
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Acute Leukemia
Interventions:	Biological: Human umbilical cord blood-derived mesenchymal stem cells Biological: Human umbilical cord blood-derived mesenchymal stem cells
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	2 Years to 19 Years Â (Child, Adult)
Phases:	Phase 1|Phase 2
Enrollment:	10
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	MP-CR-MSC003
First Received:	13-Jan-09
Start Date:	Aug-08
Completion Date:	Feb-10
Last Updated:	20-Apr-12
Last Verified:	Apr-12
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-10
Outcome Measures:	Day of neutrophil engraftment, Day of platelet engraftment, Evaluation of chimerism, Evaluation of engraftment, rate AGVHD grade
URL:	https://ClinicalTrials.gov/show/NCT00823316
Study 44:	
NCT Number:	NCT02313415
Title:	Treatment of Infertility by Collagen Scaffold Loaded With Umbilical Cord Blood-derived Mesenchyma Stem Cells
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Infertility|Intrauterine Adhesions|Endometrial Dysplasia
Interventions:	Procedure: collagen scaffold loaded|Drug: umbilical cord blood-derived mesenchymal stem cells
Sponsor/Collaborators:	The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School|Chinese Academy of Sciences
Gender:	Female
Age:	20 Years to 45 Years (Adult)
Phases:	
Enrollment:	20
Study Types:	Interventional
Study Designs:	Intervention Model: Single Group Assignment|Masking: Open Label Primary Purpose: Treatment
Other IDs:	20141206
First Received:	7-Dec-14
Start Date:	Dec-14
Completion Date:	Dec-15
Last Updated:	24-Dec-14

Last Verified:	Dec-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Jul-15
Outcome Measures:	Reduction of intrauterine scar area,the change of intrauterine adhesion The change of endometrial thickness|The change of menstrual blood volume
URL:	https://ClinicalTrials.gov/show/NCT02313415
Study 45:	
NCT Number:	NCT02812121
Title:	UC-MSC Infusion for HBV-Related Acute-on-Chronic Liver Failure
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Liver Failure
Interventions:	Drug: umbilical cord blood mesenchymal stem cells
Sponsor/Collaborators:	Sun Yat-sen University
Gender:	Both
Age:	18 Years to 65 Years Â (Adult)
Phases:	Phase 2
Enrollment:	261
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	UCBMSC|LBingliang
First Received:	27-May-16
Start Date:	Jun-16
Completion Date:	Jun-18
Last Updated:	21-Jun-16
Last Verified:	Jun-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-17
Outcome Measures:	The incidence of adverse reactions after umbilical cord blood derived mesenchymal stem cells (UC-MSC) infusions.|The survival time of patients after UC-MSC infusions.|The influence on levels of ALT (U/L) and AST (U/L) after UC-MSC infusions|The influence on levels of ALB(g/L) after UC-MSC infusions|The influence on levels of TBil (umol/L) after UC-MSC infusions|The influence on levels of INR after UC-MSC infusions|The influence on levels of MELD score, SOFA score and CTP score after UC-MSC infusions|The incidence of fatal complications after UC-MSC infusions.|Comparison of levels of NKG2A, NKG2D, NKP46, KIR2DL1, KIR2DL3, KIR3DL1, perforin, FasL, and gramzymeB among the groups after UC-MSC infusions
URL:	https://ClinicalTrials.gov/show/NCT02812121
Study 46:	
NCT Number:	NCT02304562
Title:	Umbilical Cord Blood-derived Mesenchymal Stem Cells in Regeneration of Sweat Glands and Body Repair
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Sweat Gland Diseases
Interventions:	Biological: UCB Mesenchymal Stem Cells treatment
Sponsor/Collaborators:	Chinese PLA General Hospital
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 1
Enrollment:	50
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment

Other IDs:	CHIN-PLAGH-ST-004
First Received:	21-Nov-14
Start Date:	Jan-13
Completion Date:	Dec-17
Last Updated:	1-Dec-14
Last Verified:	Nov-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	Frequency of Adverse Events|Active and inactive lesion count
URL:	https://ClinicalTrials.gov/show/NCT02304562
Study 47:	
NCT Number:	NCT02338375
Title:	Safety and Efficacy of Allogenic Umbilical Cord Blood-derived Mesenchymal Stem Cell Product
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Osteochondral Lesion of Talus
Interventions:	Biological: Cartistem
Sponsor/Collaborators:	Samsung Medical Center
Gender:	Both
Age:	20 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 0
Enrollment:	28
Study Types:	Interventional
Study Designs:	Allocation:Randomized|Endpoint Classification:Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Treatment
Other IDs:	2012-10-078
First Received:	31-Jul-13
Start Date:	Dec-12
Completion Date:	Dec-15
Last Updated:	11-Jan-15
Last Verified:	Jan-15
Acronym:	Cartistem
Results First Received:	No Study Results Posted
Primary Completion Date:	Aug-15
Outcome Measures:	evaluate the efficacy of adding allogenic umbilical cord blood-derived stem cell product(CartistemÂ®) by American Orthopaedic Foot and Ankle Society(AOFAS) HINDFOOT/ANKLE SCALE|evaluate the efficacy of adding allogenic umbilical cord blood-derived stem cell product(CartistemÂ®) by 100-mm VAS(Visual Analogue Scale)
URL:	https://ClinicalTrials.gov/show/NCT02338375
Study 48:	
NCT Number:	NCT01092026
Title:	Unrelated Umbilical Cord Blood Transplantation With Coinfusion of Mesenchymal Stem Cells
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Allogeneic Stem Cell Transplantation
Interventions:	Other: cord blood transplantation
Sponsor/Collaborators:	Universitair Ziekenhuis Brussel
Gender:	Both
Age:	15 Years to 60 Years Â (Child, Adult)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional

Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	BHS-UCB2009
First Received:	23-Mar-10
Start Date:	Nov-10
Completion Date:	Dec-17
Last Updated:	3-Jun-16
Last Verified:	May-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	treatment-related mortality|Hematopoietic recovery
URL:	https://ClinicalTrials.gov/show/NCT01092026
Study 49:	
NCT Number:	NCT02307435
Title:	Allogenic Mesenchymal Stem Cell for Bone Defect or Non Union Fracture
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Non Union Fracture|Metaphyseal Fibrous Defect
Interventions:	Biological: MSC
Sponsor/Collaborators:	Indonesia University
Gender:	Both
Age:	19 Years to 30 Years Â (Adult)
Phases:	Phase 0
Enrollment:	9
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	ISMMSC001
First Received:	7-Oct-14
Start Date:	Aug-14
Completion Date:	Dec-17
Last Updated:	1-Dec-14
Last Verified:	Dec-14
Acronym:	AMSC
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	cell viability|lower extremity functional score|disabilities of arm shoulder and hand
URL:	https://ClinicalTrials.gov/show/NCT02307435
Study 50:	
NCT Number:	NCT02587715
Title:	A Study of Allogeneic Human UC-MSC and Liberation Therapy (When Associated With CCSVI) in Patients With RRMS
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Multiple Sclerosis, Relapsing-Remitting
Interventions:	Biological: Allogeneic Human Umbilical Cord Tissue-Derived Mesenchymal Stem Cells|Other: Liberation therapy
Sponsor/Collaborators:	Novo Cellular Medicine Institute LLP
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	69
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	PRT/NOVO/2015/001

First Received:	26-Oct-15
Start Date:	Feb-15
Completion Date:	Feb-17
Last Updated:	27-Oct-15
Last Verified:	Oct-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-17
Outcome Measures:	Proportion of patients with clinical improvement in EDSS score compared to baseline |Proportion of patients with a change in either gadolinium enhancing or new T2-weighted lesions on brain MRI|Proportion of patients with reduction in T2 lesion volume on brain MRI|Proportion of patients with reduction in brain volume on MRI|Proportion of patients with clinical improvement in MSIS score compared to baseline|Proportion of patients with clinical improvement in MSFC score compared to baseline|Proportion of patients with a change in mobility and leg function as measured by the 25 foot walking test|Proportion of patients with a change in upper extremity function as measured by the Nine Hole Peg Test|Proportion of patients with a change in cognitive function as measured by the Paced Auditory Serial Addition Test (PASAT)|Proportion of patients with reduced number of relapses or freedom from progression of disease
URL:	https://ClinicalTrials.gov/show/NCT02587715
Study 51:	
NCT Number:	NCT02381366
Title:	Safety and Efficacy of PNEUMOSTEMÂ® in Premature Infants at High Risk for Bronchopulmonary Dysplasia (BPD) - a US Study
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Bronchopulmonary Dysplasia
Interventions:	Biological: Human Umbilical Cord Blood Derived-Mesenchymal Stem Cells
Sponsor/Collaborators:	Medipost America Inc.|Medipost Co Ltd.
Gender:	Both
Age:	up to 14 Days Â (Child)
Phases:	Phase 1|Phase 2
Enrollment:	12
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Prevention
Other IDs:	MD-BPD-US001
First Received:	15-Feb-15
Start Date:	Mar-15
Completion Date:	May-18
Last Updated:	22-Mar-16
Last Verified:	Mar-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jul-16
Outcome Measures:	Number of participants with adverse reactions for 84 days after treatment, Number of participants with adverse reactions between 84 days after treatment and 20 months of corrected age|Incidence of moderate/severe BPD or death at 36 weeks postmenstrual age (PMA)|Hospital Re-admission between 84 days after treatment until 20 months of corrected age|Bayley Scales of Infant and Toddler Development between 84 days after treatment until 20 months of corrected age
URL:	https://ClinicalTrials.gov/show/NCT02381366
Study 52:	
NCT Number:	NCT02237547
Title:	Safety and Feasibility Study of Cell Therapy in Treatment of Spinal Cord Injury
Recruitment:	Active, not recruiting
Study Results:	No Results Available

Conditions:	Spinal Cord Injury
Interventions:	Biological: Intravenous and intrathecal human umbilical cord tissue-derived mesenchymal stem cells and bone marrow mononuclear cells
Sponsor/Collaborators:	Translational Biosciences
Gender:	Both
Age:	18 Years to 50 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CNEI-2014-TBS-UCMSC-SCI001
First Received:	9-Sep-14
Start Date:	Sep-14
Completion Date:	Oct-19
Last Updated:	17-Oct-16
Last Verified:	Oct-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-19
Outcome Measures:	Number of patients with adverse events|Number of subjects with a change in American Spinal Injury Association (ASIA) score from baseline|Number of subjects with a change in Frankel Scale score from baseline
URL:	https://ClinicalTrials.gov/show/NCT02237547
Study 53:	
NCT Number:	NCT02181478
Title:	Intra-Osseous Co-Transplant of UCB and hMSC
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Acute Lymphoblastic Leukemia|Acute Myelogenous Leukemia, Myelodysplastic Syndromes|Myelofibrosis|Relapsed Non-Hodgkin Lymphoma|Refractory Non-Hodgkin Lymphoma|Hodgkin Lymphoma|Refractory Hodgkin Lymphoma|Relapsed Chronic Lymphocytic Leukemia|Refractory Chronic Lymphocytic Leukemia|Lymphoid Malignancies|Chronic Myelogenous Leukemia
Interventions:	Drug: cyclophosphamide|Drug: fludarabine phosphate|Radiation: total-body irradiation|Drug: cyclosporine|Drug: mycophenolate mofetil|Procedure: umbilical cord blood transplantation|Procedure: mesenchymal stem cell transplantation
Sponsor/Collaborators:	Case Comprehensive Cancer Center|National Cancer Institute (NCI)
Gender:	Both
Age:	18 Years to 75 Years Â (Adult, Senior)
Phases:	
Enrollment:	12
Study Types:	Interventional
Study Designs:	Endpoint Classification: Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CASE1Z14|NCI-2014-01316|P30CA043703
First Received:	2-Jul-14
Start Date:	Jul-15
Completion Date:	null
Last Updated:	28-Sep-16
Last Verified:	Sep-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Mar-17

Outcome Measures:	Number of patients with BM cellularity failure: Measure of feasibility|Number of patients with ANC failure without evidence of disease: Measure of feasibility|Number of patients with hematopoietic recovery without evidence of donor umbilical cord blood engraftment: Measure of feasibility|Incidence of toxicities assessed using National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0|Rate of neutrophil recovery|Rate of platelet recovery|Median time of neutrophil recovery|Median time of platelet recovery
URL:	https://ClinicalTrials.gov/show/NCT02181478
Study 54:	
NCT Number:	NCT01754454
Title:	Safety & Efficacy of UC-MSC in Patients With Acute Severe Graft-versus-host Disease
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Acute GVH Disease
Interventions:	Biological: Human Umbilical Cord Derived MSC
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	307-CTC-MSC-002
First Received:	11-Nov-12
Start Date:	Dec-12
Completion Date:	Dec-16
Last Updated:	7-Apr-16
Last Verified:	Feb-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Safety of UC-MSC in patients with acute graft-versus-host disease Efficacy of UC-MSC in patients with acute graft-versus-host disease
URL:	https://ClinicalTrials.gov/show/NCT01754454
Study 55:	
NCT Number:	NCT02054208
Title:	Safety and Exploratory Efficacy Study of NEUROSTEMÂ® Versus Placebo in Patients With Alzheimer's Disease
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Alzheimer's Disease
Interventions:	Biological: human umbilical cord blood derived mesenchymal stem cells Procedure: Ommaya reservoir insertion|Other: Normal saline 2mL
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	50 Years to 85 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	42
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety Study|Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	MP-CR-010
First Received:	29-Jan-14
Start Date:	Feb-14

Completion Date:	Feb-18
Last Updated:	6-Jan-16
Last Verified:	Jan-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-18
Outcome Measures:	Number of subjects with adverse events|Change from the baseline in ADAS-Cog|Change from the baseline in S-IADL|Change from the baseline in K-MMSE|Change from the baseline in CGA-NPI|ADAS-Cog Response Rate|Change in CDR-SOB|Change in Florbetaben-PET|Change in FDG-PET (CMRglc: regional cerebral metabolic rate for glucose)|Change in CIBIC-plus|Change from baseline in MRI (DTI mapping)|Change from the baseline in CSF biomarkers
URL:	https://ClinicalTrials.gov/show/NCT02054208
Study 56:	
NCT Number:	NCT02491658
Title:	Safety and Efficacy of UC-MSCs in Patients With Psoriasis Vulgaris
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Psoriasis Vulgaris
Interventions:	Biological: UC-MSCs
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences
Gender:	Both
Age:	18 Years to 65 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	307-PV-MSC
First Received:	24-Jun-15
Start Date:	Apr-15
Completion Date:	Dec-16
Last Updated:	3-Jul-15
Last Verified:	Jul-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Change from Baseline in Psoriasis Area and Severity Index (PASI) score at 8 weeks|Change from Baseline in Dermatology Life Quality Index (DLQI) score at 8 weeks|body temperature will be monitored for any possible infusion-related toxicities.|blood pressure will be monitored for any possible infusion-related toxicities.|Psoriasis Area and Severity Index (PASI) score|Dermatology Life Quality Index (DLQI) score
URL:	https://ClinicalTrials.gov/show/NCT02491658
Study 57:	
NCT Number:	NCT01547689
Title:	Safety and Efficiency of Umbilical Cord-derived Mesenchymal Stem Cells(UC-MSC) in Patients With Alzheimer's Disease
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Alzheimer's Disease
Interventions:	Biological: Human Umbilical Cord Derived MSC
Sponsor/Collaborators:	Affiliated Hospital to Academy of Military Medical Sciences Peking University Third Hospital
Gender:	Both
Age:	50 Years to 85 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	30

Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	307-CTC-MSC-001|2011AA020114
First Received:	5-Mar-12
Start Date:	Mar-12
Completion Date:	Dec-16
Last Updated:	18-Feb-16
Last Verified:	Feb-16
Acronym:	SEMAD
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Number of participants with adverse event|Changes from the baseline in Alzheimer' s Disease Assessment Scale-cognitive subscale(ADAS-Cog) at 10 weeks post-dose
URL:	https://ClinicalTrials.gov/show/NCT01547689
Study 58:	
NCT Number:	NCT01297205
Title:	Safety and Efficacy Evaluation of PNEUMOSTEMÂ® Treatment in Premature Infants With Bronchopulmonary Dysplasia
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Bronchopulmonary Dysplasia
Interventions:	Biological: Human Umbilical Cord Blood Derived-Mesenchymal Stem Cells
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	up to 14 Days Â (Child)
Phases:	Phase 1
Enrollment:	9
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	MP-CR-006
First Received:	11-Feb-11
Start Date:	Dec-10
Completion Date:	Dec-11
Last Updated:	3-Apr-14
Last Verified:	Apr-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Sep-11
Outcome Measures:	Number of participant with adverse reaction|Incidence of BPD
URL:	https://ClinicalTrials.gov/show/NCT01297205
Study 59:	
NCT Number:	NCT01297218
Title:	The Safety and The Efficacy Evaluation of NEUROSTEMÂ®-AD in Patients With Alzheimer's Disease
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Dementia of the Alzheimer's Type
Interventions:	Biological: Human Umbilical Cord Blood Derived-Mesenchymal Stem Cells
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	50 Years to 75 Years Â (Adult, Senior)
Phases:	Phase 1
Enrollment:	9
Study Types:	Interventional

Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	MP-CR-007
First Received:	11-Feb-11
Start Date:	Feb-11
Completion Date:	Dec-11
Last Updated:	19-Apr-12
Last Verified:	Apr-12
Results First Received:	No Study Results Posted
Primary Completion Date:	Sep-11
Outcome Measures:	Adverse event|Changes from the baseline in ADAS-cog at 12 weeks post-dose
URL:	https://ClinicalTrials.gov/show/NCT01297218
Study 60:	
NCT Number:	NCT01733186
Title:	Evaluation of Safety and Exploratory Efficacy of CARTISTEMÂ®, a Cell Therapy Product for Articular Cartilage Defects
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Degeneration Articular Cartilage Knee
Interventions:	Biological: CARTISTEMÂ®
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	18 Years and older Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	12
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	MP-0201-01
First Received:	20-Nov-12
Start Date:	Nov-12
Completion Date:	May-17
Last Updated:	1-Jun-15
Last Verified:	Jun-15
Results First Received:	No Study Results Posted
Primary Completion Date:	May-16
Outcome Measures:	Number of adverse events|Improvement in IKDC score|Improvement in knee function and pain and grade of cartilage regeneration
URL:	https://ClinicalTrials.gov/show/NCT01733186
Study 61:	
NCT Number:	NCT02580695
Title:	A Study to Assess Safety and Efficacy of Umbilical Cord-derived Mesenchymal Stromal Cells in Knee Osteoarthritis
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Osteoarthritis
Interventions:	Biological: umbilical-cord mesenchymal stromal cells|Drug: Hyaluronic Acid
Sponsor/Collaborators:	Francisco Espinoza, MD|Universidad Los Andes, Chile
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional

Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	C4COA01
First Received:	15-Oct-15
Start Date:	Dec-15
Completion Date:	Jan-17
Last Updated:	26-Sep-16
Last Verified:	Sep-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-17
Outcome Measures:	Proportion of patients who experience an adverse event|Physical function improvement measured by WOMAC OA index|Change in pain density measured by Visual analogue scale (VAS)|QoL improvement measured by SF-36|Changes in WORMS scale measured by knee MRI
URL:	https://ClinicalTrials.gov/show/NCT02580695
Study 62:	
NCT Number:	NCT01041001
Title:	Study to Compare the Efficacy and Safety of CartistemÂ® and Microfracture in Patients With Knee Articular Cartilage Injury or Defect
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Cartilage Injury|Osteoarthritis
Interventions:	Biological: Cartistem|Procedure: Microfracture treatment
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	18 Years and older Â (Adult, Senior)
Phases:	Phase 3
Enrollment:	104
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	MP-CRP-005
First Received:	29-Dec-09
Start Date:	Feb-09
Completion Date:	Jan-11
Last Updated:	19-Apr-12
Last Verified:	Apr-12
Acronym:	null
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-10
Outcome Measures:	ICRS Cartilage Repair Assessment will follow to determine the appropriate grade. The treatment will be considered efficacious if the ICRS grade drops by at least 1 grade or more from baseline to week 48.|Degree of improvement in the grade of joint pain measured on a 100-mm VAS (Visual Analogue Scale)|Grade of cartilage regeneration in patients who agreed to a biopsy during arthroscopy at week 48|Changes in WOMAC scores|Changes in IKDC Subjective Score|ICRS scores
URL:	https://ClinicalTrials.gov/show/NCT01041001
Study 63:	
NCT Number:	NCT01033552
Title:	Stem Cell Transplant for Epidermolysis Bullosa
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Epidermolysis Bullosa

Interventions:	Drug: Cyclophosphamide|Drug: Fludarabine|Drug: Anti-thymocyte globulin Drug: Cyclosporine A|Drug: Mycophenolate mofetil|Procedure: Mesenchymal stem cell transplantation|Radiation: Total body irradiation|Procedure: Bone marrow or umbilical cord blood (UCG) stem cell transplantation
Sponsor/Collaborators:	Masonic Cancer Center, University of Minnesota
Gender:	Both
Age:	up to 25 Years Â (Child, Adult)
Phases:	Phase 2
Enrollment:	75
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	MT2009-09|0911M74035
First Received:	14-Dec-09
Start Date:	Jan-10
Completion Date:	Oct-19
Last Updated:	26-May-16
Last Verified:	May-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Oct-19
Outcome Measures:	Event-free survival|Incidence of transplant-related mortality (TRM)|Pattern of biochemical improvement measured by increase in protein expression (collagen, laminin, integrin or plakin)|Quality of Life
URL:	https://ClinicalTrials.gov/show/NCT01033552
Study 64:	
NCT Number:	NCT02749448
Title:	Mesenchymal Stem Cells Therapy for Treatment of Airway Remodeling in Mustard Patients
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Pulmonary Disease
Interventions:	Other: mesenchymal stem cell
Sponsor/Collaborators:	Dr. Mostafa Ghanei|Baqiyatallah Medical Sciences University
Gender:	Male
Age:	45 Years to 65 Years Â (Adult)
Phases:	Phase 1
Enrollment:	10
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	IRCT2015110524890N1
First Received:	9-Apr-16
Start Date:	Feb-15
Completion Date:	Feb-17
Last Updated:	22-Jul-16
Last Verified:	Apr-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Nov-16
Outcome Measures:	pulmonary function testing (PFT)|St. George's Respiratory Questionnaire (SGRQ)|6 minute walk test (6MWT)
URL:	https://ClinicalTrials.gov/show/NCT02749448
Study 65:	
NCT Number:	NCT01897987
Title:	Follow-up Safety and Efficacy Evaluation on Subjects Who Completed PNEUMOSTEMÂ® Phase-II Clinical Trial

Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Bronchopulmonary Dysplasia
Interventions:	Biological: PneumostemÂ®|Biological: normal saline
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	7 Months to 7 Months Â (Child)
Phases:	Phase 2
Enrollment:	70
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	MP-CR-009-FU
First Received:	4-Jul-13
Start Date:	Jan-14
Completion Date:	Dec-16
Last Updated:	6-Jan-16
Last Verified:	Jan-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Respiratory outcome: readmission rate and duration of the hospital stay due to respiratory infection|Whether the subject is receiving medical treatments and if so, duration of the treatment (use of oxygen, steroid, or brochodilator)|Number of admissions to Emergency Room|Survival|Growth measured by Z-score|Neurological developmental status on K-ASQ, Bayley Scale, Gross Motor Function Classification System (GMFCS) for Cerebral Palsy|Deafness or Blindness|Number of adverse events|Significant changes in vital signs|Significant changes in physical exam
URL:	https://ClinicalTrials.gov/show/NCT01897987
Study 66:	
NCT Number:	NCT01828957
Title:	Efficacy and Safety Evaluation of PneumostemÂ® Versus a Control Group for Treatment of BPD in Premature Infants
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Bronchopulmonary Dysplasia
Interventions:	Biological: PneumostemÂ®|Other: Normal Saline
Sponsor/Collaborators:	Medipost Co Ltd.
Gender:	Both
Age:	up to 14 Days Â (Child)
Phases:	Phase 2
Enrollment:	70
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	MP-CR-009
First Received:	2-Apr-13
Start Date:	Apr-13
Completion Date:	Jun-16
Last Updated:	7-Apr-15
Last Verified:	Apr-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-16

Outcome Measures:	Incidence of BPD (moderate to severe) or mortality at 36 weeks PMA Intubation duration|Incidence of BPD|Survival rate|Duration of ventilator dependence|Duration of CPAP treatment|Postnatal steroid use (%) for the purpose of ventilator weaning |Cumulative duration of oxygen use|Incidence of Retinopathy of Prematurity (ROP) of Grade III or more|Retinopathy of Prematurity (ROP) that require treatment with avastin or laser|Growth velocity (Z-score)|Length of stay prior to the first discharge from the hospital|Incidence of adverse events|Clinically significant laboratory findings|Incidence of pneumothorax that require intubation|Incidence of moderate to severe pulmonary hemorrhage|Incidence of intraventricular hemorrhage of grade 3 or more
URL:	https://ClinicalTrials.gov/show/NCT01828957
Study 67:	
NCT Number:	NCT00498316
Title:	Cord Blood Expansion on Mesenchymal Stem Cells
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Myelodysplastic Syndrome|Leukemia
Interventions:	Procedure: Cord Blood Infusion|Drug: Busulfan|Drug: Fludarabine|Drug:Rituximab Other: ATG|Drug: Cyclophosphamide|Drug: Clofarabine|Radiation: Total Body Irradiation (TBI)|Drug: Melphalan|Drug: Tacrolimus|Drug: Mycophenolate Mofetil|Drug: G-CSF
Sponsor/Collaborators:	M.D. Anderson Cancer Center|ViaCell|National Institutes of Health (NIH) National Cancer Institute (NCI)
Gender:	Both
Age:	1 Year to 80 Years Â (Child, Adult, Senior)
Phases:	Phase 1
Enrollment:	125
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	2005-0781|NCI-2011-02823|RP100469 02|1P01CA148600-01A1 5R01CA061508-20
First Received:	6-Jul-07
Start Date:	Jul-07
Completion Date:	null
Last Updated:	2-Nov-15
Last Verified:	Nov-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Jul-17
Outcome Measures:	Engraftment and Time to Engraftment
URL:	https://ClinicalTrials.gov/show/NCT00498316
Study 68:	
NCT Number:	NCT02484560
Title:	Efficacy of Stem Cell Therapy in Ambulatory and Non-ambulatory Children With Duchenne Muscular Dystrophy - Phase 1-2
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Duchenne Muscular Dystrophy
Interventions:	Drug: Biological: Umbilical Cord Based Allogenic Mesenchymal Stem Cell
Sponsor/Collaborators:	University of Gaziantep|IstÄ±nye University, Cukurova University, YÄ±ldÄ±rÄ±m BeyazÄ±t University, Gaziantep Deva Hospital, Gaziantep Public Hospital
Gender:	Male
Age:	8 Years to 14 Years Â (Child)
Phases:	Phase 1
Enrollment:	10
Study Types:	Interventional

Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	56733164/203
First Received:	16-Jun-15
Start Date:	Jun-15
Completion Date:	Dec-15
Last Updated:	30-Jun-15
Last Verified:	Jun-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-15
Outcome Measures:	Degree of improvement in patients with Duchenne Muscular Dystrophy after stem cell therapy treatment administered using Northstar Ambulatory Assessment, Magnetic Resonance Imaging & Spectroscopy, muscle strength assessment equipment, and a questionnaire.
URL:	https://ClinicalTrials.gov/show/NCT02484560
Study 69:	
NCT Number:	NCT02672280
Title:	Safety and Exploratory Efficacy Study of Collagen Membrane With Mesenchymal Stem Cells in the Treatment of Skin Defects
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Wounds|Diabetic Foot Ulcers|Burns
Interventions:	Device: Medical Collagen Membrane with MSC Device: Medical Collagen Membrane
Sponsor/Collaborators:	South China Research Center for Stem Cell and Regenerative Medicine
Gender:	Both
Age:	18 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	30
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	UCMSC-3
First Received:	31-Jan-16
Start Date:	May-16
Completion Date:	Dec-17
Last Updated:	31-Jan-16
Last Verified:	Jan-16
Acronym:	SEESCMMSCTSD
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-17
Outcome Measures:	Incidence of adverse events that are related to study treatment and associated with the grafting site|Percentage of wound closure as determined|Scar outcome assessment Incidence of contracture release or revision surgeries|Incidence of increased temperature sensitivity|Incidence of paresthesias, pain, dulling of sensation assessed|Incidence and severity of infections at grafting sites|Incidence of all adverse events|Percentage area of re-grafting as determined
URL:	https://ClinicalTrials.gov/show/NCT02672280
Study 70:	
NCT Number:	NCT02786017
Title:	Injectable Collagen Scaffoldâ„¢ Combined With HUC-MSCs Transplantation for Patients With Decompensated Cirrhosis
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Decompensated Cirrhosis

Interventions:	Biological: Conventional therapy|Biological: Injectable Collagen Scaffold + HUC-MSCs
Sponsor/Collaborators:	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Both
Age:	18 Years and older Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	CAS-XDA-DC/IGDB
First Received:	25-May-16
Start Date:	May-16
Completion Date:	Dec-18
Last Updated:	25-May-16
Last Verified:	Apr-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-18
Outcome Measures:	Improvement of liver function measured by change in the model for end-stage liver disease (MELD) score|Improvement of liver function measured by change in Child-Pugh score|Change in clinical laboratory parameters of liver function|30-Day Survival|Change in the size of liver and spleen and inner diameter of spleen portal venous
URL:	https://ClinicalTrials.gov/show/NCT02786017
Study 71:	
NCT Number:	NCT02633163
Title:	MsciSLE: MSCs in SLE Trial
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Systemic Lupus Erythematosus
Interventions:	Drug: Low Dose Mesenchymal Stem Cells (MSCs)|Drug: High Dose Mesenchymal Stem Cells (MSCs)|Drug: Placebo Infusion
Sponsor/Collaborators:	Medical University of South Carolina
Gender:	Both
Age:	18 Years to 65 Years Â (Adult)
Phases:	Phase 2
Enrollment:	81
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Investigator)|Primary Purpose: Treatment
Other IDs:	MUSC-UCMSC-001
First Received:	15-Dec-15
Start Date:	Jul-16
Completion Date:	Jun-21
Last Updated:	1-Sep-16
Last Verified:	Dec-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Jun-21
Outcome Measures:	Clinical response defined by the SLE Responder Index|Change in SLEDAI score between groups|Renal and non-renal organ system flares
URL:	https://ClinicalTrials.gov/show/NCT02633163
Study 72:	
NCT Number:	NCT02755376

Title:	Development of Novel Strategy for Treatment of Anterior Cruciate Ligament (ACL) Injury Using Stem Cell
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Deficiency of Anterior Cruciate Ligament
Interventions:	Biological: Cartistem(TM)|Biological: hyaluronic acid|Procedure: ACL reconstruction
Sponsor/Collaborators:	Samsung Medical Center
Gender:	Both
Age:	20 Years to 50 Years Â (Adult)
Phases:	
Enrollment:	30
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Treatment
Other IDs:	SMC 2013-07-117-002
First Received:	22-Feb-15
Start Date:	Jan-14
Completion Date:	Dec-17
Last Updated:	25-Apr-16
Last Verified:	Feb-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Jan-14
Outcome Measures:	Bone formation on interface between bone tunnel and graft|Arthroscopic grading of graft|Telos stress X-ray|KT-2000|clinical knee scoring|Instability assessing with physical examination|Tunnel enlargement after anterior cruciate ligament reconstruction
URL:	https://ClinicalTrials.gov/show/NCT02755376
Study 73:	
NCT Number:	NCT02890953
Title:	Efficacy and Safety of PneumostemÂ® for IVH in Premature Infants (Phase 2a)
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Cell Transplantation
Interventions:	Drug: Pneumostem|Drug: Normal saline
Sponsor/Collaborators:	Samsung Medical Center
Gender:	Both
Age:	up to 28 Days Â (Child)
Phases:	Phase 2
Enrollment:	22
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	2016-06-005
First Received:	31-Aug-16
Start Date:	Sep-16
Completion Date:	null
Last Updated:	13-Sep-16
Last Verified:	Sep-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Aug-19
Outcome Measures:	Death or shunt operation|ventricular dilatation|Death
URL:	https://ClinicalTrials.gov/show/NCT02890953
Study 74:	
NCT Number:	NCT01626677

Title:	Follow-Up Study of CARTISTEMÂ® Versus Microfracture for the Treatment of Knee Articular Cartilage Injury or Defect
Recruitment:	**Completed**
Study Results:	No Results Available
Conditions:	Degenerative Osteoarthritis|Defect of Articular Cartilage
Interventions:	Biological: CARTISTEM|Procedure: Microfracture
Sponsor/Collaborators:	Medipost Co Ltd.|Dong-A ST Co., Ltd.
Gender:	Both
Age:	18 Years and older Â (Adult, Senior)
Phases:	Phase 3
Enrollment:	103
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CARTISTEM_CR_F/U
First Received:	19-Jun-12
Start Date:	Jun-12
Completion Date:	May-15
Last Updated:	6-Jan-16
Last Verified:	Sep-14
Results First Received:	No Study Results Posted
Primary Completion Date:	May-15
Outcome Measures:	Degree of improvement in knee assessments compared to the active control (microfracture)|Number of subjects with adverse events
URL:	https://ClinicalTrials.gov/show/NCT01626677
Study 75:	
NCT Number:	NCT02378974
Title:	Evaluation of the Safety and Potential Therapeutic Effects After Intravenous Transplantation of Cordstem-ST in Patients With Cerebral Infarction
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Cerebral Infarction
Interventions:	Biological: Cordstem-ST|Biological: Placebo
Sponsor/Collaborators:	CHABiotech CO., Ltd
Gender:	Both
Age:	19 Years to 80 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	18
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver, Investigator Primary Purpose: Treatment
Other IDs:	CHA-CST-101
First Received:	16-Feb-15
Start Date:	Feb-15
Completion Date:	null
Last Updated:	26-Jul-16
Last Verified:	Jul-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-18

Outcome Measures:	Number of treatment related-adverse events during the study period Improvement in clinical function as assessed by Modified Rankin Score(mRS) compared to baseline at 6 months|Improvement in clinical function as assessed by National Institute of Health Stroke Scale (NIHSS) compared to baseline at 6 months|Improvement in clinical function as assessed by Barthel Index (BI) compared to baseline at 6 months Improvement in clinical function as assessed by Brain MRI tratogram compared to baseline at 6 months
URL:	https://ClinicalTrials.gov/show/NCT02378974
Study 76:	
NCT Number:	NCT01929434
Title:	Efficacy of Stem Cell Transplantation Compared to Rehabilitation Treatment of Patients With Cerebral Paralysis
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Cerebral Palsy
Interventions:	Other: rehabilitation|Procedure: stem cell injection
Sponsor/Collaborators:	General Hospital of Chinese Armed Police Forces
Gender:	Both
Age:	1 Year to 14 Years Â (Child)
Phases:	Phase 3
Enrollment:	300
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Outcomes Assessor)|Primary Purpose: Treatment
Other IDs:	2013-05-13 CP III
First Received:	20-Aug-13
Start Date:	Oct-13
Completion Date:	Dec-15
Last Updated:	17-Jul-15
Last Verified:	Jul-15
Acronym:	CP
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-15
Outcome Measures:	Gross Motor Function Measure Score|Routine Blood Test and Biochemical Test
URL:	https://ClinicalTrials.gov/show/NCT01929434
Study 77:	
NCT Number:	NCT02274428
Title:	Phase 1 Clinical Trial of PNEUMOSTEMÂ® Treatment in Premature Infants With Intraventricular Hemorrhage
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Mesenchymal Stromal Cells
Interventions:	Drug: pneumostem
Sponsor/Collaborators:	Samsung Medical Center
Gender:	Both
Age:	23 Weeks to 34 Weeks Â (Child)
Phases:	Phase 1
Enrollment:	9
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	2014-06-103
First Received:	2-Oct-14
Start Date:	Oct-14

Completion Date:	Dec-16
Last Updated:	21-Apr-16
Last Verified:	Apr-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	unsuspected death or anaphylactic shock|Death or hydrocephalus required shunt
URL:	https://ClinicalTrials.gov/show/NCT02274428
Study 78:	
NCT Number:	NCT02672306
Title:	Safety & Exploratory Efficacy Study of UCMSCs in Patients With Alzheimer's Disease
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Alzheimer's Disease
Interventions:	Biological: UCMSCs|Biological: Placebo
Sponsor/Collaborators:	South China Research Center for Stem Cell and Regenerative Medicine Third Affiliated Hospital, Sun Yat-Sen University|Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University|Guangzhou Psychiatric Hospital
Gender:	Both
Age:	50 Years to 85 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	40
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Caregiver)|Primary Purpose: Treatment
Other IDs:	UCMSC-1
First Received:	24-Jan-16
Start Date:	May-16
Completion Date:	Oct-19
Last Updated:	29-Jan-16
Last Verified:	Jan-16
Acronym:	SEESUPAD
Results First Received:	No Study Results Posted
Primary Completion Date:	Oct-18
Outcome Measures:	Change in Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-Cog) Score|Change in Alzheimer's Disease Cooperative Study Clinician's Global Impression of Change (ADCS-CCGIC) Score|Change in Mini-Mental State Examination (MMSE) Score|Change in Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory (ADCS-ADL) Score|Change in Neuropsychiatric Inventory (NPI) Score Changes in AD Biomarkers|Change from the baseline in cerebrospinal fluid (CSF) biomarkers
URL:	https://ClinicalTrials.gov/show/NCT02672306
Study 79:	
NCT Number:	NCT02138331
Title:	Effect of Microvesicles and Exosomes Therapy on Î²-cell Mass in Type I Diabetes Mellitus (T1DM)
Recruitment:	Enrolling by invitation
Study Results:	No Results Available
Conditions:	Diabetes Mellitus Type 1
Interventions:	Biological: MSC exosomes.
Sponsor/Collaborators:	General Committee of Teaching Hospitals and Institutes, Egypt
Gender:	Both
Age:	18 Years to 60 Years Â (Adult)
Phases:	Phase 2|Phase 3
Enrollment:	20
Study Types:	Interventional

Study Designs:	Endpoint Classification: Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	666666|Cell Free MSC Exo
First Received:	12-May-14
Start Date:	Apr-14
Completion Date:	Sep-14
Last Updated:	12-May-14
Last Verified:	May-14
Results First Received:	No Study Results Posted
Primary Completion Date:	Jul-14
Outcome Measures:	Total daily insulin dose|Pancreatic Î²-cell Mass
URL:	https://ClinicalTrials.gov/show/NCT02138331
Study 80:	
NCT Number:	NCT02644447
Title:	Transplantation of HUC-MSCs With Injectable Collagen Scaffold for POF
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Premature Ovarian Failure
Interventions:	Biological: HUC-MSCs Transplantation|Biological: HUC-MSCs with Injectable Collagen Scaffold Transplantation
Sponsor/Collaborators:	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Female
Age:	20 Years to 39 Years Â (Adult)
Phases:	Phase 1|Phase 2
Enrollment:	20
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Double Blind (Subject, Outcomes Assessor Primary Purpose: Treatment
Other IDs:	CAS-XDA-POF/IGDB
First Received:	28-Dec-15
Start Date:	Oct-15
Completion Date:	Oct-18
Last Updated:	9-Apr-16
Last Verified:	Dec-15
Results First Received:	No Study Results Posted
Primary Completion Date:	Oct-18
Outcome Measures:	Safety and Tolerability assessed by Adverse Events|Number of Antral follicle development|Estradiol (E2) serum level|Follicle Stimulating Hormone (FSH) serum level|Anti-Mullerian Hormone (AMH) serum level|Pregnancy rate
URL:	https://ClinicalTrials.gov/show/NCT02644447
Study 81:	
NCT Number:	NCT02745808
Title:	Injectable Collagen Scaffoldâ„¢ Combined With HUC-MSCs for the Improvement of Erectile Function in Men With Diabetes
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Erectile Dysfunction|Type 1 Diabetes Mellitus|Type 2 Diabetes Mellitus
Interventions:	Biological: HUC-MSCs|Biological: Injectable Collagen Scaffold + HUC-MSCs
Sponsor/Collaborators:	Chinese Academy of Sciences|The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Gender:	Male
Age:	20 Years to 65 Years Â (Adult)
Phases:	Phase 1

Enrollment:	30
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Single Blind (Subject)|Primary Purpose: Treatment
Other IDs:	CAS-XDA-DEF/IGDB
First Received:	12-Apr-16
Start Date:	Sep-15
Completion Date:	Apr-17
Last Updated:	18-Apr-16
Last Verified:	Apr-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Dec-16
Outcome Measures:	Safety and Tolerability assessed by Adverse Events|Improvement in IIEF-5 (International Index of Erectile Function)|Improvement in penile colour Doppler
URL:	https://ClinicalTrials.gov/show/NCT02745808
Study 82:	
NCT Number:	NCT02000362
Title:	Safety and Efficacy of FURESTEM-CD Inj. in Patients With Moderately Active Crohn's Disease(CD)
Recruitment:	Recruiting
Study Results:	No Results Available
Conditions:	Crohn's Disease
Interventions:	Biological: Stem cells
Sponsor/Collaborators:	Kang Stem Biotech Co., Ltd.
Gender:	Both
Age:	20 Years to 70 Years Â (Adult, Senior)
Phases:	Phase 1|Phase 2
Enrollment:	24
Study Types:	Interventional
Study Designs:	Endpoint Classification: Safety/Efficacy Study|Intervention Model: Single Group Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	KSB-CD
First Received:	7-Nov-13
Start Date:	Aug-14
Completion Date:	Aug-17
Last Updated:	5-Oct-16
Last Verified:	Sep-16
Results First Received:	No Study Results Posted
Primary Completion Date:	May-17
Outcome Measures:	Number of Participants with Adverse Events|ratio of patients who is applicable to CDAI<150|the ratio of patients who reduce CDAI over 70 as contrasted with baseline value|a variation of CRP value as contrasted with baseline|a variation of CDEIS value as contrasted with baseline|a variation of MR enterographic score as contrasted with baseline|a variation of fecal calprotectin as contrasted with baseline|a variation of IBDQ score as contrasted with baseline|a variation of SF-36 score as contrasted with baseline|reduction of the number of draining fistula|all kinds of adverse effects which occur during the clinical study
URL:	https://ClinicalTrials.gov/show/NCT02000362
Study 83:	
NCT Number:	NCT02918123
Title:	Safety of FURESTEM-CD Inj. in Patients With Moderate to Severe Plaque-type Psoriasis
Recruitment:	Not yet recruiting
Study Results:	No Results Available
Conditions:	Psoriasis

Interventions:	Biological: FURESTEM-CD Inj.
Sponsor/Collaborators:	Kang Stem Biotech Co., Ltd.
Gender:	Both
Age:	19 Years to 65 Years Â (Adult)
Phases:	Phase 1
Enrollment:	9
Study Types:	Interventional
Study Designs:	Intervention Model:Single Group Assignment|Masking:Open Label Primary Purpose: Treatment
Other IDs:	KSB-PsO
First Received:	27-Sep-16
Start Date:	Nov-16
Completion Date:	Dec-20
Last Updated:	5-Oct-16
Last Verified:	Oct-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Nov-20
Outcome Measures:	number of adverse events|safety lab tests, physical examination, ECG, vital signs|variation of Cytokine, PASI, BSA
URL:	https://ClinicalTrials.gov/show/NCT02918123
Study 84:	
NCT Number:	NCT01854567
Title:	P3 Study of Umbilical Cord Blood Cells Expanded With MPCs for Transplantation in Patients With Hematologic Malignancies
Recruitment:	Active, not recruiting
Study Results:	No Results Available
Conditions:	Acute Myelogenous Leukemia|Acute Lymphoblastic Leukemia, Non-Hodgkin's Lymphoma, Hodgkin's Disease
Interventions:	Biological: Infusion of one MPC expanded cord unit and one unexpanded cord unit|Biological: Infusion of two unexpanded cord blood units.
Sponsor/Collaborators:	Mesoblast, Ltd.
Gender:	Both
Age:	up to 65 Years Â (Child, Adult)
Phases:	Phase 3
Enrollment:	240
Study Types:	Interventional
Study Designs:	Allocation: Randomized|Endpoint Classification: Safety/Efficacy Study Intervention Model: Parallel Assignment|Masking: Open Label|Primary Purpose: Treatment
Other IDs:	CB-AB006|2012-0166
First Received:	13-May-13
Start Date:	Feb-13
Completion Date:	Jul-18
Last Updated:	21-Jul-16
Last Verified:	Jul-16
Results First Received:	No Study Results Posted
Primary Completion Date:	Feb-18
Outcome Measures:	Time to Neutrophil and Platelet Engraftment|Proportion of subjects with neutrophil recovery at day 26, platelet recovery at day 60 and subjects alive at day 100|Percentage of patients with primary graft failure
URL:	https://ClinicalTrials.gov/show/NCT01854567

SECTION 8: SELECTED REFERENCES

Research

Original Investigation

Risk of Persistent Palatal Fistula in Patients With Cleft Palate

Mairaj K. Ahmed, DDS, MS; Anthony L. Maganzini, DDS, MSD; Paul R. Marantz, MD, MPH; Joseph J. Rousso, MD

IMPORTANCE Many individuals with a cleft palate also have an associated craniofacial syndrome or anomaly.

OBJECTIVE To investigate the predictive associations of persistent palatal fistulas in patients with previously repaired cleft palate.

DESIGN, SETTING, AND PARTICIPANTS We performed a case-control study of patients with cleft palate repairs from January 1, 1986, through December 31, 2000, at a major tertiary care hospital center in the Bronx, New York. The study population consisted of patients who had their primary surgery before the age of 3 years and had all their cleft-related treatment completed at the same hospital center. Palatal fistula was defined as a breakdown of the primary surgical repair of the palate, resulting in persistent patency between the oral and nasal cavities. Data collection was conducted by using the hospital centers' electronic medical records and patient tracking systems and confirmed by review of hard copies of patient records.

MAIN OUTCOMES AND MEASURES The Veau classification system was used to classify the preoperative cleft severity.

RESULTS A total of 130 patients were identified—23 patients with palatal fistula and 107 controls. A total of 12 girls and 11 boys were identified in the palatal fistula group and 56 girls and 51 boys in the control group. The mean patient age at the time of palatoplasty was 12.6 and 14.5 months in the palatal fistula and control groups, respectively. A statistically significant association was found between the outcome of fistula and severity of cleft, as defined by the Veau classification system ($P = .01$). Furthermore, for each Veau class increase, the odds of a palatal fistula increased by 2.64 (95% CI, 1.35-5.13; $P = .004$). No statistically significant associations were found between the outcome of fistula and the following independent variables: patient sex ($P = .98$), patient age at palatoplasty ($P = .82$), type of palatoplasty ($P = .57$), surgeon ($P = .15$), orthodontic treatment ($P = .59$), ear infection ($P = .30$), or clefts associated with syndromes ($P = .96$).

CONCLUSIONS AND RELEVANCE Palatal fistulas are reliably associated with severity of cleft, as defined by the Veau classification system. This knowledge gives the health care professional a more reliable method of preoperatively assessing the risk of postoperative palatal fistula in the cleft palate population.

LEVEL OF EVIDENCE 3.

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Cleft lip or palate is the fourth most common birth defect and the most common craniofacial anomaly, affecting 1 in 500 to 750 live births in the United States and totaling approximately 7500 cases per year.[1,2] Between 5% and 15% of individuals with a cleft have an associated craniofacial syndrome or anomaly.[1]

Historically, the treatment of palatal clefts was through the use of obturators. In 1837, Dieffenbach[3] first described the use of relaxing incisions to aid in palatal closure, and several techniques have evolved since then to surgically close the palate.[4,5] This method of closure has markedly improved speech, feeding, and other functional outcomes.[6-8] The drawbacks of primary closure include palatal fistulas, velopharyngeal insufficiency, deficient anterior-posterior maxillary growth, and deficient vertical midfacial development.[9,10]

Palatal fistulas are defined as a failure of healing or a breakdown in the primary surgical repair of the palate, resulting in a patency between the oral and nasal cavities. The Pittsburgh Fistula Classification System[11] includes 7 types of fistula (uvula, soft palate, junction of hard and soft palate, hard palate, junction of primary and secondary palate, lingual-alveolar, and labial-alveolar). Fistulas may be symptomatic or asymptomatic. Partially because of this, several studies[11-13] have documented vague or nonexistent descriptions of fistulas in medical records. In addition, the definition of fistulas in the literature has been ambiguous and inconsistent.[12,13]

A symptomatic fistula can lead to several problems, such as oronasal fluid and food regurgitation, malodorous discharge, rhinitis, hearing loss, audible nasal air escape during speech, and hypernasality. The incidence of fistula diagnosis during cleft palate treatment has been reported to range from 9% to 50%.[14,15] The rate of fistula recurrence ranges from 35% to 75%.[12,16]

The basis of this study is to determine a reliable method to help the physician make a preoperative, evidence-based assessment of the risk of fistula formation. This assessment is based on specific characteristics associated with the patient with cleft palate. Risk factors can be divided into factors determined during initial clinical presentation or factors related to treatment rendered. Risk factors based on clinical presentation include extent of clefting, presence or absence of syndrome, and medical or dental history. Extrinsic factors include type of palatoplasty, age at palatoplasty, operator skill or experience, feeding protocols after surgery, and orthodontic treatment. Although there are many possible untoward postoperative complications that can arise from palatoplasty, our study is limited to investigating postoperative palatal fistula.

Methods

Montefiore Medical Center Internal Review Board approval for this case-control study was granted on October 9, 2008. Patients who received cleft-related surgical care at outside institutions were excluded based on the variability of protocols and techniques among different institutions. Patients with syndrome-associated and non-syndrome-associated cleft were included. Additional exclusion criteria consisted of incomplete medical records, insufficient follow-up as defined by fewer than 3 postoperative examinations, and/or patients who had palatoplasty surgery at older than 3 years. Matching based on race was not performed because of a lack of biologic plausibility and challenges in data collections. Specifically, in our hospital system, race is a self-reported entry, which has poor levels of consensus (60%-66%).[17,18] Generally speaking, the patient population at the hospital center where this study was conducted is racially heterogenous.

Table 1. Veau Classification of Clefts

Veau Class	Description	No. (%) of Study Participants	
		Cases (n = 23)	Controls (n = 107)
I	Soft palate	0	15 (14.0)
II	Soft and hard palate	6 (26.1)	44 (41.1)
III	Soft and hard palate and unilateral prepalatal clefts	12 (52.2)	42 (39.3)
IV	Soft and hard palate and bilateral prepalatal clefts	5 (21.7)	6 (5.6)

Data collection was conducted digitally using the electronic medical records system and data mining system. Once patient demographic information was obtained, additional clinical information was obtained and confirmed by searching hard copies of patient records. Data were collected for all male and female patients with cleft palate from January 1, 1986, through December 31, 2000.

Palatal fistula was defined as a failure of healing or a breakdown in the primary surgical repair of the palate, resulting in a patency between the oral and nasal cavities that persisted for at least 1 year after surgery. Controls were those who did not have the presence of a fistula, had all cleft-related treatment at the main hospital center, and were examined at least 3 times postoperatively. The Veau classification system was used to classify the preoperative cleft severity (**Table 1**).

Exploratory univariate associations were investigated using *t* tests and 2 × 2 and 2 × 4 contingency table analysis. Those variables with $P < .20$ were included in an ordinal logistic regression model. Statistical analysis was performed using STATA statistical software, version 10 (Stata Corp).

Results

Statistical analysis was performed for 23 cases and 107 controls. As indicated in **Table 2**, a statistically significant association was found between the outcome of fistula and severity of cleft, as defined by the Veau classification system. No significant associations were found between the outcome of fistula and the following predictor variables: patient sex, patient age at palatoplasty, type of palatoplasty, orthodontic treatment, ear infection, surgeon, or clefts associated with syndromes.

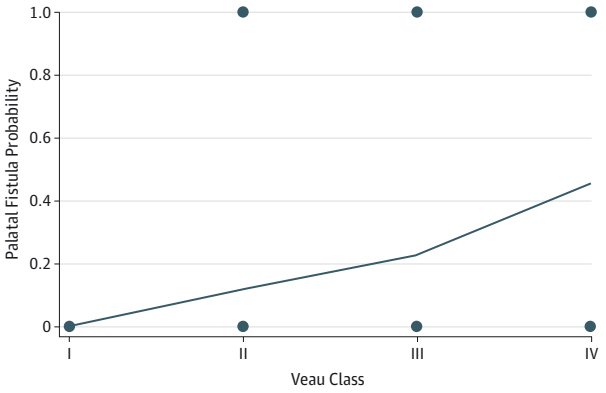
Logistic regression analysis revealed that for each Veau class increase, the odds of a palatal fistula increased by 2.64 (95% CI, 1.35-5.13; $P = .004$). This association is shown in the **Figure**. This association remained statistically significant after including the variable "surgeon" in the model.

Table 2. Summary of Univariate Analysis[a]

Variable	Cases (n = 23)	Controls (n = 107)	*P* Value
Patient sex			
Male	11 (47.8)	51 (47.7)	.98
Female	12 (52.5)	56 (52.3)	
Age at palatoplasty, mean, mo	12.6	14.5	.82
Type of palatoplasty			
Furlow	0	6 (5.6)	.57
Vomer	5 (21.7)	16 (15.0)	
von Langenbeck	1 (4.3)	3 (2.8)	
Other	17 (73.9)	82 (76.6)	
Veau class			
I	0	15 (14.0)	.01
II	6 (26.1)	44 (41.1)	
III	12 (52.2)	42 (39.3)	
IV	5 (21.7)	6 (5.6)	
Clefts associated with syndromes			
Nonsyndromic	21 (91.3)	98 (91.6)	.96
Syndromic	2 (8.7)	9 (8.4)	
Ear infection			
Yes	16 (69.6)	62 (57.9)	.30
No	7 (30.4)	45 (42.1)	
Orthodontic treatment			
Yes	4 (17.4)	14 (13.1)	.59
No	19 (82.6)	93 (86.9)	
Surgeon			
X	9 (39.1)	61 (57.0)	.15
Y	9 (39.1)	36 (33.6)	
Others	5 (21.7)	10 (9.3)	

[a] Data are presented as number (percentage) of study participants unless otherwise indicated.

Figure. Linear Association Between Veau Class and Palatal Fistula Probability



Bandwidth was 0.8.

Discussion

Our finding of increasing severity of cleft palate that resulted in increased postoperative complication rates is not surprising. This concept has been supported in many forms, with various outcome measures. The Veau classification system is commonly used as an objective measure of cleft severity and is outlined in Table 1. The general association between fistula rates and the Veau classification system has been recognized by several authors.[12,16,19-21] Among these studies,[12,16,19-21] which found an association between increased risk of fistula with higher Veau class, there was no quantified risk in terms of odds ratios. However, Jackson et al[22] found a statistically significant difference in the odds of developing postoperative fistulas when comparing preoperative Veau class IV and other classes, but this finding did not hold significance when comparing Veau class III with Veau class I and II.

To our knowledge, this is the first study to report a statistically significant quantification of the risk among individual Veau classes; for each Veau class increase, the odds of a palatal fistula increased by 2.64 times.

Because of the multifactorial nature of cleft palate care, data are contradictory regarding risk factors for fistulas. Regardless of discrepancies, the increased data from large centers continue to paint a clearer picture of optimal care of the patients with cleft palate.

Other studies[23,24] have assessed similar outcomes and associations with preoperative cleft widths. In 2009, Parwaz et al[23] found that a width of 15 mm or greater was significantly associated with risk of fistula. In assessing the development of postoperative velopharyngeal insufficiency, Lam et al[24] found that a cleft width of 10 mm increased the risk by roughly 4.5 times compared with narrower clefts. Similarly, we noted corresponding physician annotation with preoperative statements that indicated wide clefts in the patients with fistula; however, no quantifiable classification system was used to describe the width in many of these records. Therefore, we suggest objective and consistent measurements of cleft width as standard documentation in all patients with cleft palate.

This study did not find an association between the use of orthodontic treatment and fistula formation. These findings support those of Muzaffar et al[20] and contrast those of other studies.[16,25] However, orthodontic protocols, including technique and timing, vary widely among institutions, which may account for these discrepancies in study data. Regarding presurgical orthopedics, protocols of nasoalveolar molding are associated with low rates of postoperative fistula formation, as demonstrated by Dec at al.[26] This finding may be due to its role in decreasing the preoperative cleft width; therefore, we recommend that this variable should be included in any multivariable assessment of fistulas.

Although statistical significance was not obtained in regard to surgical timing, we saw an association in palatal fistula formation in those patients whose primary surgery was performed before 6 months of age. This group was relatively small (3 fistula formations in 5 patients) because of the institutional acceptance of waiting until 10 months of age before performing most primary palatoplasty operations. In addition, no statistically significant association was found between operating surgeon and fistula outcome. This finding is

in agreement with the study by Al-Nawas et al,[27] who found no significant association among patient age, experience of surgeon, and duration of surgery in early outcomes of palatoplasty. Conversely, Lu et al[28] found associations between severity of the cleft and skill of the surgeon. In our study, on comparing the 2 highest-volume surgeons to others, the others group had a nonstatistically significant fistula outcome (P = .08). The lack of significance in comparing surgeons may be a result of similar surgical techniques used among surgeons at the institution. Studies that compared differing surgical techniques have produced different results. For example, in a high-volume review, Landheer et al[29] found that 2-stage palate closures have higher rates of fistula formation (27%) when compared with a 1-stage repair (14%).

Admittedly, some factors have limited the power of this study, particularly incomplete records and the large number of patients who have had portions of their early treatment at other centers. However, inclusion of any of these patients in our study would have added too many confounding variables and delegitimized our statistically significant findings.

Bresnick et al[30] reported that patients with Treacher Collins syndrome were more likely to develop fistulas than nonsyndromic individuals. Most studies have seemingly excluded syndromic patients based on biological concerns. For example, the presence of hypotonic palatal musculature in velocardiofacial syndrome can be a confounding factor. In addition, there is some thought that syndromic cleft cases generally necessitate relatively complex care. We did not find this to be the case and found no significant association between patients with syndrome-associated clefts and those with isolated clefts. This finding is in concordance with Stransky et al,[31] who found no significant association in the rates of secondary surgery for velopharyngeal insufficiency or postoperative oronasal fistula between patients with and without Pierre Robin sequence.

Individuals with cleft palate have a higher incidence of otitis media with effusion.[32-34] Sheahan et al[35] found that 76% of patients with cleft lip and palate, 68% of patients with cleft palate only, and 16% of patients with cleft lip had a history of an ear infection or hearing loss. The research of Bluestone et al[36,37] implicated an anatomical functional obstruction of the eustachian tube at the nasopharyngeal end, where it meets 2 muscles—the levator palatini and tensor veli palatini. Because of the anatomical communication between the cavity of the middle ear and the muscles of the palate, a history of middle ear disease and subsequent surgical treatment has been postulated as a risk factor for palatal fistulas. This finding led us to research otitis media as an independent variable in palatal fistula formation, though we found no associations.

Conclusions

This study indicates that the risk of developing a persistent postoperative palatal fistula is related to the preoperative Veau class, and each increase in classification level independently increases the risk by a multiple of 2.64. Armed with this information and data, the cleft surgeon can have a reasonable understanding of the postoperative likelihood of palatal fistula in preoperatively assessing a child with a cleft palate.

ARTICLE INFORMATION

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Author Contributions: Drs Ahmed and Rousso had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Maganzini.
Acquisition, analysis, or interpretation of data: Ahmed, Marantz, Rousso.
Drafting of the manuscript: Ahmed, Rousso.
Critical revision of the manuscript for important intellectual content: Maganzini.
Statistical analysis: Ahmed, Marantz, Rousso.
Administrative, technical, or material support: Maganzini.
Study supervision: Maganzini, Marantz.

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Airway Obstruction Following Palatoplasty: Analysis of 247 Consecutive Operations

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Objective and Methods: **Between February 1987 and September 1997, 247 patients underwent primary repair of a cleft of the secondary palate by one surgeon, using the double-opposing Z-plasty (Furlow) technique. This retrospective study reviews perioperative and postoperative airway compromise among these patients. The purposes of this study were to identify factors associated with airway obstruction following palatoplasty and to analyze the management of those patients. Although infants experiencing airway problems following Wardill-Kilner and Von Langenbeck palatoplasty have been described, airway complications in a group of Furlow repair patients has not been previously reported.**

Results: **Fourteen patients (5.7%) had airway problems. The average age of these patients was 18 months, which was not significantly different from those without airway problems. Airway obstruction occurred as late as 48 hours after the completion of surgery. Twelve of the 14 patients had severe airway compromise requiring continued postoperative intubation, reintubation, or tracheostomy (one). There were no deaths. Thirteen of the 14 patients with postoperative airway problems (93%) had other congenital anomalies in addition to clefting, a named congenital disorder, or both. Seven of those 13 had Pierre Robin sequence. In contrast, only 40 of the 233 patients without airway problems (17%) had additional congenital anomalies or named disorders. Presence of other congenital anomalies was associated with a significantly increased risk of airway obstruction ($p = .005$).**

Conclusion: **Patients with cleft palate with the Pierre Robin sequence or other additional congenital anomalies had an increased risk of airway problems following palatoplasty. Awareness of this risk permits identifying those patients prior to surgery so that they can be monitored and managed appropriately, minimizing the likelihood of major complications or death.**

KEY WORDS: *airway obstruction, cleft palate, double-opposing Z-plasty, Furlow palatoplasty*

Although airway obstruction has been reported as a complication of cleft palate repair employing Wardill-Kilner or Von Langenbeck palatoplasty techniques, with or without a posterior pharyngeal flap (Lee and Kingston, 1985; Orr et al., 1987; Patane and White, 1989; Chan et al., 1995), there have been no studies of perioperative and postoperative airway compromise following the Furlow (double-opposing Z-plasty) palate repair. This study was a retrospective analysis of airway complications in a large group of consecutive Furlow palatoplasties by a single surgeon.

METHODS

All palatoplasties performed by one surgeon (G.M.S.) between February 1987 and September 1997 were reviewed. In that period of time, 247 palatoplasties were performed, all using the technique that has been described by Randall et al. (1986). All children who experienced perioperative or postoperative airway compromise following surgery were analyzed in detail. Factors that were examined included presence of associated congenital anomalies (such as micrognathia with or without the specific diagnosis of Pierre Robin sequence), duration of surgery, age at time of surgery, and severity of perioperative and postoperative airway difficulty. Chi-square analysis was used to analyze the data.

All procedures were performed using general orotracheal anesthesia and a Dingman retractor. All repairs involved a two-

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TABLE 1 Congenital Anomalies in 53 of the 247 Palatoplasty Patients

Patients	*Anomaly*
22 (8.9%)	Pierre Robin sequence
12 (4.9%)	Cardiac ± other
7 (2.8%)	Hand
6 (2.4%)	Urinary
3 (1.2%)	Other craniofacial
2 (0.8%)	Van der Woude syndrome
1 (0.4%)	CHARGE* association

* CHARGE = coloboma, heart defects, atresia choanae, retarded growth and development, genital hypoplasia, and ear anomalies.

layered closure with 4-0 Vicryl on a curved taper (RB1) needle (Ethicon, Inc., Somerville, NJ). Tongue sutures were used only in patients who showed signs or symptoms of airway edema while on the operating table. Patients were not routinely admitted to the pediatric intensive care unit (ICU) unless they had signs or symptoms of airway problems in the operating room or the postanesthesia recovery unit. Nasopharyngeal airways were not routinely used following surgery.

Results

Of the 247 patients undergoing Furlow palatoplasty during the study period, 14 (5.7%) had documented perioperative or postoperative airway compromise. Seven were boys and seven were girls. Nine of these patients (64%) underwent a palatoplasty alone or with PE tube placement; two patients (14%) had palatoplasty in conjunction with division of a previous tongue-lip adhesion; one patient (7%) underwent palatoplasty along with a lingual frenuloplasty; one patient (7%) had palatoplasty plus dental restorations; and one patient (7%) underwent palatoplasty with an orchidopexy. None of the patients had concomitant attachment of a posterior pharyngeal flap.

Of the total group (n = 247), 53 patients (21.5%) had other congenital anomalies, with or without a named genetic disorder. Twenty-two children were micrognathic and had been given the diagnosis of Pierre Robin sequence; one patient had coloboma, heart defects, atresia choanae, retarded growth and development, genital hypoplasia, and ear anomalies (CHARGE) association; two patients had Van der Woude syndrome; and 28 patients had other cardiac, urinary, hand, or craniofacial anomalies (Table 1).

Of the 14 patients with airway problems, 13 (93%) had other anomalies. Of these 13 patients, seven (54%) had Pierre Robin sequence; one patient had CHARGE association; and five patients had other central nervous system, cardiac, hand, or craniofacial anomalies. One patient had tetralogy of Fallot, agenesis of the corpus callosum, coloboma of the left eye, and strabismus; one patient had multiple craniofacial anomalies with frontonasal dysplasia and tracheal stenosis; one patient had left thumb duplication, ventricular septal defect, and patent ductus arteriosus; one patient had bilateral microtia, a lateral facial cleft, and absence of the right thumb; and one patient had microcephaly, microstomia, and bronchopulmonary dysplasia. The distribution of additional anomalies in the group of patients with obstruction is shown in Table 2.

TABLE 2 Other Congenital Anomalies That Were Present in 13 of the 14 Patients With Airway Compromise*

Patients	*Anomaly*
7 (50%)	Pierre Robin sequence
1 (7%)	Cardiac, CNS, eye
1 (7%)	Frontonasal dysplasia
1 (7%)	Bilateral microtia, facial cleft, hand
1 (7%)	Cardiac, hand
1 (7%)	CHARGE association
1 (7%)	Microcephaly, small mouth, BPD
1 (7%)	None

* CNS = central nervous system; CHARGE = coloboma, heart defects, atresia choanae, retarded growth and development, genital hypoplasia, and ear anomalies; BPD = bronchopulmonary dysplasia.

Of the 233 patients without airway problems, 40 (17%) had additional congenital anomalies or named disorders. The presence of congenital anomalies in addition to clefting was associated with a 476-fold increased risk of airway obstruction ($p = .005$).

The time of airway obstruction varied in the 14 patients. Eight of the 14 episodes of airway compromise (57%) occurred in the operating room, either during the procedure or on extubation; 3 of the 14 episodes (21%) occurred in the first 24 hours after repair; and 3 of the 14 (21%) occurred 24 to 48 hours after repair.

The severity of the obstruction determined the necessary treatment. Mild obstruction was successfully treated by positioning the patient lateral or prone, with complete resolution of the symptoms; moderate obstruction was treated by positioning but with continued symptoms, although not requiring reintubation; and severe airway compromise was treated with either continued intubation or reintubation. Of the 14 patients experiencing perioperative and postoperative respiratory compromise, one (7%) experienced mild obstruction, one (7%) experienced moderate obstruction, and 12 (86%) had severe obstruction and were reintubated or kept intubated. The patient with airway problems who did not have any other anomalies was in the severe airway compromise group. One (7%) eventually required tracheostomy. There were no deaths.

The overall mean age at palatoplasty for the 14 patients with airway compromise was 18 months, with a range of 7 to 52 months. The mean surgical duration was 145 minutes, with a range of 90 to 200 minutes. The overall mean weight at time of surgery was 9.8 kg, with a range of 7.5 to 14.6 kg. None of these parameters differed significantly between those patients with and those without airway compromise.

Case Reports

Case 1

A 2-year-old girl underwent Furlow palatoplasty and division of a previous surgical tongue-lip adhesion. The patient had multiple anomalies, including bilateral microtia, a lateral facial cleft, and absence of the right thumb. She had undergone tongue-lip adhesion for upper airway obstruction at age 4 months. Intubation for the palatoplasty was moderately diffi-

cult. Surgical duration was 95 minutes. At the completion of the surgical procedure, the patient was extubated but had significant airway compromise because of lingual edema and was reintubated in the operating room. The patient was then transferred to the intensive care unit and remained intubated for 7 days, with her course complicated by pulmonary sepsis, atelectasis, and prolonged ileus. She was extubated on the seventh postoperative day. On postoperative day 10, she was discharged home.

Case 2

A 21-month-old boy had an unrepaired wide U-shaped cleft of the secondary palate. The patient had multiple congenital anomalies and a diagnosis of Pierre Robin sequence. Previous surgical history included a tongue-lip adhesion, which was not divided at the time of palatoplasty, and release of the genioglossus muscle. There were no problems during surgery or following extubation in the operating room. The patient began experiencing airway difficulty 36 hours postoperatively and was reintubated. He remained intubated for 10 days and was unable to be weaned off the ventilator. Endoscopic examination done at that time revealed four levels of airway obstruction. Therefore, a tracheostomy was performed in addition to division of the tongue-lip adhesion. At 4-month postoperative checkup, the palate appeared intact and well healed. The tracheostomy was still present.

Discussion

Postoperative airway obstruction can range from mild episodes of stridor to severe blockage of the airway requiring intubation or tracheostomy. Airway obstruction following Wardill-Kilner or Von Langenbeck repair is well described (Lee and Kingston, 1985; Orr et al., 1987; Patane and White, 1989; Chan et al., 1995). In addition, patients with Franceschetti syndrome or Pierre Robin sequence have been reported to have increased risk for developing airway obstruction following palatoplasty. Several authors (Lee and Kingston, 1985; Wood, 1994; Lehman et al., 1995) have expressed concern over a shallow nasopharyngeal airway and insufficient maxillofacial growth at the time of palate repair in those patients. Airway obstruction can result in damage to the freshly repaired palate (because of traumatic establishment of an airway) or even death.

We used the Furlow technique of palatoplasty during this study period because of several perceived advantages. These included improved mobilization of the palatal levator muscles, lengthening of the soft palate, nonoverlapping suture lines, reduced need for secondary surgery, and improved speech results (Cohen et al., 1989; Furlow, 1990; Spauwen et al., 1992; Horswell et al., 1993). There has been little in the published literature describing airway obstruction following Furlow palate repair. Three cases of mild postoperative airway obstruction manifested as stridor have been noted, which spontaneously resolved with management by positioning (Furlow, 1986; Horswell et al., 1993). Furlow (1990) also reported one patient who required emergency intubation after repair.

The occurrence of airway obstruction following Furlow cleft palate repair, in our series, was 5.7% (14 of 247 patients experienced some degree of perioperative airway obstruction, postoperative airway obstruction, or both). Factors have been identified that seem to increase the risk of perioperative and postoperative airway obstruction. These include presence of associated congenital anomalies or a named disorder, particularly the Pierre Robin sequence, as well as history of previous airway problems.

Airway problems in patients with craniofacial anomalies are a well-recognized phenomenon (Perkins et al., 1997). Patients with Pierre Robin sequence may maintain a compromised but compensated airway, which becomes evident at the time of cleft palate surgery (Hoffman et al., 1965; Lehman et al., 1995). Our experience indicates that the presence of other congenital anomalies with or without a named syndrome poses an increased risk for the development of postoperative airway obstruction.

The ideal timing of cleft palate repair is controversial. Two opposing factors must be considered, maximizing speech outcome and minimizing airway compromise. Furlow (1990) recommends the double-opposing Z-plasty before the patient begins to speak. Some centers postpone primary repair of the hard palate in the hopes of minimizing interference with maxillary growth (Wood, 1994), though delayed repair can be associated with poor speech outcome (Jackson et al., 1983). Furlow (1990) describes the double-opposing Z-plasty as providing minimal disturbance to the hard palate, resulting in only mild malocclusion correctable with orthodontics in his patients. The mean age of the patients in this study who had airway problems following palatoplasty did not significantly differ from those without airway problems.

Much of the literature suggests that prolonged duration of surgery has a direct correlation with increased incidence of airway obstruction. In the case reports involving Wardill-Kilner and Von Langenbeck procedures that precipitated acute events of airway obstruction, surgical times are given as 3.6 hours or greater, with a mean of 4.1 hours (Musto et al., 1977; Lee and Kingston, 1985; Bell et al., 1988; Patane and White, 1989; Chan et al., 1995). In our study, the mean surgical duration was 2.4 hours, with a range of 1.5 to 3.3 hours. These operative times are within the range of other published times for the double-opposing Z-plasty (Horswell et al., 1993) as well as other techniques (Dreyer and Trier, 1984).

Published reports show that airway obstruction involving other palatoplasty procedures often occurs within the first 2 hours postoperatively. In this study, the majority of patients requiring continued intubation or reintubation (8 of 14, 57%) occurred in the operating room. Another three patients manifested in the first 24 hours after repair. However, three patients presented as late as 24 to 48 hours postoperatively.

Twelve of our 14 patients with airway problems (86%) required intubation or tracheostomy. Because of the insidious and serious nature of the airway obstruction presenting as late

as 24 to 48 hours postoperatively, careful observation for signs or symptoms of lingual and suprahyoid edema, and admission to the ICU in questionable cases, is recommended. At the first sign of serious airway obstruction, reintubation is recommended.

Several authors have proposed mechanisms by which airway obstruction following palatoplasty may occur. Excessive pressure exerted on the base of the tongue by the Dingman retractor may produce glossal hematomas, necrosis of tongue muscle, venous stasis, or lymphedema (Bell et al., 1988). Another possible source of glossal edema may be ischemia induced by the Dingman mouth gag (Senders and Fung, 1991). Lee and Kingston (1985) suggest periodic release of the mouth gag to prevent prolonged ischemia of the tongue. That might lessen airway obstruction in high-risk patients. Hyperextension of the head or Trendelenburg positioning may also contribute to impaired arterial inflow and decreased venous drainage of the tongue (Lee and Kingston, 1985; Bell et al., 1988; Chan et al., 1995).

The double-opposing Z-plasty involves more extensive surgical dissection than other techniques (Spauwen et al., 1992). The additional dissection may pose an increased risk for upper airway obstruction, particularly in the child with other craniofacial anomalies. Our study found an incidence of 5.7% of upper airway obstruction following Furlow palatoplasty. This compares with a large series of pharyngeal flap surgery, well known for precipitating airway obstruction, which found a 4% incidence of major airway obstruction, almost exclusively occurring in patients with Pierre Robin sequence or large tonsils, most of whom required emergency tracheostomy (Graham et al., 1973). Another study found a 9% incidence of postoperative upper airway obstruction within 24 hours of operation in patients following pharyngeal flap surgery (Valnicek et al., 1994).

Conclusions

Perioperative and postoperative airway obstruction following palatoplasty has been documented in relatively few studies. In our experience, the incidence and severity of airway compromise following Furlow palatoplasty was over 5% and can be anticipated in certain high-risk patients. Children with a history of associated congenital anomalies are especially prone to airway compromise. In our series, operative times were within the range of published times, and none of the patients experiencing airway difficulties was operated on at an unusually early age. Therefore, it seems prudent to monitor those patients identified as having higher risk of airway problems. Maintained intubation or reintubation at the first sign of serious airway obstruction should be considered to prevent serious sequelae. Airway morbidity following Furlow palatoplasty may stem from the more extensive surgical dissection involved in the procedure along with reduced airway reserve in children with other craniofacial anomalies, particularly micrognathia and the Pierre Robin sequence. Three of our 14 patients with airway problems had undergone simultaneous lingual frenuloplasty or division of tongue-lip adhesion. We no longer combine those procedures with palatoplasty. In addition, all of our patients with Pierre Robin sequence are now electively scheduled to spend the first postoperative night in the pediatric ICU, and we have a lower threshold for placing a tongue suture, which can be easily removed the following morning.

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Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: A preliminary report

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ABSTRACT

The purpose of this study was to evaluate the enhancing effect of recombinant platelet derived growth factor on human mesenchymal stem cells (hMSCs) in secondary alveoloplasty. Three patients with 4 alveolar defects were selected for this study. Mesenchymal stem cells were cultured from a posterior iliac bone aspirate. MSCs were mounted on biphasic scaffolds and combined with platelet derived growth factor (PDGF) in the operating room to make a triad of the scaffold, growth factor, and cells. The triads were placed in anterior maxillary cleft defects and closed with lateral advancement gingival flaps. The postoperative cleft bone volume was measured with cone beam computed tomography scans. A mean of 51.3% fill of the bone defect was calculated 3 months post-operation. Our data suggests the use of recombinant platelet derived growth factor with hMSCs may enhance the regeneration capacity of the cells.

1. Introduction

Secondary alveolar bone grafting is an essential part of the management of cleft palate patients. The commonest site for acquiring autogenous bone for grafting has been the anterior iliac crest (Eufinger and Leppänen, 2000). The use of synthetic bone substitute has been advocated for the reconstruction of small bony defects whenever the growth of the canine teeth was completed. To reduce donor site morbidity (Dawson et al., 1996; Hoard et al., 1998) and enhance bone regeneration, growth factors such as bone morphogenic proteins (BMPs) and platelet derived growth factors (PDGF) in combination with different kinds of bone substitutes were examined. Platelet rich plasma (PRP) contains many growth factors including platelet derived growth factors (PDGF), vascular endothelial growth factors (VEGF), and transforming growth factor-β (TGF-β) (Mohan and Baylink, 1991; Pierce et al., 1992; Marx et al., 1998; Marx, 2004; Moghadam et al., 2001). There are several studies that used PDGF in combination with autogenous bone grafts or synthetic bone materials to enhance bone regeneration (Pierce et al., 1992; Marx et al., 1998). The efficacy of rhBMP-2 in inter-body spinal fusion has been evaluated in several clinical studies (Burkus et al., 2003a, 2003b). Adding rhBMP-2 to animal critical sized defects has increased the amount of the new bone trabeculae and decreased the rate of wound failure (Jovanovic et al., 2007). Reduced morbidity and improved bone healing were demonstrated in older cleft patients treated with BMP-2 (Dickinson et al., 2008). With the advent of in vivo tissue engineering methods, mesenchymal stromal cells and osteoprogenitor cells were also considered as a possible treatment solution since they were capable of increasing the number of potentially osteogenic cells. Bone marrow aspirate soaked in absorbable collagen sponge was reported as an alternative method for the closure of human alveolar clefts (Gimbel et al., 2007). Mesenchymal stem cells (MSCs) loaded on biphasic hydroxyapatite/tricalcium phosphate (HA/TCP) showed enhancement of the bone regeneration in dog mandible defects (Jafarian et al., 2008). The MSCs loaded on biphasic calcium phosphate scaffolds revealed more bone formation than scaffolds carried on PRP (Khojasteh et al., 2008) when combined with MSCs, the carrying capabilities and ectopic bone formation potency of biphasic bone substitutes were also reported to be more than natural bovine derived bone mineral (Eslami et al., 2008). Lack of xenogenic growth factors such as foetal calf serum in the in vitro culture of human MSCs might have a weakening effect on the bone formation capacity of cells and may decrease the human

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applicability of these cells (Behnia et al., 2009). Mesenchymal stem cells loaded on a collagen sponge showed reduced postoperative morbidity (Gimbel et al., 2007). Adding the MSCs to the biomaterial can improve bone formation (Sauerbier et al., 2010). Combination of growth factors with human derived MSCs may strengthen these cells and encourage the clinical use of MSCs. In this study PDGF was combined with MSCs loaded on HA/TCP and applied in alveolar clefts. The platelet fibrin that could be harvested at the same time as the production of the PDGF was used to cover the defects.

2. Material and methods

2.1. Patients

Three patients with the mean age of 10 year were selected for this study. The only remaining surgical procedure in the patients was secondary alveolar bone grafting. 4 alveolar cleft were treated with this method as one of the patients had a bilateral alveolar cleft. The risks and benefits of this procedure were explained to the patients. The families agreed to the procedure despite of the absence of well-documented data to support stem cell induced bone regeneration in cleft patients. All procedures were approved by the institutional ethical committee and informed consent was obtained from all donors. Isolation and cultivation of the MSCs was performed without xenogenic supplements such as foetal calf serum (FCS). All the methods used in the cultivation and extraction of the MSCs were exactly the same as our previous projects in human subjects (Shayesteh et al., 2008; Behnia et al., 2009). Computed tomography scans were done before surgery for all the patients.

2.2. Isolation and cultivation of mesenchymal stem cells

In the cell culture lab, human MSCs were isolated (Kadiyala et al., 1997). Two weeks before surgery a bone marrow aspirate (10–15 ml) was obtained from the posterior iliac crest. The aspirate was diluted at 1:3 in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Paisley, UK). On day 1, non-adherent cells were discarded and adherent cells were washed with phosphate-buffered saline (PBS) (Gibco) and then cultured in DMEM medium with antibiotics and 20% autologous serum.

2.3. Preparation of human serum

FCS was replaced by human serum because of concerns of the ethical committee. From each patient 20 ml of whole blood was drained into blood bags (Baxter, Deerfield, IL), quickly transferred to 10 ml vacationer tubes without anticoagulants (BD, Plymouth, UK), and allowed to clot for 4 h at 4 °C–8 °C. Subsequently, the blood was centrifuged at 1800 g at 4 °C for 15 min. Serum was collected and filtered through a 0.2 m membrane (Sarstedt, Nümbrecht, Germany). Aliquots of the sterile serum were stored at −20 °C. The lab process and cultivation of the cells for each patient was performed without significant salience.

2.4. Evaluation of the mesenchymal stem cell nature of the isolated cells

2.4.1. Flow cytometry analysis

Fluorescence absorbance cell sorting (FACS) analysis was performed using standard protocols and quantification criteria. The gate to distinguish positive from negative cells was set individually for each marker. Fluorescent isothiocyanate (FITC) – conjugated monoclonal antibodies against CD34 (Miltenyi Biotech, Bergisch Gladbach, Germany), CD44, CD29, CD105, CD13 (BD Biosciences; San Diego, CA, USA) at a concentration of 2 μg/ml at 4 °C were used for 30 min. The cells stained with FITC-labelled mouse IgG were used as negative controls. The cells were washed twice with PBS and fixed with 1% Para formaldehyde. Analysis was performed on 100,000 cells per sample and the positive expression was defined as the level of fluorescence greater than 99% of the corresponding unstained cell sample.

2.4.2. In vitro osteogenic differentiation

To identify the isolated cell as the MSCs, their differentiation potential into osteogenic cells were evaluated as detailed below. Cells from third passage were provided with DMEM medium supplemented with 50 μg/ml ascorbic 2-phosphate (Sigma, Aldrich, St. Louis, MO, USA), 10 nM dexamethasone (Sigma) and 10 mM ß-glycerol phosphate (Sigma). After 3 weeks, differentiated cells were fixed for 1 h with 4% formalin and rinsed with PBS (Gibco). Mineralization of the extracellular matrix was visualized by staining with 40 mM Alizarin Red S, pH 4.2, for 5 min (Fig. 1). The osteogenic differentiation capacity of ASCs was further studied by reverse transcriptase polymerase chain reaction (RT-PCR).

2.4.3. Reverse transcription polymerase chain reaction (RT-PCR) analysis

Total cellular RNA was extracted using TRI-reagent (Sigma). Before the reverse transcription, the RNA samples were digested with DNase I (Fermentas) to remove the contaminating genomic DNA. Standard reverse-transcription reaction was performed with 2 μg total RNA using random hexamer as a primer and Revert Aid TM H Minus First Strand cDNA Synthesis Kit (Fermentas) according to the manufacture's instructions. Subsequent PCR was as following: 2.5 μl cDNA, 1X PCR buffer (AMS), 200 μM dNTPs, 0.2 μM of each primer pair and 1 unit/25 μl reaction Taq DNA polymerase (Fermentas) depending on the abundance of particular mRNA. Real-time PCR was conducted using the primers osteocalcin (OC), osteopontin (OP), PTH and β-Tubulin. Primer sequences have been described in Table 1.

2.4.4. Implant preparation

In all cases, 1 day before transplantation, implants were loaded with the cells obtained from the third subculture. HA/TCP (Reprobone, Ceramisys, Sheffield, England), polygonal 3 mm cubes were loaded with MSCs by placing 5×10^5 cells in 0.2 ml DMEM medium and blend it with the cubes. The material consisted of 60% HA and 40% TCP with a mean pore size of 300 μm–500 μm.

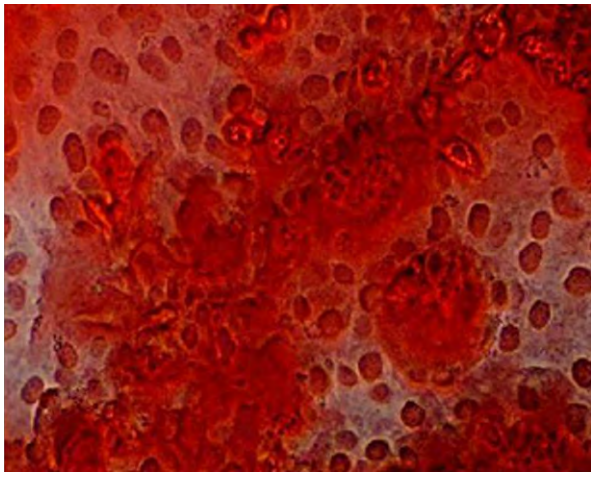

Fig. 1. Alizarin red staining of the MSCs in Osteogenic medium.

2.5. Surgical procedures

2.5.1. Incision design

Surgery was carried out under general anaesthesia. Following a crestal incision at the level of the gingival sulcus, dissections were made in the scar tissue to reach the bony surface of the cleft walls. The tissue was then elevated beneath the periosteum plane to the levels of the anterior nasal spine anteriorly, the lateral piriform rim superiorly, and to the alveolar ridges inferiorly. The flaps of the nasal floor and the oral mucosa formed the ceiling and the floor of the cleft cavity, respectively. Concomitant injection of Cefazolin (1 gr) was used during the perioperative period and followed by a 3 day course post operatively.

2.5.2. PDGF and PRF production

PDGF and PRF were prepared for each patient according to the manufacturers protocol. From each patient 30 cc of blood was drained and divided to the 6 citrated tubes. The plasma is centrifuged using digital equipment (model P.R.G.F- GAC Medicale, Spain) at a speed of 460 G for 8 min at room temperature. The plasma was separated in to 2 fractions by means of a pipette. The first 0.5 ml from this was used to produce platelet rich fibrin. The remaining plasma fraction which was located immediately above the red blood cells was aspirated in to the separated tube after centrifuging of all samples. 50 μl of 10% calcium chloride was added for each ml of aggregated plasma to activate the platelet and releasing of the growth factors. The superior fraction which contained platelet poor plasma was also collected in one tube and activated with calcium chloride. A fibrin plug could be seen from the remaining plasma after 20–30 min.

2.5.3. Scaffold implantation

The PDGF was mixed with the scaffold seeded with MSCs and made into a triple structure (Bone substitute, MSCs, PDGF). The scaffold was transferred to the defect using micro-forceps. The fibrin plug was placed over the enriched synthetic biphasic bone substitute (Fig. 2). The flap was subsequently closed in a water-tight tension free manner. 3 months after surgery the patients underwent a CT scan. The outline of 1 mm axial sections of the alveolar defect was used to determine the preoperative defect, postoperative defect, and volume of bone fill obtained by Image Pro software (NIH). The margin of the original defects could be seen in the CT scan.

3. Results

3.1. In vitro differentiation

The surface marker analysis by flow cytometry showed positive expression for adhesion molecules CD 13 (Aminopeptidase N), CD

Table 1
RT-PCR primers for bone specific gene expression analysis.

Genes	Primer sequences	Size (bp)	Annealing temperature (C°)
Osteocalcin (OC)	F 5′ GAC CAT CTT TCT GCT CAC TCT G 3′ R 5′ GTG ATA CCA TAG ATG CGT TTG TAG 3′	276	60
Osteopontin(OP)	F 5′ CAG TGA TTT GCT TTT GCC TGT TTG 3′ R 5′ GGT CTC ATC AGA CTC ATC CGA ATG 3′	377	67
PTH receptor	F 5′ GAC AAG CTG CTC AAG GAA GTT CTG 3′ R 5′ GGA ATA TCC CAC GGT GTA GAT CAT G 3′^	448	67
B-Tubulin	F 5′ GGAACATAGCCG TAAACTGC 3′ R 5′ TCACTGTGCCTGAACTTACC 3′	317	63

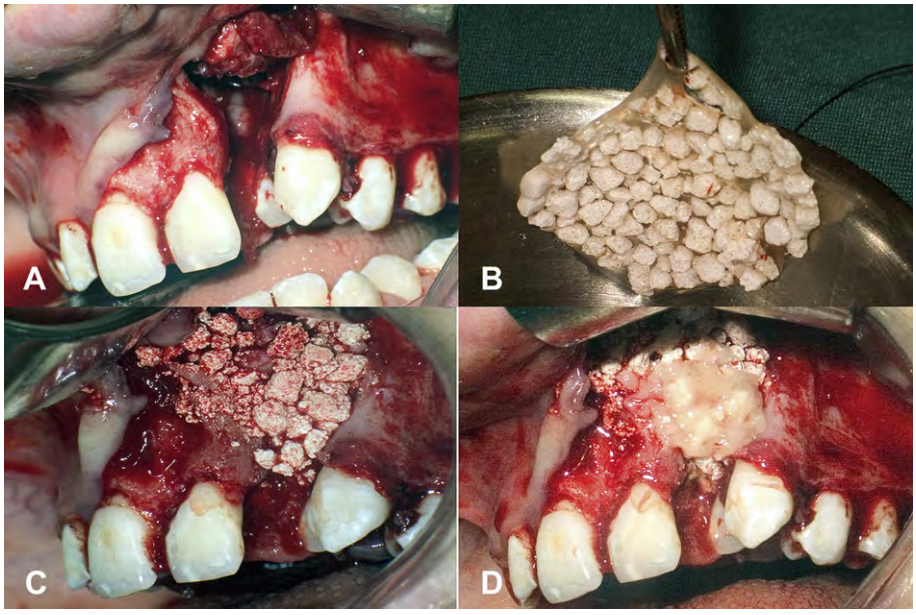

Fig. 2. (A). Exposing of the entire cleft and suturing of the nasal floor; (B). The HA/TCP scaffolds – loaded hMSCs combined with patient derived PDGF; (C). The defect was overfilled with triad structure; (D). The platelet fibrin product was placed over the grafting material.

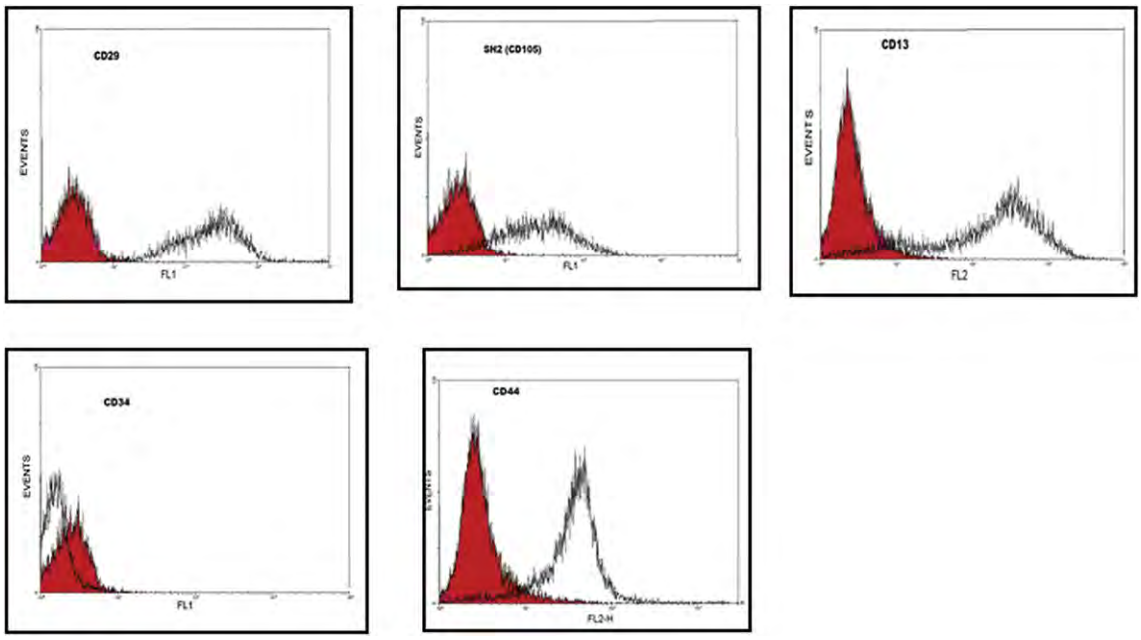

Fig. 3. Surface marker differentiation of hMSCs analysed by flow cytometric analysis.

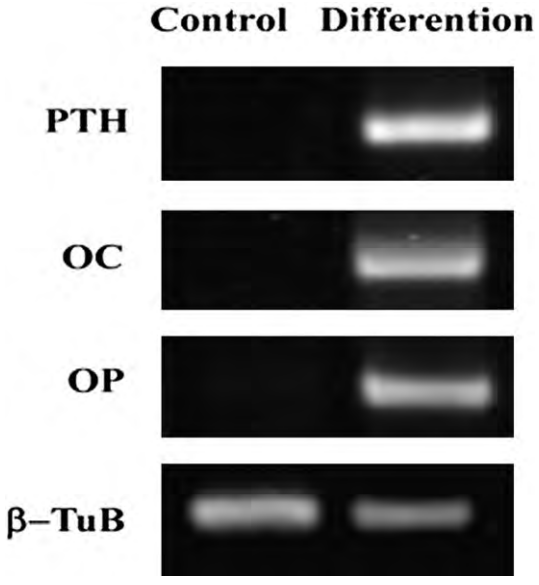

Fig. 4. Reverse transcription–polymerase chain reaction analysis. Osteopontin, Osteocalcin, parathyroid hormone expression after 3 weeks.

29 (Integrin b1), CD 44 (Hyaluronate), CD 105 (SH-2, Endoglin) and lack of expression for the CD 34 (Sialomucin-like adhesion molecule) (Fig. 3). RT-PCR confirmed elevated expression of bone associated marker osteopontin (OP), Osteocalcin (OC), parathyroid hormone (PTH) in comparison with control group after 3 weeks of osteogenic medium induction (Fig. 4).

3.2. *In vivo bone formation*

There was successful healing with no fistula or oro-nasal communication in all cases. The mean postoperative defect fill was measured 51.3% in 4 alveolar pre-maxillary clefts 3 months post operatively (Fig. 5). The patients were referred back to the department of orthodontics to start orthodontic tooth alignment (Table 2).

4. Discussion

Human bone marrow derived mesenchymal stem cells (hMSCs) appear to be a popular source of adult stem cells and nowadays are widely used in various cell therapy and tissue engineering procedures (Warnke et al., 2004). These cells have not been shown to be perfectly capable of osteogenesis in an in vitro or in vivo environment, nevertheless their behaviour is different and less predictable in a living context. Several studies carried out on animal and human models have not been able to demonstrate a significant increase in bone augmentation with the application of MSCs (Jafarian et al., 2008; Khojasteh et al., 2008; Eslami et al., 2008; Behnia et al., 2009). A previous study has shown that when compared to platelet derived growth factors, MSCs are capable of inducing new bone formation (Khojasteh et al., 2008). Defect size and site play an important role in the process of healing and osteogenesis and the anterior maxillary cleft seems to be a challenge. Pradel et al. have demonstrated the successful use of differentiated osteogenic cells for cleft repair in a case report, concluding that their method can lead to spontaneous tooth eruption on the cleft side (Pradel et al., 2008). Meanwhile, another case study on the effectiveness of MSCs for anterior maxillary cleft augmentation demonstrated disappointing results with insufficient amounts of bone formation (Behnia et al., 2009). The design of this study aimed to assess the use of MSCs loaded on HA/TCP scaffolds in combination with PDGF for alveolar cleft regeneration. The purpose was to make a triad for cell based tissue engineering (Fig. 6). Parallel with cell based strategies for in vivo bone repair, the use of growth factors such as morphogenic proteins has attracted much attention in different treatment protocols (Urist et al., 1978; Klongnoi et al., 2006a, 2006b; Herford et al., 2007; Lee et al., 2009).

Lee et al. proved an early positive effect of PRP on bone healing in secondary alveolar bone grafts, but failed to demonstrate the late effects (Lee et al., 2009). Klongnoi et al. did not report a beneficial use of PRP, combined with flourohydroxyapatite in a procedure of

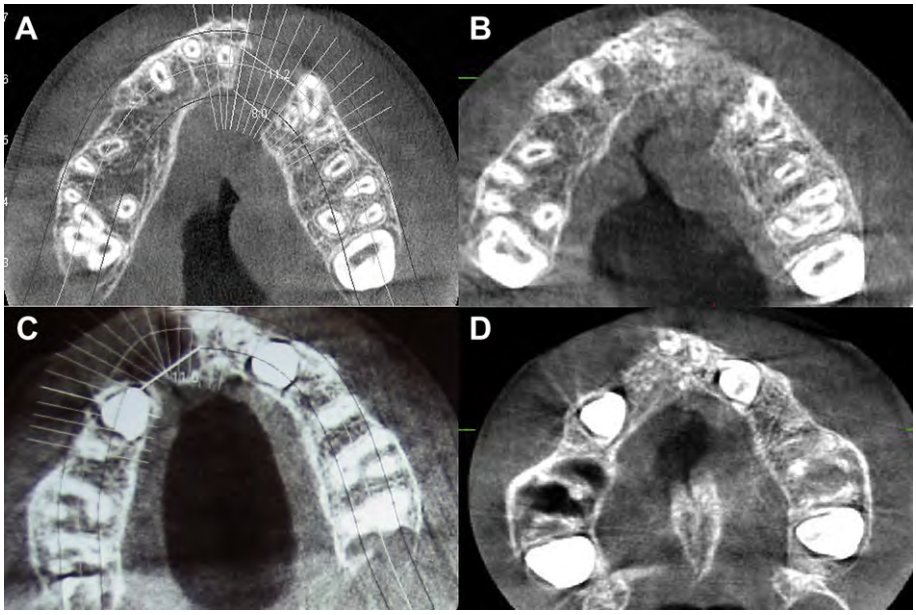

Fig. 5. Radiographic investigation 3 months after the surgery demonstrated reconstruction of the nasal floor and filling of the alveolar defect.

Table 2
The amount of new regenerate tissue in maxillary clefts.

Gender	Defect type	Age at surgery (Y.O)	3 months postoperative bone volume (%)
F	Right PMC	13	54.8
F	Right PMC	8	50.8
	Left PMC		48.3
M	Right PMC	9	51.2
		10 (Mean)	51.3 (Mean)

Abbreviation: PMC, premaxillary cleft; F, female; M, male.

sinus augmentation (Klongnoi et al., 2006a, 2006b). Various combinations of rhBMP-2 with different kinds of bone substitutes have shown promising results. Critical size animal defects have been successfully healed with rhBMP-2 (Urist et al., 1978; Jovanovic et al., 2007). However, the application of rhBMP-2 in human subjects has not been as reliable. 1 year post-operation, an adult alveolar cleft treated with rhBMP-2 showed no significant bone regeneration (Lynch et al., 2008). Herford et al. reported a bone volume ratio of 71% in children with premaxillary clefts treated with a combination of absorbable collagen membrane and rhBMP-2 (Herford et al., 2007). In this study the authors found that PDGF may have an augmenting effect on the capacity of hMSCs for bone

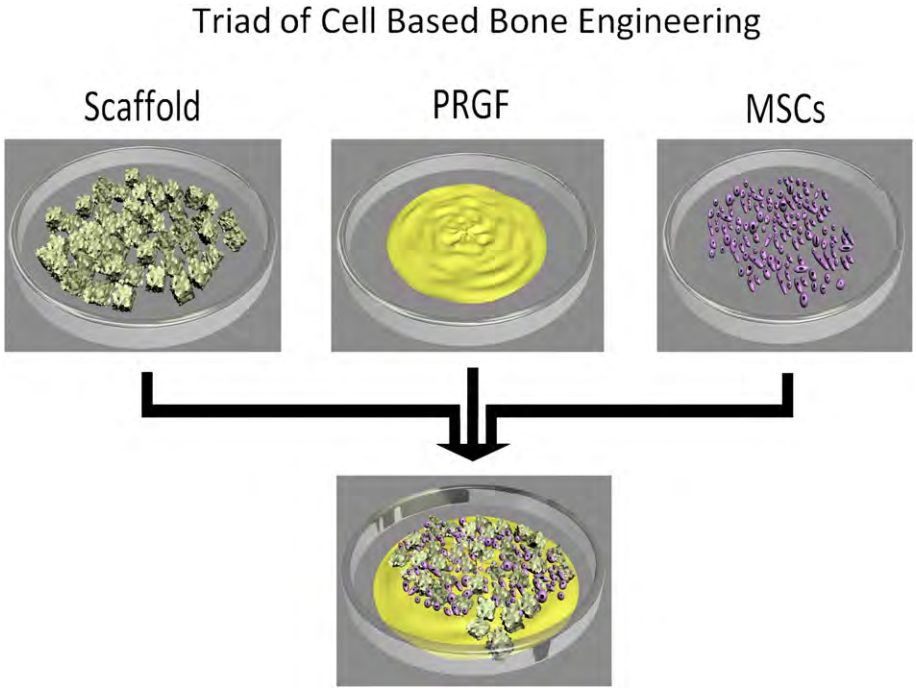

Fig. 6. Schematic of the cell based tissue engineering.

regeneration. The mean amount of regenerated bone (51.3%) achieved with the present protocol appears lower than the amount of maxillary cleft repair induced by rhBMP-2 or autogenous iliac graft (Herford et al., 2007). In addition, the amount of graft resorption in a treated cleft area is reported to be higher than other augmented maxillofacial sites (Tai et al., 2000; van der Meij et al., 2003). As a result, although the use of hMSCs with PDGF increases bone regeneration when compared with unaccompanied hMSCs (Behnia et al., 2009), the results are far from being satisfactory. The high cost of rhBMP-2 and the donor site morbidities of an iliac bone graft is the driving engine for investigators to search further for an ideal method of in vivo bone engineering. The patients in this study have not subsequently been treated with any other kind of surgical procedures, so histological evaluation of the amount of newly generated bone or the remnant part of the bone substitute could not be carried out. This study can therefore not be compared to other similar studies (Matsui et al., 2006) in a quantitative method. The second criticism of this study is the absence of a control group. This was unavoidable due to ethical issues but unfortunately has reduced the validity of the data.

5. Conclusion

A combination of human derived mesenchymal stem cells with platelet growth factors may enhance the capabilities of the cells in the clinical situation but further clinical studies are needed to find a predictable way to ensure success.

Acknowledgement

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The Therapeutic Potential, Challenges and Future Clinical Directions of Stem Cells from the Wharton's Jelly of the Human Umbilical Cord

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Abstract Mesenchymal stem cells (MSCs) from bone marrow, adult organs and fetuses face the disadvantages of invasive isolation, limited cell numbers and ethical constraints while embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) face the clinical hurdles of potential immunorejection and tumorigenesis respectively. These challenges have prompted interest in the study and evaluation of stem cells from birth-associated tissues. The umbilical cord (UC) has been the most popular. Hematopoietic stem cells (HSCs) harvested from cord blood have been successfully used for the treatment of hematopoietic diseases. Stem cell populations have also been reported in other compartments of the UC viz., amnion, subamnion, perivascular region, Wharton's jelly, umbilical blood vessel adventia and endothelium. Differences in stemness characteristics between compartments have been reported and hence derivation protocols using whole UC pieces containing all compartments yield mixed stem cell populations with varied characteristics. Stem cells derived directly from the uncontaminated Wharton's jelly (hWJSCs) appear to offer the best clinical utility because of their unique beneficial properties. They are non-controversial, can be harvested painlessly in abundance, proliferative, possess stemness properties that last several passages in vitro, multipotent, hypoimmunogenic and do not induce tumorigenesis even though they have some ESC markers. hWJSCs and its extracts (conditioned medium and lysate) also possess anti-cancer properties and support HSC expansion ex vivo. They are thus attractive autologous or allogeneic agents for the treatment of malignant and non-malignant hematopoietic and non-hematopoietic diseases. This review critically evaluates their therapeutic value, the challenges and future directions for their clinical application.

Keywords Standardization of derivation protocols · Properties and applications of Wharton's jelly stem cells · Umbilical cord compartments

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Introduction

Various types of stem cells have been isolated to date in the human from a variety of tissues including preimplantation embryos, fetuses, birth-associated tissues and adult organs. They can be broadly classified into embryonic stem cells (ESC), mesenchymal stem cells (MSC) and hematopoietic stem cells (HSC) based on biochemical and genomic markers. The plasticity of the stem cells in these three categories are also different, with the most versatile being ESCs which theoretically can be differentiated into almost all tissues in the human body and hence have been labeled as pluripotent or the 'mother of all cells'. Pluripotency in ESCs is defined as the ability of these cells to produce tissues from all three germ layers (ectoderm, mesoderm and endoderm) when transplanted into immunodeficient mice. ESCs are generated from surplus In Vitro Fertilization (IVF) embryos, MSCs from fetal and adult tissues and HSCs from the bone marrow, peripheral and umbilical cord blood (UCB). ESCs, MSCs and HSCs follow established paradigms of human development in vivo governed by the programmed pathways of their genomes.

ESCs, HSCs and MSCs from adult and fetal tissues have their own limitations. ESCs are controversial and their derived tissues pose the risks of immunorejection and tumorigenesis. To overcome the problem of immunorejection protocols were

developed where tissues could be personalized to patients by transfecting the patient's somatic cells with pluripotent genes to produce human induced pluripotent stem cells (hiPSCs) from which desirable tissues could be derived for the patient for transplantation therapy. Viral and non-viral reprogramming methods have been successfully developed but unfortunately epigenetic changes in the form of chromosomal duplications and deletions have been reported in the ensuing hiPSCs [1]. It was postulated that such genetic changes arise at three different points in the reprogramming protocol: (i) those already present in the parent somatic cell that is being reprogrammed; (ii) induced during reprogramming and (iii) induced during serial culture [2]. These genetic changes are probably brought about by in vitro manipulation as the hiPSCs are not given the opportunity to go through the natural established in vivo paradigms of human development. Additionally, hiPSCs induce tumorigenesis in immunodeficient mice and such teratoma formation is faster and more efficient than their hESC counterparts [3]. Hence, until these clinical hurdles are overcome the use of hiPSCs is limited only to the study of the pathogenesis of disease and as platform technology for drug screening and discovery.

Fetal MSCs are controversial as they are derived from human abortuses. MSCs from adult organs have limitations in terms of cell numbers and as such require expansion in vitro running the risk of loss of stemness properties, induction of artifactual chromosomal changes and problems of contamination. HSCs have limited plasticity in that they can differentiate only into blood and blood-related lineages. Also, the HSC numbers harvested from the bone marrow and umbilical cord are low and require ex vivo expansion for the treatment hematopoietic diseases in adult humans.

Stem cells from birth-associated tissues are gaining popularity and have been derived from the placenta, amniotic fluid, amniotic membrane and umbilical cord (UC) [4, 5]. Of these sources, those isolated directly from the Wharton's jelly of the umbilical cord appear to offer greater clinical utility because they are less heterogeneous and possess unique properties over bone marrow MSCs [6]. As such, they will be the focus of this review.

Embryology of the Human Umbilical Cord

Fertilization and the first 4 days of cleavage up to the early blastocyst stage takes place in the Fallopian tubes in the human. On day 5 the early blastocyst descends down into the uterus, continues its divisions, undergoes expansion to the fully expanded blastocyst stage and then implants in the uterine endometrium around day 7 to 9. The migration of cells within the expanded blastocyst results in the laying down of two distinct cell layers, a peripheral layer of trophectoderm (TE) destined to become the placenta and a cluster of approximately 30–50 cells (inner cell mass, ICM) that protrude from the inner wall of the polar TE and destined to form the entire fetus. The ICM later develops into the hypoblast and epiblast. The hypoblast gives rise to the yolk sac and allantois which eventually degenerates and the epiblast cells which are pluripotent give rise to the three germ layers (ectoderm, mesoderm and endoderm) from which the various organs and extra-embryonic membranes (amnion, chorion, placenta and UC) are formed. During further development the amnion forms an outer covering for the UC and the UC carries within it three umbilical blood vessels (two arteries and a vein) to shuttle nutrients between mother and fetus. The amnion comprises of three layers viz., inner epithelial cell layer, an intermediate non-cellular basement membrane and outer mesenchymal layer [5]. The TE forms the cytotrophoblast and syncytiotrophoblast of the placenta while the blastocoelic cavity eventually produces the exocoelom. The part of the UC closest to the fetus may therefore contain remnants of the yolk sac and allantois.

Human epiblast cells express the surface marker antigens SSEA-3 and SSEA-4 [7]. Human embryonic stem cells (hESCs) that originate from the epiblast also express SSEA-3 and -4 but not SSEA-1 [8]. Human epiblast cells also express the pluripotent genomic markers OCT3/4 and NANOG and with continued development as cells differentiate into various lineages, OCT3/4 gets downregulated [7]. Thus, since the UC lies in an intermediate position between embryo and adult on the human developmental map, evaluation of the degree of expression of the members of the entire OCT family in stem cell populations of the UC is urgently needed to understand their role in stem cell plasticity.

The Various Compartments of the Human Umbilical Cord

The human UC starts to develop around the fifth week of gestation and at term has an average length of about 50 cm [9]. From a stem cell derivation point of view, various reports in the literature describe several compartments in the human UC (Fig. 1). Stem cells have been derived in the amniotic compartment (outer epithelial layer and inner subamniotic mesenchymal layer), the Wharton's jelly (WJ) compartment, the perivascular compartment surrounding the blood vessels, the media and adventitia compartment of the walls of umbilical cord blood vessels, the endothelial compartment (inner lining of the vein) and the vascular compartment (blood lying within the umbilical cord blood vessels). All these compartments have been described as distinct regions [10] and the nomenclature used in the literature for these various compartments has been misleading and not standardized, with terms such as 'cord lining', 'subamnion', 'intervascular', 'perivascular' and 'hUVEC' being used. Stem cell populations with varied stemness

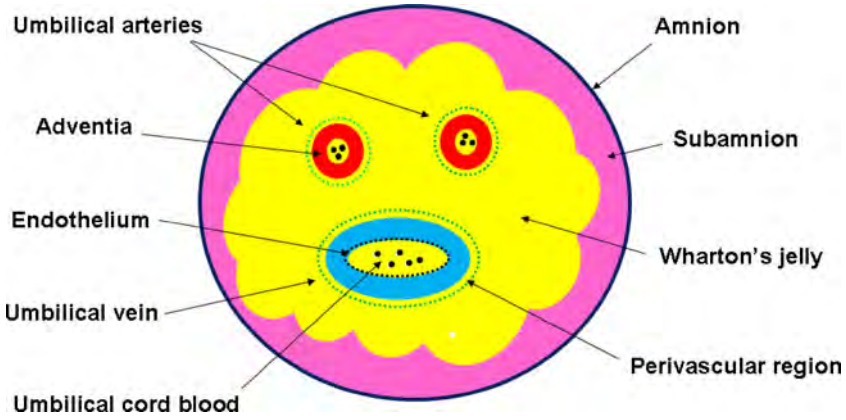

Fig. 1 Diagrammatic illustration of a cross-section of the human umbilical cord showing the compartments from which stem cells have been isolated (amnion, subamnion, Wharton's jelly, perivascular, adventiatia, endothelium and umbilical cord blood)

properties have been reported for each of these compartments [11] but the various individual derivation protocols published in the literature for stem cells from the UC are ambiguous and do not pay heed to the differences in stem cell populations between compartments. At the same time it is not known whether the stem cell populations between compartments are one and the same as there is no clear demarcation histologically between some of these compartments.

Given the reports that stem cell populations in different compartments have varied stemness characteristics the derivation protocols involving entire cord pieces containing all the compartments will result in mixed heterogeneous stem cell populations making a meaningful assessment of investigations difficult. It is therefore urgently necessary to standardize a derivation protocol for MSCs of the UC that yields defined or minimally heterogeneous cell populations.

The Diverse Methods of Derivation of Stem Cells from the Human Umbilical Cord

Several authors have reported the presence of stem cells in the various compartments of the UC using different derivation protocols. At least six different methods have been reported (Fig. 2). (i) UC pieces were first cut open, the umbilical blood vessels (which may carry with them the perivascular regions) removed and the remaining inner surface of the cord piece either scraped or squeezed with forceps to retrieve the WJ from which stem cells were harvested [12, 13] (Fig. 2a). (ii) UC pieces were cut open, umbilical blood vessels retained and only the WJ was separated. The WJ was then either directly exposed to enzymatic solutions to release the cells or cut into small pieces and then enzymatically treated [14-20]. In our laboratory we invert the cord pieces with intact umbilical vessels into an enzymatic solution (collagenase and hyaluronidase) at 37 °C for 30–45 min to facilitate detachment and loosening of the WJ into culture medium and then separate the cells from the WJ by passing the gelatinous masses through a syringe and needle [15, 17] (Fig. 2b). The stem cell populations in (i) and (ii) were referred to as human Wharton's jelly stem cells (hWJSCs) as they were derived directly from the WJ. (iii) Entire UC pieces with intact umbilical blood vessels were cut into smaller pieces and then grown as explants on plastic for a few days after which cell outgrowths from the explants were separated and cultured [21-24] (Fig. 2c). To maximise the recovery of stem cells, Tsagias et al. [25] first washed the entire length of the UC under sterile conditions to remove blood, then sterilized its surface, and with the umbilical blood vessels intact immersed the entire UC into a sterile bag containing an enzymatic solution of collagenase and hyaluronidase and incubated the bag at 37 °C for 3 h with gentle agitation. The UC was then exposed to trypsin for a further 30 min and the digested cell suspension collected by gravity. The stem cell populations in (iii) above were referred to as umbilical cord mesenchymal stem cells (UC-MSCs) (iv) The subamnion region of the UC was removed with a razor blade, cut into small pieces and grown on plastic as explants from which the cell outgrowths were separated and cultured. These stem cell populations were called subamnion or cord lining MSCs [10, 26] (Fig. 2d). These workers claimed that when separating the subamnion region from the rest of the UC it was impossible to completely remove the adjacent region underneath the amniotic epithelium and that dissection with a razor blade would leave a thin layer of mixed tissue. The same group also reported that it was difficult to exclude the contamination of Wharton's jelly-derived cells using their method of dissection. They also reported that their method was extremely time-consuming and required at least two working days after receiving the UCs and stated that it was reasonable to conclude that greater quantities of cells were available from the WJ [10]. (v) The umbilical blood vessels were removed from cord pieces, tied at either end into loops and the loops placed into an enzymatic solution for a specific period of time to allow detachment of cells from the perivascular region which are then grown in culture. These were referred to as UC perivascular stem cells (UCPVSCs) [27] (Fig. 2e).

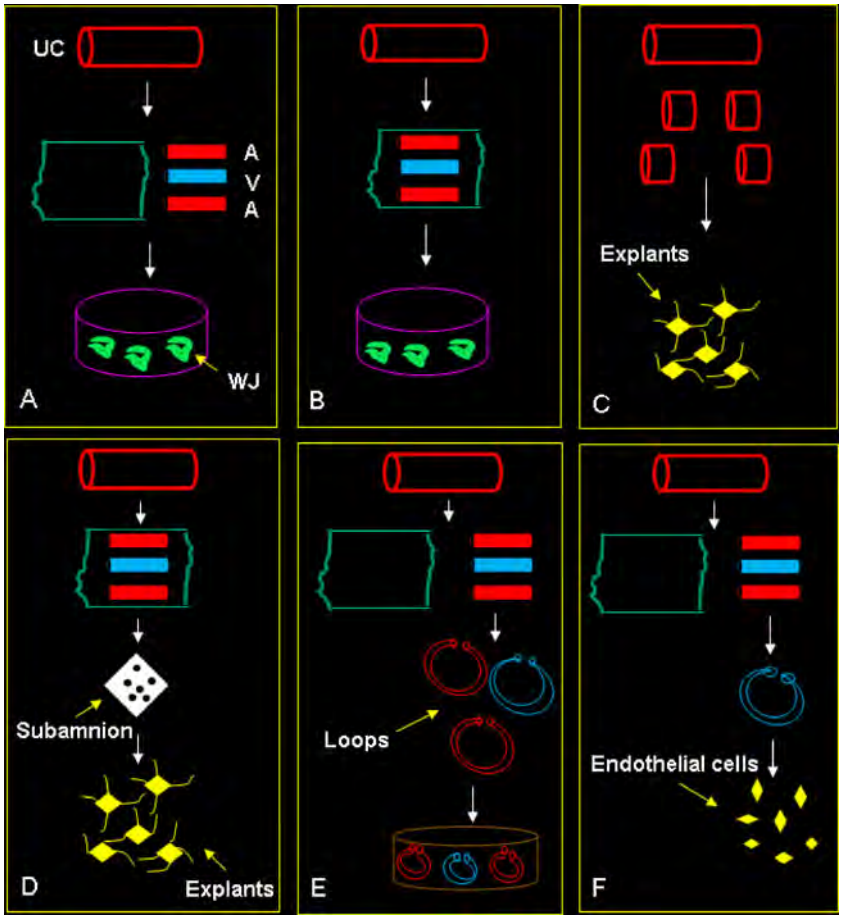

Fig. 2 Diagrammatic illustration showing six different methods of derivation of stem cells from the human umbilical cord published in the literature

(vi) Romanov et al. [28] isolated stem cells from the endothelial lining of the vein of the UC by first removing the vein and then passing through it an enzymatic solution to digest and remove the inner endothelial lining cells. The cell suspension was centrifuged to remove the enzymes and the cell pellet washed and seeded into culture medium in plastic dishes to grow the endothelial cells. The stem cells from such endothelial linings have been commonly referred to as human umbilical vein epithelial cells (hUVECs) (Fig. 2f).

Although debated, it has been claimed that MSCs usually reside in the perivascular regions of organs. In the UC however, it is very difficult to demarcate a perivascular zone from the rest of the intervascular WJ. It would therefore be prudent to keep the umbilical blood vessels intact when enzymatically separating the WJ so that the entire contents of the intervascular compartment (Wharton's jelly and perivascular cells) could be isolated. Removal of the blood vessels may result in loss of perivascular stem cell numbers from the intervascular WJ compartment as they may be firmly attached to the outer wall of the umbilical blood vessels. Watt et al. [29] reported that they observed CD146 + cells in 40 % of the cells in the perivascular regions. Recently, Kikuchi-Taura et al. [20] incubated UC pieces (with intact umbilical blood vessels) in an enzymatic mixture of collagenase and hyaluronidase for 2 h at 37 °C with intermittent shaking and then isolated stem cells from the WJ. They compared their derivation method with the method of Weiss et al. [12] (controls) which was similar except that in the controls the umbilical blood vessels were not removed. They reported large numbers of hWJSCs when the umbilical blood vessels were not removed and claimed that in vitro expansion was not required and that their fresh hWJSCs had the potential to suppress graft versus host disease. We used a similar enzymatic method to that of Kikuchi-Taura et al. [20] but separated the stem cells from the WJ by passing the gelatinous masses through a syringe and needle. We too reported large harvests of hWJSCs of approximately 4.7×10^6 cells per cm of UC [15, 17].

Recently, Bosch et al. [24] diced whole UC pieces into smaller pieces and grew them as explants. The outgrowths from the explants were separated, grown as monolayers and referred to as UC-'MSCs'. These stem cell populations may be heterogeneous and mixed as they originate from many compartments as they were not derived from a specific compartment. Based on this whole cord piece protocol these workers questioned whether UC-derived cells were true MSCs.

Even though MSCs have been reported from the various compartments of the human UC, the compartment with stem cell populations of the most optimal therapeutic value remains debatable. Robust comparisons between the stem cell populations of these various compartments so as to identify the most optimum source and subpopulation is urgently necessary for standardization and comparison of results between groups and to ensure reliability in terms of stemness properties, product quality, safety and efficiency for attaining regulatory approval for future clinical trials. Currently, stem cells from the WJ compartment appear to be the most defined with several unique characteristics.

Stem Cell Populations in the Various Human Umbilical Cord Compartments have Different Stem Cell Characteristics

It is interesting to note that differences in the properties of the stem cell populations in the various UC compartments have been reported. A differential distribution pattern of the various cytoskeletal proteins of stromal cells and extracellular matrix proteins was observed in different zones of the subamniotic stroma, WJ and the adventia of the umbilical blood vessels [30]. Comparisons were made between the MSCs derived from arterial (UCA), venous (UCV) and Wharton's jelly (UCWJ) explants of the human UC. Individual UC components from these three areas were dissected, diced into small fragments and aligned in explant cultures from which migrating cells were separated by trypsinization. UCV cells showed significantly higher frequency of colony-forming units than UCA and UCWJ. When compared for osteogenic potential, UCWJ were the least effective while UCA-derived cells developed alkaline phosphatase activity with or without an osteogenic stimulus [31]. MSCs have also been isolated in small numbers from the UCB compartment which lies within the umbilical blood vessels. However, the existence of such MSC populations in human UCB has been debated. Some workers could not isolate MSCs from UCB [32] while others claimed that UCB and peripheral adult blood were not rich sources of MSCs [33]. Some other reports stated that the typical features of hMSCs in UCB were their low counts per volume of UCB and very low proliferation rates [34-36].

The extra-embryonic membranes harbor a variety of embryonic or premature cell populations such as MSCs, endothelial stem/progenitor cells (EPCs, ECFCs) and HSCs (CD34 + and CD133+) [37]. The same authors emphasized that with respect to MSC populations from the UC, the different parts of the UC should be considered individually. La Rocca et al. [38] reported that there was a plethora of cellular sub-sources and populations that can be derived from the UC and Conconi et al. [11] described in detail the properties of these different cell populations derived by various methods. It has also been emphasized that there are a number of enzyme-mediated and enzyme-free methods described in many publications and patents to derive MSCs from the UC but there was no standardized method that was widely accepted [39, 40]. Additionally, stem cell populations from different UC compartments may represent cell populations that prefer to be differentiated along a specific lineage eg., musculoskeletal tissue engineering [41].

In situ demonstration of MSC markers on cryosections of whole UC pieces was reported by Schugar et al. [22]. After histological and immunohistochemical (IHC) analysis using primary and secondary antibodies for the various CD markers, these workers showed that the vascular regions could be clearly distinguished from the WJ matrix and epithelium. CD34+ and CD144+ cells were detected in the endothelial lining of the umbilical vessels but not in other regions of the UC. CD146 expression was observed in the vessel walls and perivascular regions. CD44+ and CD105+ cells were detected in the vessels, perivascular regions and outer WJ matrix and CD73+ cells were highest in the epithelium and subepithelial regions. These results confirm differences in stemness properties in cells of the various UC compartments. The differences are further confounded by the differences in derivation methods. For example, mechanical disruption, explant culture and dispase digestion yielded CD144+/CD146+ endothelial cells in abundance [42, 43].

Some workers claimed that of the MSCs isolated from the cord blood, WJ and perivascular regions of the UC, those from the WJ (which they included as intervascular and subamnion) offered better clinical utility because isolation frequency of colony forming unit-fibroblasts (CFU-Fs) were extremely high and delays in processing did not impact isolation [44]. The same authors stated that the MSCs from the perivascular region despite having high proliferative potential had limited transdifferentiation potential. Marker and differentiation assays indicated that the mesenchyme became more differentiated and mature from the subamnion to the perivascular regions [44, 45].

Origin of hWJSCs: How Did They Arrive in the Wharton's Jelly and What Are They Doing There?

Two possible theories can be presented as to how stem cells arrived in the WJ and what their possible role is during gestation. Wang et al. [46] carried out some elegant studies on whole human conceptuses (40 days old) that were collected through RU984-induced termination of pregnancies (TOPs). They dissected out the cells from the fetal bone marrow, yolk sac and aorta-gonadal mesonephros (AGM) and characterized them for the conventional MSC markers

recommended by the International Society for Cellular Therapy [47]. The cells in all three regions were positive for the MSC markers and based on their findings they postulated that there were two waves of migration of fetal MSCs in early human development. In the first wave, MSCs migrated from the yolk-sac and AGM via the UC to the placenta and in a second migration MSCs reverse-migrated from the placenta via the UC to home in the fetal liver and bone marrow. During these waves of migration it was suggested that some of these MSCs got trapped and resided in the gelatinous WJ of the UC [46, 48]. Their stemness characteristics appear to get modified while in their new environment making them different from bone marrow MSCs (hBMMSCs).

A second hypothesis is that the cells in the WJ are actually primitive mesenchymal stromal cells (myofibroblasts) originating from mesenchyme that was already there within the UC matrix. The role of these cells was probably to secrete the various glycoproteins, mucopolysaccharides, glycosaminoglycans (GAGs) and extracellular matrix (ECM) proteins to form a gelatinous ground substance to prevent strangulation of the umbilical blood vessels during gestation. Being primitive stromal cells and epiblast in origin they probably acquired both ESC and MSC markers at different levels of expression. Based on embryological origin and migration, the gelatinous WJ may be different from other stem cells in the UC compartments in terms of their secretory profiles. Meyer et al. [49] reported that the WJ was rich in mucoplyscacchrides and possessed a network of glycoprotein and collagen microfibrils. Several bioactive molecules (interferons, growth factors, interleukins, GAGs, cell adhesion molecules) present in the secretions released by hWJSCs [18, 50, 51] appear to be the building blocks for immunomodulatory mechanisms and tissue repair and thus can be taken advantage of for the repair of bone, cartilage and joint defects.

Wharton's Jelly Stem Cells have a Common Origin to Bone Marrow MSCs But are Different

hBMMSCs are the most popular and widely used MSCs for human stem cell research and clinical application. However, the harvesting of bone marrow MSCs is invasive and painful with the risk of infection and donor site morbidity. These features have resulted in a decline of allogeneic donations of hBMMSCs and the search for alternative MSC sources that are non-invasive.

Interestingly, even though hWJSCs and hBMMSCs may have common origins during human embryonic and fetal development, hWJSCs appear to be distinctly different and have advantages over hBMMSCs when it comes to clinical application [52]. hWJSCs resemble hBMMSCs in terms of a short-fibroblast-like phenotype [15, 17, 43, 53], non-hematopoietic surface markers [53], hypoimmunogenecity [54, 55], multipotent plasticity [38, 56-58] and expression of some markers such as CD90, CD105, CD13, CD73, CD10, CD29 CD51, CD166, CD44 and the HLS antigens HLA-A, B, C and G.

However, unlike hBMMSCs, hWJSCs do not express CD45, CD14, CD56, CD31 and CD34 at high levels and are HLA DR+[29], have higher proliferation rates, increased colony forming unit (CFU) formation and stemness characteristics that last for longer periods of time after serial passaging [17, 59]. CD68 which is classically recognised as a macrophage marker was shown to be highly expressed in hWJSCs [60]. Additionally, unlike hBMMSCs, hWJSCs express several ESC markers at different levels of expression such as the members of the OCT family, embryonic surface marker antigens (SSEA-4, Tra-1-60 and Tra-1-81), alkaline phosphatase (ALP), DNMT3B and GABRB3 and the genomic markers (SOX2, NANOG, REX2) [11, 61-64]. Based on a review of published literature, Conconi et al. [11] tabulated a detailed comparison of the different biochemical markers identified for stem cells derived by various methods from the different UC compartments [subendothelium (SE), perivascular (PV), umbilical cord lining (UCL), Wharton's jelly (WJ) and whole umbilical cord (wUC)]. They concluded that the stem cell populations from the various compartments had two common features in that they did not possess a hematopoietic profile and HLA class II antigens, but MSC and ESC markers were expressed differently in stem cell populations between compartments. CD133 and CD235a were expressed in wUC but not in the other compartments. Stem cells from UCL and partially from the PV compartments expressed CD14 which was not seen in stem cells from WJ, SE and wUC. Differences were also noted for OCT4, SSEA-4, STRO-1 and integrin-α for the stem cell populations in these different compartments. The authors claimed that the findings suggest that each part of the human UC may contain a MSC population that differs from those of other parts. In general, most primitive somatic cells possess greater growth rates in vitro than mature cells. This primitive cell characteristic appears to apply to MSCs isolated from the UC as they are highly proliferative.

hWJSCs have MSC and ESC Markers

hWJSCs share some of the stemness characteristics of both ESCs and adult MSCs as described above by possessing high level expression of MSC-CD markers and varied levels of expression of different ESC markers. They probably inherit some of the ESC markers because the UC lies in between the embryo and adult organs on the developmental map [5]. It would therefore be reasonable to refer to them as

'intermediate stem cells' that have retained some ESC markers while evolving into fully-fledged MSCs that satisfy all the minimum criteria for MSCs recommended by the International Society of Cell Therapy such as plastic adherence, self-renewal, CD-marker expression and ability to differentiate into adipocytes, chondrocytes and osteocytes [47]. Since hWJSCs originate from the epiblast theoretically they would be expected to be pluripotent. Cells that possess pluripotency usually result in chaotic differentiation of all three primordial germ layers to produce teratomas in vivo. However, hWJSCs do not produce teratomas in immunodeficient mice or immunocompetent non-human primates when transplanted at high cell doses with/without matrigel (matrigel is known to encourage teratoma formation in vivo) via various administrative routes [65, 66]. Hence, hWJSCs may have lost the property of pluripotency or their upregulated tumour suppressor genes [61] are able to downregulate their pluripotent genes, or the expression levels of their pluripotent genes are inadequate to induce tumorigenesis. Interestingly, non-tumorigenic hESCs were generated when hESCs were grown on hWJSC feeders [19] suggesting perhaps that unique proteins released by the tumour suppressor genes in hWJSCs are able to control the tumorigenic potential of hESCs. These interesting hypotheses warrant further investigation.

Cell Numbers and Growth Characteristics of hWJSCs

It is well known that with manipulation of cells in vitro there are risks of their genotype being altered. With continuous passaging cells undergo major and minor chromosomal changes and these changes occur at different passage numbers between different cell types (see human cell lines in ATCC collection, Maryland, USA). Human fallopian tubal ampullary cells started to produce polyploid chromosome complements after the 20th passage [67]. hESCs and hiPSCs were shown to develop deletions and duplications with early serial passaging [1] and Ben-David and Benvinisty [2] postulated that such chromosomal changes could be induced at several stages during the reprogramming protocol of somatic cells to hiPSCs in vitro. Thus, the genomic stability of passaged UC-MSCs must be assessed to ensure that the most stable passage, free of genomic anomalies is used for clinical application. Such changes in genotype may arise at different passage numbers between the various stem cell populations in the various UC compartments.

Thus, minimal manipulation of stem cells during ex vivo expansion would be preferred to avoid such problems. To date, our group have successfully derived 22 hWJSC lines from 22 human umbilical cords (100 %) and could consistently recover approximately 5×10^6 fresh live hWJSCs/cm of umbilical cord directly from the WJ before culture. Hence from a full-term UC of 50 cm a total of 2.5×10^8 hWJSCs could be theoretically harvested without culture. The use of a complex culture medium comprising of DMEM-high glucose+Knockout (KO) serum supplemented with bovine fibroblast growth factor (bFGF), insulin-transferrin-selenium (ITS) and L-glutamine generated high proliferation rates with mean population doubling times (PDT) of around 24 h. The stemness markers of the hWJSCs in this culture environment were retained with no chromosomal changes for at least 20 passages. hWJSCs are adherent cells that attach very well to plastic surfaces both in primary culture and passages and thus do not require feeder cells or cell attachment matrices like matrigel for their derivation and propagation. Other workers have also reported 250–300 fold increases in hWJSC numbers that were reached within 6–7 passages with a stable karyotype [45]. These workers reported that a total of $3.6\times10^6\pm6\times10^5$ viable hWJSCs/cm per sample could be obtained after primary culture and the total number of cells expanded to $11.5\times10^8\pm2.3\times10^6$ cells after subsequent passages.

The number of fresh live hWJSCs that could be harvested per cm of umbilical cord seems to vary with different reports because of differences in the derivation methods used. Wang et al. (2004) [14] obtained 25×10^3 cells/cm of human umbilical cord while Weiss et al. [12] obtained 1.5×10^6 cells/cm and Fu et al. [68] obtained 50×10^3 cells/cm of human umbilical cord. The derivation efficiency for hWJSCs from WJ is very much higher (100 %) than the derivation rates of MSCs from other compartments of the UC such as the endothelial and subendothelial layers of the umbilical veins (30 %) [28]. Also, the PDT of human umbilical cord perivascular stem cells (hUCPVSCs) was longer in a simple unsupplemented medium (60 h) resulting in the production of 10^{10} cells within 30 days of culture [27]. Lu et al. [21] showed that the mean PDT of hWJSCs grown in a super-complex medium containing DMEM (low glucose), 5 % FBS, glutamine, antibiotic-antimycotic mixture, VEGF and EGF (instead of bFGF and ITS) was 24 h and remained approximately constant until passage 10 (P10) after which it increased until passage 30 (P30).

Changes in cell surface markers of hWJSCs were observed after in vitro expansion [20]. These workers showed that CD44, CD105 and CD73 expression was increased at passage 4 (P4) and in contrast CD29 and CD90 expression did not change. Garzon et al. [69] showed that the highest viability levels for hWJSCs corresponded to the 5th and 6th passages. They also reported through gene expression analysis that this cell viability was significantly associated with pro- and anti-apoptotic genes. They suggested that there was a complex live-death equilibrium in hWJSCs maintained in culture for multiple cell passages. They concluded that the most optimal passage of hWJSCs that would be of therapeutic value was between the 5th and 6th passage.

Changes in stemness properties and protein secretions during serial culture of hWJSCs have been reported [18].

These workers characterized the proteomes of four passages (P2, P4, P8 and P12) and identified 158 unique proteins. These proteins were classified into 5 functional categories (cytoskeleton and motility, metabolism, protein biosynthesis folding and degradation, nucleotide biosynthesis, and cell signaling). They also observed that certain proteins (shootin 1, adenylate kinase 5 isoenzyme and plasminogen activator-inhibitor 2) were no longer expressed after the 2nd passage. At the end of their culture period (P12) they observed the synthesis of new proteins such as ERO1-like protein alpha, aspartyl-tRNA synthetase and prolyl-4-hydroxylase. Such protein changes between passages highlights the importance of evaluating the most optimum passage for stemness and utility of hWJSCs when it comes to clinical application.

It is also important to standardize the constituents of the culture medium that is used to derive and propagate hWJSCs as the culture environment appears to influence the nature and properties of the cells. Growth factors in the culture medium may have a positive influence on the proliferation rates of these cells in vitro. Culture media that have been used to propagate UC-MSCs have ranged from simple unsupplemented basal media to supplemented super-complex media containing a multitude of supplements. It is not known what additional benefits or detrimental effects exist when there is over-supplementation. The simple media conventionally contain the salts (DMEM), energy substrates (high or low glucose), proteins (fetal bovine serum), L-glutamine and antibiotic-antimycotic mixture [27]. The complex media are similar to the simple media but are supplemented with FGF and ITS [58]. The super-complex media contain additional nutrients such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), ascorbic acid and dexamethasone [20]. Ascorbic acid and dexamethasone are traditionally used as differentiation agents to drive stem cells towards osteogenic or chondrogenic lineages and as such it is not known whether the use of such agents will compromise the differentiation of hWJSCs into other desirable lineages. Most of the culture media that is used to grow UC-MSCs have ingredients of animal origin (xenoproteins) such as bovine brain extracts, fetal bovine serum and animal sources of insulin and FGF. If hWJSCs are to be taken to the clinic it would be mandatory to develop safe clinical grade current good manufacturing practice (cGMP) cell lines by avoiding the use of these xenoproteins and placing emphasis on human-based ingredients or supplements derived from recombinant DNA technology.

Plasticity of hWJSCs

Wharton's jelly stem cells (WJSCs) can be differentiated into many desirable tissues. When WJSCs were grown in rat neuronal conditioned medium they differentiated into CD11b microglial cells, generated neuronal proteins and the astrocyte protein GFAP was upregulated [68]. When WJSCs were exposed to salvia miltorrhiza (a shade growing perennial flowering plant) they expressed the neuronal markers β-tubulin 111, neurofilament and GFAP [70]. Several groups have reported protocols to differentiate hWJSCs into neurons [14, 15, 71]. In most of these protocols the hWJSCs were first incubated overnight with bFGF and then exposed to dimethyl sulfoxide (DMSO) and butylated hydroxyanisole (BHA). After 5–6 h the hWJSCs were treated with a mixture of potassium chloride, valproic acid, forskolin, hydrocortisone and insulin for long term induction and maintenance of neuronal differentiation [15, 17].

Several groups have also differentiated hWJSCs into bone, cartilage, and adipose cells [14, 27, 45, 72]. When hWJSCs were treated with 5-azacytidine for 3 weeks they started to exhibit the cardiomyocyte phenotype and expressed cardiac troponin 1, connexin 43, and desmin which are the conventional markers for cardiomyocyte differentiation [14]. When hWJSCs were first treated in vitro with vascular endothelial growth factor (VEGF) and bFGF and then transplanted into mouse ischaemic hearts they were shown to differentiate into endothelial cells [73]. hWJSCs exposed to a myogenic culture medium expressed the Myf-5 marker for skeletal muscle on day 7, Myo-D on day 11 and produced long multinucleated cells. When these multinucleated cells were injected into damaged rat muscles they produced the sarcomeric tropomyosin protein [72]. Pancreatic islet-like clusters have also been derived from hWJSCs for the control of diabetes [74, 75].

Immunogenecity of hWJSCs

Stem cells harvested directly from the Wharton's jelly compartment of the human UC have been shown to possess hypoimmunogenic properties that have been characterized both in vitro and in vivo. Weiss et al. [54] showed that hWJSCs inhibited a splenocyte response to concanavalin A stimulation in vitro, do not stimulate T-cell proliferation in a one-way mixed lymphocyte reaction (MLR) assay and inhibit the proliferation of stimulated T cells in a two way MLR assay. They further showed that hWJSCs express mRNA for pan-HLA-G and do not express the co-stimulatory surface antigens CD40, CD80, and CD86. These workers concluded that their results supported the view that there was no evidence of frank immunorejection of undifferentiated hWJSCs and that they would be tolerated in allogeneic transplantation settings.

When pig UC-MSCs were injected into the brains of Parkinsonian rats they were not rejected but survived in the rat brains and multiplied up to 4 weeks and produced

tyrosine hydroxylase positive neurons that expressed porcine-specific markers [76]. When hWJSCs were injected into immunodeficient rat spinal cords, the hWJSCs survived for 16 weeks post-transplantation [77]. In a more recent study, our group showed that when hWJSCs were injected into immunodeficient mice via various routes of administration (intramuscular, subcutaneous, intraperitoneal) they survived in the animals up to the termination of the experiment (20 weeks). The presence of the hWJSCs at the sites of injection were confirmed at 20 weeks by the presence of the human nuclear antigen (HNA). Analysis of the blood and spleen of the animals showed increased levels of anti-inflammatory cytokines [65].

The hypoimmunogenecity of MSCs from other compartments of the human UC derived by different methods have also been reported. The immunogenecity and immunomodulatory properties of umbilical cord lining (subamnion) MSCs were studied by Deuse et al. [78]. When these workers compared the immunogenecity of hBMMSCs and their subamniotic MSCs in immunocompetent mice the hBMMSCs exhibited a faster immunorejection response whereas in immunodeficient mice cell survival was prolonged and similar for both hBMMSCs and subamniotic MSCs. In another derivation method where diced explants of whole UC pieces were grown on plastic and the ensuing cell outgrowths separated and grown as UC-MSCs, HLA-DR and the costimulatory molecules CD80 and CD86 were not expressed by the UC-MSCs. These UC-MSCs also constitutively expressed B7-H1 (a negative regulator of T-cell activation) and its expression increased after interferon-γ treatment in vitro [55]. The immunological studies of stem cells derived from the various compartments of the human UC by different methods have been reviewed comprehensively by Conconi et al. [11] and Prasanna and Jahnavi [44].

Differences between hBMMSCs and hWJSCs in terms of immunogenecity have also been demonstrated. Mild lymphoproliferative responses to BMMSCs but none to hWJSCs were reported when these two cell types were cocultured with human peripheral blood mononuclear cells [79].

Non-tumorigenicity of hWJSCs

Using detailed microarray transcriptome profiling our group reported that hWJSCs possessed different levels of expression of pluripotent ESC genes and high level expression of a family of tumour suppressor and immunogenic genes that perhaps confer on them non-tumorigenic and hypoimmunogenic properties [61]. Other groups reported modest to high level expression of ESC markers in stem cells isolated from the subamnion, amnion and umbilical cord matrix [26, 53, 80, 81]. Given these different levels of ESC gene expression it is important that comparative studies be undertaken to evaluate the tumorigenic potential of stem cell populations from each compartment of the UC.

We reported that when hWJSCs at high doses (5×10^6 cells per injection) were transplanted with matrigel into SCID mice via three different routes and monitored for 20 weeks none of the animals developed tumors [65]. The absence of teratoma formation in the mice suggests that either the expression levels of the pluripotent genes were unable to induce teratomas or that the upregulated tumor suppressor genes are able to over-ride the expression of the pluripotent genes .

Recently, Wang et al. [66] confirmed the safety of human UC-MSCs (hUC-MSCs) in non-human primates. hUC-MSCs at doses of 2×10^6 and 1×10^7 cells/kg were injected intravenously once every two weeks for 6 weeks in cynomolgus monkeys. The toxicity of the cells were evaluated using a battery of parameters (clinical observations, histopathology, blood counts, clinical biochemistry, urine analysis, bone marrow smears and immunology). No stem cell transplantation-related toxicity was reported and all the injection sites and organs studied were normal with no prevalence of tumours.

Furthermore, injection of UC-MSCs or their derived tissues into xenograft diseased rat models resulted in engraftment and good functional outcome with no immunorejection or tumorigenesis [56].

Clinical Applications of hWJSCs and Its Extracts (Conditioned Medium and Cell-free Lysate)

Cell-based Therapies

hWJSCs have specific use for cell based therapies, as an anticancer agent and as stromal support for the expansion of CD34+ cells in cord blood banks. Fan et al. [56] reviewed several studies that undertook the preclinical validation of UC-MSCs or its derived tissues in diseased animal models. In all these studies the UC-MSCs differentiated and engrafted with successful functional outcome in vivo in rat models for cerebral ischemia, intracerebral hemorrhage, spinal cord injury, Parkinson's disease, retinal disease, Type 1 diabetes and myogenic disease.

Anticancer Effects

Many groups have reported that hWJSCs, its conditioned medium (hWJSC-CM) and cell-free lysate (hWJSC-CL) exhibit anticancer effects on solid tumors and are therefore attractive candidates for future cancer therapies [51, 82-88]. When hWJSCs were injected intravenously into severely combined immunodeficient (SCID) mice with mammary adenocarcinomas they migrated to metastatic tumor sites in

the lungs suggesting their homing abilities [80]. When rat hWJSCs were injected intravenously or intra-tumorally into rats with mammary carcinomas they completely abrogated cancer cell growth in 34–38 days compared to controls [84]. Similar findings were later observed in the human. When hWJSCs were administered intravenously 8 days after tumor transplantation in a human mammary adenocarcinoma xenograft rat model they homed to metastatic tumor sites in the lungs and reduced tumor burden [83, 85]. Engineered hWJSCs expressing human interferon-β were also shown to abrogate mammary adenocarcinoma growth in animal models [89]. The anticancer effects of hWJSCs are mediated via cell-to-cell and/or non-cellular contact mechanisms. One of the mechanisms involved in the inhibition of mammary adenocarcinoma cells by hWJSCs in vitro was through entosis [85]. These authors showed with time lapse imaging hWJSCs being first engulfed by mammary adenocarcinoma cells and then disintegrating within the cancer cells leading to apoptosis of the mammary adenocarcinoma cells [87]. The extracellular matrix of WJSCs was also shown to inhibit mammary adenocarcinoma cell proliferation by secretion of dickkopf-1 and suppression of the Wnt signalling pathway [86].

Artifactual growth advantages of one cell type over the other in vitro make coculture (the interaction of two cell types) studies sometimes unreliable. As such there has been recent interest in the evaluation of acellular agents harvested from hWJSCs (hWJSC-CM and hWJSC-CL) for the inhibition of cancer cell growth. Such acellular agents would be less controversial to regulatory bodies and more attractive as patentable technology for clinical application due to the lack of cell therapy induced cancer risks.

Our group examined the inhibitory effects of hWJSC-CM and hWJSC-CL on three cancer cell lines [breast adenocarcinoma (MDA-MB-231), ovarian carcinoma (TOV-112D) and osteosarcoma (MG-63)] [51]. The cancer cells were exposed to either 50 % hWJSC-CM or hWJSC-CL (15 μg/ml protein) for 48–72 h and morphologic changes, cell proliferation, cell cycle, gene expression, cell migration and cell death evaluated. The anticancer effect was most severe on the MG-63 osteosarcoma cell line. The growth of all three cancer cell lines was inhibited with morphologic changes ranging from cell shrinkage to blebbing and vacuolation. Based on MTT and BrDU assays, inhibition of cancer cell growth ranged from 2–6 % and 30–60 % for hWJSC-CM and hWJSC–CL respectively. The transwell migration assay also showed inhibition rates of 20 %–26 % and 31 %–46 % for hWJSC-CM and hWJSC-CL respectively for all three cancer cell lines. The sub-G1 and G2/M phases were increased in cell cycle assays and Annexin V-FITC and TUNEL positive cells were seen in the ovarian and breast cancer cells suggestive of apoptosis. The presence of anti-BECLIN1 and anti-LC3B antibodies seen with the osteosarcoma cells suggested an autophagic mechanism of cell death. There was an upregulation of the pro-apoptotic BAX gene and downregulation of the anti-apoptotic BCL2 and SURVIVIN genes in all three cancer cell lines and the autophagy genes (ATG5, ATG7, BECLIN1) were upregulated in the osteosarcoma cells. We concluded that hWJSC-CM and hWJSC-CL possessed tumour inhibitory properties and may be useful therapeutic anticancer agents [51].

Various molecules in conditioned media and cell-free lysates influence the initiation of transcriptional activity, differential expression of functional genes and reprogramming of specific cell types [90, 91]. However, the exact mechanisms as to how these molecules alter cell fate are not well known. Our data showed that the cell-free lysate had greater tumour inhibitory activity than the cell conditioned medium. This could be attributed to regulatory molecules present within the hWJSC matrix rather than the molecules secreted by hWJSCs into the culture medium because of varying sizes and cellular trafficking.

hWJSCs secrete a wide variety of bioactive soluble molecules including cytokines, glycosoaminoglycans (GAGs), hyaluronic acid (HA), chondroitin sulphate, cell adhesion molecules and growth factors [18, 51, 92, 93]. Cytokines are known to influence cell cycle regulation of cancer cells and induce growth attenuation and apoptosis. Matsuzuka et al. [94] demonstrated that hWJSCs expressing the interferon-β gene significantly attenuated bronchioalveolar carcinoma xenografts in SCID mice. In our xenotransplantation studies we showed increased expression of IL-16 in hWJSCs [65] and high concentrations of interleukins (IL-1a, IL-6, IL-7, IL-8) in hWJSC-CM [51]. We postulated that hWJSC-CM and hWJSC-CL exert their influence on cell cycle regulation leading to cell cycle arrest through cytokine-mediated mechanisms.

It was also reported in a xenograft model that human mammary carcinomas produced in mice with cancer stem cells (CSCs) isolated from the MDA-MB-231 breast cancer cell line underwent regression when injected intra-tumorally with hWJSCs. It was postulated that the inhibitory effects on the human CSC-induced tumours were via inhibition of phosphoinositide 3-kinase and AKT signaling mechanisms [88]. We also observed that hWJSCs do not transform into a tumour-associated fibroblast (TAF) phenotype when exposed to breast and ovarian cancer cells unlike hBMMSCs and as such do not participate in tumour formation [95].

The above data showed that hWJSCs and its extracts have paradoxical anticancer properties. This is quite an exceptional feature because the claims of anticancer properties of MSCs in general have been controversial. Furthermore, small animal xenograft models to study complete abrogration of tumours usually provide inconclusive results as most animal care guidelines do not encourage the maintenance and monitoring of animals with tumours for long periods of time because of undue distress to the animals. As such, larger xenograft animal models preferably non-human

primates will provide more conclusive evidence that hWJSCs and/or its extracts abolish tumours completely. Although the above studies showed that hWJSCs and/or its extracts were able to shrink tumours in both size and volume, complete abolishment of the tumours need to be demonstrated with doses that match the human if this novel approach to cancer therapy is to be adopted in clinical settings. Also, the actual molecules responsible for the anticancer effects of hWJSCs need to be closely examined through rigorous exosomal microRNA and proteomics studies.

hWJSCs and Its Extracts Support for Ex Vivo Expansion of HSCs

Confluent cell monolayers from three primary cell types (hBMMSCs, hUVEC and hWJSCs) were compared as feeder stromal cell support for the expansion of hematopoietic cells isolated from UCB [96]. Mononuclear cell expansion was 30–60 fold, colony-forming cell expansion 20-40-fold, and cobblestone area-forming cell expansion was 10-50-fold in the presence of these monolayers. After evaluating the immunological properties of these three primary feeder cell types the authors concluded that all three cell types may be suitable for use in clinical settings for UCB-CD34+ expansion with hWJSCs being the preferred choice because of easier and more efficient methods of isolation. The authors added that the choice of feeder stromal support should rest on whether an autologous coculture is wanted or not.

Our group showed through time lapse imaging that in the presence of hWJSCs and hWJSC-CM, HSCs put out pseudopodia-like outgrowths, became highly motile, migrated towards and attached to the surfaces of hWJSC monolayers and underwent proliferation [50]. After 9 days of culture, MTT and trypan blue proliferation and viability assays showed significant increases in HSC numbers. FACS analysis showed significantly greater numbers of $CD34^+$ cells compared to controls. We also reported that in the presence of hWJSC-CM, HSCs produced the highest number of colonies (CFU assay) and all the six typical classifications of colony morphology suggestive of normal hematopoiesis were observed [50]. Proteomic analysis of the hWJSC-CM showed significantly greater levels of interleukins (IL-1a, IL-6, IL-7 IL-8), SCF, HGF and ICAM-1 compared to controls suggesting that they may be the agents involved in the HSC and CD34+ expansion [51].

The above preliminary studies demonstrate the exceptional properties of hWJSCs over other MSC types. More in-depth controlled studies are required showing significant fold increases in HSC numbers that would be of therapeutic value before the results are definite and can be applied in clinical settings. If this is definitely known then we propose that cord blood banks freeze from the same umbilical cord autologous hWJSCs at the same time when cord blood HSCs are being frozen for future ex vivo HSC expansion and cell based therapies of the patient when required (Fig. 3).

Challenges and Future Directions

Based on the results of in vitro laboratory studies and preclinical animal validation already carried out on hWJSCs by

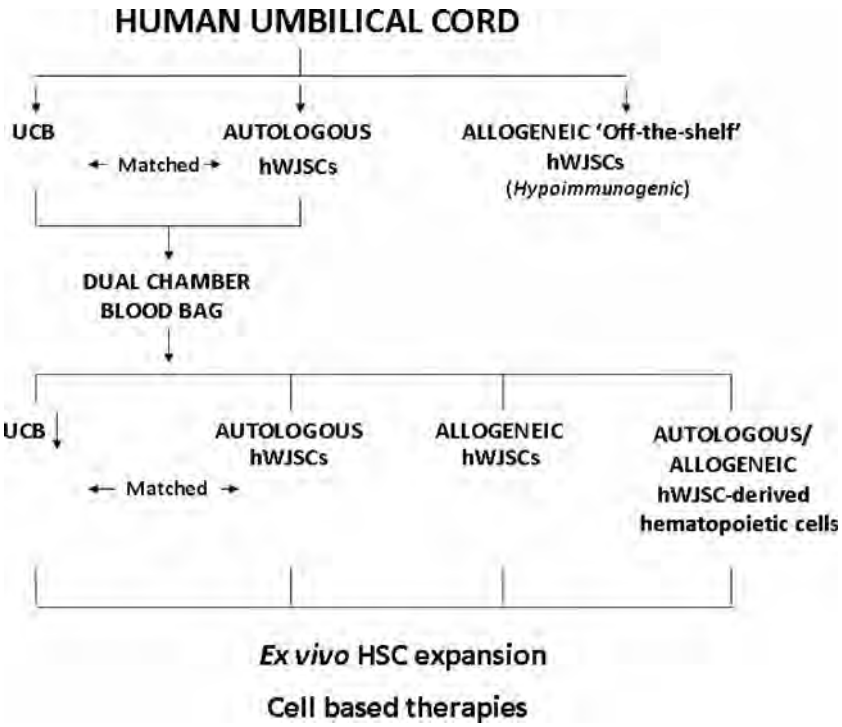

Fig. 3 Proposed model for storage and use of human Wharton's jelly stem cells in cord blood banks for autologous and allogeneic cell based therapies

various groups it appears that hWJSCs and/or its extracts (hWJSC-CM and hWJSC-CL) may be ideal agents for the treatment of malignant and non-malignant diseases beyond the hematopoietic system. The major challenge is to translate and confirm whether the same results obtained pre-clinically will be observed in human clinical settings. To meet this objective the next step would be the preparation and storage of clinical-grade hWJSCs and its extracts in current good manufacturing practice (cGMP) conditions to ensure that they are safe for clinical application. Thereafter, such cGMP-compliant hWJSCs/extracts could be used first in Phase 0 clinical trials to confirm patient safety and improved functional outcome before proceeding to Phase II and III trials. Given the fact that work has already shown that hWJSCs are safe and non-tumorigenic in both laboratory animals and non-human primates it may not be difficult to obtain approval from regulatory bodies to administer fresh or early passaged cGMP-compliant hWJSCs to patients in Phase 0 trials. As a first step to taking these agents to the clinic it may be quicker to obtain regulatory approval for hWJSC extracts rather than hWJSCs because of lesser concerns of any cell-associated cancer risks.

The routes of administration, cell numbers, volumes and duration of dosages are some of the factors that remain challenges and need to be worked out to bring about effective treatment. It is also not known whether for cell based therapies hWJSCs need to be differentiated first in vitro into the desirable tissue of choice before transplantation or whether they can be transplanted directly into the patient allowing differentiation and engraftment to take place in vivo. It is also not known whether any improvements in functional outcome will be mediated via engraftment of differentiated tissues or via paracrine effects as is the case with autologous bone marrow MSC transplantation. Another major challenge is to evaluate in clinical trials whether allogeneic hWJSCs will engraft successfully in the human as they have been shown to have hypoimmunogenic properties.

Although it has been shown in laboratory animal xenograft models that certain tumors shrink in size after exposure to hWJSCs/extracts it has not been possible in most studies to monitor such animals for long periods of time to evaluate complete tumor abolishment because of possible distress caused by the tumors to the animals. Therefore, to confirm complete abolishment of tumors with hWJSCs/extracts it may be important to evaluate dose-time studies on larger non-human primate models where monitoring can be done for longer periods of time until the tumors are completely abrogated. However, the published results of many studies showing the positive anticancer effects of hWJSCs/extracts may be adequate justification to make a case for human clinical application at least in Phase 0 trials. The tumors that have been studied thus far could be first shrunk with the administration of cGMP grade hWJSCs/extracts and then followed with chemotherapy or surgery. It is not known whether hWJSCs/extracts specifically target cancer stem cells (CSCs) residing within tumors. Future anti-cancer studies should be aimed in this direction which if positive may change the paradigms of cancer therapy where hWJSCs/extracts are used to target CSCs that usually reside at the core of tumors rather than at peripheral tumorigenic cells that are the actual progeny of the CSCs.

The derivation and storage of autologous hWJSCs/extracts on the same day that UCB-HSCs are frozen from the same UC will serve as an ideal adjuvant in cord blood banks for future personalized cell based therapies and expansion of HSCs for the patient and immediate family. Autologous hWJSCs/extracts from the same umbilical cord have the advantage of being matched to the same patient avoiding any immunorejection problems. Also, since hWJSCs have been shown to be hypoimmunogenic, allogeneic sources of hWJSCs/extracts from donor umbilical cords would also serve as useful 'off-the-shelf' cells for the same purposes. It is convenient to freeze and store the hWJSCs/extracts in the second chamber of a dual chamber blood bag with the first chamber reserved for the same patient's UCB. Since hWJSCs are highly proliferative the frozen hWJSCs could be thawed and confluent monolayers established within a few hours as stromal support for expansion of HSCs or be used for cell based therapies. hWJSC-CM or hWJSC-CL being cell-free liquids could also be separately frozen in ampoules or cryovials in the liquid nitrogen vapor phase and after thawing be used immediately. Currently, most cord blood banks discard and do not freeze UCB samples that have low HSC counts. Storage of the hWJSCs/extracts provides an opportunity to salvage such samples as they can be expanded with the hWJSCs/extracts.

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Predictors of complication for alveolar cleft bone graft

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Abstract

We have analysed the predictors of postoperative complications and the need for reoperation after grafting of the alveolar cleft from one specialised cleft centre. The data were obtained from hospital casenotes of patients operated on from December 2004 to April 2010, with a minimum one-year follow-up from the final operation. Independent variables included postoperative complications and the need for reoperation. Conditional variables were sex, age, type of cleft, sides affected, donor area, type of graft material, and the presence of an erupted tooth in contact with the cleft. A total of 71 patients had bone grafted on to the alveolar cleft. The following associations were found to be significant: postoperative complications and need for reoperation ($p = 0.003$); age and complications ($p = 0.002$); affected side and complications ($p = 0.006$); age and reoperation ($p = 0.000$); sex and reoperation ($p = 0.001$); and type of cleft and reoperation ($p = 0.001$). Proper attention should be given to all the variables and risk factors to overcome the many obstacles that might have an adverse influence on a successful outcome of alveolar bone grafting for patients with clefts.

Keywords: Cleft lip; Cleft palate; Tissue donors; Age groups; Complications; Autogenous graft

Introduction

Orofacial clefts are the most common congenital deformities of the head and neck, and present in about 1/700 live births across the world. They may develop during the second and third weeks of pregnancy as a result of disturbed differentiation of the primordial cell layer and be associated with genetic and environmental factors related to formation of the lip and palate.[1,2]

Several surgical interventions are required to optimise the restoration of speech, feeding, and masticatory function, and adequate development of the facial skeleton.[3–5] Alveolar bone grafting is usually done before the eruption of the permanent maxillary canine or the lateral incisor tooth to provide alveolar continuity, adequate closure of the oronasal fistula, support for the nasal base, and bony support for permanent tooth eruption or eventual prosthetic rehabilitation.[3,4,6–13]

Several variables may interfere with the outcomes of bone grafting of the alveolar cleft. Details of the patient (such as age, status of tooth eruption on the cleft side, details of the cleft, and the patient's general health), surgical wound conditions (overall oral health, quality and amount of soft and hard tissue adjacent to the cleft, blood supply, donor site, and scar tissue from previous operations) and technical characteristics (graft material, and the surgeon's experience) may all play a part in the outcomes.[4,14–16]

Whenever a postoperative complication develops the surgical outcome may be compromised and reoperation might be necessary. The need for reoperation increases the

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overall costs of treatment, exposes the patient once more to risks related to the operation and general anaesthesia, and might even be a reason for the patient or family to refuse further treatment. Reoperation will also result in formation of more scar tissue and further compromise the local blood supply and healing of hard and soft tissue.[5,17]

In this study we investigate the predictors of postoperative complications and the need for reoperation from a specialised cleft centre to provide useful information so that we can anticipate and prevent, or properly deal with and overcome, complications after alveolar bone grafts.

Methods

We retrospectively evaluated the medical records of consecutive patients who had alveolar cleft bone grafts at the Cleft & Craniofacial Centre of the Hospital Geral Universitário (affiliated with the University of Cuiabá – UNIC) to find out the incidence of postoperative complications and the reasons for reoperation related to patients operated on within the period December 2004 and April 2010, with a minimum postoperative follow-up of one year. The study followed the principles of the Declaration of Helsinki, and we had the informed consent of patients or their legal guardians. The study was approved by the Ethics in Research Committee of the University of Cuiabá under the number 068 CEP/UNIC – protocol 2010-066.

Two surgeons (one staff and one resident) were responsible for recording and evaluating the results of the operations. The following variables were recorded: sex; age (12 years or younger, or over 12 years old); type of cleft according to an anatomical classification (clefts of the preincisive foramen or the transincisive foramen); sides affected (unilateral or bilateral); donor area (anterior iliac crest or intraoral); type of corticocancellous bone graft (particulate, block, or mixed); and erupted tooth in contact with the cleft (present or absent). Independent variables were reported for postoperative complication (yes or no), and the need for reoperation (yes or no).

The significances of differences were assessed using BioStat 2009 (AnalySoft 2011, Brazil). Independent variables were tested against conditional variables using McNemar's test (2×2 contingency tables) or the chi square test (3×2 and 4×2 contingency tables), and probabilities of less than 0.05 were accepted as significant.

Results

We studied a total of 71patients who had alveolar cleft bone grafting procedures (Table 1).

Postoperative complications developed in 29 patients (41%). These included exposure of the graft associated with wound dehiscence ($n = 13$), infection of the wound with purulent discharge ($n = 8$), or resorption of the graft as reported at the orthodontic follow-up ($n = 8$).

Table 1
Descriptive variables ($n = 71$).

Variable	No. (%) of patients
Sex	
Male	32 (45)
Female	39 (55)
Age (years)	
12 or younger	24 (34)
Over 12	47 (66)
Type of cleft	
Preincisive foramen	32 (45)
Transincisive foramen	39 (55)
Sides affected	
Unilateral	58 (82)
Bilateral	13 (18)
Donor area	
Anterior iliac crest	49 (69)
Intraoral	29 (41)
Type of graft	
Particulate	22 (31)
Block	16 (23)
Mixed	28 (39)
Unknown	5 (7)
Erupted tooth cleft	
Present	21 (30)
Absent	43 (60)
Unknown	7 (10)

Correlation between conditional variables and the presence of reported postoperative complications are shown in Table 2. Twenty patients required reoperation (28%). Correlations between conditional variables and the need of reoperation are shown in Table 3. Not all patients who required reoperation had a postoperative complication. Among patients who had a further operation, 17 had a reported postoperative complication, whereas the other 3 required reoperation without any complication reported after the first alveolar bone graft (Table 4).

Discussion

Successful outcomes of grafting of an alveolar bone cleft are defined as long-term preservation of alveolar bone in the area of the cleft; adequate functional support for the nasal structures; eruption, integrity, and periodontal support of the permanent teeth at the site of the cleft; and the ability to place osseointegrated dental implants when required.[8,13] While previous research has focused on the success rate of the bone graft, we know of few studies that have discussed specific complication rates (reported to be between 15% and 40%), which might significantly influence the need for reoperation.[3,9,10,12–14,18,19]

Wound dehiscence can lead to infection or exposure of the graft, or both, and may be caused by an excessive amount of bone being grafted into the cleft, tension in the soft tissue after closure of the wound, local trauma postoperatively, or poor compliance with postoperative oral hygiene.[2,7,11,20,21] Lack of blood supply and nutritional support from the overlying

Table 2
Correlation between conditional variables and the presence of reported postoperative complications ($n = 71$).

Variable	No complication ($n = 42$)	Complication ($n = 29$)	p value
Sex			
Male	18	14	0.11
Female	24	15	
Age (years)			
12 or younger	16	8	0.002
Over 12	26	21	
Type of cleft			
Preforamen	22	10	0.07
Transforamen	20	19	
Sides affected			
Unilateral	33	25	0.006
Bilateral	9	4	
Donor area			
Iliac crest	26	23	0.26
Intraoral	16	6	
Type of graft			
Particulate	13	9	0.08
Block	11	5	
Mixed	15	13	
Unknown	3	2	
Tooth adjacent			
Yes	11	10	0.16
No	25	18	
Unknown	6	1	
Reoperation			
Yes	3	17	0.003
No	39	12	

Table 3
Correlation between conditional variables and the need for reoperation ($n = 71$).

Variable	No reoperation ($n = 51$)	Reoperation ($n = 20$)	p value
Sex			
Male	24	8	0.001
Female	27	12	
Age (years)			
12 or younger	20	4	<0.0001
Over 12	31	16	
Type of cleft			
Preforamen	26	6	0.001
Transforamen	25	14	
Sides affected			
Unilateral	41	17	0.18
Bilateral	10	3	
Donor area			
Iliac crest	32	17	0.74
Intraoral	19	3	
Type of graft			
Particulate	17	5	0.16
Block	11	5	
Mixed	19	9	
Unknown	4	1	
Tooth adjacent			
Yes	11	8	0.16
No	34	11	
Unknown	6	1	
Complications			
Yes	12	17	0.003
No	39	3	

soft tissues over the bone graft and the presence of direct exposure to the contaminated oral and nasal environments are major problems related to exposure of the bone graft, which may lead to infection or resorption of the bone graft, or both, with a diminished final bone volume.[22]

Although a postoperative complication might be related to the need for reoperation (85% of the cases that needed reoperation in the present study also had a postoperative complication), this might not necessarily result in failure of the graft if the wound was debrided properly and antibiotics given systemically to allow adequate wound healing.[7,9,23]

Infection at the site of the graft might have an adverse effect on the surgical outcome. In this study, 6/8 cases with reported postoperative infections required reoperation. Strategies to ensure a higher success rate using adequate oral hygiene with careful wound irrigation and the prolonged use of antibiotics should be helpful for cases at increased risk of infection.[11,12]

An inherent disadvantage of the autogenous bone graft is its known resorption potential, which varies between 24% and 51% after the first year.[7,10,21] While the resorption rate has traditionally been evaluated by conventional radiographs, the accuracy of computed tomographic 3-dimensional evaluation makes it the most reliable method.[3,6,10,12,15,20,24] As adequate bone volume at the site of the graft is routinely evaluated by the orthodontist in the postoperative phases, a request for reoperation from the orthodontist is also one of the many determinants of a successful bone graft.[19] The resorption rate at the grafted site might also be influenced by infection, the presence of a tooth at the site of the cleft, eruption of a tooth through the grafted bone, the quality and quantity of grafted bone, and even pressure from soft tissues overlying the grafted area.[2,7,16,20] Reoperation was indicated for resorption of bone in 19 (27%) of the patients in this study. Although this is a large number, it confirms the results of other studies as it indicates a 73% success rate, which is consistent with the mean reported success rates of alveolar bone grafting.[3,8,12,19,20,24]

Three patients required reoperation without any reported complication. Possible reasons include insufficient bone

Table 4
Descriptive analysis of postoperative complications and the need for reoperation.

Variable	No reoperation	Reoperation
Resorption	4	4
Mean (SD) age (years)	11 (±8.1)	11 (±3.1)
Exposed graft	6	7
Mean (SD) age (years)	14 (±3.3)	24 (±7.3)
Infection	2	6
Mean (SD) age (years)	12 (±2)	23 (±7.8)

The mean (SD) age for patients that presented with infection and requiring reoperation was 23 (±7.8) years.

grafted during the first operation, or late bony resorption found during orthodontic treatment. Appropriate recording of data is critical to elucidate the reason for a further operation, and this requires compliance from the entire multidisciplinary team. Despite the importance of having a pattern to score the success of bone grafting, this study did not use an exclusive classification criteria. Most orthodontists on our team use the Kindelan scale, but they do not always have the score of their evaluation in patients' notes, and radiographs were not always available during the review of casenotes, which is why this manuscript is based not on radiographs but rather on the report of complications and the need for reoperation.

Age was an important factor that influenced the incidence of postoperative complications and the need for reoperation. The mean (SD) age for patients that presented with infection and requiring reoperation was 23 (??) years, which might be related to periodontal problems, the presence of dental caries, and poor compliance with instructions about oral hygiene.[11,20,21]

Patients who required reoperation were significantly older than the patients who, even with a postoperative complication, did not require further intervention. According to published reports, the best age for bone grafting is during the mixed stage of dentition, generally between 5 and 12 years of age.[8,9,11,20] While traditionally secondary alveolar bone grafting would be before eruption of the canine tooth or a viable maxillary lateral incisor, some surgeons prefer to do it before eruption of the central incisor.[3,8,13] On the other hand, tertiary alveolar bone grafting (those grafts done after completion of the second stage of dentition) has not resulted in the same successful outcomes, and has even compromised the psychological health of neglected patients.[3,9,12,15,17,18,20]

Sex might have a role in the incidence of cleft lip, or palate, or both, but this was not the focus of this study.[3] Although sex was found to have a significant effect on the need for reoperation, this might not reflect a common clinical occurrence.

There were no significant differences among grafts from intraoral donor sites and grafts harvested from the anterior iliac crest. Although there is a continuous evolution of tissue engineering that is searching for an effective alternative to autologous bone grafts, costs are high and nothing has yet been found to be superior to autogenous bone grafts.[25] Attention might be drawn to the size of bony particles and to the quantity of grafted bone, in that an inadequate bone graft will fail to promote enough new bone, whereas excessive bone grafting will compromise wound closure and the density of newly formed bone. Excessive crushing of the bone graft might also increase its resorption rate through cell death and consequently diminish the final volume of bone.[2,16]

The width of the alveolar cleft is a concern for survival of the graft, because alveolar bone grafting in bilateral clefts has been reported to be more critical than in unilateral clefts.[3,12,16,19,20,23] Another anatomical consideration is related to the type of the cleft that has already been considered to influence outcomes.[20] Blood flow and velocity seem to be decreased on the cleft side in unilateral clefts, which might influence postoperative revascularisation at the centre of the graft, particularly in wide bilateral clefts.[5,12] The results presented here did show that bilateral clefts had a significantly higher rate of complications, whereas transforamen clefts were more associated with reoperation and a tendency for complications than preforamen clefts.

Teeth in the area of the alveolar cleft can influence the outcomes of grafting in both good and bad ways. Healthy teeth (no caries or periodontal disease) maintain adequate bony height and may even help to guide the formation of new bone.[10] However, teeth with periodontal disease at the cleft area can be a potential source of infection.[10] Deciduous teeth should be extracted, when required, about 4 weeks before alveolar bone grafting, to allow enough time for healing and renewal of the mucosa.[13,14]

Although we have not considered them here, important variables such as socioeconomic status of the patient's family, experience of the surgeon, severity of the deformity, deficiency of soft tissue, amount of scar tissue adjacent to the alveolar cleft, pregraft orthodontic treatment (including expansion), and compliance with the proposed treatment plan all have a role in the final surgical outcome.[12,14,15,18,24]

Considering that the multidisciplinary cleft centre team in this study initiated activity in 2004, the complication rate and the need for reoperation might also reflect a learning curve related to the maturation of a professional team and the development of a department. Patients' heterogeneity and surgical variables should be understood as limitations to the study. The number of patients over 12 years old also reflects a considerable number of patients with cleft lip, or palate, or both, whose conditions were neglected.

Strategies to optimise results include providing an alveolar bone graft at the cleft site during the mixed dentition stage with intraoperative attention to both the soft and hard tissues, water-tight closure of the nasal floor and the oral mucosa to prevent direct open exposure of the bone graft, and tension-free closure of soft tissue. Proper attention should be given to many variables and risk factors to overcome the many obstacles that might adversely influence a successful outcome of alveolar bone grafting for the patient with a cleft.

In our assessment of predictors for complications after alveolar bone grafting, we have shown the importance of the association between postoperative complications and need for reoperation; age and complications; affected side and complications; age and reoperation; sex and reoperation; and type of cleft and reoperation. These findings should assist clinicians in the preoperative assessment of these variables, the prevention of postoperative complications, and proper management of problems that might adversely influence a successful outcome of alveolar cleft grafting. We plan a prospective study in the near future to evaluate possible improvements in outcomes with the benefit and use of the results of this present study.

Conflict of interest

There is no conflict of interest associated with the submitted research.

Ethics statement

The study followed the principles of the Declaration of Helsinki, gathered informed consent from patients or their legal guardians, and was approved by the Ethics in Research Committee of the University of Cuiabá under the number 068 CEP/UNIC – protocol 2010-066.

Author contributions

Conception and design of study/review/case, and the acquisition of data for laboratory or clinical/literature series were performed by Alexandre Meireles Borba and Carolina Silvano Vilarinho da Silva. Analysis and interpretation of data collected were performed by Alexandre Meireles Borba and Alvaro Henrique Borges. Drafting of article and/or critical revision were performed by Alexandre Meireles Borba, Alvaro Henrique Borges, Mariana Aparecida Brozoski, Maria da Graça Naclério-Homem and Michael Miloro. The final approval and guarantor of manuscript were Alexandre Meireles Borba, Alvaro Henrique Borges, Carolina Silvano Vilarinho da Silva, Mariana Aparecida Brozoski, Maria da Graça Naclério-Homem and Michael Miloro.

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How Accurate Is CBCT in Measuring Bone Density? A Comparative CBCT-CT In Vitro Study

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ABSTRACT

Purpose: Recently, cone beam computed tomography (CBCT) has become widely used for oral and maxillofacial imaging. Twenty dry mandibles were CBCT and conventional multislice CT scanned to evaluate if there is a statistically significant difference between the bone density values they produce, defined as gray density values, and to determine any correlation between them.

Materials and Methods: Using software and a radiographic template, the CT and CBCT scan images were overlapped, and two data sets were created, each one giving the respective gray values (voxel value [VV] or Hounsfield unit [HU]) of the same area with the same spatial coordinates. For the statistical analysis, *t*-test, Pearson's correlation, and Pearson's *r* were used.

Results: The differences between the CBCT (VV) and CT (HU) gray density values were statistically significant ($p \leq .05$), whereas the Pearson's correlation coefficients and Pearson's *r*-values demonstrated a statistically significant linear correlation between VV and HU gray density values.

Conclusion: The lower radiation dose and reduced costs of CBCT make this a useful substitute for CT; however, this study has shown that, in order to more accurately define the bone density with CBCT, a conversion ratio needs to be applied to the VV.

KEY WORDS: bone density, computed tomography, cone beam, Hounsfield value

INTRODUCTION

Over the past three decades, osseointegrated dental implant therapy has had successful outcomes, but some clinical reports have indicated a higher success rate when dental implants have been inserted in the mandible rather than in the maxilla.[1,2]

Clinical studies have also shown that a greater failure rate is associated with poorer volume and/or density of the bone.[3]

The mechanical properties of the bone are an important factor in osseointegration, which determines the primary implant stability.[4]

Several studies have proposed a variety of methods for assessing bone density, but these involve evaluation either at the time of implant site preparation or subsequent to implant placement.[3]

In recent years, the use of a computed tomography (CT) scan has been common for preoperative quantitative and qualitative assessment of implant sites, and the Hounsfield unit (HU) is routinely used to determine the bone density objectively.[3,5,6]

Even more recently, due to the need for less expensive image acquisition protocols or for scanners with lower radiation dose, cone beam CT (CBCT) has been widely employed for oral and maxillofacial imaging,[7] as

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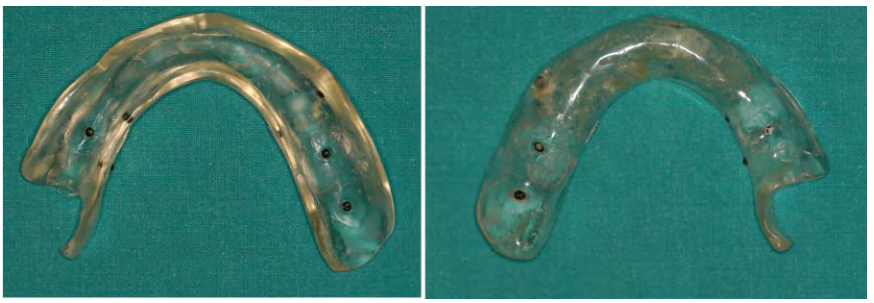

Figure 1 Radio-transparent resin template with nine lead circular radiopaque shots used as landmarks.

it seems to provide good spatial resolution, gray density range, and contrast, as well as a good pixel/noise ratio.[8]

With CBCT, the dimensional accuracy is also comparable with CT, but unlike CT, the gray density values of the CBCT images (voxel value [VV]) are not absolute.[8]

In fact, CT could be calibrated using as a reference the density values of the air (–1,000 HU) and pure water (0 HU); otherwise, CBCT does not consent to be calibrated, and the values, which are based on the difference of gray scale, are already preset by the manufacturer.

The purposes of this study were the following:

1 to evaluate if there is a statistically significant difference between the measurements of bone density values, defined as gray density values, collected when using CBCT (VV) or CT (HU) in implant planning;
2 to determine if there is a correlation between the different gray density values measured through a CBCT (VV) and through a CT (HU).

The hypothesis of this study is that CBCT is a reliable method to evaluate the bone density of the implant sites, but it is necessary to use a conversion ratio to convert the CBCT gray values into CT.

MATERIALS AND METHODS

To evaluate the accuracy of CBCT in determining bone density, the gray density values of specific anatomical specimen areas at the same spatial coordinates were measured using CBCT (VV) and CT (HU).

This method allowed us to obtain comparable images of the same area under investigation.

The protocol employed in this in vitro study consisted of an integrated sequence that involved the following series of steps:

1 Creation of a radio-transparent resin template with nine lead circular radiopaque shots to be used as landmarks to ensure a perfect overlap (Figure 1).
2 Execution of CBCT and CT scans for all anatomical specimens (20 dry mandibles; Figure 2), employing the same template for both types of scan (Figure 3).

A spiral CT machine (Siemens SOMATOM®, Erlangen, Germany) was used. The CT parameters used were tube voltage of 120 kV, tube current of 72 mAs, high-resolution bone kernel, 0.5-mm nominal slice thickness, 0.5-mm interval, and 0.5-mm pitch. Calibration was performed to ensure that the air was defined as –1,000 HU.

The CBCT used (Soredex SCANORA® 3D, Tuusula, Finland) had an amorphous-silicon, flat-panel image detector and offered a cylindrical volume of reconstruction up to 13 × 14.5 cm with a 14-bit gray density, 0.250-mm pixel size, 90-kV tube voltage, 0.25-mm

Figure 2 One of the dry mandibles used as an anatomical specimen.

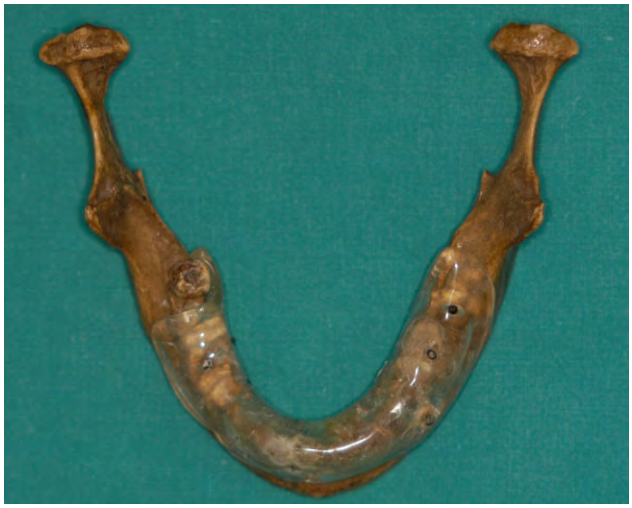

Figure 3 The dry mandible and the resin template used for cone beam computed tomography and computed tomography scans.

nominal slice thickness, 15 mAs, and 40-s exposure time. Unlike CT, the CBCT scanner employed factory-defined gray density attenuation.

All acquired data were saved in Digital Imaging and Communications in Medicine (DICOM) format.

The quantified gray density values of the planned volume were measured and expressed as VV and HU in the CBCT and CT groups, respectively.

3 Overlapping of the DICOM images in order to have the same spatial coordinates for both scans.

The CT DICOM images were imported using software (Mimics®, Materialise, Leuven, Belgium), and the "image registration" tool was used. With the "image registration" tool, it is possible to fuse two data sets by doing a landmark point-based registration (nine lead shots); thus, after importing CBCT DICOM images, a perfect overlap was obtained (Figure 4).

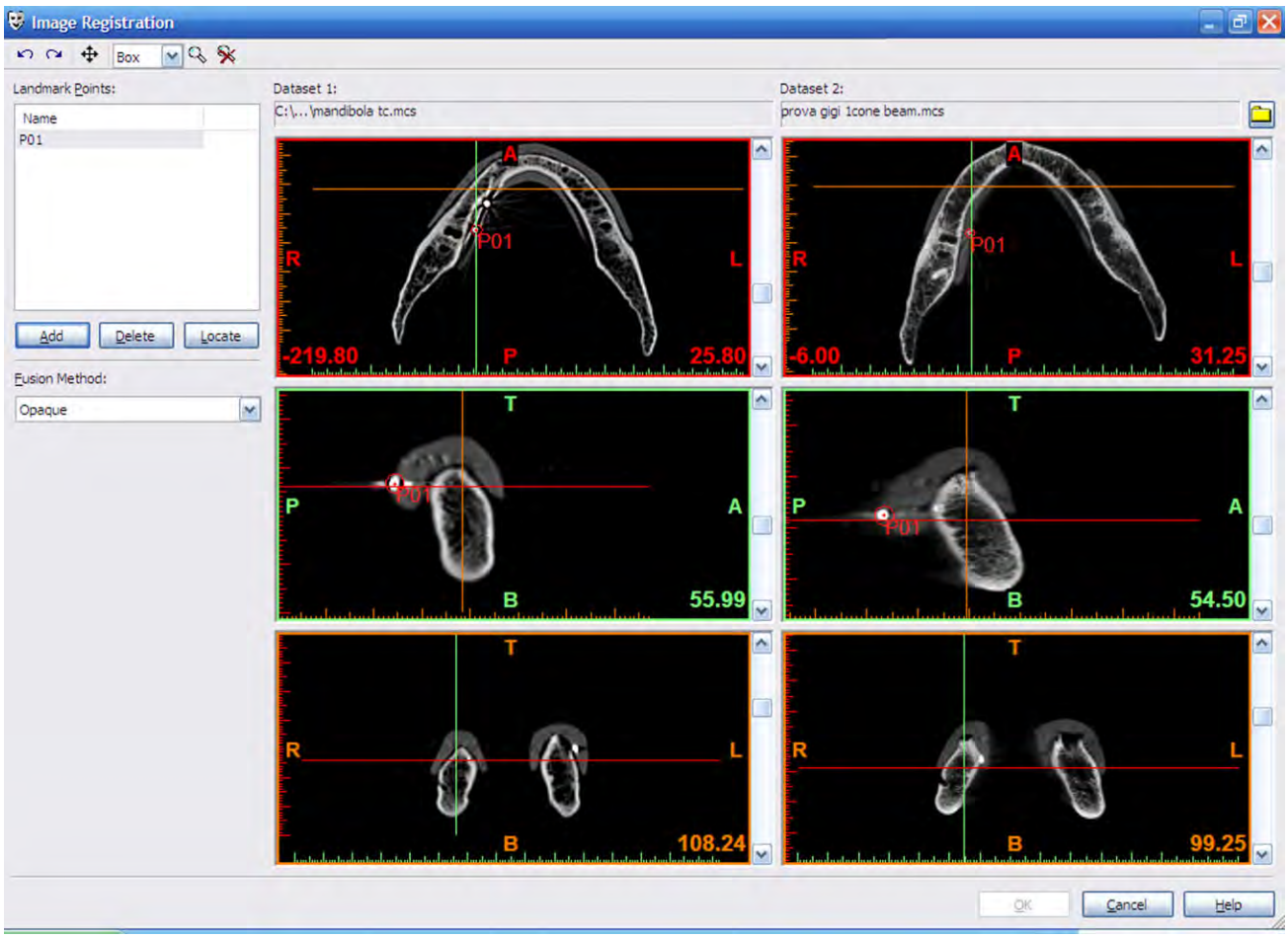

Figure 4 Overlapping of two data sets (cone beam computed tomography and computed tomography) of Digital Imaging and Communications in Medicine images, using the Mimics® software "image registration" tool.

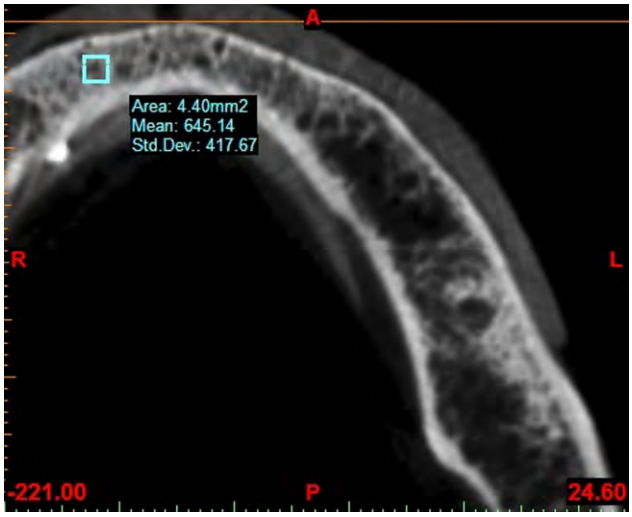

Figure 5 Determination of cancellous bone cone beam computed tomography gray density values (voxel value [VV]). The drawn square shows a mean value of 645.14 VV. The coordinates of the square are indicated below the figure in red.

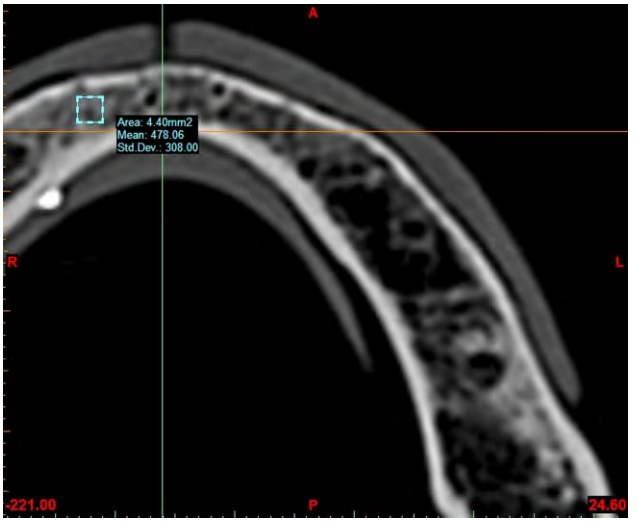

Figure 6 Determination of cancellous bone computed tomography gray density values (Hounsfield unit [HU]). It should be noted that the area and the coordinates of the drawn square are the same as in Figure 5 (–221.00, 24.60). The mean gray density value recorded was 478.06 HU.

This software runs until it finds the exact overlap between the images of CT and CBCT scans and does not require any intervention by the examiner, thus excluding any possible human measurement error.

The final result was the creation of two sets of data relating to the same areas (spatial coordinates): one set gave gray values in terms of HU, while the other set gave values in terms of VV.

4 Calculation of the gray density values for CBCT (VV) and CT (HU) images.

A square with the same spatial coordinates was drawn for both data sets, and the gray density value within the square was determined. For each anatomical specimen, 30 measurements were made (10 in the cancellous-cortical bone, 10 in the cancellous bone, and 10 in the cortical bone), and the gray density values were determined in the following six groups, which were paired according to the area under investigation:

Group A1: cancellous-cortical bone CBCT gray density values (VV);
Group A2: cancellous-cortical bone CT gray density values (HU);
Group B1: cancellous bone CBCT gray density values (VV) (Figure 5);
Group B2: cancellous bone CT gray density values (HU) (Figure 6);
Group C1: cortical bone CBCT gray density values (VV) (Figure 7);
Group C2: cortical bone CT gray density values (HU) (Figure 8).

Statistical Analysis

The volume gray density values of the groups were analyzed using the *SPSS*® for Windows software (Statistical Package for Social Science, IBM Corporation, Armonk, NY, USA).

Descriptive statistics of gray density values (HU and VV) consisting of the mean, standard deviation, and minimum–maximum were calculated for each study group.

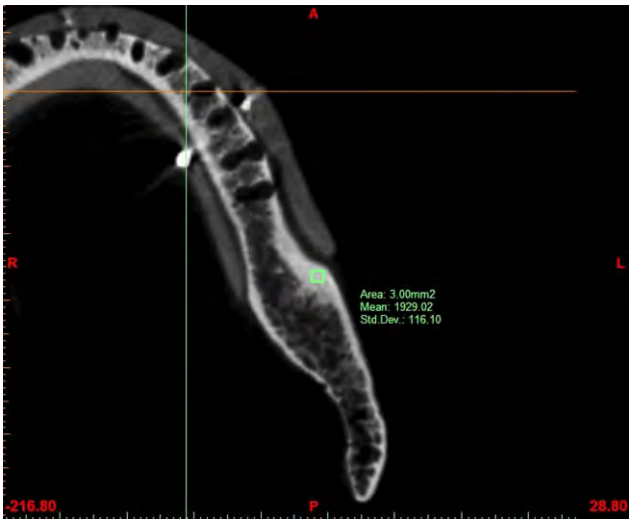

Figure 7 Determination of cortical bone cone beam computed tomography gray density values (voxel value [VV]). The drawn square shows a mean value of 1,929.02 VV. The coordinates of the square are indicated below the figure in red.

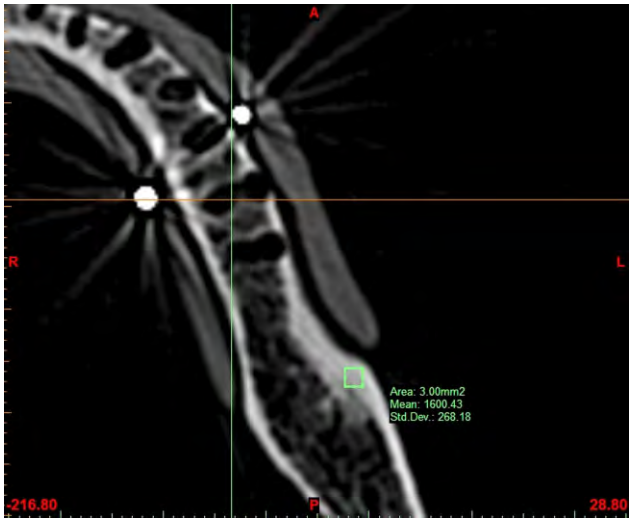

Figure 8 Determination of cortical bone computed tomography gray density values (Hounsfield unit [HU]). It should be noted that the coordinates of the drawn square are the same as in Figure 7 (–216.80, 28.80). The mean gray density value recorded was 1,600.43 HU.

The *t*-test was used to determine if there was a statistically significant difference between the two paired groups (group A1 vs A2, group B1 vs B2, and group C1 vs C2). Significance was set at $p \leq .5$.

Correlation between gray density values (HU and VV) of different groups was tested using the Pearson's correlation coefficient.

The Pearson's *r*-values were used to assess the magnitude of the covariance, regardless of the gray values of the CBCT and CT measurement sites. All measurement sites were used as the computational unit.

Again, a *p* level below .05 was accepted as statistically significant.

RESULTS

Quantitative data of each paired group were described with mean values, minimum–maximum values, and standard deviation (Table 1).

When using the *t*-test, the differences in the gray density values between the CBCT and CT groups were statistically significant for all paired groups (Table 2).

The Pearson's correlation coefficients demonstrated a statistically significant correlation between the compared groups (Table 3).

The Pearson's *r*-values showed a linear correlation between the gray density values of the CBCT and CT.

In scatter plots, the clusters shaped by the CBCT and CT gray values indicate the presence of a linear association between them (Figure 9, A–C).

The presence of a close linear correlation consented to determine the conversion ratio to transform the gray density values of CBCT (VV) to that of CT (HU).

In particular, in the present study, the conversion ratio was approximately 0.7 (0.7 × values of CBCT = values of CT).

DISCUSSION

The relationship between CBCT- and CT-based gray density values (VV and HU) was analyzed in this study.

Evaluation of the bone density prior to the insertion of implant may be of critical importance, especially when multiple implants are planned. In some cases, in fact, as a result of the disuse atrophy, the mineral content of the alveolus in totally and partially edentulous jaws may have decreased dramatically, resulting in an increased risk of implant placement into the compromised areas.

In a recent review aimed to survey the definition of bone tissue characteristics and methods of assessing them in studies of dental implant planning and placement, Ribeiro-Rotta and colleagues[9] concluded that there is a diversity of classifications of bone tissue characteristics and of methods used to examine and assess jawbone tissue.

TABLE 1 Descriptive Statistics: Mean, Minimum–Maximum Values, and Standard Deviation

	Mean	Maximum	Minimum	SD
Group A1 (VV)	1,053.31	2,700.77	88.37	490.15
Group A2 (HU)	744.35	1,890.54	61.86	366.70
Group B1 (VV)	816.62	1,110.00	645.00	84.29
Group B2 (HU)	572.45	777.00	436.00	58.42
Group C1 (VV)	1,505.26	2,006.00	1,019.00	151.02
Group C2 (HU)	1,354.00	1,896.00	840.00	143.19

HU = Hounsfield unit; VV = voxel value.

TABLE 2 *t*-Test Regarding the Differences between the CBCT and CT Gray Density Values

	Sig.	Difference between Means	Standard Error
Group A1 vs group A1	.000*	308.96	61.21
Group B1 vs group B2	.000*	244.17	10.25
Group C1 vs group C2	.000*	151.26	20.81

*Statistically significant ($p \leq .05$).
CBCT = cone beam computed tomography; CT = computed tomography.

The authors[9] suggested a strong need for future uniformity in the design of implant studies. Similar assessment methods, classification system, and measurement units are essential prerequisites for comparing the results of different studies and for improving the understanding of treatment outcomes in relation to different bone characteristics.

CT has been widely used to evaluate the dimension and density of the bone as it provides quantitative and qualitative data of the medullary and cortical bone.[3,5,6,10–13]

With CT, bone density measurements are given in HU based on density values for air (−1,000 HU) and pure water (0 HU). The cortical bone ranges from +1,000 to +1,600 HU values.[11]

Due to its relatively low cost and reduced radiation dose, CBCT has become more widely used for oral and maxillofacial imaging, providing good spatial resolution, gray density range, and contrast, as well as a good pixel/noise ratio.[7]

In CBCT, the dimensional accuracy is also comparable with CT, but in contrast to CT, the gray density values of the images (VV) are not absolute.[8]

Arisan and colleagues,[8] in a recent study aimed at determining the relationship between CT- and CBCT-based gray density values, revealed gray density values ranging from 167 to 989 HU and from 229 to 1,042 VV.

In the present study, the gray density values measured in the CBCT groups were higher than those measured in the CT groups, results that were similarly reported in another study.[14]

The reason for this phenomenon was attributed to various technical factors such as x-ray beam hardening, scattered radiation, and "projection data discontinuity-related effect," all of which resulted in a decrease in the dynamic contrast of the CBCT scanners compared with multislice CT.[8]

According to Arisan and colleagues,[8] the effect of beam hardening is more pronounced when the radiopacity increases, which can explain the significant

TABLE 3 Pearson's Correlation between CBCT and CT Groups

		Group A1	Group A2	Group B1	Group B2	Group C1	Group C2
Group A1	Pearson's correlation		0.977*				
	Sig.		.000				
	n		100				
Group A2	Pearson's correlation	0.977*					
	Sig.	.000					
	n	100					
Group B1	Pearson's correlation				0.931*		
	Sig.				.000		
	n				100		
Group B2	Pearson's correlation			0.931*			
	Sig.			.000			
	n			100			
Group C1	Pearson's correlation						0.978*
	Sig.						.000
	n						100
Group C2	Pearson's correlation					0.978*	
	Sig.					.000	
	n					100	

*Statistically significant ($p \leq .05$).
CBCT = cone beam computed tomography; CT = computed tomography.

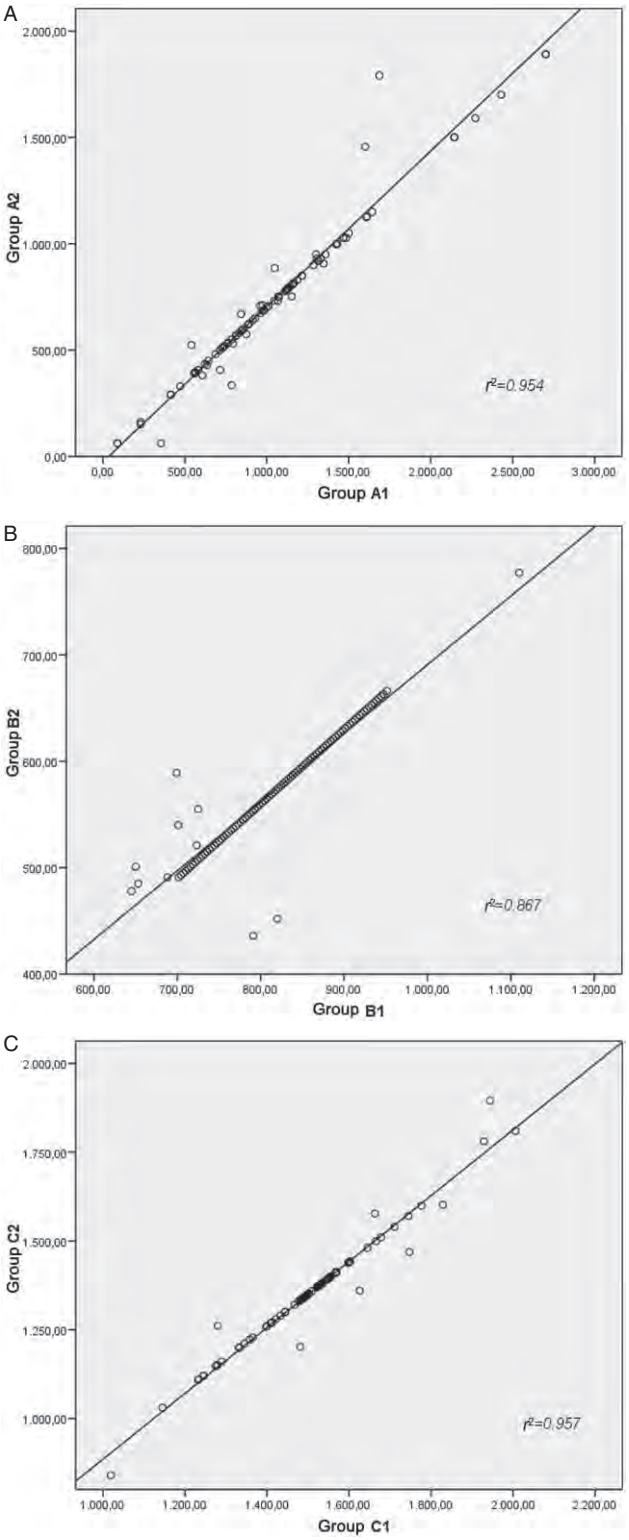

Figure 9 *A–C*, Scatter plots visually identify the relationship between the cone beam computed tomography and computed tomography gray values in the study groups. In each study group, the points follow a linear pattern that indicates a high linear correlation. The high values of r^2 indicate that the points are close to the straight line. The linear relationship is strong if the points are close to the straight line.

differences between the HU and VV in the cortical bone compared with the more similar results found in the trabecular, low-density maxillary bone.

In contrast to Arisan and colleagues,[8] the results of the present study did not show smaller differences between measurements taken in areas of the bone marrow compared with those in areas of the cortical bone.

In a recent investigation conducted by Naitoh and colleagues, the relationship between VVs obtained from CBCT and bone mineral densities (BMDs) obtained from multislice CT was evaluated in the mandible.[14]

A high-level correlation between VVs of CBCT and BMDs of multislice CT was observed ($r = 0.965$).

Also, the same authors[14] transformed the BMDs of CBCT from the VVs in one hundred twenty-eight implant sites using a regression line, and then the absolute difference between the values and BMDs of multislice CT was calculated. The difference was from 1 to 182 mg/cm^3 HA, with a mean of 46 mg/cm^3 HA (SD 36).

This high correlation between VV of CBCT and BMDs of multislice CT was close to that reported in a previous study conducted by Aranyarachkul and colleagues.[15]

These authors,[15] comparing HU density recordings made using the conventional quantitative CT (QCT) method with HU density recordings made with the quantitative CBCT (QCBCT), observed that QCBCT bone density values were generally higher than the corresponding QCT recordings. The relationships between the QCT and QCBCT values were close, as demonstrated by the Pearson's correlation coefficients, which ranged from 0.92 to 0.98.

Unlike the protocols used in the cited research,[14,15] where a single arbitrarily chosen cross-sectional image of the designated implant area was referred for the quantification of the gray density values, the methodology of the present study is rather sophisticated using dedicated software that allows the exact overlap between the images of CT and CBCT scans and does not require any intervention by the examiner, thus excluding any possible human measurement error.

The present study also demonstrates the possibility of correlating the gray density values recorded by CT and CBCT; in fact, a correlation between VV of CB CT and HU values of multislice CT was observed.

More specifically, in this study, the conversion ratio between the two gray values was determined and defined

equal to 0.7; thus, to convert the CBCT gray values into CT, it is necessary to multiply CBCT values by 0.7.

This conversion ratio, moreover, is approximate and may vary based on the CBCT used; a conversion ratio between CT and CBCT gray density values has never been proposed before, and comparable data are not present in the literature.

Whether the CT or the CBCT values are closer to the corresponding histological bone densities remains to be learned, and this topic will be addressed, relating both CT and CBCT gray values to histological measurements of bone density.

CONCLUSION

This study demonstrated that the utilization of a CBCT to evaluate the bone density of implant sites can be useful due to its lower radiation dose and lower costs; however, the surgeon needs to be aware that VVs are not absolute values. Nevertheless, there is a linear correlation between the gray density values of CBCT and CT, which allows the surgeon to convert CBCT gray density values (VV) into absolute values (once the correct conversion rate has been established) and, in so doing, ensure a more successful result.

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Regenerative Technologies for Craniomaxillofacial Surgery

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Regenerative medicine has recently enjoyed a tremendous amount of basic and translational research activity. A convergence of technologies is occurring that has afforded opportunities previously not possible with conventional surgical reconstructive techniques. These discoveries are making their way into current surgical practice. Patients requiring complex reconstructive surgery in the craniomaxillofacial region typically benefit from local or regional flaps, nonvascularized grafts, microvascular tissue transfer, and substitute alloplastic materials to restore function and form. In these clinical situations, grafting procedures or alloplastic substitute materials serve as best-case replacements for resected, injured, or congenitally missing tissues. However, in many cases, ideal reconstructive goals, such as a complete return to original form and function, are not completely achieved. Regenerative techniques, currently in clinical use and at the translational research stage, hold the promise of custom-tailored constructs that have the potential to regenerate tissue in the host without major donor-site morbidity. These techniques can provide better structure, esthetics, and function compared with the best of currently available options. This article presents the most recent concepts in craniomaxillofacial regenerative medicine and reviews the multiprong approach to restoring architecture using novel "smart" multifunctional scaffolds, cellular technologies, growth factors, and other novel regenerative strategies.

There is a need for predictable reconstructive techniques for surgeons to use in patients with complex injury, congenital malformation, or defects from ablative surgery. Reconstructive goals have not been entirely met using current techniques—even by the best of reconstructive surgeons using the most recent techniques.[1] Traditional techniques focus on providing tissue from the local anatomic region to compensate for the lost tissue or providing tissue from another region of the body and retrofitting this anatomy to yield the desired form. The craniofacial region has different specific functional demands, such as protection of the brain and optic tracts, breathing, mastication, speech, and hearing. In addition, the craniofacial region is important for social acceptance and self-esteem.[2] A surgeon must plan for reconstructions and be mindful of the functional and esthetic requirements to achieve success.

Regenerative medicine and tissue engineering aim to provide custom constructs that become integrated fully in the local anatomy and provide ideal form and function once they are in the host. Regenerative medicine can be used to recruit local tissues to produce the desired tissue—ideally in a manner in which the structure and form are esthetically and functionally useful. The use of commercially available recombinant bone morphogenic protein (BMP) products serve as an example of this concept.[3] Currently, proteins can be delivered to "grow" bone at a given site and regenerate lost bony tissue.[4] However, the field is taking these concepts several steps further. Examples of current strategies consist of a biodegradable scaffold embedded with stems cells to produce bone

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regeneration after mandibular resection or use of advanced calcium phosphate-based cements that are biomimetic and tailored with nano-functional attributes offering the capability to temporally release proteins, medications, or genes that drive the regenerative process locally.[5-9]

The growth in technologies related to this field has propelled the possibilities forward and new reconstructive options are becoming a reality. The convergence of different technologies has been instrumental in contributing to recent developments in this area. This article discusses these advances, including the basic concepts of regenerative medicine for the craniomaxillofacial region.

Regenerative Medicine: An Interdisciplinary Field

The basic premise of regenerative medicine or tissue engineering is that a practitioner can provide a new construct to replace lost tissue—whether that tissue be bone, skin, mucosa, tendon, cartilage, heart muscle, liver, entire solid organs, or composite tissues.[10,11] Different terms have been used to describe activities involved in repairing and regenerating tissues, in whole or part by using cells, proteins, matrices, signaling molecules, or other strategies. *Regenerative medicine, reparative medicine*, and *tissue engineering* have been used, somewhat interchangeably, to describe these activities. As with many advances, the process of defining these efforts is more accurately described as incremental process and systematic discovery, rather than a specific sentinel event or seminal published work. Many discussions, articles, and symposia have contributed to the current understanding of the field. However, one can point to several areas of discovery to explain the current direction in the area of craniofacial regeneration.[12-14]

Thus far, biomaterials have been used as replacement tissues, and grafting is performed to reconstruct defects in the craniofacial region. Synthetic vascular grafts, resorbable collagen matrix, synthetic bone cements, and allogeneic transplants have been used to serve as replacement tissues for those that were diseased, lost to injury, or lacking in some way owing to deformity or defect.[10,12-16] In some cases, autogenous grafts can be used to replace lost tissue. These techniques have worked reasonably well, but have considerable downsides. When Urist[4] first produced exogenous bone with the help of BMP, it became clear that it was possible to engineer a process within tissues using the local milieu and its complex cellular signaling environment to produce a desired tissue response. Although these attempts have been directed at producing only some bone at a few select locations, they have been some of the first successful attempts at tissue engineering in the craniofacial region. Other tissues have been repaired or regenerated, including skin and bone, using different techniques, such as expanded neonatal cell lines or stem cell transplants[17] (Fig 1). The challenge of late has been to combine different technologies to control the response in a particular defect. Currently, a collision of bioscience, bioengineering, biomaterials science, and clinical surgery is occurring in an attempt to find workable constructs or bioreactors using the body's ability to recapitulate itself to produce the desired regenerated tissue.

Basic Principles of Regenerative Medicine

Regenerative medicine or tissue engineering is an interdisciplinary, translational field that applies the principles of bioengineering to the development of biological substitutes that restore, maintain, or improve tissue function.[10] To regenerate new tissues within a specific environment, 3 basic tools are essential—cells, a scaffold, and signaling molecules. Some or all of these can be provided by the engineered construct. In some instances, proteins, signaling molecules, and matrices can be used to drive the body's response in the desired direction for regeneration. Concerted efforts in the craniomaxillofacial region are being explored using different techniques, such as bone regeneration with proteins, cellular technologies, synthetic matrices, and vascular biomimetic systems.

BONE REGENERATION

The area of regenerative medicine that has received the most attention for the craniomaxillofacial region is bone regeneration with cellular techniques, biomaterial replacement, and signaling molecule use. Autogenous grafting has been considered the standard for bone replacement in the craniomaxillofacial region. Surgical specialists have been looking for bone substitutes to avoid donor-site morbidity and provide a more convenient way to regenerate defects whether they are from congenital deformities, acute trauma, chronic nonunion, or resection of pathology. Allogeneic bone grafts are suitable, to some extent, for more simple defects but still have drawbacks, such as cost, less than ideal mechanical properties, risk of disease transmission, and the need to procure the material from limited cadaveric specimens. For most serious craniomaxillofacial defects, allografts and xenografts have a small role if one compares outcomes with autogenous sources. For example, grafting a maxillary or alveolar cleft site is typically performed with autogenous bone from the iliac crest. Previous attempts to use allogeneic

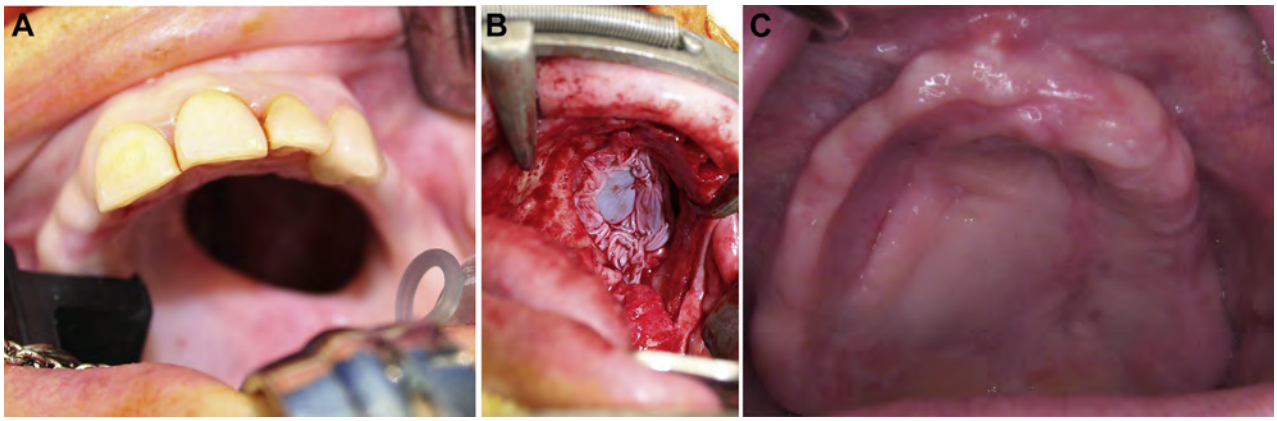

FIGURE 1. *A*, A woman with a chronic fistula from cocaine use and presumed granulomatosis disorder who had multiple failed attempts by other surgeons to close a large oral, nasal, and antral fistula and refused temporalis flap or microvascular tissue transfer techniques. *B*, She consented to the use of cultured and expanded neonatal cells on a bilaminar collagen membrane in conjunction with a 1-layered closure of mucosa on the oral side. *C*, The wound healed completely without any evidence of even a small fistula.

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bone grafts or xenografts have not had the success rates seen with autogenous grafting, and as a result, these techniques are seldom used. Any option must surpass the success of autogenous grafting and provide a solution that also limits morbidity.[18,19]

To engineer a substitute, it is necessary to regenerate bone in a manner by which the material(s) can survive through the initial phases of healing and implantation. This means that bone must form within a short period and that the cellular, biochemical, and biomechanical challenges be overcome in a given region. For example, the cranial vault is essentially a non–weight-bearing and nonfunctional bone compared with the mandible. A critical-size defect of the cranial vault is likely to sustain less biomechanical force than a critical-size defect of the mandible. Although the cranium and mandible have excellent blood supply in the region, bacterial contamination is much more of an issue in the mandible than in most areas of the cranium. Hence, the 3-dimensional (3D) construct that provides the structural support for the reconstruction must be suited to meet the biomechanical demands and provide an appropriate environment for regeneration. Autogenous grafting has performed this fairly well, but even microvascular tissue transfer often fails to provide the ideal 3D structure that completely restores the defect back to full functionality and meets the esthetic demands. Procedures that have provided BMP-2 to a defect site often produce bone, but in an unpredictable fashion with considerable side-effects, including swelling. New efforts are aimed at designing novel 3D functional scaffolds exhibiting all the desired biofunctional attributes of biocompatibility, bioactivity, safety, internal and external micro- and macrostructures combined with the desired spatial and temporal pharmacokinetic transport response. As a result, novel biocompatible scaffolds are available that serve not only as a home for cells and proteins, but also as smart delivery systems to address some of these shortcomings (Fig 2A-C).

Scaffolds

Tissue in the craniomaxillofacial region is varied in composition and in its simplest form consists of a matrix and different cell types.[20] The matrix represents a 3D structure, or scaffold, for cells, which provides them with a specific environment and architecture for a given functional purpose.[21] The structure also serves as a reservoir of water, nutrients, cytokines, and growth factors. When one applies these concepts to tissue engineering in the craniofacial skeleton to restore function or regenerate bone tissue, the scaffold will act as a temporary matrix, or template, for cell proliferation, extracellular matrix deposition, bone regeneration, and remodeling until the mature bony tissue is regenerated.[10,22,23] During this process, the scaffold acts as a template for vascularization.[24,25] Recently, different new materials have been used as a matrix for bone regeneration, including ceramics, synthetic and natural polymers, and composites.[20] Examples have included demineralized bone, collagen, proteins, fibrin, and various forms of calcium phosphate.[10,20] Nanostructured forms of calcium phosphates that can bind and condense plasmid DNA and attach growth factors, including 3D architectures of tailored natural polymer-based gels and cements, exploit the enhanced bioactivity and resorption potential. These novel cements also are being studied as next-generation systems for tissue engineering.[26-29]

The scaffolds ideally need to accomplish several goals, which include biocompatibility, appropriate mechanical strength, and appropriate degradation. If

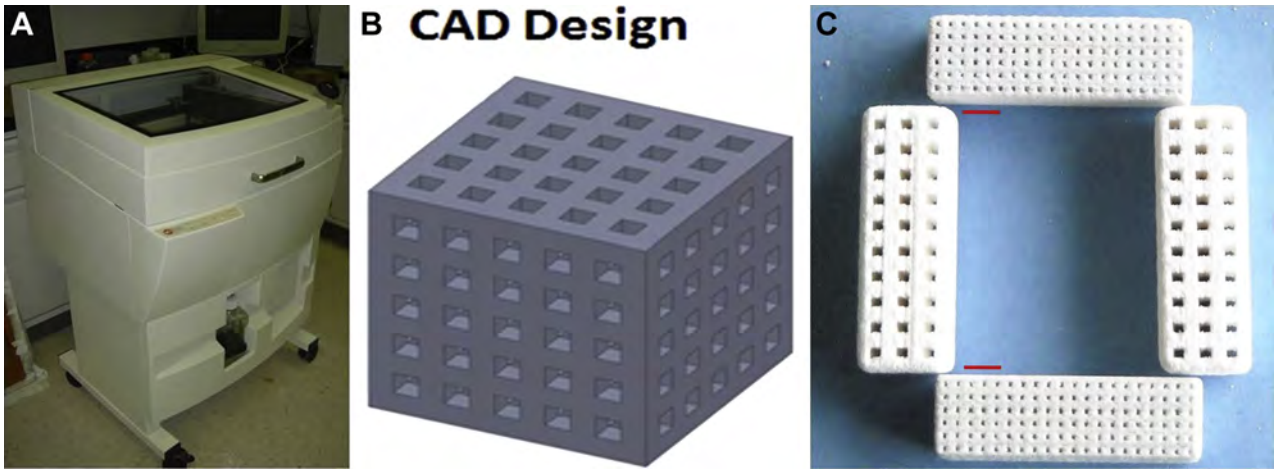

FIGURE 2. *A*, A 3-dimensional inkjet printer that can print different materials, including metal structures or calcium phosphate cement material from standard imaging software. *B*, Digital Imaging and Communications in Medicine (DICOM) images can be imported into the software, where the scaffolding can be adjusted and redesigned for printing. *C*, Custom constructs can be printed for use with proteins, cells, or other materials for regenerating tissues. CAD, computer-assisted designed.

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a material lacks biocompatibility, then it elicits host responses that destroy the materials.[30] In addition, the surface of the scaffolds must support appropriate cell interaction. Because proliferation of most mammalian cell types is anchorage dependent, scaffolds must provide a suitable surface for cell attachment, proliferation, differentiation, and migration.[31] This can be achieved by the use of materials that are derived from, or mimic, the micro-physiologic environment.[20,26-29]

A scaffold also must have adequate tensile and compressive strengths. These requirements might vary in different areas of the craniomaxillofacial complex. For example, a zygomatic bone or cranial vault reconstruction does not typically require that the construct endure a heavy functional load. However, the mandible routinely requires substantial strength to endure various loads in multiple directions and dimensions. It is essential that the scaffold exhibit acceptable strength to execute the biological cues needed for regeneration. In vitro, the scaffolds also should have sufficient mechanical strength to withstand hydrostatic pressures and to maintain the spaces required for cell ingrowth and matrix production.[32] In vivo, the mechanical properties of the implanted construct ideally should match those of living bone, so that an early function of the reconstructed site can occur.[23]

In addition, the degradation rate should be matched with the regeneration rate of the newly forming tissue in an optimal fashion. The scaffold should exhibit nontoxic degradation. For example, polylactic acid constructs typically degrade by hydrolysis during the Krebs cycle and release carbon dioxide and water as their byproducts. Porosity and interconnectivity also are important for an efficient and effective diffusion of nutrients and gases. These properties aid in the appropriate removal of metabolic waste resulting from the cells that regenerate into the scaffold. Owing to the metabolic demands of the bone, high rates of mass transfer are expected with the regenerative process.[33] The pore size of the scaffold material plays an important role in cell proliferation and cell distribution throughout tissue regeneration.[34] Some have suggested an optimal pore size of 200 to 400 μm.[35] Others have suggested that pore sizes up to 200 μm in polyester membranes result in the best bone ingrowth.[36] The size of the pore has an important effect on its mechanical integrity and its ability to perform under functional demands.[21] Pores and voids disrupt the continuity of the solid phase and subsequently decrease the solid-phase density. This weakening of the solid phase and decreased mass density across the surface and bulk result in defects at the atomic and molecular levels, causing the material to fail more easily under decreased stresses compared with the more dense (less porous) material. The authors and others are working to maximize the strength of the solid phase and maintain porosity in the range of 70% with biologically acceptable pore sizes. Thus far, this has been a challenge when combining materials science and clinical applicability of bone substitutes, scaffolds, and various materials used in regenerative approaches.

Growth Factors

Growth factors are proteins that act as signaling molecules on an appropriate cell to carry out a desired function. These proteins activate the cellular communications network and influence functions such as cell

proliferation, matrix deposition, and differentiation of tissues.[37] Growth factors have been shown to play a key role in bone and cartilage formation, fracture healing, and the repair of other musculoskeletal tissues.[38] Abnormalities in the genes that code for these proteins cause different craniofacial skeletal dysostoses (ie, Apert syndrome, Crouzon syndrome, and the achondroplasia syndromes). The binding of a growth factor to its receptor initiates intracellular signaling that will lead to different events, such as the promotion or prevention of cell adhesion, proliferation, migration, and differentiation. This typically occurs by upregulating or downregulating the synthesis of proteins and receptors.[38-40] Much like other tissues, bone has many different growth factors that are active in its formation and remodeling processes. BMPs, transforming growth factor-β (TGF-β), fibroblast growth factors, insulin growth factors I and II, and platelet-derived growth factors are the most commonly studied. Some of these have been evaluated for their inductive potential as regenerative medicine adjuncts.[3-7,38-42]

In 1965, Urist[4] made the observation that demineralized bone matrix could produce bone formation when placed in subcutaneous tissue. This capability was later attributed to BMP.[4,42] Currently, the BMPs are grouped into the TGF-β superfamily by virtue of their similarities in protein structure and sequence homology. BMPs are closely associated with the bone matrix and are expressed during the early phases of fracture healing.[43] Their role is to recruit mesenchymal stem cells (MSCs) to the healing site and then differentiate them into the osteogenic lineage for bone deposition. Although many BMPs have been described, BMPs 2, 4, 6, and 7 are the most well studied in craniofacial biology and are considered to have the greatest potential for regeneration.[40,44,45]

Cellular Approaches

Cells are important during the integration of regenerative materials used for reconstruction and for the long-term viability of any implanted material or device. These cells might be provided initially with the "graft" or recruited into the construct during the early regenerative process. Different tissues and defect sizes will have different demands, and the interaction of the cells, signaling molecules, and scaffold are vastly different for soft tissue, bone, or other complex tissues. In addition, as the scaffold changes or is resorbed in the regenerative process, the cellular components likely change in different ways. The sequence of these events can be quite complicated and represent a major challenge when designing regenerative techniques for the craniomaxillofacial region. Different cellular technologies and approaches have been used to achieve regeneration in the craniomaxillofacial region.

Among the ways to provide cells to a defect is simple bone autotransplantation. Free and nonvascularized bone grafts have been a mainstay of reconstruction for different craniomaxillofacial defects for decades. These procedures were tried as an empirical approach and at the time seemed ideal for regenerating bone because bone marrow is rich in osteoprogenitor cells and osteogenic precursors—including the ability to secrete BMPs.[22] The procedure involves procuring bone marrow from a donor site (eg, iliac crest, cranium, rib, tibia, or mandible) and transplanting it into the defect site with enough soft tissue coverage to allow the local tissues to eventually provide vascular supply to the graft. Autogenous grafting is relatively simple and inexpensive and has limited morbidity in most cases. However, the limited sources and relative scarcity of osteogenic cells after aging, disease, and irradiation ultimately limit its widespread use for all defects or compromised sites.[46,47] In addition, these types of grafting procedures rarely heal in a manner that achieves the ideal 3D structure to replace the defect.

MESENCHYMAL STEM CELL TECHNOLOGY

MSCs are immature and undifferentiated cells that are obtained from bone marrow and the periosteum. They have the capacity to achieve extensive replication without differentiation and they possess multilineage developmental potential, making them a powerful source of cells.[48,49] MSCs can be subdivided into adult stem cells (ASCs) and embryonic stem cells (ESCs). ESCs are usually isolated from the inner wall of preimplantation blastocysts. These embryonic cells in the early developmental stages are more proliferative and are pluripotent owing to their indefinite amplification, without the risk of dedifferentiation.[50] ASCs reside in the fully differentiated or adult tissues. ASCs have been procured from the bone marrow, periosteum, muscle, fat, brain, dental pulp, and skin.[51-58] Ethical issues, immune rejection uncertainty, and uncontrolled differentiation make ESC use challenging.

In addition to their differentiation potential, MSCs have other important attributes. It has been suggested that these cells might possess unique immune effects that might render them immune privileged or play immunosuppressive roles, which would make them suitable for allogeneic or xenogeneic transplantation. An important issue clinicians face when using MSCs is how to induce them while controlling their differentiation into the desired cell type. Key to this process is the clear understanding and reproducibility of methods to isolate desired populations of MSCs. The process by which these cells undergo expansion and differentiation into different cell lineages is quickly being understood such that cell lineages that produce bone, fat, cartilage, muscle, and tendon can be produced. These

techniques have been used to regenerate bone in cranial vault defects, cartilage in fetal tracheas, and cardiac muscle after acute myocardial infarction.[59-63] Currently, considerable work is focused on these concepts and using these technologies to provide live cells to a craniomaxillofacial wound defect to regenerate tissues without a major immune response.

DIFFERENTIATED OSTEOBLASTS AND PERIVASCULAR CELLS

These cells are committed mesenchymal cells that have been directed down the osteogenic lineage to push the cell type closer toward the final type desired. Using this type of approach has the potential advantage of achieving rapid repair of defects because the cells are already differentiated. The main disadvantage is that they have a limited capacity for proliferation because they can perform only a certain number of replication cycles before the problem of dedifferentiation arises. The presence of these cells could drive the process locally and recruit additional cells from the host to continue the process.

Human perivascular cells also possess multilineage progenitor properties. These pericytes were purified from skeletal muscle, pancreas, adipose tissue, and other organs and were found to be myogenic in vivo and ex vivo, regardless of their tissue of origin.[64-66] More importantly, these human perivascular cells, when sorted from diverse human tissues and cultured over the long term, can give rise to adherent, multilineage progenitor cells that exhibit the features of MSCs.

PERIOSTEUM REGENERATION WITH HUMAN BONE MARROW STROMAL CELL SHEETS

Cell sheet engineering is an emerging technology that is being researched for tissue regenerative therapies, such as myocardial tissue repair and cornea regeneration.[67,68] This technique involves culturing cells to hyper-confluence and inducing matrix production for the formation of a robust tissue sheet.[69] At this point, tissue sheets can be peeled from the dish with forceps or released from the dish if cultured on a thermo-responsive polymer.[70] The periosteum is a tissue sheath surrounding the bone; therefore, the addition of a cell sheet around a bone defect or regenerative scaffold could provide an avenue for periosteum engineering. Some studies have shown promising results for bone regeneration using cell sheet techniques; however, the formation of a functional periosteum had not yet been structurally or molecularly characterized until the authors' work in this area.[71-74]

Bone marrow stromal cells (BMSCs) are a promising cell source for bone engineering because they can be easily isolated from autologous tissue and contain a population of ASCs with osteogenic capacity.[75] The authors hypothesized that engineered BMSC sheets could be used for the formation of a morphologically and molecularly relevant periosteum-like tissue. As mentioned earlier, calcium phosphates comprise the mineral component of vertebrate hard tissues, and therefore these biomimetic materials are often used for scaffolding for bone regeneration.[76,77] The authors performed a study in which calcium phosphate scaffolds were wrapped in cell sheets formed from human BMSCs (hBMSCs) and the constructs were subcutaneously implanted into mice for 8 weeks[78,79] (Fig 3). The development of bone-like and periosteum-like tissues was histologically assessed.

Hematoxylin and eosin staining showed that control scaffolds lacking tissue sheet wraps supported the infiltration of host mouse cells that generated a dense connective tissue within the scaffold. The addition of hBMSC tissue sheet wraps facilitated the formation of a bone-like tissue around the perimeter of the cement containing osteocyte-like cells. Furthermore, cuboidal osteoblast-like cells were seen organized in rows on the outer surface of the regenerated bone-like tissue, which is characteristic of a functional periosteum[80-82]; this type of structure was not present in the control samples. Immunohistologic staining was

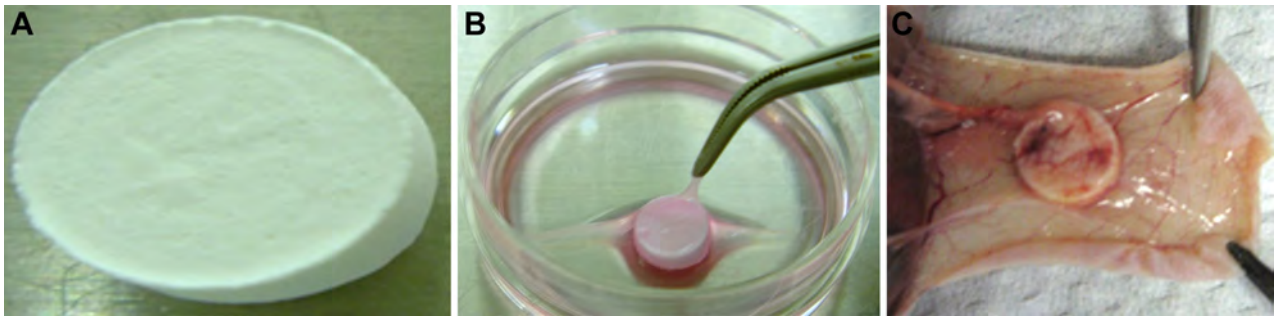

FIGURE 3. *A*, Calcium phosphate before manipulation with cells. *B*, Calcium phosphate being enveloped with a cell sheet. *C*, Calcium phosphate scaffold with a cell sheet wrap after explantation showing the development of the periosteum.

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performed to localize periostin expression to further verify periosteum formation. A layer of tissue on the outer surface of the regenerated bone-like structure strongly expressed periostin in constructs containing hBMSC sheet wraps. Together, the expression of periostin and the organization of osteoblast-like cells on the outer surface of the bone-like tissue indicate the regeneration of a functional periosteum-like tissue.

To their knowledge, the authors' report was the first to describe the generation and characterization of a functional periosteum-like tissue in an engineered construct. Studies have reported periosteum engineering by combining hBMSCs with collagen gel scaffolding or matrix from small intestinal submucosa or periosteum engineering using hBMSC cells sheets.[71,74,83,84] However, the focus of the analyses in these studies was the regeneration and characterization of bone-like tissues; the formation of a functional periosteum was not assessed. Although the formation of bone tissues is of great importance for regenerative therapies, the generation of a functional periosteum indicates that the regenerated bone is actively forming in a natural manner and signifies continued radial bone growth. In a previous study, a periosteum-like tissue formed naturally on a 3D scaffold-less bone-like construct engineered from rat BMSCs and was morphologically characterized by the organization of osteoblast-like cells on the bone surface.[78,79] In this report, the analysis of periosteum formation was similarly morphologically assessed and further verified by protein expression.[79] The authors' study conclusively showed the formation of a regenerated functional periosteum by the combination of an hBMSC sheet with a calcium phosphate cement scaffold. This could be a promising method of cell delivery and engineered periosteum formation for bone regenerative therapies.

The field of stem cell biology is changing rapidly and the understanding of stem cell differentiation or dedifferentiation of somatic cells is expanding greatly. Recent advancements have transformed mature skin cells into pluripotent cells by inserting just 4 genes (Oct3/4, Klf4, Sox2, and c-Myc) into the cell nucleus. These cells were called *induced pluripotent stem cells*.[64-66] Although more basic research is required to acquire knowledge about these cells, they are considered a major therapeutic possibility. Cellular therapy is changing rapidly and future use of these advanced technologies for craniofacial regeneration could hold great promise for patients.

Vascular Regeneration

One of the most intriguing aspects of tissue engineering has been the challenge of encouraging neovascularization—particularly for large defects. Although it has become quite straightforward to provide a large mass of cells to a defect in a given scaffold, providing an adequate blood supply to sustain those cells throughout the "graft" has proved difficult. In addition, providing a conduit for application of growth factors within a wound for temporal release throughout the regenerative process has been challenging. Different approaches have been considered to address these concerns. Much is understood about the process of normal vascular development, neoangiogenesis in the adult, and the genes that are involved in vascular development.[85,86] There is a complex dynamic that occurs during vascular development, including extrinsic influences, flow physics, hypoxia, and other factors.[87,88] Many scientists working in this area believe that once the issue of vascular regeneration is adequately addressed, it will open a door of opportunity not yet seen for regenerative medicine. This is the major challenge for larger constructs.

The process of neovascularization involves the interaction of endothelial cells and their formation of a primitive blood-vessel plexus that becomes a network of arteries, veins, and their associated capillaries. This process is believed to be tightly regulated with various signal pathways and involves the interaction of the local environment and the regulation of genes important in vascular development. The vascular endothelial growth factors (VEGFs) involved in this process are believed to drive this system forward in the proper manner. As such, the simple deposition of one particular type of VEGF at a high concentration within a wound is not likely to reproduce a complex vasculature system. The temporal release of these and other factors in the proper environment make creating a vasculature that could support a regenerative tissue construct challenging. Moreover, there are some pathologic processes that likely involve inappropriate VEGF upregulation, such as arteriovascular malformations, tumor growth, and aneurysm formation. Understanding this complex mechanism is the key to developing a tissue-engineered construct that will provide appropriate vascular support.

VEGF appears to have a key role in tissue development and regenerative processes. It has been well described as a part of the cascade controlling bone development during the promotion of vascular structures—particularly in the process of bone healing by acting on bone-forming cells.[86-90] Taking advantage of this growth factor has proved more challenging that just delivering it to a site with the expectation that vascular tissues will regenerate. There are dose-and-effect concerns and the importance of the temporal release of factors during a healing and regeneration phase that is appropriate. Kaigler et al[91] reported

some interesting work in this area, including the use of a VEGF-based scaffold that showed improved neovascularization and bone regeneration in a critical-size rat calvarial defect exposed to radiation. Continued work in these areas is likely to produce factors that optimize vascular proliferation and provide clinicians with ways to optimize vasculature within tissue-engineering constructs.

Regenerative medicine is poised to affect craniomaxillofacial surgery in major ways in the near future. Advances in the understanding of signaling molecules, scaffolds, and cellular technology are already influencing regenerative possibilities, and many of these technologies are being evaluated in large animal models and in initial clinical studies. Currently, signaling molecules and proteins are being used to drive local tissues to produce bone. Novel materials are being evaluated as improved scaffolds with better functionality to serve as delivery systems of growth factors, proteins, and genes in a controlled efficient manner, in addition to better mechanical properties and improved biocompatibility. Cellular technologies are providing some options for regeneration of tissues in the craniomaxillofacial skeleton. In addition, the understanding of neovascularization is helping tailor approaches to designing regenerated tissues. Additional technologies being developed in the laboratory, such as resorbable metals to hold the constructs, also could be helpful. Surgeons who work in the craniomaxillofacial region must have a clear understanding of these concepts, and it is fertile ground for translational science in craniomaxillofacial surgery.

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Early Complications After Cleft Palate Repair: A Multivariate Statistical Analysis of Patients

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Abstract: This study presents a large consecutive institutional experience with primary cleft palate repairs. The purpose of this study was to determine the incidence of early complications after cleft palate surgery in a series of nonsyndromic children treated at the authors' comprehensive cleft center. This retrospective analysis includes 709 consecutive patients with cleft palate treated by 6 different staff surgeons at Guwahati Comprehensive Cleft Care Center between April 2011 and December 2012. Secondary cases were excluded from this study. The patients were initially followed up between 1 week and 1 month after surgery. The overall incidence of early complications was determined, and the effect of the extent of clefting, the type of repair, the age at repair, and the operating surgeon were analyzed. Early complications in this study include dehiscence of the wound, fistula formation, hanging palate, and total or partial flap necrosis. There was a 2.4% rate (17/709) of takeback to the operating room in the immediate postoperative period for control of bleeding, although no blood transfusions were required. The incidence of postoperative fistulas in this series was 3.9% (20/512). There was a statistically significant increase in the incidence of cleft palatal fistula for Veau IV clefts, but there were no significant differences with respect to operating surgeon, patient sex, patient age, and type of palatoplasty. The complication and fistula rate is consistent with other published reports from developed countries and provides evidence for the value of this model for surgical delivery in the developing world.

Key Words: Complication, palatoplasty, fistula

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Cleft palate management is complex. There is no current agreement on the appropriate treatment strategy.[1] The central goals of cleft palate repair are to achieve a complete anatomical and functional closure with normal speech, no regurgitation of fluids or food into the nasal cavity, lack of maxillary growth disturbance, and minimization of hearing loss. Numerous centers have reported on their results after cleft palate repair, with varying results. Fistula formation after primary repair has been reported to range from 3% to 50%, with most with recent reports quoting rates of 5% to 25%.[2,3] The occurrence of complications after palate repair compromises the goals of surgery and complicates future management for the treatment team.[4] Palatal fistulas often require operative repair because they interfere with speech, result in nasal regurgitation, and negatively impact oral hygiene. Secondary surgery to close palatal fistulas creates additional palatal scarring and is associated with alarmingly high recurrence rates.[5,6] Clearly, surgical techniques that reduce postoperative palatal fistula risk offer significant benefits to patients.

To our knowledge, this is the largest study of its kind to review early postoperative results of cleft palate surgery in the developing world and provides insights into strategies for improved outcomes.

METHODS

This retrospective analysis includes 709 consecutive nonsyndromic patients with cleft palate treated by 6 staff surgeons at Guwahati Comprehensive Cleft Care Center between April 2011 and December 2012. Institutional review board approval was obtained for this study. All documents relating to diagnosis, surgical details, and follow-up were analyzed. Secondary cases were excluded from this study. Patients received their initial follow-up examinations between 1 week and 1 month after surgery, and those who did attend their follow-up were excluded from the study. Extent of clefting was described according to Veau classification (Table 1).[7] The overall incidence of early complications was determined. Early complications in this study are those complications that were evident in first month of the repair, including dehiscence of the wound, fistula formation, and total or partial flap necrosis. Cleft palatal fistula was defined as a failure of healing or a breakdown in the primary surgical repair of the palate. In keeping with recent fistula studies, postoperative fistulas were recorded when breakdown of the primary palate repair occurred along a suture line posterior to the incisive foramen. For fistulas, the location and size were noted if accurate information was available from the patients' clinical records. The size of fistulas was graded as small (1–2 mm), medium (3–5 mm), and large (>5 mm).

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This study was carried out at Guwahati Comprehensive Cleft Care Center (Assam, India), located at Mahendra Mohan Choudhury Hospital, Guwahati.

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TABLE 1. Veau Classification

Class	Site Involved
I	Soft palate
II	Soft palate and hard palate
III	Soft palate and hard palate and unilateral cleft of primary palate
IV	Soft palate and hard palate and bilateral cleft of primary palate

Fistula locations were designated as hard palate, junction of hard and soft palate, and soft palate.

Subsequently, the patients with and without early postoperative complications were compared with respect to age at time of surgery, sex, extent of cleft, type of palatoplasty, and operating surgeon. The emphasis was to determine if any of these variables resulted in higher incidence of complications. A χ^2 analysis was used to examine the associations between discrete variables.

RESULTS

There was a 2.4% rate (17/709) of take-back to the operating room in the immediate postoperative period for control of bleeding. There were no blood transfusions.

Five hundred twelve of 709 patients attended their follow-up appointments for an overall follow-up rate of 72.2%. These patients included 257 boys and 255 girls with ages ranging from 11 months to 36 years at the time of surgery. Twenty of 512 patients had complications resulting in fistula formation, for a total complication rate of 3.9%.

There were 3 fistulas classified as small (1–2 mm), 4 as medium (3–5 mm), and 13 as large (>5 mm). Nine fistulas were located in the hard palate, 5 at the junction of the hard and soft palate, 5 located in the soft palate, and 1 complete dehiscence involving the entire hard and soft palate.

Of all patients included in this series, 14.1% had palates classified as Veau I, 35% as Veau II, 40.6% as Veau III, and 10.4% as Veau IV. Complication rates were 2.8% (2/72) for Veau I, 2.2% (5/179) for Veau II, 3.8% (8/204) for Veau III, and 11.3% (6/53) for Veau IV (Table 2). The increased incidence of complications with Veau IV palates was statistically significant ($\chi^2 = 9.416$, $P = 0.024$).

Regarding type of palate repair, there was not a statistically significant difference in complication rates between the various techniques. The complication with 2-flap palatoplasty was 4.0% (17/428), which is by far the most commonly utilized repair at our center and typically performed with a radical intravelar veloplasty. There were no complications after Dorrance 1-flap repair (0%, 0/59), which we often prefer for Veau I and II clefts. Limited incision palatoplasty resulted in a 13.3% complication rate (2/15), whereas 25% (2/8) of patients receiving von Langenbeck repairs had postoperative complications.

No statistically significant differences in complication rates were identified with respect to operating surgeon, patient sex, patient age at palatoplasty, or type of palatoplasty (Tables 3–6).

TABLE 2. Veau Classification Correlation With Fistulas

Fistula	Veau I (n = 72)	Veau II (n = 179)	Veau III (n = 208)	Veau IV (n = 53)
Yes	2.8%	2.2%	3.8%	11.3%
No	97.2%	97.8%	96.2%	88.7%

$\chi^2 = 9.416$, $P = 0.024$.

We conclude that there is a significant association between fistula and Veau classification ($P < 0.05$).

TABLE 3. Operating Surgeon Correlation With Fistulas

Fistula	Surgeon 1 (n = 78)	Surgeon 2 (n = 54)	Surgeon 3 (n = 102)	Surgeon 4 (n = 89)	Surgeon 5 (n = 105)	Surgeon 6 (n = 84)
Yes	5.1%	3.7%	4.9%	6.7%	1.9%	1.2%
No	94.9%	96.3%	95.1%	93.3%	98.1%	98.8%

$\chi^2 = 5.201$, $P = 0.391$.

We observe that there is no significant association between fistula and the surgeons ($P > 0.05$).

Postoperative fistulas were distributed throughout the palate. There was 1 complete dehiscence (1/20; 5%), and otherwise 45% (9/20) of fistulas were located in the hard palate, 25% (5/20) at the junction of the hard and soft palate, and 25% (5/20) in the soft palate (Fig. 1).

DISCUSSION

This is the only institution in the state of Assam in Northeast India that provides comprehensive cleft care and serves a population of 31 million people.[8,9] With 87% of the population residing in rural and isolated conditions, patients are very poor and travel very far to reach the center. Although less than ideal, the follow-up rate of 72.2% is considered acceptable, given the distances, terrain, and socioeconomic limitations of the patient population. This is also higher than published figures from groups under similar circumstances.[10–13]

We report a 3.9% rate of early complications after primary cleft palate repair, a rate that is favorable in comparison to numerous rates quoted in the literature. We used multiple regression analysis to examine simultaneously the influence of type of palate repair, age at palate closure, sex, type of cleft, and surgeon on the subsequent development of cleft palate fistulas. The only factor leading to a statistically significant increase in complication rates was Veau IV palates, which is in agreement with prior studies.

The majority of reported data comes from centers in the developed world, where patients are referred to cleft teams as infants. Although controversy lingers and various treatment algorithms exist, cleft teams almost always proceed with palatal closure by 2 years of age.[14,15] Most algorithms now also involve presurgical orthopedics, and some centers use a 2-stage palate repair to decrease the size of the defect.[16,17]

Because of the complex socioeconomic challenges of our patients, they often do not present until much later and with much more severe clefts. In this series, 92.4% (473/512) of patients presented for surgery after 2 years of age, and none received preoperative orthopedics (Fig. 2). Despite this, our results compare favorably with numerous studies including patients with a much lower median age.[18–20]

We also found no statistically significant difference in complications in regard to patient age within our cohort, despite the fact that primary palatoplasty in older children and adults is significantly more challenging.[21–23] The clefts are wider, and palatal segments are more vertically displaced; mucoperiosteal flaps are more

TABLE 4. Sex and Correlation With Fistulas

	Male (n = 257)	Female (n = 255)
Yes	3.5%	4.3%
No	96.5%	95.7%

$\chi^2 = 5.219$, $P = 0.640$.

We observe that there is no significant association between fistula and sex of the patient ($P > 0.05$).

TABLE 5. Age at Repair and Correlation With Fistulas

Fistula	11 mo to 2 y (n = 39)	2–5 y (n = 89)	6–10 y (n = 124)	11–15 y (n = 126)	16–20 y (n = 64)	21–36 y (n = 69)
Yes	5.1%	1.1%	2.4%	4.0%	6.3%	7.2%
No	94.9%	98.9%	97.6%	96%	93.7%	92.8%

$\chi^2 = 5.893$, $P = 0.317$.

We observe that there is no significant association between fistula and the age of patient at repair ($P > 0.05$).

adherent to underlying bone, and they have an increased propensity for bleeding.[24]

We also found no statistically significant difference among the 6 staff surgeons in regard to early postoperative results. The technique preferred by all of our surgeons for repair of complete cleft palate (Veau III and IV) is a 2-flap technique with intravelar veloplasty as described by Sommerlad.[25] For clefts of the soft and posterior hard palate (Veau II), we typically prefer a single-flap palatoplasty as described by Dorrance and Bransfield[26] and had zero complications in this series with this technique.

The technique for incomplete clefts of the soft palate varies, including 2 flaps with intravelar veloplasty, von Langenbeck with intravelar veloplasty, Dorrance procedure with intravelar veloplasty, and Furlow double opposing Z-plasty.

We believe that postoperative fistulas correlate strongly with suture line closure tension, and numerous maneuvers are used for adequate mobilization of all tissues for a tension-free repair. A tempting mistake is to perform inadequate mobilization with narrow or incomplete clefts, and we do not hesitate to mobilize tissues fully. We also stress delicate tissue handling and do not directly grasp mucosal edges, finding that we can maneuver the flaps by grasping the muscle and internal surfaces. Cautery is minimized and applied with precision using a pointed tip.

After elevation of the mucoperiosteal flaps, both flaps are skeletonized on the greater palatine arteries, with division of oral fibers from the tensor veli palatine that extend toward the oral mucosa posterior to the pedicles. We do not divide the tensor muscle and do not intentionally fracture the pterygoid hamulus. If there is inadequate flap mobilization at this point, then we divide the periosteum on either side of the pedicles in order to achieve pedicle lengthening. This is achieved with 2 parallel incisions approximately 2 mm on either side of the greater palatine vessels in order to release them from the adherent and restrictive periosteum. Impressive mobilization can be achieved with this maneuver, resulting in bilateral mucoperiosteal flaps that can then be advanced medially without tension even in the widest of clefts. Closure always includes both a nasal layer and oral layer. With very wide clefts, the nasal layer on both sides can be extensively mobilized by completely releasing these flaps from the nasal surface of the palatal shelves and continuing the dissection to release the flap from the lateral nasal wall in the region of the inferior nasal meatus. This allows for extensive mobilization of the nasal mucosa, allowing for complete closure in cases of wide bilateral clefts with atrophic vomer. Sutures are tied with cephalic-facing knots in order to evert the wound edge into the nasopharynx. After the intravelar veloplasty, closure of the oral layer is performed with everting sutures. One or 2 sutures are used to incorporate both oral nasal layers to oppose them and obliterate the intervening dead space. We use a tongue stitch in all patients, and if there is any difficulty with bleeding we suture in a palate pack to minimize risk of postoperative bleeding.

Our treatment protocol involves primary repair of the cleft palate at age of presentation, with a minimum age of 1 year. This conservative lower age threshold is to minimize our anesthetic risk profile in these vulnerable patients. A single perioperative dose of

TABLE 6. Type of Repair Correlation With Fistulas

Fistula	2-Flap Palatoplasty (n = 428)	Dorrance 1-Flap Repair (n = 59)	Limited Incision (n = 15)	von Langenbeck (n = 8)
Yes	5.1%	3.7%	4.9%	6.7%
No	94.9%	96.3%	95.1%	93.3%

$\chi^2 = 0.268$, $P = 0.965$.

We observe that there is no significant association between fistula and type of repair ($P > 0.05$).

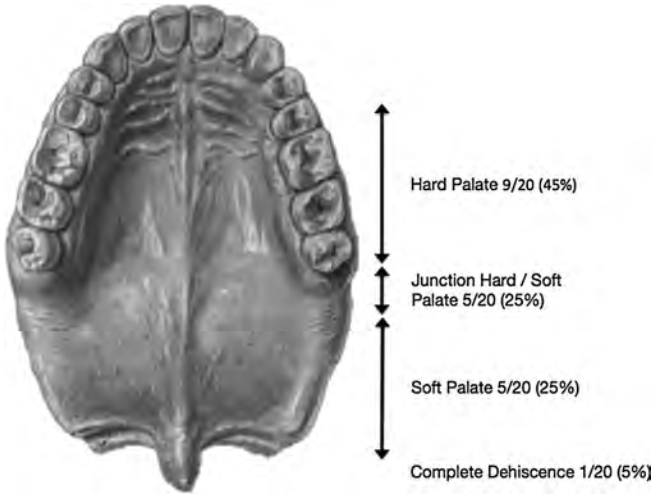


FIGURE 1. Distribution of postoperative fistulas.

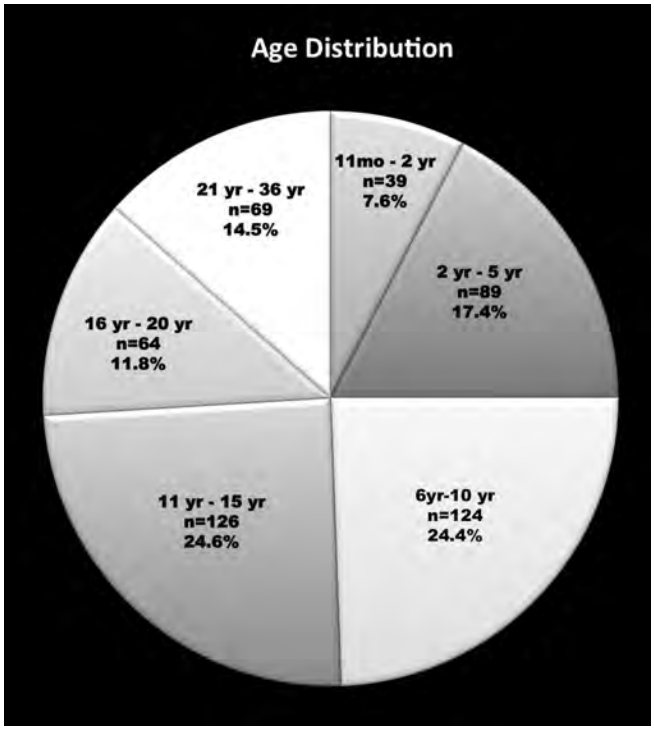
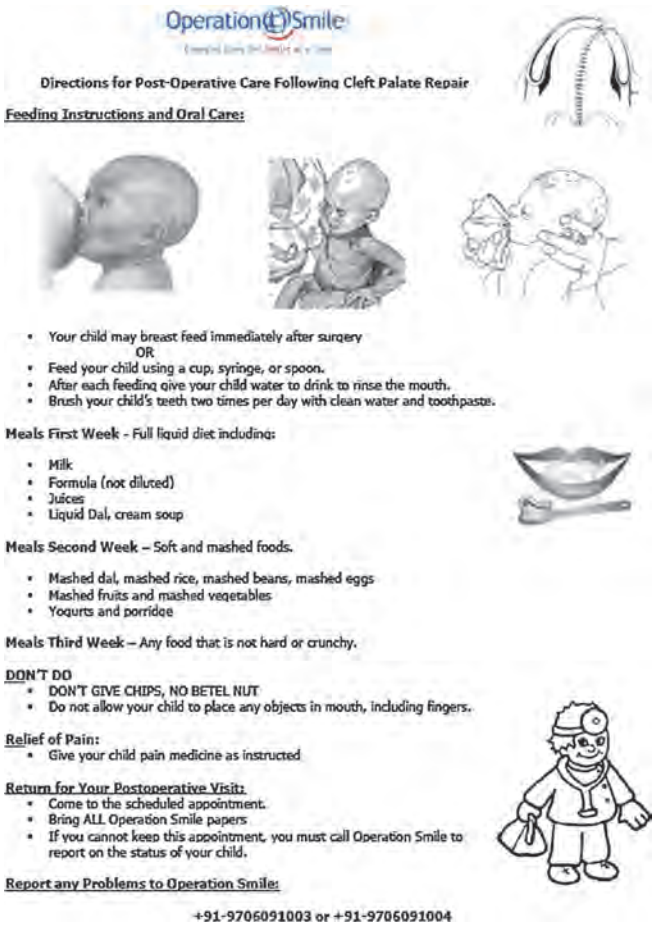


FIGURE 2. Age distribution at the time of primary palate repair.


Operation Smile

Directions for Post-Operative Care Following Cleft Palate Repair

Feeding Instructions and Oral Care:

- Your child may breast feed immediately after surgery
  OR
- Feed your child using a cup, syringe, or spoon.
- After each feeding give your child water to drink to rinse the mouth.
- Brush your child's teeth two times per day with clean water and toothpaste.

Meals First Week - Full liquid diet including:

- Milk
- Formula (not diluted)
- Juices
- Liquid Dal, cream soup

Meals Second Week – Soft and mashed foods.

- Mashed dal, mashed rice, mashed beans, mashed eggs
- Mashed fruits and mashed vegetables
- Yogurts and porridge

Meals Third Week – Any food that is not hard or crunchy.

DON'T DO

- DON'T GIVE CHIPS, NO BETEL NUT
- Do not allow your child to place any objects in mouth, including fingers.

Relief of Pain:

- Give your child pain medicine as instructed

Return for Your Postoperative Visit:

- Come to the scheduled appointment.
- Bring ALL Operation Smile papers
- If you cannot keep this appointment, you must call Operation Smile to report on the status of your child.

Report any Problems to Operation Smile:

+91-9705091003 or +91-9705091004

FIGURE 3. Postoperative information sheet for the patients.

intravenous antibiotics is given, and 5 days of amoxicillin is given after surgery. We do not use arm restraints; we feel that they are totally unnecessary. Postoperative pain is controlled almost universally with acetaminophen, and we rarely require narcotics after cleft palate surgery. After repair, patients and parents participate in a standardized patient education program. This includes standardized protocols followed by all surgeons, and intensive patient education sessions administered by the nurses prior to discharge. All patients receive discharge instruction sheets with pictographs in order to improve patient understanding and compliance (Fig. 3). Infants can be breast-fed immediately after surgery, and older children and adults take full liquids using a cup, syringe, or spoon. Oral hygiene is stressed, and patients are instructed to drink water to rinse the mouth and to brush their teeth 2 times a day with clean water and toothpaste. Our diet protocol includes full liquids for the first week, soft and mashed foods for the second week, and a normal diet starting the third week after surgery to include any food that is not hard or crunchy. The vast majority (>90%) of patients are discharged home on postoperative day 1. Patients follow up at periods of 1 week, 1 month, and 6 months after surgery. We find this approach to be tremendously helpful, and a study by our group has demonstrated the decrease in complications that result from standardized postoperative protocols combined with intensive patient education programs.[27]

Our results compare favorably with those published out of respected cleft institutions in developed countries, despite the vulnerability of cases and complexity of cases. Our findings are consistent with numerous studies demonstrating improved quality for complex procedures through use of high-volume hospitals.[28,29] We confirm this volume-outcome relationship for cleft palate repair, and provide further support for this model of surgical care delivery in the developing world.[30–32]

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Outcomes Article

Measuring Quality of Life in Cleft Lip and Palate Patients: Currently Available Patient-Reported Outcomes Measures

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Background: Patient-reported outcomes in cleft lip and palate treatment are critical for patient care. Traditional surgical outcomes focused on objective measures, such as photographs, anatomic measurements, morbidity, and mortality. Although these remain important, they leave many questions unanswered. Surveys that include aesthetics, speech, functionality, self-image, and quality of life provide more thorough outcomes assessment. It is vital that reliable, valid, and comprehensive questionnaires are available to craniofacial surgeons.
Methods: The authors performed a literature review to identify questionnaires validated in cleft lip and palate patients. Qualifying instruments were assessed for adherence to guidelines for development and validation by the scientific advisory committee and for content.
Results: The authors identified 44 measures used in cleft lip and palate studies. After 15 ad hoc questionnaires, eight generic instruments, 11 psychiatric instruments, and one non-English language questionnaire were excluded, nine measures remained. Of these, four were never validated in the cleft population. Analysis revealed one craniofacial-specific measure (Youth Quality of Life–Facial Differences), two voice-related measures (Patient Voice–Related Quality of Life and Cleft Audit Protocol for Speech–Augmented), and two oral health–related measures (Child Oral Health Impact Profile and Child Oral Health Quality of Life). The Youth Quality of Life–Facial Differences, Child Oral Health Impact Profile, and Child Oral Health Quality of Life questionnaires were sufficiently validated. None was created specifically for clefts, resulting in content limitations.
Conclusions: There is a lack of comprehensive, valid, and reliable questionnaires for cleft lip and palate surgery. For thorough assessment of satisfaction, further research to develop and validate cleft lip and palate surgery–specific instruments is needed. (*Plast. Reconstr. Surg.* 128: 518e, 2011.)

Cleft lip and palate is one of the most common congenital abnormalities encountered.[1] Surgeons treating these patients strive to achieve excellent aesthetic and functional outcomes to improve their patients' quality of life.[2] Traditional outcomes research for cleft surgery has focused on objective measures, such as anatomic measurements and clinical photographs, as well as morbidity and mortality.[3] Although these factors remain important, they are no longer sufficient on their own. Patient-reported outcomes measures that include aesthetic results, speech, functionality, self-image, incorporation into society, and quality of life provide surgeons with a more comprehensive assessment of surgical outcomes.

To appropriately evaluate these surgically relevant outcomes, well-developed and well-validated patient questionnaires are required.[4] Ad hoc questionnaires that were not formally developed or psychometrically tested may ask reasonable questions; however, their reliability or ability to produce consistent and reproducible scores and validity, or ability to measure what is intended to be measured, cannot be ensured.

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In the absence of cleft lip and palate–specific validated questionnaires, many studies employ generic instruments that measure health-related quality of life in diverse patient populations.[5–10] Although many of these instruments have been shown to be reliable, they are less likely to be sensitive to all of the issues specific to the cleft lip and palate condition and the changes resulting from surgical treatment. Further, they often lack the ability to measure change over time, a quality known as responsiveness.[11] Reliability, validity, and responsiveness are the three key psychometric properties necessary in the creation of health status instruments.[11]

The treatment of cleft lip and palate affects many domains related to a patient's quality of life, including appearance, speech, social integration, self-image, and physical and psychological functioning.[3,12–15] Instruments related to just one domain, such as speech, cannot fully assess the effect of cleft lip and palate on a patient. To investigate the various dimensions of cleft lip and palate, it is necessary to develop a cleft-specific instrument that considers all of these domains.[16]

The use of reliable, valid, and responsive patient questionnaires is vital to evaluate the impact of cleft lip and palate surgery on the lives of these patients. Ad hoc instruments are inadequate because they lack proven reliability and validity. Generic instruments may be insufficient because they are not sensitive to all of the cleft-specific domains and may not be responsive to surgical change. Thus, the primary objective of this study was to identify existing cleft lip and palate–specific instruments that have undergone formal development and validation. The secondary objective was to systematically evaluate these measures to determine the extent to which they meet internationally established criteria based on guidelines set by the Scientific Advisory Committee of the Medical Outcomes Trust for health-related outcome measures.[17,18] This review will identify the best instruments currently available. It will also guide efforts to develop new cleft-specific instruments that can be used in future studies.

METHODS

An electronic bibliographic search was conducted to find relevant questionnaires using the following search terms: cleft, cleft surgery, quality of life, patient satisfaction, and questionnaire. Searches were performed in the following databases: MEDLINE, CINAHL (Cumulative Index to Nursing and Allied Health), and PsycINFO (Psychological Abstracts) from the inception of each database to September of 2010. Limits were placed to exclude non-English citations. All instruments included in the review were identified as patient-reported outcome questionnaires measuring quality of life and/or patient satisfaction specifically in cleft lip and palate patients who had undergone validation in the cleft lip and palate population.

To find relevant instruments not detected in the electronic search, a follow-up review of article references was performed as well as an electronic review of the *Cleft Palate-Craniofacial Journal* from January of 1990 until September of 2010. All instruments cited in the articles were evaluated for evidence regarding their development and validation criteria. Questionnaires not validated in the cleft lip and palate patient population were excluded. If the validation information was not published, the corresponding author was contacted and queried.

The remaining questionnaires were assessed for their adherence to international guidelines for health outcomes instrument development and validation as delineated by the Scientific Advisory Committee of the Medical Outcome Trust.[17] Content domains encompassed by each questionnaire were also appraised. A flow diagram of our search strategy is presented in Figure 1.

RESULTS

Our systematic literature review demonstrated that there are a limited number of patient-reported outcome measures that have been validated in the cleft lip and palate population. We identified 44 instruments used in cleft lip and palate studies. After 15 ad hoc questionnaires,[7,19–26] eight generic instruments,[6,10,27–30] 11 psychiatric instruments,[8,12,31–36] and one non–English language questionnaire[37] were excluded, nine cleft lip and palate relevant measures remained. Of these, four were never validated in the cleft population. Analysis of the remaining five measures revealed that there is one craniofacial specific measure, the Youth Quality of Life–Facial Differences questionnaire,[38–40] two voice-related measures, the Patient Voice–Related Quality of Life survey[41] and Cleft Audit Protocol for Speech–Augmented,[42] and two oral health-related measures, the Child Oral Health Impact Profile[43,44] and Child Oral Health Quality of Life questionnaire,[45–47] that were validated in the cleft population. Evaluation of their development and validation is represented in Table 1. Assessment of the scope of their content is summarized in Table 2. An outline of the ages in which these questionnaires were validated is presented in Table 3.

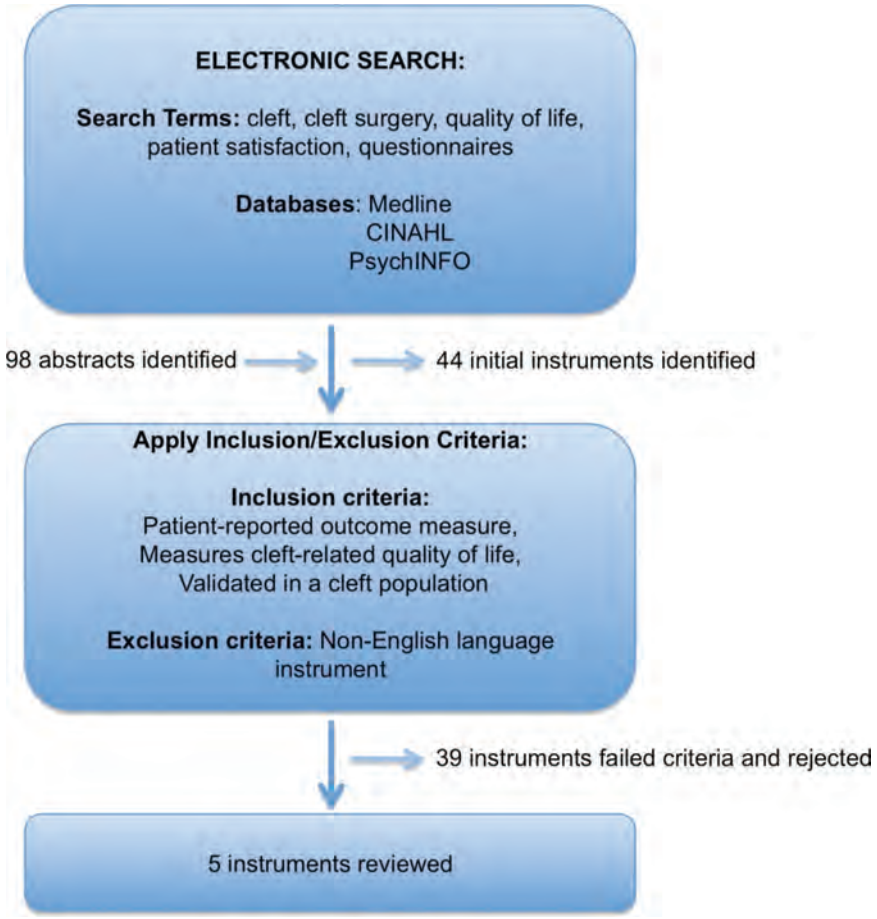

Fig. 1. Flow diagram of search strategy.

Craniofacial Specific Quality of Life Questionnaire

Youth Quality of Life–Facial Differences Questionnaire

The Seattle Quality of Life Group associated with the University of Washington developed a questionnaire to assess the quality of life in people with facial differences. Designed for children 11 to 18 years old, the Youth Quality of Life–Facial Differences questionnaire defines "facial difference" as a noticeable congenital or acquired craniofacial condition. It consists of 48 questions across five domains: stigma, negative self-image, positive consequences, negative consequences, and coping. These items were generated through expert opinion, patient and parent interviews and focus groups, literature search, formal item reduction with factor analysis, and psychometric testing.[38–40] Psychometric evaluation of this questionnaire involved testing of its conceptual and measurement model, internal consistency, reproducibility, validity, respondent burden, and alternate modes of administration. This instrument showed good acceptability, internal consistency reliability, and reproducibility (Cronbach's alpha less than 0.7 in all domains), and a principal components analysis supported the hypothesized domain structure. Construct validity was supported by hypothesis testing within the scale and when compared with the Youth Quality of Life–Research Version, generic youth quality-of-life survey. Overall, the Youth Quality of Life–Facial Differences questionnaire is a carefully constructed and validated survey that assesses facial appearance and social-emotional domains in adolescents with facial differences. It is not specific for cleft patients, however, and may miss several key issues that are important to this population, such as cleft palate–related function or concerns regarding treatment.

Speech and Voice-Specific Questionnaires

Pediatric Voice-Related Quality of Life Survey

The Pediatric Voice-Related Quality of Life survey is a parent proxy instrument designed to evaluate the voice-related quality of life of pedi-

Table 1. Patient-Reported Outcome Measures for Cleft Patients: Development and Validation Criteria

	Oral HQOL Specific		Voice Specific		Craniofacial Specific
Method/Evaluation	**COHIP**	**COHQOL**	**PVRQOL**	**CAPS-A**	**YQOL-FD**
Item generation					
Patient interviews					●
Literature	●	●	●	●	●
Expert opinion	●			●	●
Develop conceptual model	●	●			●
Item reduction					
Expert opinion	●				●
Item redundancy	●	●			●
Endorsement frequencies	●	●			●
Missing data	●	●			●
Factor analysis	●				●
Tests of scaling assumptions					●
Psychometric analyses					
Acceptability	●	●	●	●	●
Internal consistency reliability	●	●	●		●
Item total correlations					
Interrater reliability				●	
Validity					
Within scale	●	●	●		●
Comparison with other measures	●	●	●		●
Hypothesis testing responsiveness	●	●	●		●

HQOL, Health-Related Quality of Life; COHIP, Child Oral Health Impact Profile; COHQOL, Child Oral Health Quality of Life questionnaire; PVRQOL, Patient Voice–Related Quality of Life survey; CAPS-A, Cleft Audit Protocol for Speech–Augmented; YQOL-FD, Youth Quality of Life–Facial Differences questionnaire.

Table 2. Patient-Reported Outcome Measures for Cleft Patients: Content Analysis

	Oral HQOL Specific		Voice Specific		Craniofacial Specific
Domain	**COHIP**	**COHQOL**	**PVRQOL**	**CAPS-A**	**YQOL-FD**
Facial appearance					
Satisfaction with appearance	●				●
Noticeable change in appearance					
Affects self esteem	●				●
Affects social contact/activities	●				●
Concern about scarring					
Speech					
Voice quality/understandability	●	●	●	●	
Voice strength/volume			●		
Communication/talking			●		
Satisfaction with voice			●		
Interference with social activities/school	●		●		
Strain during speech			●	●	
Oral					
Oral health/symptoms	●	●			
Functional					
Eating/swallowing (jaw function)	●	●			
Hearing					
Breathing through nose	●	●		●	
Food/drink passage from mouth to nose					
Pain/discomfort	●				
Social-emotional					
Depression/anxiety	●	●	●		
Avoid smiling/speaking in front of others	●	●			
Experienced teasing	●	●			●
Worry what others think	●	●			●
Self-image/self-esteem	●	●	●		●
Anger/frustration	●	●	●		●
Sensation of loss of control					●
Shyness	●	●	●		●
Treatment/surgery					
Satisfaction with treatment/surgery	●				
Perceived need for treatment					
Anxiety regarding treatment/surgery	●				

HQOL, Health-Related Quality of Life; COHIP, Child Oral Health Impact Profile; COHQOL, Child Oral Health Quality of Life questionnaire; PVRQOL, Patient Voice–Related Quality of Life survey; CAPS-A, Cleft Audit Protocol for Speech–Augmented; YQOL-FD, Youth Quality of Life–Facial Differences questionnaire.

Table 3. Patient-Reported Outcome Measures for Cleft Patients: Age Ranges

	Oral HQOL Specific		Voice Specific		Craniofacial Specific
Validated for	COHIP	COHQOL	PVRQOL	CAPS-A	YQOL-FD
Parents		●	●	●	
Children					
4–7 years					
7–11 years	●				
11–14 years	●	●			●
14-18 years					●

HQOL, Health-Related Quality of Life; COHIP, Child Oral Health Impact Profile; COHQOL, Child Oral Health Quality of Life questionnaire; PVRQOL, Patient Voice–Related Quality of Life survey; CAPS-A, Cleft Audit Protocol for Speech–Augmented; YQOL-FD, Youth Quality of Life–Facial Differences questionnaire.

atric patients. It is a 10-item instrument adapted from the adult Voice-Related Quality of Life questionnaire.[41] The use of parent proxies for this survey is argued to be necessary, particularly for young children, because the parents are thought to better understand the scope of the child's problem and parental concern is often the driving force behind seeking treatment for speech issues.[41] Previous studies by Hartnick et al. have shown that administration of voice surveys to parent proxies is a reliable means of determining voice-related quality of life in children.[48,49] The Pediatric Voice-Related Quality of Life survey measures both social-emotional and physical-functional aspects of voice and speech issues. Psychometric evaluation of this survey showed good acceptability and internal consistency reliability (Cronbach's alpha = 0.96, test-retest reliability weighted κ value = 0.8), and the survey was validated in a general pediatric otolaryngology population by assessing convergent and discriminant validity. Overall, this questionnaire is based solely on the literature, is well validated, and evaluates voice-related concerns.

Cleft Audit Protocol for Speech–Augmented

The Cleft Audit Protocol for Speech–Augmented is a parent proxy instrument based on the Cleft Audit Protocol for Speech designed to assess speech in pediatric cleft patients.[42] Three cleft speech experts at the Regional Cleft Center in the United Kingdom developed the 14 speech parameters measured in the questionnaire. After a review of the literature, they developed parameters including intelligibility, voice, hypernasality, hyponasality, nasal emission, nasal turbulence, nasal friction, grimace, noncleft speech errors, specialist intervention, and four categories of cleft-type characteristics: anterior, posterior, nonoral, and passive. Psychometric evaluation of the augmented protocol through two pilot studies showed good acceptability and ease of use and good/very good interrater reliability in seven sections and moderate interrater reliability in three sections.[42] Overall, this survey examines specific aspects of cleft-related speech based solely on literature and expert opinion and has been psychometrically evaluated only in a small sample population.

Oral Health–Related Quality-of-Life Questionnaires

Child Oral Health Impact Profile

The Child Oral Health Impact Profile was designed to assess self-reported oral-facial well-being in children aged 8 to15 years. Development of the questionnaire involved multiple stages and revisions, including initial item generation using literature review and expert opinion, followed by formal item reduction using face and content validity testing, impact evaluation, and factor analysis. The final questionnaire consists of 34 items and five conceptual domains: oral health, functional well-being, social/emotional well-being, school environment, and self-image.[43] Psychometric evaluation of the Child Oral Health Impact Profile showed good acceptability and internal consistency reliability (Cronbach's alpha = 0.91, test-retest reliability intraclass correlation coefficient = 0.84).[44] Discriminant validity testing also supported the questionnaire's ability to reveal differences in oral health–related quality of life in children with craniofacial anomalies compared with those without such conditions. Overall, this survey is well developed (although it excluded patient interviews for item generation), valid, and reliable and assesses several issues important in the cleft patient population.

Child Oral Health Quality of Life Questionnaire

The Child Oral Health Quality of Life questionnaire was designed to measure self-reported oral health–related quality of life in children aged 11 to 14 years. It includes four domains: oral symptoms, functional limitations, emotional well-be-

ing, and social well-being. Originally a 37-item questionnaire, it was shortened to a 16- and an 8-item questionnaire for use in clinical settings.[45] Development of this instrument involved literature review and expert opinion followed by item reduction based on the input of children with dental caries (a pediatric dentistry group), malocclusions (an orthodontic group), and clefts of the lip and/or palate (an orofacial group).[46] An item impact method and stepwise regression were used to shorten the original questionnaire.[45] Psychometric evaluation of the long and short forms showed good acceptability, internal consistency reliability (Cronbach's alpha less than 0.7 in both), and discriminant and construct validity.[45,46] A parent proxy form of the survey was also developed and found to be valid and reliable.[47] Overall, this survey is well developed, valid, reliable, and assesses many social-emotional and oral-functional aspects of clefts.

DISCUSSION

In our review, we identified five questionnaires assessing quality of life and/or patient satisfaction validated in the cleft lip and palate patient population. The quality of these surveys varied with respect to their development, validation, and content. Of these questionnaires, the Youth Quality of Life–Facial Differences questionnaire, Child Oral Health Impact Profile, and Child Oral Health Quality of Life questionnaire were sufficiently validated according to guidelines.[17,18] All of these measures have some content limitations, specifically relating to surgical experience and satisfaction and cleft-specific functional issues. This is in part due to the fact that none of these questionnaires, with the exception of the Cleft Audit Protocol for Speech–Augmented, was created with the cleft population specifically in mind.

Questionnaire Development

Of the five questionnaires in our review, only the Youth Quality of Life–Facial Differences questionnaire was created using patient interviews as a component of item generation, and only three of the five had a published formal item reduction process. Although expert opinion and literature review are essential aspects of item generation, patient interviews are valuable sources of input for the creation of these questionnaires. Patient interviews permit the identification of issues of most importance to patients that may not be considered significant by surgeons and other health care providers. By not including patient interviews as part of the survey generation, it may be inferred that the issues of most importance to the patient may not be included among the questions asked. Therefore, thoroughly analyzed patient interviews are an important aspect of item generation in the creation of these patient-reported outcome measures. Formal item reduction is also a vital part of questionnaire generation. This includes factor analysis, item redundancy, endorsement frequencies, and other psychometric techniques that ensure that only statistically strong questions are included in the final measure. These are important steps in the creation of strong, statistically relevant questionnaires.

Psychometric Evaluation

Of the five questionnaires, four were comprehensive in their psychometric analysis. Psychometric testing is vital for the assessment of validity, reliability, and responsiveness of a measure, and these data are essential for researchers to ensure the precision of surveys that they intend to use in their own studies.[17,18] Only the Cleft Audit Protocol for Speech–Augmented was deficient in psychometric evaluation, as it was tested only in a small sample population and only for interrater reliability.[42] The other four questionnaires had extensive psychometric tests published. The only measure that has not been assessed in any of the questionnaires was responsiveness, the ability to detect change over time, which is an important aspect of outcomes research, particularly for patients currently undergoing treatment.[11,17]

Questionnaire Content

With respect to content, no individual questionnaire assessed all of the domains expected to be important for patients with cleft lip and palate. This is likely due in part to the fact that only the Cleft Audit Protocol for Speech–Augmented, a speech-specific measure, was created specifically for the cleft population. This may also be due to the lack of patient interviews included in the development of the majority of the surveys. Facial appearance was assessed only in the Child Oral Health Impact Profile and Youth Quality of Life–Facial Differences questionnaire; however, change in appearance secondary to surgery or other treatment was not assessed in any of the measures. Speech was well addressed by the Patient Voice–Related Quality of Life survey, and issues of voice quality/understandability were also brought up in the Child Oral Health Impact Profile, Child Oral Health Quality of Life ques-

tionnaire, and Cleft Audit Protocol for Speech–Augmented. Oral health and symptoms were addressed in the Child Oral Health Impact Profile and Child Oral Health Quality of Life questionnaire only. Cleft palate–related functional issues were poorly represented throughout the five questionnaires. Only the Child Oral Health Impact Profile and Child Oral Health Quality of Life questionnaire included assessment of jaw function, whereas the Child Oral Health Impact Profile also evaluated pain and discomfort. Breathing through the nose was addressed in Child Oral Health Impact Profile, Child Oral Health Quality of Life questionnaire, and Cleft Audit Protocol for Speech–Augmented. Social-emotional factors were well represented in the Child Oral Health Impact Profile, Child Oral Health Quality of Life questionnaire, Patient Voice–Related Quality of Life survey, and Youth Quality of Life–Facial Differences questionnaire, with many aspects of the patient experience included in these measures. Only the Child Oral Health Impact Profile addressed the issues of patient experience with treatment and surgery. Each questionnaire provides partial information on specific domains of the cleft experience; thus, various combinations of questionnaires or the addition of supplemental, unvalidated questions may be required for a full assessment, which may be challenging and time-consuming in clinical settings. Therefore, there is a need for the development of a more comprehensive measure.

CONCLUSIONS

The goal of this review was to identify and assess currently available patient-reported outcome measures for cleft lip and palate patients based on the guidelines outlined by the Scientific Advisory Committee of the Medical Outcomes Trust.[17] The results highlight the lack of comprehensive, valid, reliable, and responsive instruments with which to measure patient-reported outcomes in cleft lip and palate surgery.

There are measures currently available that may be clinically useful to surgeons and researchers caring for cleft lip and palate patients interested in assessing patient-reported outcomes. It appears, however, that fully evaluating all domains of the cleft treatment experience would require the use of a combination of these patient-reported outcomes measures or the addition of more, unvalidated questions to fill in potential gaps.

To allow for thorough assessment of satisfaction and quality of life in these patients following surgery, further research to methodically develop and validate new instruments specifically for the cleft lip and palate population is necessary. Specifically, the inclusion of patient interviews to identify issues of most significance to the cleft population, as well as formal item reduction to ensure that only items of significance are included in the final questionnaire, is important for survey generation. To create comprehensive, valid, reliable, and responsive measures, it is vital that future efforts to create cleft lip and palate–specific, patient-reported outcomes measures follow the guidelines outlined by the Scientific Advisory Committee of the Medical Outcomes Trust and by Cano et al.[11,17] These guidelines support a three-phase approach of item generation, item reduction, and psychometric analyses that is summarized in Figure 2.

The creation of a patient-reported outcomes measure for the cleft lip and palate population is inherently complicated due to the variety of defects encompassed by this condition and the range of ages of these patients. When attempting to create a cleft-specific patient-reported outcomes measure in the future, researchers must be cognizant of the challenges presented by both the differences in defects seen within this population of patients and the changes in cognitive development as these patients age. This review suggests

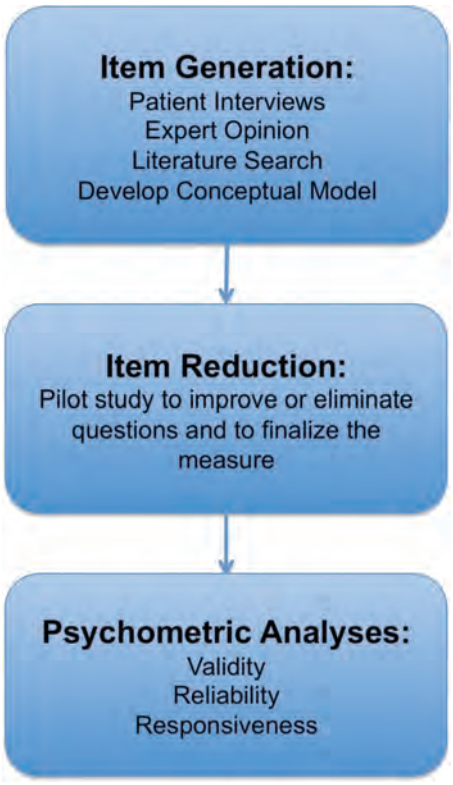

Fig. 2. Three phases of patient-reported outcome measure development.

that the creation of a valid and reliable cleft-specific survey would be a valuable addition to both patient care and clinical research evaluating the impact of surgery and treatment on cleft lip and palate patients' quality of life.

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Long-Term Results after Secondary Bone Grafting of Alveolar Clefts

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The aim of this study was longitudinally to evaluate the treatment results after secondary bone grafting in 224 cleft patients with an observation period of more than four years. The patients were divided into three groups according to age and eruption stage of the canine at the time of surgery. Group A included 94 patients with a mean age of 10 years, operated before eruption of the canine; group B included 72 patients with a mean age of 13.1 years operated after eruption of the canine; and group C included 58 patients operated after the age of 16 years (mean age, 20.4 years). The evaluation of the treatment results included longitudinal comparison of marginal bone level, periodontal status on cleft-related teeth, dental status in the bone grafted region, esthetical and functional properties of the reconstructed alveolar process, as well as the influence on growth of the maxilla. The marginal bone level was found to be significantly higher among unilateral cleft lip and palate (UCLP) and bilateral cleft lip and palate (BCLP) patients in the youngest groups as compared to the other groups. The number of UCLP and BCLP patients who could be treated without bridgework was significantly higher in the youngest age group than in the other groups, as were the esthetic and functional properties of the reconstructed alveolar process. External root resorption occurred in 17 patients in groups B and C. No influence of the procedure on sagittal growth of the maxilla could be demonstrated, whereas the anterior facial height was reduced. The length of the maxilla was significantly greater in group A than group B, where shorter maxillas were found compared to the control group. The study demonstrates that significantly better results are achieved with secondary bone grafting if the treatment is performed before eruption of the canine.

An important goal in the treatment of cleft patients is to normalize the anatomy of the cleft alveolar process. One of the treatment procedures used to achieve this goal is bone grafting the defects in the alveolar process. The recommended timing for this operation has varied. Some years ago, bone grafting in early childhood was advocated; however, the long-term results from many centers showed serious growth disturbances in the maxilla as a sequel to this treatment.[1,2] During recent years, several reports on the treatment results after secondary bone grafting using iliac crest bone have been published.[3–15] Based on these studies, there seems to be a trend towards bone grafting between six and 12 years of age.

In a comparative short-term follow-up study of 293 cleft palate patients subjected to secondary and late secondary bone grafting, the results were most promising among the patients operated before eruption of the canine.[16] The aim of this study was to

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Table 1. Age at Operation, Sex Distribution, and Observation Period

Group	A (n = 94)	B (n = 72)	C (n = 58)
Mean age at operation (yr, mo)	10,0	13,10	20,4
Range (yr, mo)	7,5–14,10	11,0–15,9	16,1–39,0
Sex			
Female	24	26	21
Male	70	46	37
Mean observation period (yr, mo)	7,0	6,5	6,0
Range (yr, mo)	4,1–9,10	4,2–9,1	4,0–12,3

evaluate the long-term treatment results in 224 of these patients, and longitudinally to compare the achieved marginal bone level on cleft related teeth.

Materials and Methods

The criterion for entering the study was a dentate individual with residual cleft(s) in the alveolar process(es).[16] The results of treatment recorded in the study represent the findings at least four years following the first operation involving secondary bone grafting. Among the patients who were classified as failures after the short-term evaluation,[16] some have been reoperated. These patients were included in this study, but the findings before reoperation were registered as the treatment result. In the short-term study,[16] a number of patients who received maxillary osteotomies simultaneously with the bone grafting were included. In the present study these patients have been excluded.

The material has been divided into three groups with regard to eruption stage of the canine and age at the time of operation (Table 1). Group A represents secondary bone grafting before eruption of the canine, group B secondary bone grafting after eruption of the canine, and group C late secondary bone grafting. The age at surgery, observation period, and sex distribution among the various groups appears in Table 1.

The distribution of the diagnoses of cleft lip and alveolar process only (CLA), unilateral cleft lip and palate (UCLP), and bilateral cleft lip and palate (BCLP) is shown in Table 2.

Before bone grafting, all patients received orthodontic treatment, including transverse expansion of the maxilla with a fixed orthodontic appliance. After surgery and eruption of all permanent teeth, the orthodontic treatment was continued.[17] All postoperative examinations were performed at Aarhus Cleft Palate Institute.

Evaluation of the treatment results included:

1. Assessment of the marginal bone level on teeth adjacent to the cleft by means of intraoral films and four scores (Fig. 1). The tooth with the lowest marginal bone level was used as the score for the patient. In patients with bilateral clefts, separate evaluation was made for each side

2. Periodontal evaluation. Periodontal condition on teeth adjacent to the cleft was assessed by measuring gingival recession from the cementoenamel junction and periodontal pockets from the gingival margin by probing. The amount of attached gingiva was also assessed. In some patients a buccal rotational flap was used which, in a number of cases, resulted in lack of attached gingiva. The effect of lack of attached gingiva on the marginal bone level was investigated

3. Dental evaluation. The teeth adjacent to the cleft were examined for external root resorption and retention of canines was examined by intraoral radiographs. Whether the treatment had been finished by orthodontics alone or with a combination of orthodontics and bridgework was also recorded

4. The esthetic and functional properties of the reconstructed alveolar process were assessed by an index with scores of 1–4, indicating the morphology of the alveolar process in the bone grafted region. Score 1 indicated the optimal, score 2 the acceptable (i.e., minor reduction of the height of the alveolar process making an esthetic and functional rehabilitation of the patient possible), score 3 indicated unacceptable possibilities for rehabilitation, and score 4 indicated that rehabilitation of the patient was impossible without reoperation.

In the evaluation of the marginal bone level and the esthetic and functional properties of the reconstructed alveolar process, each cleft was classified by the highest total score. In patients with bilateral clefts, the treatment result was evaluated for each cleft

5. Maxillary growth. The cephalometric analyses were made on lateral cephalograms, using selected reference points with special reference to midface growth. Cephalograms of 36 UCLP patients from group A, 31 UCLP patients from group

Table 2. Distribution of Diagnoses Among the Various Groups of Patients

Group	A (n = 94)	B (n = 72)	C (n = 58)	n (n = 224)
Cleft lip and alveolar process only	19	8	2	29
Unilateral cleft lip and palate	55	52	44	151
Bilateral cleft lip and palate	20	12	12	44

B, and a control group of 27 UCLP patients who had received the same treatment except bone grafting were compared. The sagittal dimension was evaluated by the angles SNA, SNPr, SNB, and SNPg, the vertical dimension anteriorly by N-ANS and posteriorly by S-PNS. The antero-posterior position of the maxilla was recorded with the linear measurements Ar-PNS, Ar-ANS, and the length of the maxilla with ANS-PNS. Finally, the inclination of the upper and lower jaw was recorded by the angles SNL-NL and SNL-ML.

Statistical Analyses

The statistical analyses of marginal bone level, esthetic, and functional properties of the reconstructed cleft region and the effect of a buccal rotational flap was made by the Kruskal-Wallis one-way analysis of variance. The longitudinal comparison of marginal bone levels was done with a chi-square test, comparing the three groups of UCLP and BCLP patients, respectively. The number of clefts without change in bone level were compared with the number of clefts where a reduced bone level was found after long-term follow-up. The number of clefts in the various groups where the treatment was finished with/without bridgework was analysed as a three-dimensional contingency table using GENSTAT software. The material was tested for the absence of pairwise interactions between the three factors—age group, diagnosis, and abscence/presence of bridgework—

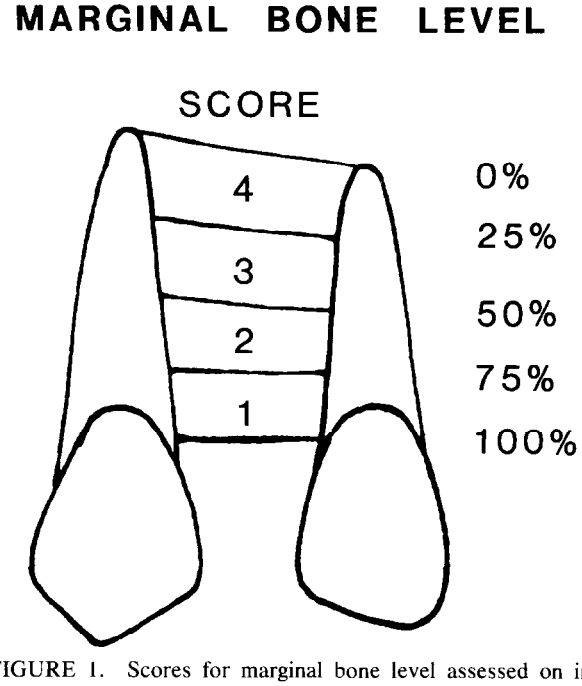

FIGURE 1. Scores for marginal bone level assessed on intraoral films.

Table 3. Marginal Bone Level on Teeth Adjacent to Cleft

Score	1	2	3	4	
Cleft lip alveolar process only					
Group A (n = 19)	18	1	0	0	
Group B (n = 8)	5	3	0	0	
Group C (n = 2)	2	0	0	0	
Unilateral cleft lip palate					
Group A (n = 55)	40	13	2	0 *	†
Group B (n = 52)	26	18	3	5 ‡	†
Group C (n = 44)	18	13	6	7	†
Bilateral cleft lip palate					
Group A (n = 40)	28	9	2	1 *	‡
Group B (n = 24)	7	10	4	3 ‡	‡
Group C (n = 24)	6	12	1	5	‡

* $P < 0.01$, † $P < 0.001$, ‡ $P < 0.0005$.

and differences between the age groups and diagnoses were then analysed. Comparison of the cephalometric data was done with Student's t-test. Statistical significance was defined at the level of $P < 0.05$.

Results

The distribution of scores for marginal bone level after an observation period of more than four years is shown in Table 3. Comparison of the marginal bone level among the three groups of UCLP patients and the three groups of BCLP patients showed significant differences ($P < 0.001$ and $P < 0.0005$, respectively); the best results were seen in the youngest groups of patients. Among UCLP patients, significant differences were found between groups A and B ($P < 0.01$) and between groups A and C ($P < 0.0005$). Comparison of the BCLP patients showed significant differences between groups A and B ($P < 0.001$) and between groups A and C ($P < 0.0005$). In Table 4, the marginal bone level in patients with UCLP and BCLP is compared longitudinally with the data for the same patients in the previous short-term study.[16] This comparison showed no significant differences in marginal bone level between the groups. In 24% of the clefts, a reduction of the marginal bone level was found after long-term follow-up as compared to short-term follow-up.

Gingival recession was found in three teeth adjacent to the cleft in all UCLP patients from group B. Periodontal pockets >3 mm were only seen in two teeth adjacent to the cleft. In 94 of the operated clefts, buccal rotational flaps were used during the operation (Table 5). There was lack of attached gingiva in 62 of these clefts. In 32 of the 62 clefts, a

Table 4. Changes of Marginal Bone Levels on Teeth Adjacent to Cleft From Short-Term (*) to Long-Term Follow-up

Change in Score	No Change	1–2	2–3	3–4	1–3	1–4	2–4
Unilateral Cleft Lip and Palate							
Group A (n = 55)	44	9	2				
Group B (n = 52)	41	7	1		2	1	
Group C (n = 44)	32	5	5		1	1	
Bilateral Cleft Lip and Palate							
Group A (n = 40)	32	5			2	1	
Group B (n = 24)	18	4	1				1
Group C (n = 24)	18	4				2	

No significant differences.
* Data from previous study.[16]

gingivoplasty with a free palatal mucosa graft was performed three to six months after the bone grafting procedure. This gingivoplasty resulted in significantly better marginal bone level scores than found among the patients who did not receive gingivoplasty ($P < 0.0005$).

External root resorption occurred in 17 patients, all in groups B and C. Sixteen of the resorptions were seen on the facial surface of the canines at the cementoenamel junction. To date, five of the 16 canines have been extracted, the others are being treated endodontically with calcium hydroxide in the root canal. Retention of canines was seen among 20 of the 55 UCLP patients (36%), and six of the 20 BCLP patients (15%) in group A.

The number of patients in the various groups where the treatment could be finished orthodontically without bridgework are listed in Table 6. The pairwise interactions between the variables were not significant ($P < 0.98$). The difference in treatment results between the diagnostic groups was significantly different ($P < 0.0005$), as was the difference between the three age groups ($P < 0.005$).

The scores for the esthetic and functional properties of the reconstructed alveolar process are shown in Table 7. Comparison of the treatment results among the three groups of patients with UCLP and BCLP showed significant differences ($P < 0.0005$). Comparison of UCLP patients in groups A and B; A and C and B and C all showed significant differences ($P < 0.05$, $P < 0.0005$, and $P < 0.0005$). Among the BCLP patients significant differences were demonstrated between groups A and B and A and C ($P < 0.0005$).

No significant influence on sagittal maxillary growth could be shown whether the bone grafting was performed before (group A) or after eruption of the canine (group B) (Table 8). Comparison with a control group of UCLP patients who received the same treatment except bone grafting did not show any significant differences. The anterior facial height measured from N to the anterior nasal spine (N-ANS) showed significantly less growth in groups A and B as compared to the nonbone grafted control group ($P < 0.001$). The maxillary length (ANS-PNS) was significantly shorter in group B than in group A ($P < 0.05$) and the control group ($P < 0.001$).

Discussion

This study has demonstrated significantly better long-term results of secondary bone grafting among UCLP and BCLP patients regarding marginal bone level if bone grafting is performed before eruption of the canine. Comparison of the marginal bone level after short-term observation[16] and the present findings showed no significant differences, indicating that a reasonably safe estimate of the final treatment result can be obtained after a rather short observation period as used in the previous study.[16]

The indications for treatment as well as the methods for evaluation used have been previously discussed.[16,17] With the surgical method used in this study,[18] the preferable time for the operation to be performed is before eruption of the canine. This is in accord with other reports on the subject.[3,8–12,14] Periodontal complications were rare in this study, and as reported by Hinrichs et al.,[13] "the bone grafting resulted in a satisfactory periodontal support for cleft associated canines." The importance of attached gingiva facially to the reconstructed alveolar process has previously been noted.[11,13,17]

It appears from Table 5, that 30 patients with a postoperative lack of attached gingiva developed a significant reduction of the marginal bone level compared to a group of patients in whom a secondary gingivoplasty was performed. Therefore, if a buccal rotational flap used during the bone grafting leads to a postoperative lack of attached gingiva, this has to be reestablished by a secondary gingivoplasty three to six months later.

Table 5. Effect of Buccal Rotational Flap in 94 Clefts on Amount of Attached Gingiva and Marginal Bone-Level Score

Marginal Bone Level Score	1	2	3	4
Lack of attached gingiva (n = 62)				
Patients not reoperated with gingivoplasty (n = 30)	6	9	7	8
Patients reoperated with gingivoplasty (n = 32)	21	10	1	0

* $P < 0.0005$. *

Operation before eruption of the canine in a number of cases led to retention of the tooth, that then had to be surgically exposed and orthodontically assisted in its eruption, a finding that has been reported previously.[9,13] The advantages gained by bone grafting before eruption of the canine, however, are of such importance that failure of the canines to erupt is considered a minor clinical problem.

External root resorption appeared in a number of cases in the older groups of patients (B and C), a complication that has previously been reported after bone grafting in cleft patients by Ames et al.[19] That iliac crest cancellous bone has the potential to induce external root resorption has previously been demonstrated when it was applied in intraosseous periodontal defects.[20–22] Probably the development of resorption is due to a damage of the periodontal ligament and/or cementum on the root surfaces.[23] External root resorption is a serious complication that may lead to a loss of the tooth. No cases of external resorption have been seen in the youngest group of patients to date. This indicates that the problem can be avoided if the patients are operated before eruption of the canine.

A significantly higher number of clefts in the youngest group of patients as compared to the older groups in this study could be treated without the need of bridgework. Previous reports have drawn attention to the facilitated rehabilitation of cleft patients after bone grafting.[8,10,14] Comparison of the esthetic and functional properties of the reconstructed alveolar process demonstrated significantly better results in group A among patients with UCLP and BCLP as compared to the other groups; this finding also points toward operating before eruption of the canine.

The final influence of secondary bone grafting on maxillary growth cannot be determined until growth has ceased. As the youngest group of patients was operated at an average of 10 years of age, and the subsequent mean postoperative observation period was 6.5 years, these results must be considered as preliminary. The cephalometric investigation of maxillary growth demonstrated a significant reduction in anterior midface height in both groups and a reduced maxillary length in group B. The sagittal maxillary growth was not affected by the bone grafting. Groups A and B were operated at a mean age of 10 and 14 years of age, respectively. As the sagittal and transverse anterior maxillary growth normally is completed at the age of eight to nine years,[24] this may explain the unaffected growth. The antero-posterior position of the maxilla registered by the linear measurements Ar-PNS and Ar-ANS was similar in the investigated groups. The length of the maxilla was significantly less in group B than in the other groups. The greater maxillary length found in group A may be explained by an early commencement of the orthodontic treatment after surgery. Most cleft lip and palate patients need a sagittal orthodontic expansion of the upper jaw. When the alveolar process is bone grafted at an early age, this orthodontic correction of the sagittal malocclusion may be performed shortly after the operation in the early mixed dentition and, thereby, very likely cause a longer maxilla.

The vertical growth of the maxilla was affected anteriorly in groups A and B. As the vertical growth of the maxilla normally continues beyond the age of the investigated groups,[24–26] the reduced vertical maxillary growth may be a result of the extensive mobilization of the palatal mucosa performed during surgery.[18] The mobilization of the palatal mucosa is done to enable the flaps to be sutured without tension and avoid dehiscence. In general, this technique has proven to be successful, as

Table 6. Number (and %) of Clefts in the Various Groups Where the Treatment was Finished Without Bridgework

Group No. of Clefts	A (n = 114)	B (n = 84)	C (n = 70)
Cleft lip alveolar process only	16/19 (84%)	6/8 (75%)	1/2 (50%)
Unilateral cleft lip and palate	27/55 (49%)	14/52 (27%)	4/44 (8%)
Bilateral cleft	19/40 (48%)	6/24 (25%)	3/24 (13%)

Comparison of diagnostic groups: $P < 0.0005$; comparison of age groups: $P < 0.005$.

Table 7. Esthetic and Functional Properties of the Reconstructed Cleft Region

Score	1	2	3	4
Cleft lip alveolar process only				
Group A (n = 19)	19	0	0	0
GroupB (n = 8)	8	0	0	0
Group C (n = 2)	2	0	0	0
Unilateral cleft lip palate				
Group A (n = 55)	50	4	1	0
Group B (n = 52)	39	10	0	3
Group C (n = 44)	18	14	5	7
Bilateral cleft lip palate				
Group A (n = 40)	36	2	2	0
Group B (n = 24)	11	10	0	3
Group C (n = 24)	8	11	0	5

Comparison UCLP groups A, B, and C, $P < 0.0005$; groups A and B, $P < 0.05$; groups A and C, $P < 0.0005$; groups B and C $P < 0.0005$.

Comparison BCLP groups A, B, and C, $P < 0.0005$; groups A and B, $P < 0.0005$; groups A and C, $P < 0.0005$.

Table 8. Cephalometric Analysis of the Influence of Secondary Bone Grafting on Maxillary Growth

	Group A (n = 36)		Group B (n = 31)		Control (C) (n = 27)				
	Mean	SE	Mean	SE	Mean	SE	A vs B	A vs C	B vs C
SNA	73.43	0.662	73.47	0.519	74.64	0.553	ns	ns	ns
SNPr	76.28	0.642	76.04	0.463	77.08	0.538	ns	ns	ns
SNB	75.01	0.573	74.75	0.674	76.34	0.647	ns	ns	ns
SNPg	76.97	0.608	76.88	0.736	78.78	0.741	ns	ns	ns
N-ANS	51.99	0.614	51.36	0.602	54.85	0.898	ns	‡	‡
S-PNS	43.25	0.528	43.83	0.563	45.08	0.779	ns	ns	ns
Ar-ANS	87.71	0.800	86.90	0.772	88.72	0.763	ns	ns	ns
Ar-PNS	36.91	0.563	37.89	0.660	37.05	0.836	ns	ns	ns
ANS-PNS	51.10	0.534	49.30	0.718	52.23	0.618	*	ns	‡
SNL-NL	10.98	0.571	10.28	0.654	11.32	0.844	ns	ns	ns
SNL-ML	36.61	0.988	36.09	0.997	36.26	1.294	ns	ns	ns

* $P < 0.05$; †$P < 0.01$, ‡$P < 0.001$; ns, not significant.

documented by the treatment results achieved in the youngest groups of patients. Following a reduced vertical maxillary growth a more prognathic and anteriorly rotated mandible should be expected; however, such jaw relations were not found at a significant level. The unfavourable effect upon maxillary growth might be reduced, either by a less extensive mobilization of the palatal flaps, or by postponing the operation until growth has ceased. However, as demonstrated in this report, significantly better treatment results are obtained by operating before eruption of the canine and by starting the orthodontic treatment early after surgery. It seems that this treatment may compensate for the retardation in growth of the length of the maxilla.

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Review

Stem cell regenerative therapy in alveolar cleft reconstruction

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ABSTRACT

Achieving a successful and well-functioning reconstruction of craniofacial deformities still remains a challenge. As for now, autologous bone grafting remains the gold standard for alveolar cleft reconstruction. However, its aesthetic and functional results often remain unsatisfactory, which carries a long-term psychosocial and medical sequelae. Therefore, searching for novel therapeutic approaches is strongly indicated. With the recent advances in stem cell research, cell-based tissue engineering strategies move from the bench to the patients' bedside. Successful stem cell engineering employs a carefully selected stem cell source, a biodegradable scaffold with osteoconductive and osteoinductive properties, as well as an addition of growth factors or cytokines to enhance osteogenesis. This review highlights recent advances in mesenchymal stem cell tissue engineering, discusses animal models and case reports of stem cell enhanced bone regeneration, as well as ongoing clinical trials.

Contents

1. Introduction

Cleft lip and palate is a congenital defect with the overall prevalence of 7.94 per 10,000 live births (Tanaka, Mahabir, Jupiter, & Menezes, 2012) and wide variability of clinical expression and severity, from orbicularis oris muscle defect to cleft lip, alveolar

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cleft and cleft palate alone. (Dixon, Marazita, Beaty, & Murray, 2011) Alveolar cleft is a result of an improper fusion of the maxillary prominences around the 5th–6th week of gestation, that is caused by both environmental and genetic factors (Coots, 2012; Molina-Solana et al., 2013 FebMolina-Solana, Yáñez-Vico, Iglesias-Linares, Mendoza-Mendoza, & Solano-Reina, 2013). It comprises a heterogeneous group of defects with large differences in volume and shape (Bugaighis et al., 2010). Affected children suffer from speech and hearing disorders, their academic achievements might be affected as well (Flynn & Lohmander, 2014; Knight, Cassell, Meyer, & Strauss, 2014). The correction procedure is aimed at closing the oronasal fistula and providing adequate support for tooth eruption through restoration of maxillary arch continuity, which provides conditions for proper dentition and occlusion development. (Bajaj et al., 2003 NovBajaj, Wongworawat, & Punjabi, 2003) The repair of the alveolar cleft can be conducted at different phases of dentition. Adequate timing of correction could promote further maxillary growth and reduce future craniofacial abnormalities. (Farronato, Kairyte, Giannini, Galbiati, & Maspero, 2014) Bone grafting is considered to be the gold standard with the cancellous bone harvested from the anterior iliac crest. However, the procedure is invasive and carries a potential risk of complications including pain, bleeding, infection, fracture or even late-occurring problems such as scarring, chronic pain, paresthesia or even gait abnormalities (Gimbel et al., 2007) and (Moreau, Caccamese, Coletti, Sauk, & Fisher, 2007). Moreover, the failure rate is about 15% (Paganelli et al., 2006; Schultze-Mosgau, Nkenke, Schlegel, Hirschfelder, & Wiltfang, 2003). Further orthodontic treatment is a necessity due to malalignment dentition and midfacial retrusion (Toscano, Baciliero, Gracco, & Siciliani, 2012). Bearing in mind all the above-mentioned complications and behavioral implications accompanying cleft palate, alternative approaches to autologous bone grafting are needed (Hunt, Burden, Hepper, & Johnston, 2005). Tissue engineering, a strategy of tissue regeneration employing combined use of biomaterials and biological molecules, arises as a new therapeutic option. It includes use of biodegradable scaffolds, an addition of growth factors and barrier membranes, as well as the use of stem cells. The craniofacial tissue development is closely related to interaction between stem cells and growth factors and thus they represent an interesting field for therapeutic use. Bone regeneration in patients with cleft palate is a challenging task as the newly formed bone must have adequate mechanical properties to endure a significant amount of pressure in the orofacial area.

2. Stem cells

Mesenchymal stem cells (MSC), multipotent stromal cells, are the one considered to be most promising in tissue engineering. Their minimal identifying criteria have been declared by the International Society for Cellular Therapy (Dominici et al., 2006). These cells must be plastic-adherent during culture in standard conditions, express cell surface markers such as CD105, CD73 and CD90 and no CD45, CD34, CD14, CD11b, CD79α, CD19 or HLA-DR. They must be proven to differentiate into osteoblast, adipocyte and chondroblast lineage. MSC are supposed to act not only through direct bone formation, but also due to paracrine effects: releasing cytokines, producing extracellular matrix and promoting angiogenesis. MSC in combination with biomaterials, carry a great potential that has already been proven in animal studies and on first human cases (Yoshioka et al., 2012; Tanimoto et al., 2013; Zhang et al., 2011; Ou, Jian, & Lin, 2007; Pourebrahim et al., 2013; Korn, Schulz, Range, Lauer, & Pradel, 2014; Hibi, Yamada, Ueda, & Endo, 2006; Pradel, Tausche, Gollogly, & Lauer, 2008; Behnia et al., 2009; Behnia, Khojasteh, Soleimani, Tehranchi, & Atashi, 2012; Stanko et al., 2013; Chai et al., 2006). Several aspects have to be taken into consideration when planning stem cell-enhanced bone regeneration.

2.1. Selecting stem cell donor

As for now, autologous stem cell sources are the one being used in tissue engineering, however, their acquisition requires prior harvesting procedure and generates drawbacks in both time and patient comfort. The idea of stem cell tissue-engineered product promptly available for off-the-shelf application is promising. However, an allogeneic source will be needed to fulfill such a concept. MSC can potentially be applied in an allogeneic setting as the mesenchymal stem cell immunophenotype with no major histocompatibility complex (MHC) II and low MHC I expression is said to be weak or non-immunogenic (Law & Chaudhuri, 2013). Host response to autologous, allogeneic and xenogeneic bone marrow-derived MSC (BM-MSC) was evaluated by Pigott, Ishihara, Wellman, Russell, and Bertone (2013). Stem cells were delivered intra-articularly to 6 five-year-old horses. Inflammatory cytokine release was present in all cases, however, host immune response upon re-exposure was detected only with xenogenic material. Further studies are needed to asses MSC immunogenicity, as some of the preclinical experiments show conflicting results. (Knaän-Shanzer, 2014) Third party MSC have been successfully used in a few clinical settings. They are applied to treat Graft-*versus*-Host Disease after hematopoietic stem cell transplantation in immunocompromised patients (Introna et al., 2014). First promising results regarding use of allogeneic umbilical cord blood-derived MSC (UCB-MSC) on 9 patients with bronchopulmonary dysplasia with mean gestational age of 25 weeks and weight of about 800 g have already been published (Chang et al., 2014). In a clinical trial evaluating safety and efficacy of a transendocardial delivery of autologous and allogeneic BM-MSC in treating ischemic cardiomyopathy, no significant alloimmune reactions were reported (Hare et al., 2012). No clinical studies concerning allogeneic MSC and bone regeneration have been carried out, however, a few studies on animal models have been conducted. Tsuchida, Hashimoto, Crawford, Manske, & Lou, (2003) attempted a repair of a rat femoral defect with bone morphogenetic protein 2 (BMP-2) and BM-derived allogeneic stem cells with short-term tacrolimus immunosuppression, and demonstrated equal effectiveness to the autologous source. Dighe, Yang, Madhu, Balian, & Cui (2013) study on a mice model showed that allogeneic MSC could be more efficient in bone regeneration if recipient T-cell and INF-γ production as a host response was inhibited. Another experiment (Liu et al., 2013a) combined allogeneic MSC that were differentiated until forming cell sheet of 6–7 layers with calcined bovine bone to repair critical size bone defect in osteoporosis rat model. No immunosuppression was applied. Osteogenic potential of allogeneic adipose tissue-derived MSC (AT-MSC) was assessed on canine cranial defects and the therapeutic effect was comparable to the one achieved with autologous cells. The study did not include immunosuppression, however, no host immune response was noted (Liu et al., 2013b). Comparative analyses of MSC allogeneic and autologous sources in bone regeneration have been conducted in a few studies. Allogeneic BM-MSC applied in an ovine critical-sized tibial bone defect showed results comparable to the autologous source (Berner et al., 2013). Similar outcome was achieved with allogeneic BM-MSC on a cancellous bone granulate scaffold in a rabbit model of critical-sized radius bone defect. Follow-up with both micro-computed tomography (μCT) and histological evaluation showed no major differences between autologous and allogeneic source (Kang et al., 2014).

2.2. Mesenchymal stem cell sources

The question of stem cell therapy entering the clinic is no longer valid. However, as science progresses, more and more new obstacles arise. Investigators have to pay great respect to the variability of stem cell sources, carefully selecting the one most compatible with their study, as it may affect the results in a great manner. Non-invasive donation, ease of isolation, high proliferation and differentiation capabilities should be among the most important attributes of an ideal stem cell source. Moreover, stem cells used for bone tissue engineering should be able to integrate with the scaffold and differentiate successfully into osteogenic lineage. MSC have been isolated from multiple tissues including bone marrow, adipose tissue, embryonic tissue, amniotic fluid, cord blood, cord tissue, tooth, skeletal muscle, dermis, cornea or even menstrual fluid (Branch et al., 2012; Khoury, Alcayaga-Miranda, Illanes, & Figueroa, 2014; Crisan et al., 2008). The differences between stem cell populations are currently being investigated.

2.2.1. Bone marrow-derived stem cells

Bone marrow, as the first source reported to contain mesenchymal stem cells, is the one being most thoroughly examined. Differentiation of BM-MSC into osteogenic, chondrogenic, adipogenic, hepatogenic, cardiogenic and neurogenic lineage was documented (Sousa et al., 2014). Matsuo, Yamazaki, Takase, Aoyagi, & Uchinuma (2008) created a hybrid-type bone substitute with the use of cryopreserved MSC and autologous serum, and placed it subcutaneously in a nude mice. The follow-up showed good osteogenic potential of cryopreserved cells and thus the potentiality of banking them for bone grafting. However, invasive donation procedure (although with reduced donor site pain in comparison to traditional autologous bone grafting), as well as reports concerning age-related deterioration in proliferation and differentiation capabilities, may hinder its clinical use (Gimbel et al., 2007; Wu et al., 2014). First studies did not show any differences concerning BM-MSC potential and aging (Stenderup, Justesen, Eriksen, Rattan, & Kassem, 2001; Justesen, Stenderup, Eriksen, & Kassem, 2002; Leskelä et al., 2003), but more recent papers on both human and animal BM-MSC showed that the stem cells do undergo the process of senescence and thus their differentiation and proliferation capabilities decrease with the donor age (Kretlow, Jin, Liu, Zhang, Hong, & Zhou, 2008). Bellows et al., (Bellows, Pei, Jia, & Heersche, 2003) proved reduced self-renewal capability of rat osteoprogenitors, while Tokalov et al. (2007) showed age-related decrease of MSC population on a rat model. Mareschi et al. (2006) expanded BM-MSC from both adult and children, and observed differences in cell growth with favorable population doubling time in the pediatric donors and different cell morphology in adults, probably resulting in the decreased proliferation capacity. In another experiment (Stenderup, Justesen, Clausen, & Kassem, 2003) MSC were differentiated to the maximal life span and a decrease in viability and population doubling rate of human adult-derived MSC was detected with no differences in the mean telomere length in early passages. Mesenchymal stem cells derived from maxillofacial sources are thought to have higher proliferative capacity and bone formation ability (Liu, Hu, & Wang, 2014). They can be easily obtained and thus are attractive as an autologous stem cell source. One of its major advantages is that maxilla-derived stem cells proliferation potential is probably unaffected by donor age, and only population doubling time seems to increase with donor age (Kellner et al., 2014). BM-MSC have already been used in the alveolar cleft regeneration on animal models (Yoshioka et al., 2012; Tanimoto et al., 2013; Zhang et al., 2011; Ou et al., 2007; Pourebrahim et al., 2013; Korn et al., 2014) and in first clinical cases (Hibi et al., 2006; Pradel et al., 2008; Behnia et al., 2009; Behnia et al., 2012; Stanko et al., 2013; Chai et al., 2006). BM-MSC were by many investigators found suitable for craniofacial tissue engineering including calvarial bone defects (Koob et al., 2011; Stockmann et al., 2012; Jiang, Liu, Zhang, Wu, & Shang, 2012; Lima et al., 2013; Stephan et al., 2010) and both maxillary (Rickert et al., 2011) and mandibular (De Kok, Drapeau, Young, & Cooper, 2005; Khojasteh et al., 2013; Abukawa et al., 2009; Warnke et al., 2004; Hernández-Alfaro, Ruiz-Magaz, Chatakun, Guijarro-Martínez, 2012) bone reconstruction.

2.2.2. Adipose tissue-derived stem cells

AT-MSC are known to have high proliferation abilities and they can be easily obtained by lipo-aspiration (Peng et al., 2008). They have been proven to differentiate into various lineages including osteogenic (Zuk et al., 2001; Gimble & Guilak, 2003). The concern for age-related deterioration of stem cell properties is logical for adipose-derived MSC as well, especially that studies report opposing results. Shi, Nacamuli, Salim, & Longaker (2005) study on an animal model focused on osteogenic potential and found no age-related changes in contrast to the adipogenic potential, but another investigation showed that osteogenic potential dominates at later passages (Wall, Bernacki, & Loboa, 2007). However, other studies revealed contradictory results (Stolzing & Scutt, 2006; Stolzing, Jones, McGonagle, & Scutt, 2008). Kim et al. (2012) suggested that expression of transcription factors changes with stem cell age resulting in a reduction of osteogenic potential and increase of adipogenic capabilities. Chung et al. (2013) implied that subpopulation of $CD90^{+}$ AT-MSC is more capable of new bone formation, while other researchers augmented osteogenesis with microRNA/BMP-2 viral transduction or co-culture with BM-MSC (Liao et al., 2014; Kim, Park, & Im, 2014). An important question of pediatric AT-MSC properties has been raised by Guasti et al. (2012). The investigators examined MSC derived from abdominal adipose tissue and confirmed its highly proliferation and differentiation properties with an ability of selective osteogenic, but not chondrogenic differentiation. In preclinical studies AT-MSC were found compatible with various scaffolds including titanium dioxide in simvastatin-enhanced bone engineering, collagen I/beta-tricalcium phosphate (β-TCP) and bioceramic/collagen scaffold (Pullisaar, Reseland, Haugen, & Brinchmann, 2014; Yang, Huang, Wang, Dang, & Wang, 2013; Daei-Farshbaf et al., 2014). An interesting study by Benazzo et al. (2014) revealed that trabecular titanium acts as both scaffold and osteogenesis inducer. The conducted *in vivo* studies showed that AT-MSC are compatible with hydroxyapatite (HA)/tricalcium phosphate (TCP), poly(lactic-co-glycolic acid) (PLGA) as well as bioactive glass or β-TCP scaffolds (Hicok et al., 2004; Cowan et al., 2004; Levi et al., 2010; Sándor et al., 2014). AT-MSC were also successfully used in combination with fibrin glue in a 7-year-old patient with large calvarial traumatic defect (Lendeckel et al., 2004).

2.2.3. Embryo-derived stem cells

Embryonic stem cells are the one derived from the inner mass of blastocyst during embryogenesis. In contrast to other stem cells, they have superior proliferation capabilities with high osteogenic potential and lowest immunogenicity, and they are the only ones able to differentiate into tissues of all germ layers (Zhang et al., 2012; Tremoleda et al., 2008). Their osteogenic and chondrogenic potential was compared with that of BM-MSC and calvaliar osteoblasts. Calcium sequestration was higher, however with different mineral distribution, another mechanism of deposition and expression of housekeeping genes (Liu et al., 2014d). Co-culture of ESC with MSC-conditioned medium up-regulated gene expression of mesodermal lineage and enhanced osteogenic differentiation (Evans, Swain, Gentleman, Gentleman, & Stevens,

2012). Lee et al. (2014) successfully regenerated cranial defects with embryo-derived stem cells and calcium phosphate cement in a rodent model. However, use of human embryo-derived stem cells stirs ethical concerns and has higher potential for tumorigenicity and genome instability (de Wert & Mummery, 2003; Shand, Berg, & Bogue, 2012).

2.2.4. Amniotic-derived stem cells

Amniotic-derived stem cells can be isolated from the placenta or amniotic fluid (AF-MSC), for example during amniocentesis, with no harm to the embryo. Their biology is close to that of embryo-derived stem cells, and they are superior to adult stem cells as regards proliferation and differentiation capacity. A study by Sun et al. (2010) revealed good osteogenic properties of AF-MSC in combination with BMP-7 and nanofibrous scaffold. AF-MSC were also compatible with porous medical-grade poly-epsilon-caprolactone (Peister, Deutsch, Kolambkar, Hutmacher, & Guldberg, 2009) and collagen scaffold (Maraldi et al., 2013). A few studies augmented osteogenic potential of AF-MSC by: co-culture with dental pulp stem cells (De Rosa et al., 2011), upregulation with calcimimetics (Di Tomo et al., 2013), or addition of herbal-derived substances such as curculigoside (Liu, Li, & Yang, 2014) or naringin in a dose-dependent manner. (Liu, Li, & Yang, 2014) One of the recent studies suggested that differentiation towards osteogenic lineage could be modified by Wtn signaling pathway (D'Alimonte et al., 2013).

2.2.5. Cord blood-derived stem cells

Umbilical cord blood is a well-known source of stem cells, not only hematopoietic, but also endothelial progenitor and mesenchymal (Phuc et al., 2012). UCB-MSC are close in differentiation and proliferation capacity to embryonic stem cells and thus seem to be very expandable. (Kern, Eichler, Stoeve, Klüter, & Bieback, 2006) Harvesting procedure is easy and ethically non-controversial, but isolation rate is poor and the number of collected cells is limited (Jin et al., 2013a). UCB-MSC have been proven to differentiate towards osteogenic, chondrogenic, adipogenic, hepatogenic and cardiogenic lineage (Sousa et al., 2014) and have the greatest anti-inflammatory effect. (Martins, Paiva, Morgado, Gomes, & Pais, 2009) In preclinical *in vitro* and *in vivo* models UCB-MSC were tested with partially demineralized bone matrix for bone tissue regeneration. They were proven successful when osteogenically induced for regeneration of athymic rats parietal bone (Liu et al., 2010).

2.2.6. Wharton's jelly-derived stem cells

Wharton's jelly, a part of the umbilical cord, surrounds and protects blood vessels. It is composed of low number of cells and high amount of extracellular matrix, including high amounts of hyaluronic acid, collagen, and sulphated proteoglycans. It is a good source of growth factors and an abundant source of MSC (Wang et al., 2004; Sobolewski, Małkowski, Bańkowski, & Jaworski, 2005). Wharton's jelly-derived MSC (WJ-MSC) have high proliferation capabilities and their biology is close to the embryo-derived stem cells. (Trivanović et al., 2013). However, they do not have such tumorigenic potential and the donation procedure is completely noninvasive. Moreover, WJ-MSC are weakly immunogenic and they maintain their privileged immune status after differentiation, thus they make a good potential allogeneic source of beforehand culture and processing (Liu et al., 2012; La Rocca et al., 2013). Their osteogenic potential has been confirmed (Baba et al., 2012) in a few *in vitro* studies with different scaffolds: fibrin, collagen/calcium phosphate and 3-dimensional nanofibrous structures (Baba et al., 2013; Gauthaman et al., 2013; Karadas et al. 2014Karadas, Yucel, Kenar, Torun Kose, & Hasirci, 2014). Chen et al. (2013) compared osteogenic potential of WJ- and BM-MSC by seeding them onto calcium phosphate cement and applying to cranial defects artificially created in athymic rats, and revealed that WJ-MSC were as effective as BM-MSC in both new bone and vessel formation.

2.2.7. Dental tissue-derived stem cell sources

Dental pulp stem cells (DPSC) have similar properties and differentiation abilities to those derived from bone marrow, however, they are more bounded to odontogenic lineage (Gronthos, Mankani, Brahim, Robey, & Shi, 2000). Their viability, proliferation capability and function remains sufficient up to 14 passages (Martin-Piedra et al., 2014). The pro-angiogenic properties of DPSC were proven *in vitro* and *in vivo*, however, the detailed cascade of stem cell differentiation is not yet fully understood (Bronckaers et al., 2013; Hilkens et al., 2014). DPSC have been proven to have adipogenic, myogenic, neurogenic, as well as odontogenic potential (Gronthos et al., 2000; Alipour et al., 2010; Zhang et al., 2008; Arthur, Rychkov, Shi, Koblar, & Gronthos, 2008). It has been reported that dental stem cells could be of neural-crest origin and thus possess a superior multilineage differentiation potential, since during embryo development they undergo transition from neuroectoderm to ectomesenchyme (La Noce et al., 2014). They represent a promising source of stem cells for craniomaxillofacial tissue engineering with their high developmental plasticity and independent gene regulation (Ibarretxe et al., 2012). So far, they have been used in the prevention of postoperative bone loss. (d'Aquino et al., 2009) Preclinical studies showed that DPSC could augment bone formation through effective neovascularization (Yamada, Ito, Nakamura, Ueda, & Nagasaka, 2011) Unfortunately, animal studies did not distinctly show any benefit from DPSC therapy (Ji et al., 2010; Park, Jeon, & Choung, 2011). An interesting study by Yazid, Gnanasegaran, Kunasekaran, Govindasamy, & Musa, 2014) compared immunological properties of DPSC derived from healthy and inflamed deciduous teeth and revealed that only healthy dental pulp should be considered as a stem cell source. Stem cells derived from inflamed dental pulp of deciduous teeth did not fulfill the criteria for typical MSC characteristics and had impaired inhibition of T-cell proliferation and pro-inflammatory cytokines secretion. Moreover, they expressed lower percentage of HLA-ABC and G, which is indispensable during pregnancy and probably plays a role in developing allograft acceptance (Menier et al., 2010). DPSC were tested with silicon biomaterial, which gave positive results concerning quick transplantation *in vivo* or long-term *in vitro* differentiation and proliferation on 36 nm porous scaffold with a different chemical treatment (Yamada et al., 2011). Mesenchymal stem cells can also be harvested from periapical follicle, periodontal ligament, pulp tissues of human exfoliated deciduous teeth, root apical papilla, tooth follicle or even periapical cyst (Collart-Dutilleul et al., 2014; Navabazam et al., 2013; Jeon et al., 2014; Marrelli, Paduano, & Tatullo, 2013; Morsczek, 2015; Arthur et al., 2015) An interesting graphic presentation of tooth-derived stem cell sources that deepens understanding of dental stem cells was proposed by Saito, Silvério, Casati, Sallum, & Nociti (2015).

Dental tissue-derived stem cells might find application in regeneration of tooth, dental pulp, periodontal tissue or even neural or other nondental tissues, for an excellent review see Liu et al. (2015) or Xiao & Nasu (2014).

2.2.8. Induced pluripotent stem cells and cancer stem cells

Induced pluripotent stem cells (iPS) are adult somatic cells reprogrammed with use of specific genes encoding transcription factors (Takahashi & Yamanaka, 2006) that have a potential to differentiate into tissues of all germ layers, which makes them an attractive stem cell source for tissue engineering (Amabile & Meissner, 2009). Their differentiation into osteogenic lineage has

been confirmed as well (Tashiro et al., 2009). iPS were found promising in periodontal tissue regeneration, including alveolar bone (Duan et al., 2011). They have been found compatible with several scaffolds including silk (Duan et al., 2011; Ye et al. 2011), calcium phosphate cement (Tang et al. 2014), HA/TCP (Li & Niyibizi, 2012) and poly(ε-caprolactone) (PCL) (Jin et al., 2013b). A few papers comparing iPS properties for bone tissue engineering have been published. Wang et al. reported on similar potential of iPS as well as BM-MSC and WJ-MSC (Wang et al., 2015). Ardeshirylajimi found iPS superior to AT-MSC in case of proliferation rate and mineralization despite lower Runx2 level (Ardeshirylajimi, Soleimani, Hosseinkhani, Parivar, & Yaghmaei, 2014). iPS therapeutic potential is promising, since they are patient-specific and can be generated from various sources. However manipulation carried out to create iPS has low efficiency and stirs safety concerns (Fu et al., 2014).

Cancer stem cells (CSC), a type of cancer cells that predict neoplasm aggressiveness, might origin from adult stem cells, since they both share properties of self-renewal and differentiation. Another hypotheses include mature cancer cell dedifferentiation and reprogramming during iPS formation, which is similar to transformation of normal cells into malignant cells. CSC are heterogenic subpopulation influenced by its microenvironment and capable of plasticity (Islam, Qiao, Smith, Gopalan, & Lam, 2015). In-depth studies of tissue-engineered cancer models and CSC give insight into metastatic activity, chemioresistance and relapse (Ricci, Moroni, & Danti, 2013). In the future, CSC may serve as therapeutic targets for novel treatment methods (Schulenburg et al., 2013; Podberezin, Wen, & Chang, 2013).

3. Scaffolds

Biological tissue substitutes show a great potential in regenerative medicine. Proper selection of scaffold material plays a critical role in potential success or failure of undertaken therapy (Kinoshita & Maeda, 2015). Stem cells act as seed cells and scaffold is used as an extracellular matrix. The biological, chemical and mechanical characteristics of the scaffold should be carefully considered as they affect stem cell properties and are responsible for long-term transplant viability. An ideal scaffold should be able to interact with stem cells and provide a 3-dimentional environment capable of enhancing stem cell proliferation and differentiation abilities (Murphy, O'Brien, Little, & Schindeler, 2013). The scaffold design should balance osteoconductive and mechanical properties, as well as promote vascular ingrowth. It should be non-immunogenic and able to biodegradate within a period of time (Garg, Singh, Arora, & Scaffold, 2012).

Scaffolds are typically divided into three groups: ceramics, synthetic polymers and natural polymers. The first group comprises one of the most widely used materials in bone regeneration (calcium phosphate, TCP and HA) with advantages such as good biocompability and osteoconductivity. Microstructured β-TCP, without stem cells, was used by de Ruiter et al. (2014) in 7 patients with unilateral alveolar cleft with satisfactory results. Synthetic polymers such as poly(alpha-hydroxy acid) polymers can be adjusted to the desired characteristics. Even their degradation rate can be controlled by changing the composition. However, during degradation synthetic polymers release acidic products and thus could negatively affect the regenerating tissue. In contrast, natural polymers do not release toxic by-products and are biocompatible, but lack adequate mechanical properties. They can be modified into biomimetic scaffolds by adding, for example, ceramic phase to improve their mechanical properties (Murphy et al., 2013).

Some studies have risen a concept of bioactive scaffolds derived from natural sources that would be able to boost stem cells by delivering growth factors (Hodde, 2002). Jiao et al. (2014) hypothesized that cryopreserved dentin matrix could serve as a scaffold for dental-derived stem cells and preserve its beneficial biological and mechanical properties. Despite up to 6 months of preservation in liquid nitrogen, the scaffold has shown promising features regarding mechanical characteristics and induction of odontogenesis of dental follicle stem cells. Cryopreservation would facilitate scaffold production and its banking could be useful for regenerative medicine to ensure prompt availability of the product (Costa, Dias, Reis, & Gomes, 2012). Another approach is demonstrated by Li et al. (2013) who transducted adipose-derived stem cells with lentiviral vector to induce production of osteogenic factors. The rodent experimental group showed new bone formation in an artificially created defect which was confirmed histologically.

Scaffold usability is determined not only by its material per se, but also porosity (Mastrogiacomo et al., 2006). Small pores might limit cell migration and inflow of nutrients, while large impair cell adhesion (Phipps, Clem, Grunda, Clines, & Bellis, 2012; Melchels et al., 2010; Kasten et al., 2008). Sicchieri, Crippa, de Oliveira, Beloti, & Rosa (2012) suggested that combination of different size pores should supply adequate properties for new bone formation. In-depth studies of scaffold architecture showed that it can significantly affect the results of bioengineering (Lawrence & Madihally, 2008). An *in vitro* study (Huri, Ozilgen, Hutton, & Grayson, 2014) with human AT-MSC and PCL scaffold showed that porosity influenced stem cell osteogenic properties and distribution pattern. A study by Sanzana et al. (2014) led to a conclusion that not only porosity, but pore architecture and scaffold microstructure influences *in vivo* bone formation. A relation between pore architecture and MSC viability was also found. (Domingos et al., 2013). Moreover, scaffold microstructure should be adjusted to the chosen stem cell population, as different seeding cells have different requirements for successful bioengineering (Wittenburg, Flade, Garbe, Lauer, & Labudde, 2014; Duan et al., 2013), and thus investigators combine and enrich scaffolds to achieve optimal environment for bone formation (Miao, Tan, Li, Xiao, & Crawford, 2008; Pang et al., 2013; Hou et al., 2014). *In vitro* culture methods are known as well to affect stem cell differentiation on a scaffold (Mygind et al., 2007; Bjerre, Bünger, Baatrup, Kassem, & Mygind, 2011).

Before clinical application *in vitro* studies optimizing scaffold characteristics and cell-scaffold interaction should be carried out. Understanding 3-dimensional interactions during scaffold colonization and manipulating with scaffold properties to adjust them for cell-specific preferences would allow to achieve better results both *in vitro* and *in vivo*. A tremendous advancement in tissue engineering with latest innovations in biomaterials has led to creation of an anatomically shaped porous bone graft through three-dimensional printing technology, that could be potentially seeded with stem cells (Temple et al., 2014).

4. Enhancing cell-scaffold combination

Supplementation of growth factors to boost osteogenesis through manipulating *in vivo* signaling processes is a third component of successful bone engineering triad, which is represented in Fig. 1. Researchers have found numerous factors affecting bone formation such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), TGF-β, insulin-like growth factor-1 (IGF-1), BMP-2, bone morphogenetic protein-7/osteogenic protein-1 (BMP-7/OP-1), Wtn proteins, parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) (Gothard et al., 2014). The keys to successful bone augmentation include growth

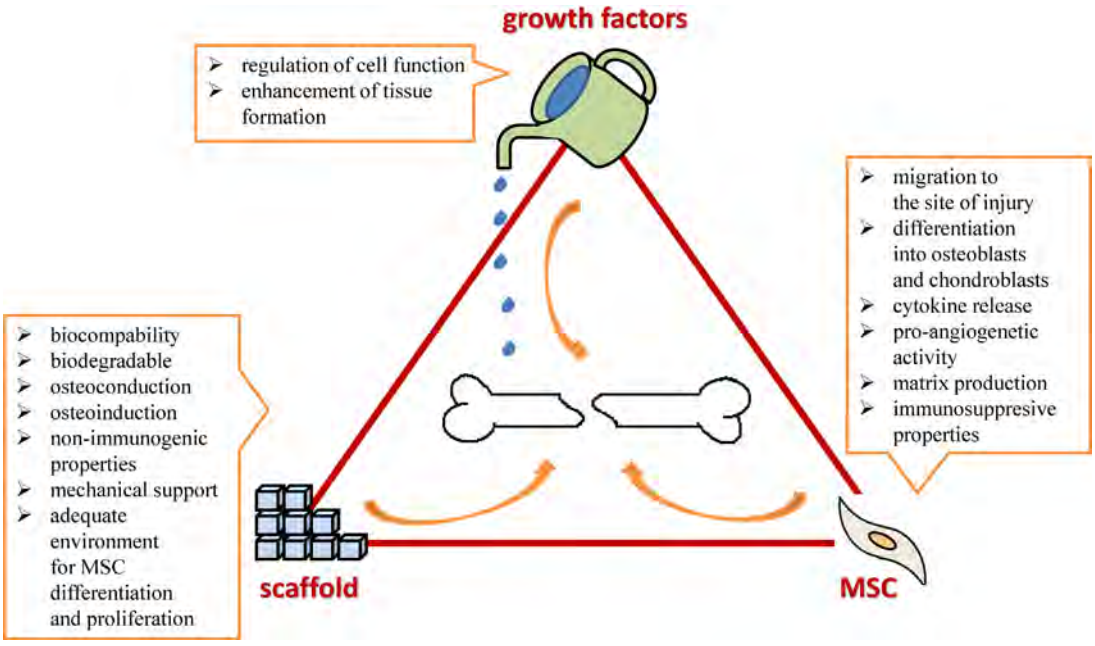

Fig. 1. Schematic graph showing stem cell triad in bone tissue engineering.

factor dosage, release kinetics and compatibility with stem cell source and scaffold.

Platelet-rich plasma (PRP) and BMP are widely used in enhancing bone regeneration (Oyama, Nishimoto, Tsugawa, & Shimizu, 2004; Lee et al., 2009; Marukawa, Oshina, Iino, Morita, & Omura, 2011; Dickinson et al., 2008Alonso et al., 2010). PRP is an autologous and cost-effective source, that can be easily obtained and contains multiple growth factors that could serve for MSC expansion (Bieback et al., 2009). It is thoroughly examined, safe and clinically applied in various settings. PRP-enhanced bone regeneration is considered controversial in the view of conflicting results obtained from multiple studies. Different approaches and study designs hinder clear interpretation. A study by Wen et al. (2014) revealed that PRP could enhance WJ-MSC proliferation capability and ability to differentiate into osteogenic lineage both *in vitro* and on an animal model. The most pronounced results were achieved with 10% PRP solution. PRP was tested in combination with (silicon stabilized) HA/β-TCP scaffold for calvariar defect regeneration in an animal model. μ-CT analysis revealed more prominent new bone formation in the group with addition of PRP, compared to scaffold alone. Moreover, *in vitro* studies showed different tissue reaction in comparison with the study group: recruitment of progenitor cells to the site of injury following an initial phase of pro-inflammatory response. Investigators demonstrated that PRP enhanced late stage bone regeneration with osteoinductive effects that lasted beyond 8 weeks in contrast with the reference group (El Backly et al., 2014). PRP improved bone formation in artificially-induced alveolar defects when combined with bioactive glass foams. (Dutra, Pereira, Serakides, & Rezende, 2008). It was also found suitable for expansion and osteogenic induction of WJ-MSC (Wen et al., 2014). On the other hand, the study by Luaces-Rey et al. (2010) comprising 20 patients with cleft palate who underwent secondary alveoloplasty, did not reveal any differences between control and study group who received PRP and autologous bone. However, the procedure did not involve scaffold or stem cells and study groups were small.

Use of BMP family members in tissue engineering shows an increasing tendency despite their high cost (Melchels et al., 2010). Recombinant human forms of BMP-2 and BMP-7 are commercially available. They belong to the TGF-β superfamily and take part in regulating osteogenesis and promote new vessel formation together with VEGF and FGF (Chenard, Teven, He, & Reid, 2012; Bai et al., 2014). Moreover, BMP-2 has been proven to promote endogenous stem cell mobilization and enhance homing to the site of the defect (Akiyama et al., 2014). However, adverse effects were also reported including ectopic bone formation, osteolysis and postoperative swelling (Zhang et al., 2014; Oryan, Alidadi, Moshiri, Bigham-Sadegh, 2014). Several authors have described use of recombinant human BMP in treating patients with alveolar clefts with results comparable to autologous bone grafting (Neovius et al., 2013) (Canan et al., 2012; Fallucco & Carstens, 2009). However, a study conducted by Neovius et al. (2013) had to be terminated because of severe gingival swelling in patients receiving effective doses of BMP-2.

The combination of PRP and BMP was found to be successful by several investigators. A study by Chen et al. (2012) revealed that the combination of PRP and BMP-2 was superior in terms of osteogenic differentiation of AT-MSC. Synergistic effect was based on the BMP-2 influence over early and final stage of osteogenic differentiation. Tomoyasu et al. hypothesized that not only BMP, but also PRP contributes to a synergistic effect through enhancing BMP-dependent differentiation (Tomoyasu et al., 2007).

Another advance in enhancing tissue engineering is construction of drug-releasing constructs aimed at mimicking natural restorative processes. They can serve as vehicles for the delivery of multiple bioactive molecules (Chung & Park, 2007). An adequate composition of hybrid scaffold will be the key for successful regeneration. Optimal combination of angiogeneic and growth factors could boost stem cell adhesion, proliferation and differentiation (Huang, Lee, & Loo, 2014). Mandal and Kundu (2009) created calcium alginate embedded fibroin 3D scaffolds capable of releasing two different substances with various kinetics, from a single delivery vehicle, and achieved prolonged, sustained release of bioactive molecules. Another approach to drug-eluting constructs was proposed by Wray et al. (2012), who aimed at controlling scaffold properties by composing a silk-based scaffolds with hollow channels that imitate physiologic vasculature. This design would enable pre-vascularization and subsequent optimization of tissue regeneration by enhancing nutrient delivery to the cells. Moreover, it could also serve as vehicle for controlled delivery

of bioactive agents. An interesting study carried out by Shi et al. (2010) showed that mesenchymal stem cells can be osteogenically induced by controlled release of drugs from PLGA sintered microsphere scaffolds. Superior osteogenic properties were confirmed with high expression of typical genes and proteins. Several other studies have enhanced stem cell osteogenic properties with drug-delivering scaffolds (Lee et al., 2013; Irmak et al., 2014). Among latest novelties are strategies to model drug elution in response to stimuli from surrounding environment or upon induction. Drug delivery triggers include alternations in pH and temperature, light-based strategies or even wireless electronic devices (Costa, 2015). The above-mentioned strategies could lead to creating a multiple-factor delivery system guiding MSC differentiation intro various tissues and finding application not only in bone tissue engineering but also in the field of neurology (Kang et al., 2012) or cardiology (Spadaccio et al., 2009; Kurobe, Maxfield, Breuer, & Shinoka, 2012).

Manipulation of osteogenic gene expression is another strategy implied to enhance bone regeneration. MSC have already been transduced with Sonic Hedgehog and stimulated with Wnt-4 and BMP2. A viral-mediated delivery of osteogenic genes has been found to promote bone regeneration as well (Greene & Kirshner, 2015).

Another interesting area in bone tissue engineering is the use of microRNA, a small non-coding posttranscriptional regulator that plays a vital role in different biological processes including osteoblast proliferation and differentiation (Sriram, Sainitya, Kalyanaraman, Dhivya, & Selvamurugan, 2015). Several studies showed that microRNA manipulation affects differentiation of MSC (Zeng et al., 2012; Mariner, Johannesen, & Anseth, 2012; Hamam, Ali, Kassem, Aldahmash, & Alajez, 2015). Moreover, biomaterials applied in tissue engineering were found to have an influence on microRNA expression level. Understanding their interactions would led to enhancement of bone tissue engineering.

5. Preclinical studies on animal models

Yoshioka et al. (2012) aimed at bone regeneration with autologous BM-MSC and carbonated hydroxyapatite (CAP) particles on a canine model. The authors used carbonated hydroxyapatite that contains about 3–5% of carbonate ions with an aim to increase scaffold biocompatibility. They created artificial jaw clefts in 3 beagle dogs and noted no spontaneous recovery. CAP with BM-MSC were transplanted on the one side and CAP alone on the other, PLGA barrier membrane was used to stabilize biomaterial in the operating field. There were no adverse effects after the procedure. The follow-up was based on radiographic and histological examination. The radio-opacity on the experimental site was increasing, whereas in the control site it had a decreasing tendency, which indicates new bone formation with BM-MSC and CAP together. Histological observation indicated that the number and size of CAP particles on the experimental site decreased and no fibroblastic cells were detected compared to the control site. New bone formation on the MSC/CAP site was confirmed with histological samples. The study also evaluated the number of capillary vessels, which turned to be significantly greater with stem cells usage. Efficient vascularization is important for new bone formation and paracrine stem cell activity could boost it through VEGF secretion. The experiment resulted in complete closure of the cleft on the experimental site that was comparable with the original height of alveolar ridge and allowed for subsequent orthodontic tooth movement (Tanimoto et al., 2013).

Zhang et al. (2011) investigated whether, on a canine model, autologous bone marrow-derived and osteogenically induced mesenchymal stem cells combined with porous β-TCP could repair bone defect and thus allow for succeeding orthodontic tooth movement. Researchers created 14 alveolar defects in seven 24-week-old beagle dogs. One dog was left without intervention as a control and example of spontaneous healing. The remaining 12 alveolar defects were divided into 3 groups with different therapeutic interventions: MSC combined with β-TCP, β-TCP alone and autologous bone harvested from iliac crest. Orthodontic tooth movement was undertaken 8 weeks after the procedure and lasted 12 weeks. The follow-up consisted of sequential fluorescent labelling and radiographic evaluation. Twenty weeks after the procedure animals were put through histological and histomorphometric analysis. The results showed that MSC combined with β-TCP were as effective as autologous bone and superior to β-TCP alone, and that MSC allowed for proper physiological function and successful tooth movement with adequate bone support. There were no adverse effects observed apart from soft tissue edema lasting up to 3 days after the procedure.

Ou et al. (2007) combined autologous BM-MSC with collagen and transplanted it then into a canine model of the alveolar cleft. Three-dimensional CT and histological analysis at 12 weeks after the procedure revealed full regeneration at the width of the defect and slightly smaller height of the newly formed bone compared to the control group. It was concluded that these method could be applied clinically to treat alveolar cleft (Ou et al., 2007).

Pourebrahim et al. (2013) have chosen AT-MSC as seeding cells and HA/β-TCP as a scaffold for bone tissue engineering. The study was conducted on four mongrel dogs with mean age of 22 months. Autologous bone harvested from tibia was used as a control group. There were no adverse effects after the procedure. The follow-up consisted of histological and histomorphometric analysis. Mean bone formation on the experimental side was lower at 15 and 60 days after the procedure and amounted to 5% and 70%, respectively (*versus* 45% and 90% in the control group). However, active bone formation at the stem cell side was still present at 60 days. Poorer results achieved with stem cells could be due to use of adult mongrel dogs and thus, adult-derived stem cells with less proliferation and differentiation capabilities.

An interesting and key issue of whether we should differentiate stem cells into osteogenic lineage or not was taken up by Korn et al. (2014). The study was conducted on 72 Lewis rats divided into 4 groups: HA/β-TCP/silica matrix with undifferentiated stem cells, HA/β-TCP/silica matrix with osteogenic differentiated stem cells, HA/β-TCP/silica matrix alone and blank control. Follow-up study, which consisted of histomorphometric analysis and computed beam tomography scan, revealed reduction of the defect size most prominent and statistically significant with undifferentiated stem cells.

Other studies (Han et al., 2013; Ito, Yamada, Naiki, & Ueda, 2006; Yamada, Ueda, Naiki, & Nagasaka, 2004) focused on alveolar bone regeneration around dental implants with the aim to achieve adequate stabilization important for aesthetic occlusion reconstruction. Experiments were conducted on canine models using bone marrow or peripheral blood-derived MSC in combination with fibrin and platelet-rich plasma, platelet-rich plasma alone or composite of calcium sulfate and nano-hydroxyapatite with collagen. Overall results showed that stem cell tissue engineering is a promising method for improving results of dental implantology.

So far, there have been no studies evaluating long-term stability of tissue engineered bone in alveolar cleft repair. However, Kuznetsov, Huang, Marshall, Robey, & Mankani (2008) analyzed results of stem cell-induced bone repair up to 19 months of observation. Autologous BM-MSC were combined with HA/TCP scaffold and transplanted into a dog model of mandibular atrophy. Follow-up studies showed that applied therapy provided a long-lasting functionality of the augmented bone. CT scans performed

during follow-up period showed a constant increase in the mineral bone density and new bone formation with decreasing volume of transplanted material.

6. Stem cell engineering in a clinical setting

The first clinical use of stem cells for bone tissue engineering was published by Hibi et al. (2006) The patient was a 9-year-old female with 10×13 mm alveolar cleft, who was born with unilateral cleft lip and palate and underwent surgical correction at the age of 2 months. Bone marrow aspiration was done in local anesthesia. Mesenchymal stem cells were cultured for 4 weeks and then differentiated into osteogenic lineage. During operation alveolar cleft was supported with a titanium-mesh plate and then MSC, PRP and calcium chloride solution with thrombin were applied using a syringe. No complications were recorded. Nine months after the procedure, 79.1% of the bone was regenerated, and the canine and the lateral incisor erupted forcing out the mesh plate.

Pradel et al. (2008) reported physiologic spontaneous tooth eruption after tissue-engineered secondary osteoplasty in a 10-year-old male. Patient's past medical history consisted of two surgical interventions due to unilateral cleft lip, alveolar and palate. Bone marrow was chosen as a stem cell source and harvested from maxilla under local anesthesia. The defect was filled with osteoblast-like cells seeded and cultivated on a bovine collagen matrix. There were no complications during the postoperative period. Eight months after transplantation there was new bone formation, which allowed for spontaneous teeth migration. The follow-up radiographic studies after 18 months showed complete defect closure allowing for orthodontic treatment.

Behnia et al. (2009, 2012) published two papers describing a few case reports of stem cell bone engineering. The first two cases were a 14-year-old female and a 10-year-old male. The girl had surgically corrected unilateral cleft lip and palate with small bony discontinuity in the alveolar region and a small fistula. The boy had larger alveolar defect and presented with poorer results of previous surgical interventions. Both patients underwent pre-surgical orthodontic treatment and were qualified for secondary osteoplasty. Stem cells were derived from bone marrow and seeded on demineralized bone matrix (DBM) and calcium sulphate scaffold, which was subsequently confirmed by scanning electron microscopy (SEM). After 2 months nasal floor was integrated, which was evaluated with apanoramic view. Mean postoperative defect fill was estimated at 34.5% in the first, and 25.6% in the second patient. Both patients were qualified for sequential orthodontic tooth movement (Behnia et al., 2009). Further research on tissue engineering resulted in stem cell-enhanced secondary alveoplasty in 3 patients (including 1 with bilateral cleft), with mean age of 10 years (Behnia et al., 2012). This time Behnia et al. (2012) used HA/TCP scaffolds and cultivated them with BM-MSC adding recombinant PDGF in the operating room. The procedure was uneventful. The nasal floor was reconstructed leaving no fistula, and the mean quantity of regenerated bone amounted to 51.3% at 3 months post the procedure, which allowed for further orthodontic treatment.

Stanko et al. (2013) have undertaken a repair of 10×7 mm unilateral complete cleft with symptomatic oronasal fistula in a 25-year-old man. MSC were harvested from patient's iliac crest under local anesthesia and differentiated on a collagen membrane. After the biomaterial placement, a "platelet gel" (composed of platelet-rich plasma and HA particles coagulated together) was added. After the wound closure, MSC and PRP were applied transmucousally. The postoperative course was uneventful and the patient was discharged from hospital shortly after surgery. Ten weeks later he did not present any symptoms of the oronasal fistula and CT scan revealed bone formation in the area of the defect.

Chai et al. (2006) reported bone tissue engineering with osteogenically induced autologous BM-MSC and partly demineralized bone matrix in 7 patients with alveolar cleft. Stem cells were cultured on scaffold for a week before clinical application. The follow-up with 3D-CT at 3 months after surgery showed formed bone, which was maintained up to 1-3 years (Chai et al., 2006).

A comparison of strategies applied in human and animal studies on stem cell regenerative therapy for alveolar cleft reconstruction, as well as clinical trials described below, is presented in Table 1.

7. Ongoing clinical trials

Currently, there are 2 clinical trials assessing stem cell contribution to bone regeneration. Both focus on autologous stem cell sources, one involves μ-CT, which can give more detailed insight into newly formed tissue.

The trial conducted by the University of Michigan (Cell Therapy for Craniofacial Bone Defects, NCT01616953) is an ongoing open label randomized interventional study with an experimental arm, composed of Ixmyelocel-T and a control arm with autogenous bone grafting. Ixmyelocel-T is a product name of autologous bone marrow-derived CD90+ CD14+ stem cells produced and expanded trough bioreactor (Pagni et al., 2012). Up to 20 people aged between 18 and 60 years and fulfilling missing tooth criteria will be enrolled, including 10 with alveolar defects secondary to cleft palate. The follow-up of bone regeneration will be carried out with histological analysis and μCT at 4 months after the procedure. The dental implants will be loaded at the same time and followed for stability after 6 months. The study is still ongoing.

Another trial is carried out by Sirio-Libanes Hospital in Brazil (Use of Mesenchymal Stem Cells for Alveolar Bone Tissue Engineering for Cleft Lip and Palate Patients, NCT01932164) and currently recruiting participants for a single group assignment study, in which 5 patients aged between 7 and 12 years with 2/3 of the root of the canine tooth formed and previous dental arch alignment, will undergo tissue engineered alveolar grafting. MSC will be derived from dental pulp of extracted deciduous tooth, and seeded on a scaffold composed of collagen and HA. New bone formation will be measured by CT scan during 3 months following the procedure, and the quality of tissue engineered bone will be evaluated though observation of canine tooth eruption in the regenerated area.

8. Critical points in stem cell bone engineering

One of the major barriers for long-term bone stability and functionality is effective vascularization. The presence of vessel networks is indispensable for nutritional support and proper healing. Therefore, strategies promoting vascularization, such as growth factor delivery, co-culturing systems or scaffold design, will lead to acceleration of tissue regeneration (Roux, Cheng & Brey, 2015; Unger, Dohle, & Kirkpatrick, 2015). Angiogenesis is a complicated process requiring multiple factors, paracrine MSC activity and its ability to exude, inter alia, VEGF and stromal-derived factor 1 (SDF1) that could potentially augment new bone formation. In a study by Kim et al. (2014) angiogenesis was improved by use of co-culture of AT-MSC and BM-MSC. Zou et al. (2012) made a step further and decided to transduce BM-MSC using lentiviral vector to overexpress hypoxia-inducible factor-1 α with the aim to boost transcription of angiogenic genes. Vascular network formation was promoted resulting in enhanced new bone formation. To avoid complicated manipulations, a careful selection of tissue engineered components with adequate pro-angiogenic

Table 1
Comparsion between studies on stem cell regenerative therapy for alveolar cleft reconstruction. Abbreviations: BM, bone marrow; HA, hydroxyapatie; TCP, tricalcium phosphate; CT, computed tomography; CA, clinical assessment; MSC, mesenchymal stem cells; SEM, scanning electrone microscopy; CAP, carbonated hydroxyapatite; PLGA, poly(lactic-co-glycolic acid); AT, adipose tissue; PRP, platelet rich plasma; PDGF, platelet-derived growth factor.

Study	Donor	Cell source	Cell dose/density	Osteogenic induction	Scaffold type	Scaffold size	Cell-scaffold incubation time	Mean pore diameter
Korn et al. (2014)	Allogenic	BM, Femur	1×10^6/mL per scaffold portion	Yes	87% of 60%HA/40%β-TCP and 13% of silica matrix	0.6×4.0 mm	24 h	No exact data
Zhang et al. (2011)	Autologous	BM, Iliac crest	2×10^7 cells/mL	Yes	β-TCP	1.5–2.5 mm	4 h	450 ± 50 μm
Yoshioka et al. (2012)	Autologous	BM, Iliac crest	1×10 (Bajaj et al., 2003 Nov) cells/well	No	CAP	600–800 μm	None	No data
Pourebrahim et al. (2013)	Autologous	AT, Scapular subcutanues tissue	5×10^6 cells/$3 \times 3 \times 3$ mm scaffold	Yes	60%HA/40%β-TCP	$3 \times 3 \times 3$ mm	21 days	200-800 μm
Ou et al. (2007)	No data available	BM	No data available	No data available	Collagen	x	48 h	x
Hibi et al. (2006)	Autologous	BM, Iliac crest	Total dose of 5×10^7	Yes	x	x	x	x
Pradel et al. (2008)	Autologous	BM, Maxilla	No data	Osteoblast-like cells	Resorbable bovine collagen matrix	$0.3 \times 1 \times 1$ cm	3–4 days	x
Behnia et al. (2009)	Autologous	BM, Iliac crest	5×10^5 in 0.2 ml medium	No	Demineralized bone matrix, calcium sulphate	No data	1 day	no data
Behnia et al. (2012)	Autologous	BM, Iliac crest	5×10^5 in 0.2 ml medium	No	60%HA/40%TCP	3 mm cubes	1 day	300-500 μm
Stanko et al. (2013)	Autologous	BM, Iliac crest	No data	No	Collagen membrane and HA as a part of platelet gel	0.5 mm diameter (HA)	3 weeks on collagen membrane, HA added at operating room	no data
Chai et al. (2006)	Autologous	BM, Iliac crest	No data available	Yes	Partly demineralized bone matrix	No data available	1 week	No data available
NCT01616953	Autologous	BM, Iliac crest	No data available	No, cell expansion: ixmyelocel-T	x	x	No data available	x
NCT01932164	Autologous	Deciduous dental pulp	No data available	No	Collagen and HA	No data available	No data available	No data available

Study	Membrane	Growth factors	Subject	Number of subjects	Age	Control groups	Defect	Follow up	Maximum observation time	Results
Korn et al. (2014)	No	No	Lewis rats	72	Adult	(1) Scaffold with undifferentiated stem cells (2) scaffold alone (3) blank control	3 mm diameter in anterior maxilla	Cone-beam CT, histological and histomorphometric analysis, CA	6 weeks	Remaining defect volume: 5.00 ± 0.84 mm^3 (scaffold + induced BM-MSC)[a], 4.08 ± 1.36 mm^3(scaffold + BM-MSC)[b], 5.50 ± 1.05 mm^3 (scaffold alone), 6.86 ± 3.21 mm^3 (blank control)[a,b]
Zhang et al. (2011)	No	No	Beagle dogs	7	24-week-old	(1) Scaffold alone (2) autologous bone obtained from icliac bone (3) blank control	Alveolar defects extending to the nasal floor, $10 \times 5 \times 15$ mm	SEM for cell seeding, sequential fluorescent labelling, radiographic observation, histological and histomorphometric analysis, CA	20 weeks	(1) Alveolar height: $73.60\% \pm 6.51\%$ (scaffold+BM-MSC)[a] $72.42\% \pm 8.72\%$(autologous bone)[b] $56.31\% \pm 7.72\%$ (scaffold alone)[a,b] (2) tooth movement: 5.345 ± 0.936 mm (scaffold+BM-MSC)[a], 4.665 ± 0.483 mm (autologous bone)[b], and 6.986 ± 1.412 mm (scaffold alone)[a,b]
Yoshioka et al. (2012)	PLGA	No	Beagle dogs	3	3-moth-old	(1) Scaffold alone	5×10 mm	Radiographic observation, histological analysis, CA	6 months	(1) Higher radiopacity of regenerated bone at experimental side (2) Decreasing number and size of CAP particles, higher number of new capillary vessels and new bone formation at experimental side (3) Perfect closure of the defect after 6 months at the experimental site
Pourebrahim et al. (2013)	No	No	Mongrel dogs	4	Adult, mean age of 22 months	(1) Autologous bone obtained from tibia	15 mm wide, with penetration to the nasal cavity	SEM for cell seeding, histological and histomorphometric analysis, CA	60 days	New bone formation at 15 and 60 days: 15%[a] and 70%[b] (scaffold + BM-MSC), 45%[a] and 96%[b] (autologous bone)
Ou et al. (2007)	No	No	Dogs	12	No data available	Yes, however no exact data available	No data available	3-d CT, Histological analysis, CA	12 weeks	Defect width on the experimental site is comparable to the positive control group, however defect height is less than control
Hibi et al. (2006)	Titanium mesh	PRP, human thrombin,	Human	1	9-year-old female	x	10×13 mm	CT, CA	9 months	New bone formation of 79.1% and canine eruption at 9 months after the procedure

Table 1 (*Continued*)

Study	Membrane	Growth factors	Subject	Number of subjects	Age	Control groups	Defect	Follow up	Maximum observation time	Results
		calcium ions								
Pradel et al. (2008)	No	No	Human	1	10-year-old male	x	x	Radiographic observation, CA	18 months	(1) tooth movement at 8 months after the procedutr (2) canine eruption at 18 months after the procedure
Behnia et al. (2009)	No	No	Human	2	14-year-old female and 10-year-old male	x	No exact data	SEM for cell seeding, radiographic observation, CT, 1-mm cornal sections, CA	4 months	(1) nasal floor integrity at 2 months after the procedure (2) $<$ 50% bone regeneration in both cases
Behnia et al. (2012)	No	PDGF	Human	3	3 patients, mean age of 10 years	x	No exact data	Cone beam CT, CA	3 monts	(1) mean new bone formation of 51.3%
Stanko et al. (2013)	No	PRP, calcium ions	Human	1	25-year-old man	x	Unilateral complete cleft with oronasal fistula, 10×7mm	CT, CA	10 weeks	Initial bone formation at 10 weeks and no clinical signs of oronasal fistula
Chai et al. (2006)	No data available	No data available	Human	7	Unknown	x	No data avaiable	3-d CT, CA	3 years	New bone formation in 3 month after the procedure which was stable up to 3 years of follow-up
NCT01616953	No	No	Human	10 with alveolar cleft	18–80 years	(1) autologous bone (randomized study)	Specified in inclusion criteria	Histological analysis, μCT, CA	10 months	Study will be completed by April 2016
NCT01932164	No	No	Human	5	7–12 years	x	Specified in inclusion criteria	CT, CA	No exact data	Study will be completed by March 2016

[a] Same letters indicate statistically significant differences.
[b] Same letters indicate statistically significant differences.

properties and vascularization potential should be made. Another approach for enhancing vascularization is mechanical stimulation that can be achieved by use of bioreactors to ensure adequate distribution of nutrients and cells within the scaffold. More advanced methods, such as microfabrication, are being introduced to control scaffold properties and mimic native tissue construction (Nguyen et al., 2012). Vascularized bone grafts could be one of the most promising strategies, however several hurdles such as osteogenic and vascular integration are yet to be overcome (Mercado-Pagán, Stahl, Shanjani, & Yang, 2015).

The proper selection of stem cell source, scaffold and growth factors is crucially important for successful bone regeneration. The selection should be made not only based on their sole properties, but according to their ability to act as a group. Interactions in tissue-engineered products depend on scaffold composition, pore structure and its overall porosity that should mimic the bone microenvironment. Selecting an improper construct will negatively influence stem cell regenerative properties (Polo-Corrales, Latorre-Esteves, & Ramirez-Vick, 2014). Even stem cell seeding technique, cell-scaffold incubation time and culture conditions greatly influence the final outcome (Hasegawa et al., 2010; Rajan et al., 2014).

The possibility of cryopreservation of a bioengineered product is interesting. However, it may influence not only stem cell viability and adhesion to the scaffold, but also mechanical properties of the construct (Costa et al., 2012; Xu, Liu, & Cui, 2014).

Another obstacle is that we still lack long-term data on the functionality of the bioengineered constructs and first of all, short-term results are based on case reports with large diversity of applied strategies. A problem of vast heterogeneity of follow-up strategies was also brought up by Janssen et al. who performed excellent review on similar topic, but with focus on different aspects (Janssen, Weijs, Koole, Rosenberg, & Meijer, 2014). A more detailed follow-up with histological and imagining studies, including μCT, is indispensable. Patient satisfaction with the procedure and quality of life should be assessed as well.

To conclude, vast heterogeneity of studies and complicated interplay between all the components hinders the choice of seeding cells, scaffold and growth factors, and underlines the need of preclinical studies before clinical application. Safety record and previous use in human trials must be considered as well.

The idea of stem cell-based tissue engineering for treating alveolar cleft is promising and has strong scientific rationale and provides hopeful results of preclinical studies and first case reports. Tissue-engineered products holds a great promise for replacing autologous bone grafting and improving the quality of life of patients with alveolar cleft.

Conflict of interest

The authors declare no conflict of interest.

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Stromal cells and stem cells in clinical bone regeneration

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Abstract

Stem-cell-mediated bone repair has been used in clinical trials for the regeneration of large craniomaxillofacial defects, to slow the process of bone degeneration in patients with osteonecrosis of the femoral head and for prophylactic treatment of distal tibial fractures. Successful regenerative outcomes in these investigations have provided a solid foundation for wider use of stromal cells in skeletal repair therapy. However, employing stromal cells to facilitate or enhance bone repair is far from being adopted into clinical practice. Scientific, technical, practical and regulatory obstacles prevent the widespread therapeutic use of stromal cells. Ironically, one of the major challenges lies in the limited understanding of the mechanisms via which transplanted cells mediate regeneration. Animal models have been used to provide insight, but these models largely fail to reproduce the nuances of human diseases and bone defects. Consequently, the development of targeted approaches to optimize cell-mediated outcomes is difficult. In this Review, we highlight the successes and challenges reported in several clinical trials that involved the use of bone-marrow-derived mesenchymal or adipose-tissue-derived stromal cells. We identify several obstacles blocking the mainstream use of stromal cells to enhance skeletal repair and highlight technological innovations or areas in which novel techniques might be particularly fruitful in continuing to advance the field of skeletal regenerative medicine.

Introduction

Bone has an innate propensity to regenerate following traumatic injury. Upon fracture, resident stromal, stem and progenitor cells work in tandem with pro-inflammatory and anti-inflammatory macrophages[1,2] and circulating blood cells[3] to orchestrate a complex signalling cascade that leads to scarless healing.[4] In spite of this tremendous capability, a number of clinical indications remain that require therapeutic intervention to facilitate bone repair and regeneration. Autologous bone grafting, in which bone from another part of the

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W.L.G., B.P.H. and J.M.G. researched data for the article and wrote the article. W.L.G., B.A.B., E.M., T.F. and J.M.G. made substantial contributions to discussions of the content. All authors reviewed and/or edited the manuscript before submission.

body is transplanted to the defect site, remains the gold standard; however, this approach is associated with numerous drawbacks, including donor-site morbidity, the availability of limited grafting material and compromised bone quality in patients with osteoporosis.[5] Bone-tissue engineering (BTE) has been developed as a potential alternative to overcome the critical shortcomings associated with autografts and allografts. In general, BTE involves the use of various combinations of cells, growth factors and/or cytokines, and bioactive carriers (scaffolds and/or hydrogels). Even though it has been ~30 years since the first efforts in this area,[6] few BTE techniques have translated into clinical practice and none of them has become the standard of care in regenerative medicine.

This Review focuses specifically on the successes and challenges of using stromal or stem cells in the clinical translation of BTE techniques. Some controversy remains over the specification of adipose-tissue-derived and bone-marrow-derived progenitors as stem cells. Although the authors consider that each of the two descriptions has merits, these cells will be referred to in the remainder of this Review as stromal cells. Currently, the role of transplanted stromal cells in mediating regeneration remains poorly understood, particularly in the clinical trials that have been conducted. The original premise of many early *in vitro* and preclinical studies was that transplanted cells would undergo differentiation and morphogenesis to form the regenerated tissue; however, this paradigm has been challenged by experimental findings documenting that very few regenerative cells actually survive following transplantation.[7] In spite of the clear benefits associated with cell delivery, the poor mechanistic understanding of stem-cell-mediated regeneration is an obstacle to optimizing regenerative approaches. Animal models have the potential to provide some insight; however, many of the available models do not effectively recapitulate the clinical situation, which is either due to the size of the defects or the timing of cell delivery relative to when the defect was created. In addition to the lack of mechanistic insight, logistical, regulatory and technical challenges continue to limit the clinical application of stromal and stem cells for skeletal regeneration. In this Review, we briefly discuss the history of stromal cells, their use in clinical trials, the challenges facing their widespread implementation and current approaches to bone regeneration that are based on stromal and stem cells. This Review also highlights novel technologies and future studies that are needed to establish stromal-cell-mediated and stem-cell-mediated BTE as a standard component of clinical care.

Stromal cells

Historical and developmental relationships

Pioneering reports in the 1960s by Alexander Friedenstein and colleagues at the University of Moscow laid the foundations for the modern era of multipotent-stromal-cell and mesenchymal-stem-cell (MSC) research.[8–10] Friedenstein's team was the first to demonstrate that bone marrow contains fibroblast-like stromal cells, termed mechanocytes, which are capable of osteogenic differentiation and are necessary for the creation of the haematopoietic microenvironment or niche.[8] Additionally, the researchers demonstrated that similar cell populations are present in the thymus, liver and other organs. These findings were advanced in the 1980s by the demonstration that primary cultures of bone-marrow-derived stromal cells are adipogenic and chondrogenic.[11,12] During the same period,

techniques for the long-term culture of primary bone marrow cells were developed.[13,14] Adherent stromal-cell populations, which are required to support proliferation and differentiation of haematopoietic progenitors and stem cells, were also shown to be capable of mediating adipogenesis in the presence of glucocorticoid-containing horse serum.[15] In pursuit of these findings, haematologists across the globe developed clonal cell lines from adherent stromal cells in long-term cultures, which were isolated from bone marrow, spleen, liver and other tissues capable of supporting B-cell lymphopoiesis.[16–18] When co-cultured in the presence of stromal cell clones, haematopoietic stem cells and progenitor cells (which routinely died in culture) were able to proliferate and differentiate as a result of the release of as yet unidentified growth factors. In many cases, stromal-cell clones went on to be used as critical reagents for the isolation and characterization of haematopoietic cytokines, such as IL-7 and IL-11.[19–21]

The stromal-cell field was advanced in the early 1990s by the adoption of the term ‘mesenchymal stem cells’ to classify the adherent bone marrow cells that are characterized by their ability to differentiate along the adipocyte, chondrocyte, osteoblast, skeletal myocyte and tenocyte pathways.[22,23] Several research groups were among the first to generate monoclonal antibodies against the human bone-marrow-derived mesenchymal-stromal and stem-cell (MSC) surface antigens STRO-1, CD73 (which targets the Src homology [SH] 3 and SH4 domains) and CD105 (which targets the SH2 domain).[24,25] Some researchers have argued that MSCs do not meet the scientific standards required to define them as stem cells, as no reports have documented their ability to be serially passaged through multiple recipients whilst retaining tissue-generating functionality. As a compromise, in 2006, the International Society for Cellular Therapy issued a consensus statement defining MSCs as ‘multipotent stromal cells’ on the basis of the following criteria: firstly, capability of plastic adherence and self-renewal in culture; secondly, staining positive for CD73, CD90 and CD105, and staining negative for CD11B or CD14, CD19 or CD79α, CD34, CD45 and HLA-DR; and thirdly, differentiating along the adipocyte, chondrocyte and osteoblast pathways *in vitro*.[26] At present, these criteria have been used to define MSCs in the majority of the published literature; however, questions have been raised as to whether or not this *in vitro* evidence is sufficient for the characterization of MSCs as stem cells. The growing number of MSC filings at the FDA have employed an ever-widening array of surface antigenic biomarkers.[27] The inconsistency of these characterizations has been complicated by the fact that an increasing percentage of MSC products are derived from tissues other than bone.[27] The FDA consortium has suggested that additional bioactivity assays, which involve proteomic analysis of membrane proteins, and adipocyte differentiation be used to identify and define MSCs that are isolated from these various tissues.[27–31] Some researchers have long advocated that the *in vivo* criteria should constitute the ‘gold standard’ for MSC definition.[32] These researchers and others have documented the robust ability of bone marrow MSCs to form mineralized bone that contains a haematopoietic marrow after insertion on a hydroxyapatite or related scaffold and implantion subcutaneously in rats.[32] Although the pharmaceutical and biotechnology industries continue to pursue *in vitro* surrogate assays, the *in vivo* assay of bone differentiation remains the definitive standard for many academic laboratories.[33]

Dependence on tissue of origin

Multiple independent studies have isolated cells with MSC-like characteristics from amniotic, placental and umbilical-cord tissues, as well as adult adipose, dental, dermal and skeletal muscle tissues.[34–43] Tissue-derived MSCs have been isolated and/or identified, in part, on the basis of their adherence properties to tissue culture surfaces and by flow cytometric sorting on the basis of the surface antigens they express.[24,43–45] Perivascular cells isolated from multiple tissues on the basis of their expression of CD146, chondroitin sulphate proteoglycan 4 (commonly known as NG2) and platelet-derived growth factor receptor β display clonal multipotency and express mesenchymal markers, which suggests that stromal cells and pericytes are functionally equivalent.[46] A limited number of studies have directly compared stromal cells that were isolated from distinct tissues. Although failing to demonstrate substantial differences in immunophenotype or morphology, an initial comparison of cells that were isolated from adipose tissue, bone marrow and umbilical cord found a greater frequency of colony-forming units in adipose-tissue-derived cells than in bone-marrow-derived and umbilical-cord-derived cells and greater proliferative and inferior adipogenic capacities in umbilical-cord-derived cells than in adipose-tissue-derived and bone-marrow-derived cells.[42] A later analysis determined that although adipose-tissue-derived stromal cells (ASCs) shared multiple features in common with their bone-marrow counterparts, ASCs display subtle differences in their immunophenotypic profile.[47] These differences have been encapsulated in a joint International Society for Cellular Therapy and International Federation for Adipose Therapeutics and Science consensus statement.[48] ASCs have demonstrated osteogenic potential *in vitro* and *in vivo* both in preclinical models and in clinical trials.[34,49–55] *In vitro* studies have demonstrated that the extent of ASC osteogenesis can be enhanced by manipulating the concentrations of ascorbate and dexamethasone in cell-culture media.[56] Nevertheless, some studies have highlighted concerns that the osteogenic capacity of ASCs is significantly lower than that of MSCs isolated from bone.[57,58] In spite of this concern, the ease of accessibility and relative abundance of ASCs in comparison to that of MSCs confer practical advantages and have contributed to the continued interest in the clinical use of these cells in bone regeneration.[52,54,59] Whether or not the tissue of origin regulates the epigenetic memory of MSCs and their subsequent differentiation potential remains to be determined.[60]

Skeletal-muscle-derived stem cells (MDSCs) are distinct from the resident satellite cells, which are stimulated upon muscle damage to repair the tissue. MDSCs, isolated from muscle tissues on the basis of expression of CD34 and apoptosis regulator Bcl-2, demonstrate robust osteogenic capacity and thus have potential for use in skeletal repair.[61] Similarly to MSCs and ASCs, the MDSC population exhibits multipotency, can regenerate various tissues *in vivo* and can secrete a number of trophic factors that are capable of stimulating endogenous repair.[62,63] However, as a therapy for bone defects, MDSCs lag behind ASCs and MSCs and they are not currently being investigated in clinical trials.

Skeletal regeneration

The field of skeletal regeneration continues to face multiple challenges that would potentially benefit from approaches that involve cell-based therapeutics. Of these

challenges, the most common is acute trauma, which accounts for the majority of orthopaedic surgical procedures in the USA and internationally. The body responds to trauma by initiating a cascade of inflammatory and regenerative events. Sequentially, these actions include local and systemic release of proinflammatory cytokines, homing of immune cells to the site of injury, soft-tissue inflammation and oedema, mobilization of osteogenic progenitor cells, local release of bone morphogenetic proteins, callus formation, bone remodelling and eventual bone replacement. A rationale exists for introducing exogenous MSCs during one or more of these events. Immediately following the acute injury (hours to days), MSCs can dampen or modulate local and systemic inflammatory responses by producing immunosuppressive factors, such as transforming growth factor β, prostaglandin E2 and indoleamine 2,3-dioxygenase 1 (commonly known as IDO). Additionally, the release of stromal cell-derived factor 1 (also known as SDF-1) and other cytokines from MSCs can alter the types of immune cells that are recruited to the site of injury. During intermediate periods (from days to weeks) following the injury, MSCs can contribute to the repair process by differentiating into chondrocytes and osteoblasts, thereby augmenting the recruitment of local endogenous osteoprogenitor cells. Although whether exogenous MSCs have a substantial benefit when introduced late (from weeks to months) following acute trauma remains to be determined, at least one instance in which late delivery of MSCs can be beneficial is delayed union or non-union of bone.

Non-union can occur in up to 15% of cases of complex trauma as a result of mechanical factors, as seen in comminuted fractures with multiple bone fragments; infection, as seen with bacterial contamination of the injury site or a patient's underlying viral diseases (for example, hepatitis and HIV); smoking and other tobacco-related or drug-related toxins; and endocrine disorders, such as type 2 diabetes mellitus, obesity, osteopenia and osteoporosis. Finally, exogenous MSCs have the potential to benefit treatment of bone-related tumours, such as Ewing sarcoma, osteosarcoma and metastatic bone disease. The introduction of a healthy exogenous MSC population might enable bone metabolism to recover more rapidly following chemotherapy, radiation and/or surgical ablation of the tumour by improving the local microenvironment. Additionally, MSCs that are genetically modified to deliver specific proteins, radioisotopes or microRNAs can be used as antitumour vectors owing to their ability to home to sites of active primary or meta-static cancers.[64–71] Nevertheless, as MSCs can promote the proliferation of breast, prostate and other tumours *in vitro* and *in vivo*,[72,73] preclinical safety studies need to be performed before using native or genetically modified MSCs in the context of bone tumours. The safety concerns regarding the use of ASCs or MSCs, which include tumour formation in bone regeneration applications, are significantly lower than those regarding the use of pluripotent stem cells (embryonic stem cells or induced pluripotent stem cells). To date, MSCs and ASCs have been used in a small number of studies for bone regeneration in humans (Table 1). In the majority of studies, autologous cells (with or without prior expansion *in vitro*) were either directly injected into the defect site or injected with the aid of biomaterial carriers. Owing to the immunoprivileged characteristics of ASCs and MSCs, a number of clinical trials are also currently being performed with genetic cells.

Clinical trials

Craniofacial bone regeneration

Ectopic vascularized bone formation—The treatment of large craniofacial defects presents unique challenges owing to the complex 3D geometry of bone. The current gold standard for the treatment of large bone defects is autologous bone transplantation, which can be vascularized or nonvascularized. In nonvascularized bone, the lack of vasculature can lead to graft resorption with resultant loss of the geometric structure of bone. To transplant vascularized, autologous bone, the surgeon has to painstakingly dissect out suitable portions of the patient's own iliac crest, fibula or ribs, shape them into an approximate anatomical shape and then use microsurgical techniques to restore the blood supply. This immediate supply of oxygen and nutrients is critical for graft survival and long-term integration. Consequently, one clinical approach to utilize BTE grafts incorporates an *in vivo* cultivation period. The grafts are implanted into large, highly vascularized muscle tissues (for example, latissimus dorsi[74] or rectus abdominis[75]) for several months to facilitate vascular ingrowth and the development of a vascular paedicle suitable for microsurgical anastomosis. To achieve this vascular growth, a preshaped titanium mesh is used to enclose mineralized matrix (for example, autograft cancellous bone chips or xenograft bone blocks), osteoinductive recombinant human (rh) growth factors (such as rhBMP-2 or rhBMP-7) and cells (Figure 1). Both bone-marrow aspirates for mandibular reconstruction[74] and ASCs expanded using good manufacturing practice (GMP) standards to treat maxillary defects[75] have been successfully used to regenerate craniofacial bone with this methodology. The resulting bone had sufficient structural integrity to support dental implants 4 months following surgical reconstruction of the defect with the BTE graft.[75]

In situ bone formation—In spite of the considerable successes of the two-step process described above, ectopic bone formation requires additional surgeries, which increases the risk of comorbidities and involves a substantial investment in time to facilitate new mineral deposition in the graft. Therefore, several groups have taken a single-step approach to form new, structurally sound bone matrix in orthotopic sites. Treatment of a 10 cm anterior mandibular defect (left after tumour excision) using additive manufacturing recreated the exact geometry of a patient's mandible from radiographic images.[76] A titanium mesh was then prefabricated with the customized patient geometry and filled with β-tricalcium phosphate (β-TCP) granules that were soaked in rhBMP-2 for 48 h before implantation of ASC cultures (expanded under GMP conditions). By 10 months post-surgery, sufficient new bone had developed to support dental implants. A similar *in situ* approach was used to treat calvarial defects in a child with multiple fractures.[77] The surgical team applied milled bone from the iliac crest, ASCs isolated from fat tissue harvested from the gluteal region and autologous fibrin glue, which was used to hold the cells and milled bone grafts in place. The patient displayed bone regeneration within 3 months of surgery. Although the patient numbers were limited and the studies were not randomized with double-blinded controls, these case reports indicate that MSCs can be used successfully to repair defects in nonweight-bearing craniofacial bones.[76,77] In both cases, a titanium mesh was used to provide the regenerated bone with the appropriate anatomical geometry, whereas the granules, cancellous bone chips or bone blocks were osteoconductive and osteoinductive.

Advanced biomaterial scaffolds capable of integrating these structural elements and biological signals can also be used to guide new bone growth (discussed elsewhere[78–81]).

Distal tibial non-union

In addition to the treatment of craniofacial defects, which requires highly invasive procedures, the clinical relevance of stromal cells in minimally invasive procedures has also been assessed. Specifically, non-unions and delayed fracture healing, in which a deficiency of fracture repair exists, are ideal situations in which to harness the regenerative potential of stromal and/or stem cells. In a randomized controlled clinical study, which included 24 patients who were considered to be at low risk of non-unions of the tibia, the prophylactic effects of MSCs in expediting fracture healing were assessed.[82] Autologous MSCs (~10^8) isolated from the iliac crest and peripheral blood were injected into the fracture site together with platelet-rich plasma (containing 1.1×10^9 platelets) and allograft demineralized bone matrix. This treatment resulted in a significant reduction in the time to union, from 3.0 months to 1.5 months in the intervention group of patients who received the biological composite compared with the control group of patients who did not receive this treatment. Additionally, subcutaneous grafting of a portion of the injectable composites into immunodeficient mice resulted in bone formation; however, as the authors were unable to assess the origin of the newly formed bone *in vivo*, the precise role of MSCs in promoting the improved response remains unclear.

Osteonecrosis of the femoral head

Another potential minimally invasive application of stromal cells is the treatment of osteonecrosis of the femoral head (ONFH).[83–86] Nontraumatic ONFH is a debilitating skeletal disorder that can lead to collapse of the femoral head and the need for total hip replacement. In 2004, a double-blind nonrandomized study was conducted to assess the effect of delivering autologous bone marrow mononuclear cells (following core decompression of the lesion) to patients with stage I or stage II ONFH.[84] Cells were injected directly into the defect site via a trephine without any scaffold or hydrogel to enhance retention at the site. Within 3 months, a statistically significant reduction in the lesion-volume:femoral-head-volume ratio was observed in the cell-grafting group compared with the untreated control group. Further decreases in this volume ratio occurred by 24 months, which indicated the possibility of a slight progression in healing over time. At 60 months of follow-up, the number of patients who progressed to fracture in the cell-grafting group was markedly decreased compared with those in the control group;[83] however, the use of core decompression might have introduced additional trauma to the region. A less-invasive approach, in which iliac-crest-derived bone marrow mononuclear cells (BMMCs) are delivered through the medial circumflex femoral artery (using fluoroscopy to locate the injection site) was subsequently developed.[85] The 62 patients in this study were all treated with autologous BMMCs, which enabled the safety, but not the efficacy, of the treatment to be assessed. One limiting factor in these approaches is the low concentration of MSCs in bone-marrow aspirates from the iliac crest. This limitation was overcome and the safety and efficacy of the approach was demonstrated in a randomized trial of 100 patients by use of culture-expanded autologous BMMCs that were isolated from the subtrochanteric region (aspirated through the decompression tunnel) together with iliac-crest-derived BMMCs.[86]

Limitations, challenges and opportunities

Animal models

The available clinical data strongly support an enhanced regenerative effect of stromal cell delivery to the site of bone defects. However, advancing these approaches in order to bring them into the realm of standard clinical care requires insight into the underlying mechanisms of cell-mediated effects. Animal models provide the best proxy to investigate cellular mechanisms. Small rodent models are, traditionally, the first choice to assess *in vivo* responses. Cranial defects in mice have been used to demonstrate bone regeneration following the delivery of ASCs.[87–89] By use of fluorescent *in situ* hybridization staining of female (donor) chromosomes in male recipient mice,[89] up to 99% of new bone was seen to be formed by the transplanted cells. An important consideration, however, is that the mouse calvarium is <500 μm thick and, therefore, oxygen gradients throughout the graft that might lead to the generation of hypoxic regions in the core are not a complication. Consequently, cell survival in this model is not heavily dependent on revascularization, which does not mimic the reality of the clinical situation. The model of femoral defects in rats shows greater reliance on sufficient revascularization to facilitate bone healing than that in mice.[90] Using this model, transplanted cells were confirmed to enhance regeneration even though they are not incorporated into the new bone.[7] Such reports provide indirect evidence that the primary mechanism through which transplanted stem cells mediate tissue repair might involve the secretion of paracrine factors that stimulate the recruitment and activation of endogenous stem cells.[91,92]

The critical dependence on trophic factors to facilitate regeneration rather than to direct differentiation, tissue morphogenesis and integration of transplanted cells is further supported by studies in which blocking VEGF signalling impaired stem-cell-mediated bone repair.[93–95] Hypoxia, acting through the hypoxia-inducible factor 1 (HIF-1) transcription factor, reduced the expression of vascular endothelial growth factor receptor 1 (VEGFR-1; commonly known as FLT-1) on bone marrow MSCs.[94] Similarly, *in vitro* studies using murine muscle-derived stem cells showed that overexpression of soluble FLT-1, a VEGF antagonist, promoted chondrogenesis in pellet cultures.[95] Overexpression of soluble FLT-1 improved articular cartilage repair *in vivo*, whereas overexpression of VEGF-A_{165} (the main biological isoform of VEGF-A) led to arthritic changes in the joint, which were consistent with hypertrophic cartilage formation.[95] Furthermore, gain-of-function and loss-of-function expression studies in the mouse embryo have shown that VEGF, released by the limb bud mesenchyme, is required for the development of the skeletal vasculature.[93]

Long-bone defects in larger animal models such as dogs and sheep might be better approximations of the clinical scenario than similar defects in mouse. Treatment of 3 cm full-thickness, segmental defects in 6–7-year-old sheep by use of polycaprolactone–tricalcium phosphate (PCL–TCP) scaffolds with nonautologous, culture-expanded MSCs or rhBMP-7 has been reported.[96] In this study, rhBMP-7 elicited the greatest new bone formation, whereas sheep that received MSCs showed similar healing to the group that received PCL-TCP only. Large animal models might also be used to assess bone healing in skeletally immature animals. For example, a porcine model was used to evaluate the effect

of BMP-7 delivery on bone healing in an immature pig.[97] The porcine model has also been used to assess the effect of direct or indirect ASC delivery to noncritical-sized bone defects in the mandible.[98] None of these models precisely mimics the challenges involved in regeneration of large craniofacial defects, non-unions or ONFH in humans. At this time, no single species has been identified as the 'gold standard' preclinical animal model for human skeletal regeneration. Consequently, future studies to investigate the mechanisms of stromal-cell-mediated bone repair will continue to rely on a variety of small and large animal models.

Cell survival and vascular integration

Widespread adoption of BTE grafts as the clinical standard of care for the treatment of massive bone defects requires the development of simple and effective techniques to rapidly vascularize grafts and enhance the survival of transplanted stromal cells. To address this need, multiple groups have investigated methods of prevascularization to harness the proangiogenic potential of endothelial cells, which are co-cultured with pro- osteogenic stromal cells. Endothelial cells have the ability to self-assemble into primitive capillary-like networks in response to proangiogenic growth factors in permissive hydrogel environments. Ideally, these nascent networks can anastomose with blood vessels, which infiltrate the defect site from its periphery. Once perfused, the resulting blood flow stimulates maturation and subsequent pruning of the vasculature. In purely vascular tissue-engineering systems, these nascent networks have been shown to remain viable for up to 1 year after intervention.[99] Several groups have employed this technique using endothelial cells and either MSCs or ASCs to engineer vascularized bone grafts in preclinical models.[100–104] The main limitation of this technique is the extensive *in vitro* manipulation and precultivation required to facilitate blood vessel development and mineralization. Angiogenesis and osteogenesis are tightly coupled processes during bone development and healing. However, the development of vascularized osteogenic grafts has resulted in the requirement for separate cultivation conditions for cells, followed by a reintegration phase. The extensive manipulation and use of multiple culture conditions and growth factors might continue to substantially limit the clinical utility of this approach. Cell pre-aggregation methods, which enhance cell survival following transplantation[105–107] and harness the potential of stromal cells to self-assemble into complex tissues,[4,108] might facilitate translation of this approach into clinical practice. These techniques could be combined with the development of biomaterials capable of time-released, serial delivery of angiogenic factors followed by delivery of osteogenic factors; biomaterials with these attributes have already been described.[109–113]

Rapid vascularization is essential for graft viability. In the absence of an immediate vascular supply to BTE grafts in the defect site (as was the case in the clinical trials conducted to date), transplanted cells are immediately exposed to severe hypoxia and ischaemia. To minimize the negative effect of hypoxia, several groups have investigated the potential of localized oxygen delivery using perfluorocarbons or peroxide-based scaffolds. Perfluorocarbons are capable of delivering oxygen for 2–3 h before becoming depleted.[114] Revascularization occurs over a period of 1–2 weeks in clinically sized grafts and bone formation takes months. In spite of this obvious mismatch, delivery of oxygen for just a few

hours after transplantation resulted in statistically significantly enhanced bone formation within 6 weeks in a murine model.[115] In contrast to perfluorocarbons, oxygen-generating peroxide scaffolds are able to continuously supply oxygen for weeks to months. The main drawback of peroxides is that they produce reactive oxygen species (ROS), which are detrimental to cell viability. Thus, methods to improve biomaterial design for localized oxygen delivery remain a promising opportunity for future research to enhance the outcomes of cell-based therapies.

An alternative approach for delivering oxygen along with the scaffold is to prime cells to be resistant to low-oxygen conditions. MSCs and ASCs are known to be highly resilient in hypoxic conditions and, under these conditions, they upregulate expression of the HIF-1α pathway. The transcription factor HIF-1α controls both the production of angiogenic growth factors and cytokines and the ability of vascular cells to respond to these proteins. Additionally, HIF-1α activates the expression of multiple proangiogenic cytokines, including SDF-1, placenta growth factor and angiopoietins,[116,117] which are required for physiological vascularization and which cannot be effectively stimulated by VEGF signalling through VEGFR-2 alone. HIF-1α modulates metabolic responses to hypoxia in order to maintain homeostatic levels of cellular energy, pH and the redox state. These responses include cellular adaptations regulated by downstream HIF-1α signalling that limit the production of ROS during periods of hypoxia; upregulation of levels of glucose transporters and glycolytic enzymes; and modified protein expression that facilitates pH homeostasis. Interestingly, the expression of HIF-1α in osteoblasts has been identified as a mechanism for coupling angiogenesis and osteogenesis in native bone.[118–120] By culturing cells in low-oxygen conditions or chemically inducing upregulation of levels of HIF-1α before transplantation, cell survival and homing of the cells following transplantation (mediated by upregulation of levels of CXCR4) can be augmented, which thereby enhances the regenerative properties of the cells, as has been shown in cardiovascular applications.[121,122]

Cell dosing and optimal concentrations

Cell numbers for transplantation are often determined empirically through intuition on the basis of prior experience or from *in vitro* data. In clinical applications for which the size and geometry of the graft needs to be customized, it is impossible to ascertain optimal cell numbers *a priori* without knowledge of the mechanisms of cell-mediated bone regeneration. However, mechanistic data derived from preclinical models can be used to develop computational approaches that model bone repair as a function of initial bone condition.[123] Computational models can be used predictively to rigorously define the number of cells and mode of delivery required for any specific application.

In vitro expansion

In cell culture, the term hypoxia is used to describe oxygen levels lower than the 20% found in the atmosphere in which cells are cultured *ex vivo*. However, average oxygen levels within tissues in the body can be as low as 5%. Consequently, culturing cells *ex vivo* exposes them to hyperoxic conditions, which might lead to elevated levels of intracellular ROS production. Another drawback of extended *ex vivo* culture is the potential for

development of genetic and epigenetic mutations as a consequence of rapid cell division. Thus, methods that employ culture-expanded allogenic or autologous cell sources require robust assays to monitor and validate biological changes that might negatively influence the safety and subsequent efficacy of cell-based therapies.

Genetic modification of stromal and stem cells

Stromal and stem cells can be transduced to overexpress growth factors (in particular, BMPs[124]) and transcription factors (such as sonic hedgehog[125]) in order to enhance the efficacy of promoting new bone formation. This approach has been shown to be efficacious in small-animal models and its potential has been reviewed elsewhere.[126,127] However, genetically modified stromal cells and stem cells remain a highly experimental model, which has proven difficult to advance through the regulatory process as a result of substantial safety concerns.

Approved cell-based products

The most established commercial cell product available for orthopaedic disabilities is Carticel® (Genzyme, USA), which uses culture-expanded, autologous chondrocytes for the treatment of degenerated articular cartilage. The use of MSCs or ASCs rather than chondrocytes can be advantageous owing to the potential to obtain larger quantities of cells and because it is associated with less donor-site morbidity. Although studies assessing the role of cell adhesion in improving chondrogenic outcomes with MSCs have been reported,[128] no comparable commercially available cell products for bone regeneration exist. Osteocel® (NuVasive, USA), which retains viable MSCs within bone allografts, is now available for spine fusion applications. Trinity® Evolution™ (Orthofix, Netherlands Antilles) is a similar product for spine and other orthopaedic conditions. Lately, culture-expanded allograft MSCs have been used in a clinical trial to treat meniscectomies.[129] Such clinical successes might herald the routine use of allograft cell-based products for bone regeneration.

To achieve widespread usage, cell-based products must be readily available at all levels of the healthcare system. This advance will require the development, validation and standardization of guidelines and protocols for the shipment and storage of cell therapeutics. Fortunately, the decades of experience obtained, and the advances made, by blood and transfusion centres serve as a foundation for the nascent MSC field. Nevertheless, multiple questions remain to be addressed. At present, no national or international infrastructure of facilities to manufacture and ship cells for skeletal regeneration to points of care exists. Few hospitals or universities are equipped with certified current GMP laboratories that are suitable for the isolation, expansion, characterization and processing of MSCs. Although private current GMP contract research organizations have emerged to fill this gap, their numbers and locations are limited. Furthermore, cells are being produced, stored and shipped under multiple environmental conditions, ranging from room temperature to −196° C (by use of liquid nitrogen); however, not all hospitals or outpatient surgical centres have the capability of storing cell products reliably at low temperatures. Owing to the fact that regulatory authorities and clinical research societies have not yet agreed on definitions for the quality assurance and control of MSC products, the distribution of skeletal regenerative cells across national borders is difficult. Such issues related to international standardization

remain an obstacle to the continued growth of the cell-therapy field. Another difficulty relates to obtaining regulatory approval from the FDA for combination products, such as cells combined with growth factors and other bioactive biomaterials (for example, osteoinductive scaffolds). Without appropriate precedence, the safety standards of these approaches remain undefined, a challenge compounded when one considers the huge costs associated with animal testing.

Conclusions

Stem-cell-mediated bone regeneration provides a number of potential therapeutic advantages to the use of autograft tissues. Currently, there is a large amount of preclinical and clinical data that support the delivery of stromal and stem cells to defect sites to enhance bone repair and regeneration. The successful clinical applications of MSCs and ASCs firmly establish proof-of-concept of the clinical feasibility of complex, multistage surgical processes, as well as the indications requiring minimally invasive approaches. The number of ongoing clinical trials with both MSCs and ASCs (Table 1) bodes well for the future of cell-based therapies. Therefore, what are the obstacles limiting the more extensive use of stromal cells and stem cells in the clinic and what steps need to be taken in order to bring ASC-mediated and MSC-mediated bone repair up to the standard of care currently reserved for autografts? In the near term, more prevalent therapeutic use of stromal and stem cells for skeletal regeneration does not require any major changes in methodology. The techniques for cell delivery involving hydrogel encapsulation in combination with a mineralized component have been well established over the past 30 years. The critical restriction at this stage is the development of internationally recognized, standardized regulatory guidelines that define the minimum safety criteria and, consequently, robust methods for cell expansion, storage and shipping that minimize or eliminate potentially harmful changes in the cells' genetic make-up whilst retaining their potency. The growing number of cell products on the market will facilitate movements in this direction.

However, in the longer term (that is, beyond the next 10 years), elevating stromal-cell-mediated and stem-cell-mediated bone regeneration to the standard of clinical care requires technological advances that maximize cell retention, viability, homing (for systemic delivery), vascular network formation, osteogenic differentiation capacity and tissue assembly properties. These advances require a fundamental understanding of the functioning of cells following transplantation. Put simply, are transplanted cells actively undergoing tissue morphogenesis and integration or are they merely mediators, emitting signals to recruit and stimulate their endogenous counterparts into action? The answer might be context-dependent and simple techniques, such as cell aggregation (reviewed elsewhere[130,131]) might profoundly influence the fate of cells after transplantation. Similarly, continued research into cell–biomaterial interactions (particularly *in vivo* interactions in immunocompetent animals) that spawn novel techniques for oxygen delivery[132,133] or growth-factor tethering, retention and presentation[111] might profoundly enhance regenerative outcomes. Most critically, systematic studies that deepen our level of understanding to an extent that we can model predicted regenerative outcomes on the basis of specific input parameters will ultimately facilitate the creation of customized therapies

that are founded on rational design and usher in a new standard of care in the field of regenerative medicine.

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Key points

- Stromal cells and/or stem cells can be isolated from different tissues on the basis of plastic adherence and surface-antigen profiles, thereby providing opportunities for bone regeneration
- The regenerative potential of therapies that are based on adipose-tissue-derived and bone-marrow-derived mesenchymal stromal or stem cells is being tested clinically for the treatment of craniofacial bone defects, tibial non-unions and osteonecrosis of the femoral head
- Although most approaches in this area use autologous cells, allogeneic sources that include commercially available allograft cell-based products are being investigated
- Widespread use of cell-based products requires the development and standardization of guidelines and protocols for the shipment and storage of cell therapeutics
- Despite strong clinical data, which indicates enhanced regenerative outcomes following stromal-cell or stem-cell transplantation, further insight is needed into the mechanisms of action of these strategies
- Opportunities exist to develop technologies that improve cell survival, morphogenesis and functionality to advance cell therapy as standard care for the treatment of bone defects

Review criteria

The articles selected for this Review were identified by searching PubMed, ISI Web of Knowledge and ClinicalTrials.gov. Search terms used included, but were not limited to: “stromal cells”, “stem cells”, “MSCs”, “ASCs”, “muscle-derived stem cells”, “pericytes”, “bone tissue engineering”, “oxygen”, “hypoxia”, “VEGF”, “BMP”, “HIF”, “angiogenesis”, “osteogenesis”, “craniofacial bone”, “non-union”, “osteonecrosis”, “spine” and “gene delivery”. Often, the names of renowned researchers within the field were combined with key words in Boolean searches. Articles spanning the late 1960s to the mid 1990s were used to write the brief history of the field. Known clinical trial reports were researched and articles from those reference lists were used to find other clinical trial reports. Major advances that continue to affect current paradigms were cited to describe the development of the field over time without considerations of publication date. A major focus of the article concerns developing trends and novel technologies. Hence, many of the studies cited were published within the past 5 years. In citing other review articles on specific topics, the most recent reviews were used. All cited articles were published in the English language.

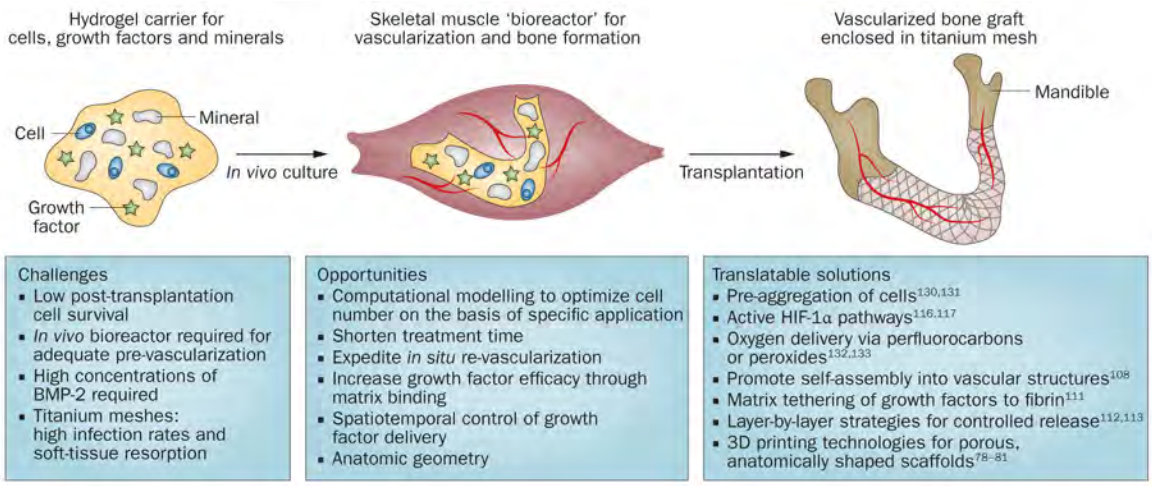

Figure 1.

Generalized clinical approach for stem-cell-based regeneration of large craniofacial bone defects. Adipose-tissue-derived stromal and/or stem cells are mixed with growth factors (such as BMP-2) and combined with mineral blocks in a preshaped titanium mesh, cultured *in vivo* and transplanted to repair the bone defect (the mandible is shown as an example). Abbreviations: BMP-2, bone morphogenetic protein 2; HIF-1α, hypoxia-inducible factor 1α.

Table 1

Clinical trials in which stromal cells were used for skeletal regeneration

Indication	Cell source	Cell processing and delivery	Clinical trial
MSC			
Non-union of bone	Autologous	Direct injection	NCT00512434,[134] NCT01206179,[135] NCT01429012,[136] NCT01788059[137]
		Implantation with carrier	NCT00250302,[138] NCT01435434,[139] NCT02177565,[140] NCT01626625,[141] NCT01842477,[142] NCT01725698,[143] NCT01958502[144]
ONFH	Autologous	Direct injection	NCT02065167,[145] NCT01700920,[146] NCT01544712[147]
		Implantation with carrier	NCT01605383[148]
Other (spine fusion, osteoarthritis)	Autologous	Direct injection	NCT01210950[149]
		Implantation with carrier	NCT01552707,[150] NCT01389661[151]
	Allogeneic	Direct injection	NCT01603836,[152] NCT02172885,[153] NCT00186914[154]
		Implantation with carrier	NCT00001391,[155] NCT01207193,[156] NCT00221130[157]
ASC			
Non-union of bone	Autologous	Implantation with carrier	NCT01532076[158]
	Allogeneic	Direct injection	NCT02140528[159]
ONFH	Autologous	Direct injection	NCT01643655[160]
Other (spine fusion, osteoarthritis)	Autologous	Direct injection	NCT01501461,[161] NCT01739504,[162] NCT01585857,[163] NCT01885819,[164] NCT02241408,[165] NCT01885832,[166] NCT02142842,[167] NCT01947348[168]
		Implantation with carrier	NCT01633892,[169] NCT01645722,[170] NCT01218945[171]

Abbreviations: ASC, adipose-tissue-derived stromal cell; MSC, bone-marrow-derived stromal cell; ONFH, osteonecrosis of the femoral head.

SPECIAL TOPIC

Fistula Incidence after Primary Cleft Palate Repair: A Systematic Review of the Literature

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Background: The development of an oronasal fistula after primary cleft palate repair has a wide variation reported in the literature. The aim of this review is to identify the *reported* oronasal fistula incidence to provide a benchmark for surgical practice.
Methods: A systematic review was undertaken to investigate the incidence of fistula. Multiple meta-analyses were performed to pool proportions of reported fistulae, in each data set corresponding to the continent of origin of the study, type of cleft, and techniques of cleft palate repair used.
Results: A total of 9294 patients were included from 44 studies. The overall incidence of reported fistula was 8.6 percent (95 percent CI, 6.4 to 11.1 percent). There was no significant difference in the fistula incidence corresponding to the continent of origin of each study or the repair technique used. The incidence of fistula in cleft lip–cleft palate was 17.9 percent, which was significantly higher (p = 0.03) than in cases of cleft palate alone (5.4 percent).
Conclusions: Palatal fistulae were more likely to occur in cases of combined cleft lip–cleft palate, compared with cleft palate alone. The authors would recommend the prospective examination and recording of all fistulae to a standardized classification scheme. (*Plast. Reconstr. Surg.* 134: 618e, 2014.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, III.

The risk of development of an oronasal fistula after primary cleft palate repair is well known. The literature reports a wide variation in incidence, ranging from 0 to 77.8 percent,[1,2] and the presence of a fistula is one of the important factors indicating the early outcomes of surgery.[3] Palatal fistulae are located in the secondary palate connecting the oral and nasal cavities,[4–6] including fistulae of the hard palate, the transition between hard and soft palates, and the soft palate.[5–9] However, other authors also record fistulae of the primary palate, including linguoalveolar and labioalveolar fistulae,[10–14] although this will include intentional, unrepaired fistulae. Some groups have attempted to standardize the nomenclature associated with palatal fistulae to reduce ambiguity in reporting.[14,15] Rather than the actual classification, it may be used to report small, or asymptomatic fistulae that do not require surgical intervention, which may be deficient in the literature. The continuity of care from birth to adulthood may not be the standard of care worldwide, and the long-term follow-up may reveal the late presentation of fistulae.[16] As such, the *true* incidence of oronasal fistula may be underreported in the literature.

Postoperative oronasal fistula occurs because of a failure of normal palatal wound healing after surgical repair.[11,17] It may be related to patient factors such as age at operation,[4] type and extent of cleft,[17] and associated syndromes.[18] Operative factors such as experience of the operating surgeon,[4,11] tension at the site of repair, bleeding, and infection[19] have also been implicated. Fistulae of significant sizes can lead to nasal air escape, difficulty with articulation, and nasal regurgitation of food, all of which may require repair. With a wide range of surgical techniques, schedules for palate repair, and multiple postoperative management plans, there is little consensus and uniformity worldwide in the approach to this

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problem. Current ongoing multicenter studies have set the objective to identify best practices.[20]

At present, there is little high-quality evidence available to determine the optimum conditions that prevent fistula formation. The aim of this review is to identify the *reported* oronasal fistula incidence in the literature, to interrogate the data for possible factors that may influence fistula development, and to provide a standard for surgical practice to be benchmarked against.

PATIENTS AND METHODS

Data Sources

We conducted a systematic literature review of publications in English of the following electronic databases: Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, MEDLINE, and Embase. The following key words were used: (cleft) AND (palate) AND (fistula) AND (repair OR palatoplasty). The date range was from January 1, 2000, to January 9, 2013.

Study Selection

Articles were included if they met the following criteria:

- Population: Human participants with nonsyndromic cleft palate who underwent primary cleft palate repair.
- Intervention: Case series, cohort studies, controlled trials, and randomized controlled trials of patients undergoing primary repair of cleft palate; all ages at intervention; all surgical repair techniques; prospective or retrospective data collection.
- Outcome: Number of reported oronasal fistulae as a proportion of the entire cohort. A fistula was defined as an unintentional connection between the oral and nasal mucosa of the secondary palate arising after primary cleft palate repair, which may be symptomatic or asymptomatic.

Study selection was performed through two levels of screening. In the first level, abstracts were reviewed for the exclusion criteria (summarized in Table 1).[1,2,5,9,13,17,21–57] In the second level screening, all articles filtered through the first level were read in their entirety and the same inclusion and exclusion criteria were applied. Only studies that successfully passed both levels of screening were included in our analysis. The present investigation is reported using the Meta-analysis Of Observational Studies in Epidemiology guidelines for metaanalyses of observational studies.[58]

Assessment of Methodologic Quality

The methodologic quality of randomized controlled trials was assessed using the Detsky scale.[59] The methodologic quality of nonrandomized studies was assessed using the Methodological Index for Nonrandomized Studies[60] instrument. We appraised each study and calculated a Detsky score from a maximum of 20, or a Methodological Index for Nonrandomized Studies score from a maximum of 16 for noncomparative studies and 24 for comparative studies. Studies that received at least 75 percent of the maximum Methodological Index for Nonrandomized Studies or Detsky score were considered to be high quality, which is consistent with previous research.[60–63]

Data Extraction

We recorded data using Microsoft Excel (Microsoft, Corp., Redmond, Wash.). The extracted data are summarized in Table 1. Articles were included if a subgroup of patients fulfilling the exclusion criteria (e.g., Pierre Robin sequence) could be extracted from the reported cohort. The minimum length of follow-up was recorded as in the study by Becker and Hansson.[24]

Data Synthesis and Analysis

We performed multiple meta-analyses to pool proportions of reported fistulae in each data set corresponding to the continent of origin of the study, type of cleft, and techniques of cleft palate repair used. We first transformed proportions by means of the Freeman-Tukey double arcsine method[64,65] and then calculated the pooled proportions as the back-transform of the weighted mean of the transformed proportions, using a random effects model with a 95 percent confidence interval: we tested the significance of heterogeneity between studies using the Cochran Q test[66] and selected the random effects model. We conducted a meta-regression based on a random effects logistic model for proportion of fistulae between each data set. Analysis of pooled proportions was performed and presented using StatsDirect (StatsDirect Ltd, Cheshire, United Kingdom), and IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, N.Y.) was used for meta-regression. The rest of the data were summarized and reported in a descriptive manner. The threshold considered for statistical significance was $p < 0.05$.

Table 1. Inclusion and Exclusion Criteria Applied to the Screened Articles and Data Selected for Extraction

	Inclusion Criteria	Exclusion Criteria	Data Extracted
Population	Patients undergoing primary cleft palate repair	Any secondary cleft surgery (e.g., fistula repair, revision palatoplasty)*	No. of patients
	Nonsyndromic cleft palate	Diagnosis of cleft palate as part of a sequence or syndrome*	
	All types of cleft palate	Other craniofacial clefts*	Type of cleft palate
	Human participants	Nonhuman studies	
Intervention	Randomized and nonrandomized studies; noncomparative studies; case series	Single case reports; review articles	Year of publication; continent of origin of population; type of study; years of study
	Unique cohort with oronasal fistulae previously unreported in the literature	Cohort with oronasal fistulae previously reported in the literature*	
	Study cohort of >20 patients	Study cohort of <20 patients	
	English language literature	Primary language other than English	
	All surgical repair techniques and schedules		Type of surgical repair technique; age at intervention
	Prospective or retrospective data collection	Mean length of follow-up <2 mo	Minimum length of follow-up
Outcome	Patients with unintentional postoperative oronasal fistulae	Intentional linguoalveolar or labioalveolar fistulae*	No. of patients with oronasal fistulae

*Article was included if this subset of patients could be excluded from the report.

RESULTS

Study Selection and Assessment of Methodologic Quality

The literature search identified 181 potential articles. After removal of duplicates and application of inclusion and exclusion criteria, 65 articles progressed to the second level of screening. After full review of each selected article, 44 were included in the final analysis (Fig. 1). There were five randomized controlled trials[35,37,42,48,55] and 39 nonrandomized studies,[1–3,5,9,13,17,21–34,36,38–41,43–47,49–54,56,57] of which 10 were comparative studies and 29 were noncomparative. The mean Detsky score for the randomized controlled trials was 14, with two studies considered to be of high quality.[48,55] The mean Methodological Index for Nonrandomized Studies score for noncomparative studies was 8, with two studies considered as high quality[23,38]; and the mean Methodological Index for Nonrandomized Studies score for comparative studies was 11, with none considered as high quality (Appendix).

Data Extraction

A total of 9294 patients were included from the selected studies. The continent of origin of the studies was Europe in 13 cases (n = 2943 patients), the Americas in 15 cases (n = 4688 patients), Asia in 13 cases (n = 1452 patients), and Africa in three cases (n = 211 patients) (Fig. 2). Although the year of publication was limited to January 1, 2000, onward, studies reported on patient cohorts undergoing primary cleft palate between 1954[33,34] and 2012.[24] In the majority of cases, cleft type was presented either descriptively (bilateral cleft lip–cleft palate, unilateral cleft lip–cleft palate, cleft of the palate in isolation, or cleft of the soft palate including submucous cleft palate) or using the Veau classification.[67] When reported, the type of cleft was unilateral cleft lip–cleft palate in 50.5 percent of cases, bilateral cleft lip–cleft palate in 15 percent, cleft of the palate in isolation in 18.7 percent, and cleft of the soft palate including submucous cleft palate in 15.8 percent. The age at which primary repair of the cleft palate was performed varied greatly, from 2 months[5] to 37.2 years.[36] Age at surgery was presented in multiple formats, including age range, mean age, median age, and modal age, and as such no valid comparisons can be made or conclusions drawn about the optimum time of intervention.

Palatal repair techniques were classified as bipedicled mucoperiosteal flaps (e.g., von Langenbeck repair), unipedicled mucoperiosteal flaps (e.g., Veau-Wardill-Kilner repair), vomerine flaps, or direct approximation (when no flaps or relaxing incisions were necessary). In some studies, multiple techniques were performed within the same cohort, which might have reflected surgeon preference or clinical indication. From the studies that recorded the technique of hard palate repair, 16 exclusively used unipedicled flaps and 14 exclusively used bipedicled flaps. A further five studies used both

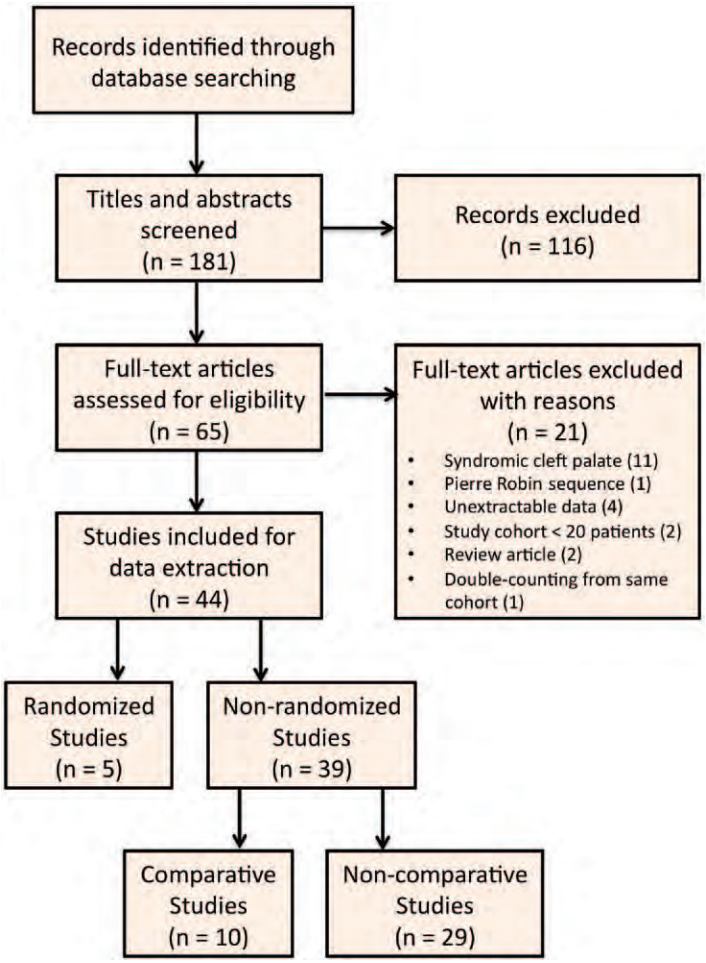

Fig. 1. Flow diagram depicting the search strategy for inclusion of articles in the systematic review.

unipedicled and bipedicled flaps with data that were presented so that they could be extracted from the overall cohort, resulting in 21 patient cohorts having unipedicled flaps (n = 3497 patients) and 19 patient cohorts having bipedicled flaps (n = 4241 patients).

The reported incidence of fistulae ranged from 0[1,31,46,53,56] to 35.5 percent,[5] and there was no significant correlation with study cohort size (Fig. 3). The fistula rate was recorded as those requiring surgical repair in 10 studies (n = 1636 patients) and in the remaining studies was either undefined or included prospective evaluation for postoperative fistulae. The minimum length of follow-up was recorded in 23 studies and ranged from 1 week to 72 months. The follow-up, when recorded, was presented in multiple formats (range, mean, and median) and thus the minimum length is shown to allow standardization.[24]

Data Synthesis and Analysis

Pooled estimates of the proportions of oronasal fistulae were calculated from the data set. The overall incidence of reported postoperative fistulae was 8.6 percent (95 percent CI, 6.4 to 11.1 percent) (Fig. 4). Separate data sets were created based on continent of origin of the study, type of cleft, and techniques of cleft palate repair used. The pooled proportion of fistulae was 9.9 percent (95 percent CI, 6.3 to 14.1 percent) from European studies, 7.3 percent (95 percent CI, 3.7 to 12 percent) from American studies, and 8.1 percent (95 percent CI, 4.1 to 13.3 percent) from Asian studies. There was no significant difference in the fistula incidence between these data sets.

The pooled proportions of fistulae from studies investigating cohorts of patients with cleft palate in isolation (Veau class 1 and 2; n = 1201 patients) was 5.4 percent (95 percent CI, 2 to 10.3 percent); and cohorts of combined cleft lip–cleft palate, with no patients with cleft palate in isolation (Veau class 3 and 4; n = 1556 patients), was 17.9 percent (95 percent CI, 11.6 to 25.3 percent). The incidence of fistula in Veau classes 3 and 4 was

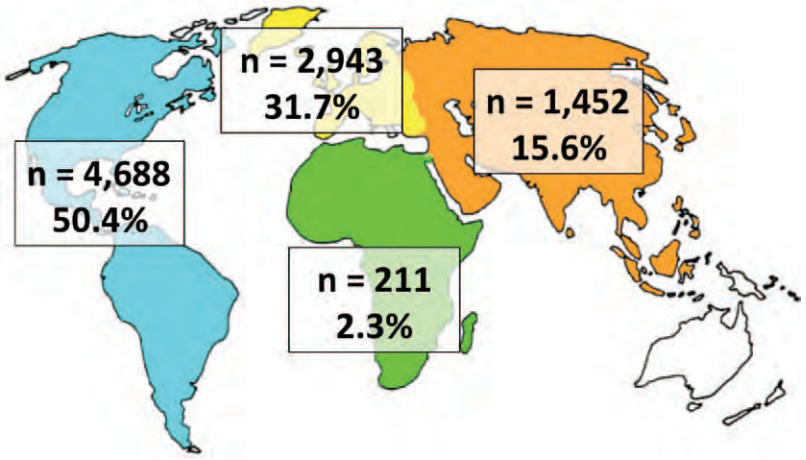

Fig. 2. Continent of origin of the included studies. The data are presented as total number of patients and as a proportion of the total cohort (percent).

significantly higher than in cases of Veau classes 1 and 2 ($p = 0.03$). The pooled proportion of fistulae from studies using a unipedicled flap technique for palate repair was 6.2 percent (95 percent CI, 4.3 to 8.4 percent), whereas in those studies using a bipedicled repair technique, the proportion was 10.2 percent (95 percent CI, 6.3 to 15 percent). Although there was an observed trend toward a lower incidence of fistula with the unipedicled techniques, this was not statistically significant.

DISCUSSION

This is, to our knowledge, the first systematic review of the worldwide literature to investigate the incidence of cleft palate fistulae. There was poor reporting of results, with only four studies overall (9.1 percent) considered to be of high quality, as assessed by validated instruments. Articles were selected with stringent inclusion and exclusion criteria: the exclusion of small cohorts of fewer than 20 patients was applied to exclude studies that may not have recorded a fistula by chance. This was based on recently reported fistula incidence, from two large cohort studies, of approximately 5 percent.[27,41] Patients with syndromic clefts were excluded because of small numbers and a reported high fistula incidence,[2,18] which could have skewed the overall results. Because up to 50 percent of patients diagnosed with Pierre Robin sequence may have other underlying syndromes,[51] these too were excluded. Because of the long study period in most reports (mean, 12.6 ± 9.7 years), parallel articles from the same cohort were excluded to remove any potential double counting. With the exclusion of articles published before 2000, an attempt was made to analyze contemporary practice, although a proportion of studies did include data from the previous century.

It must be acknowledged that the incidence of postoperative oronasal fistulae is based on the *reported* data, and most likely does not represent the true figure, as small or asymptomatic fistulae may not have been acknowledged. The incidence of the different types of cleft (e.g., cleft of the palate in isolation, unilateral cleft lip–cleft palate, or bilateral cleft lip–cleft palate) was similar to previously published data.[68] When the operative procedure was classified into either a unipedicled or bipedicled flap technique,

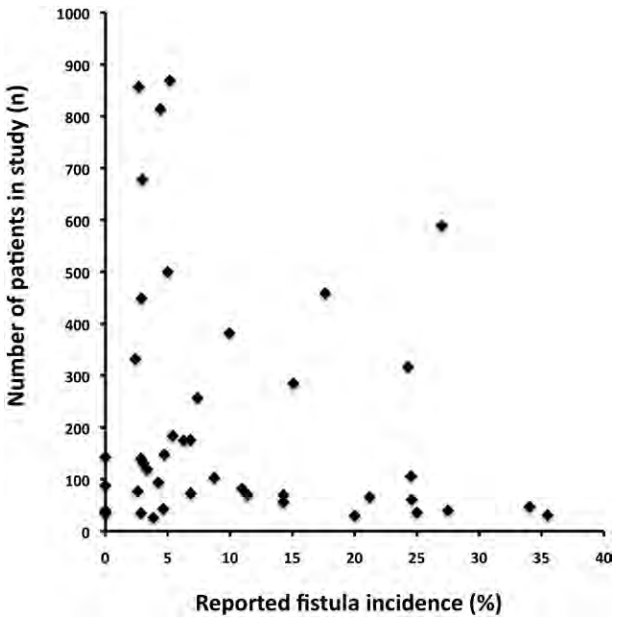

Fig. 3. Reported incidence of oronasal fistula for individual studies. The data are presented as the proportion of fistulae as part of the total cohort (percent) plotted against the number of patients in the cohort.

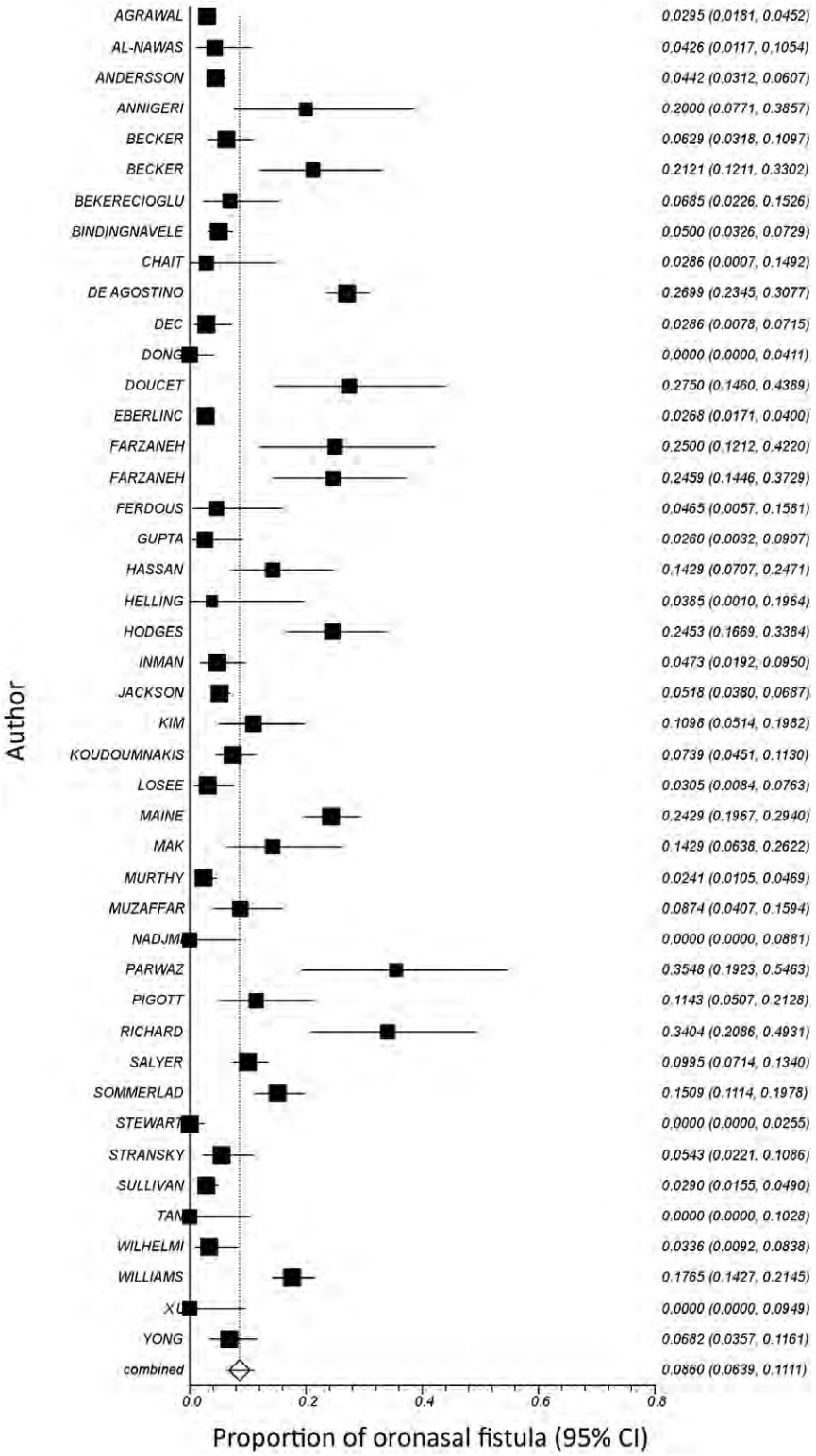

Fig. 4. Forest plot showing the proportion of reported oronasal fistulae after primary cleft palate repair (9294 patients in 44 studies). Individual studies and their results are given in the body of the figure; the summary statistic of the random effects model shows the incidence of oronasal fistula (0.0860) when all studies are combined in the meta-analysis model.

a comparison could be made, but no significant difference in fistula rate was recorded, although there was a trend for a lower fistula incidence with unipedicled flaps, in keeping with previous studies.[69] Although there is a reduced incidence of fistula formation, which may be related to decreased tension at the repair site, the larger denuded area of palate has been shown to lead to poorer outcomes in terms of maxillary growth and scarring.[70,71] We must acknowledge that the crude classification of techniques into unipedicled or bipedicled techniques does not take into account subtle variations within each method, such as the use of vomerine flaps,[72] or the extent of relaxing incisions.

The majority of study groups consisted of patients with highly variable clinical findings and surgical management plans; thus, no comment can be made on the optimum treatment schedule. Few studies were limited to a single operative technique, surgeon, or cleft type. The only significant influence of fistula occurrence was the cleft subtype and therefore, indirectly, the cleft width (although this was not specifically analyzed). This has been previously shown by Cohen et al.[11] in their study of 129 patients, and by Muzaffar et al. in their study of 103 patients.[17]

No significant difference in the incidence of fistula formation was noted between populations from Europe, the Americas, or Asia. The crude incidence in the Americas was 9.9 percent, which was significantly higher than the crude incidence in the European (6.6 percent) and Asian (6.3 percent) cohorts. Because of the variation of fistula incidence within the American cohort (the three highest fistulae rates being from South American studies[29,44,55]), when the random effects model was applied, this dropped to 6.9 percent and no longer was significant. The random effects model has the advantage of providing an estimation of the mean distribution of effects while still including effects from smaller studies, and not placing too much weight on large studies, which may be lost in a fixed effects model.[73] When the whole study cohort is examined, the crude fistula incidence (8.5 percent) closely resembles the incidence when the random effects model is applied (8.4 percent).

The process of meta-analysis can be criticized because of the inclusion of all relevant material: the good, the bad, and the indifferent.[74] Effects can be multivariate and data summary may not be homogenous. In an effort to combat this, stringent inclusion and exclusion criteria have been applied to provide the best account of the available data. The optimum treatment schedule or operative technique cannot be concluded from this study because of the heterogeneity of the reported results and as such was not an aim of this analysis.

CONCLUSIONS

We have found that the only statistically significant effect on nonintentional palatal fistula formation is the presence of a cleft of both lip and palate, when compared with clefts of the palate in isolation. We would recommend the following: prospective examination and recording of all fistulae to a standardized classification scheme[14]; and length of follow-up, recorded and presented as a range (minimum to maximum) with a calculated mean. Multicenter randomized controlled trials that focus on treatment schedule and operative technique are required to optimize the management of cleft lip and palate.

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APPENDIX. OUTCOME MEASURES AND QUALITY (MINORS AND DETSKY) SCORES FOR EACH STUDY IN THE META-ANALYSIS

Reference	Quality Assessment	Quality Score	Quality Rating
Agrawal and Panda, 2006[21]	MINORS	2/16	Low
Al-Nawas et al., 2013[22]	MINORS	12/24	Low
Andersson et al., 2008[9]	MINORS	2/16	Low
Annigeri et al., 2012[23]	MINORS	12/16	High
Becker et al., 2013[24]	MINORS	10/16	Low
Becker et al., 2000[25]	MINORS	9/24	Low
Bekerecioglu et al., 2005[26]	MINORS	8/16	Low
Bindingnavele et al., 2008[27]	MINORS	8/24	Low
Chait et al., 2002[28]	MINORS	2/16	Low
de Agostino et al., 2014[29]	MINORS	4/16	Low
Dec et al., 2013[30]	MINORS	4/16	Low
Dong et al., 2012[31]	MINORS	14/24	Low
Doucet et al., 2013[32]	MINORS	12/24	Low
Eberlinc and Kozelj, 2012[3]	MINORS	10/16	Low
Farzaneh et al., 2009[33]	MINORS	15/24	Low
Farzaneh et al., 2008[34]	MINORS	14/24	Low
Ferdous et al., 2010[35]	Detsky	13/20	Low
Gupta et al., 2011[36]	MINORS	7/16	Low
Hassan and Askar, 2007[37]	Detsky	12/20	Low
Helling et al., 2006[38]	MINORS	12/16	High
Hodges, 2010[39]	MINORS	10/16	Low
Inman et al., 2005[40]	MINORS	11/16	Low
Jackson et al., 2013[41]	MINORS	10/16	Low
Kim et al., 2009[42]	Detsky	14/20	Low
Koudoumnakis et al., 2012[43]	MINORS	8/16	Low
Losee et al., 2008[44]	MINORS	10/16	Low
Maine et al., 2012[45]	MINORS	10/24	Low
Mak et al., 2006[2]	MINORS	8/16	Low
Murthy et al., 2009[13]	MINORS	8/16	Low
Muzaffar et al., 2001[17]	MINORS	8/16	Low
Nadjmi et al., 2013[46]	MINORS	10/24	Low
Parwaz et al., 2009[5]	MINORS	10/16	Low
Pigott et al., 2002[47]	MINORS	10/24	Low
Richard et al., 2006[48]	Detsky	16/20	High
Salyer et al., 2006[49]	MINORS	8/16	Low
Sommerlad, 2003[50]	MINORS	6/16	Low
Stewart et al., 2009[1]	MINORS	9/16	Low
Stransky et al., 2013[51]	MINORS	8/16	Low
Sullivan et al., 2009[52]	MINORS	6/16	Low
Tan et al., 2012[53]	MINORS	10/16	Low
Wilhelmi et al., 2001[54]	MINORS	10/16	Low
Williams et al., 2011[55]	Detsky	15/20	High
Xu et al., 2007[56]	MINORS	6/16	Low
Yong et al., 2010[57]	MINORS	10/16	Low

MINORS, Methodological Index for Nonrandomized Studies.

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Gingivoperiosteoplasty

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KEYWORDS

• Gingivoperiosteoplasty • Nasoalveolar molding • Alveolar cleft • POPLA

KEY POINTS

- Gingivoperiosteoplasty (GPP) replaces alveolar cleft soft tissue with a gingivoperiosteal tunnel that facilitates bone healing without the need for bone grafting.
- Skoog's descriptions of "boneless bone grafting" popularized the technique in cleft care.
- The reported negative impact of the Latham device on dentofacial growth has led to the development of nasoalveolar molding (NAM), a passive, noninvasive, molding process.
- NAM-GPP prerequisites include appropriate cleft anatomy to allow alveolar bony approximation.
- GPP seals the cleft nasally, orally, and labially by mucoperiosteal flaps.
- GPP advocates report the elimination of secondary alveolar bone grafting at mixed dentition as a benefit.
- Critics voice concerns over iatrogenic facial growth restriction and malocclusion.
- Data available on NAM-GPP (with appropriate patient selection and technique) are favorable but long-term outcome studies are required.

Gingivoperiosteoplasty (GPP) removes the soft tissue barrier within an alveolar cleft and replaces it with a gingivoperiosteal tunnel that facilitates bone healing through guided tissue regeneration (GTR) without the need for bone grafting and its associated donor site morbidity. The importance of the periosteum in bony healing has been well documented by several investigators[1,2]; however, Ollier[3] is most often credited with first emphasizing the osteogenic potential of the periosteum. This is especially true for patients at a younger age.[4] Dahlin and colleagues[5] were first to demonstrate union of critical bony defects by creating a Teflon tunnel to guide bone regeneration while impairing soft tissue in-growth and fibrous nonunion. Although the osteogenic properties of mucoperiosteum in healing a cleft palate were initially recognized by Langenbeck[6] in the 1800s, it was not until Tord Skoog's[7] descriptions of primary GPP or "boneless bone grafting" in the 1960s that the technique became popularized in cleft care. Successful GTR following a GPP depends on the integrity of the guiding tunnel to restrict fibrous in-growth, the presence of viable periosteum in the created flaps, and the age of the patient.

HISTORICAL PERSPECTIVE

Skoog[8] described the creation of local mucoperiosteal flaps with oxidized regenerated cellulose. Though not every patient grew bone initially, with repeated periosteal flaps, all patients eventually formed a bony bridge. Without presurgical molding, this method required extensive, often repeated, subperiosteal maxillary dissection to close the alveolar cleft, with an associated negative impact on facial growth. In the 1970s and 1980s, Ritsilä and colleagues,[9] and Rintala and Ranta,[10] reported the outcomes of free tibial periosteal grafts. During a 6-year period, they treated

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67 patients with Skoog's maxillary periosteal flap technique and 23 with free periosteal grafts. Although bony formation was noted in 64% of patients treated with the Skoog technique and 85% of those treated with the tibial periosteum graft technique, secondary bone grafts were required in 72% and 73% of each group, respectively. There was a lateral cross-bite in all patients. A similar finding with the Skoog technique was reported by Renkielska and colleagues.[11] They found 50% of patients had Goslon scores of 4 or 5 with need for orthognathic surgery and the technique fell out of favor.

The popularization of presurgical orthopedics ushered in the next phase in GPP care. Millard's presurgical orthopedics, periosteoplasty, and lip adhesion (POPLA) approach involved presurgical orthopedics with the "Latham device" followed by lip adhesion and GPP at 3 to 4 months of age.[12] Active mechanical presurgical approximation of the alveolar edges allowed for less subperiosteal dissection, but critics of the POPLA approach note a 40% to 42% maxillary vertical growth disturbance of the maxilla[13] and 100% of patients with cross-bites that were often difficult to correct orthodontically.[14]

NAM-GPP

The potential negative impact of the active direct force of the Latham device on dentofacial growth led to the development of a more passive molding process, nasoalveolar molding (NAM), as described by Grayson and colleagues.[15] NAM is described as guiding early alveolar growth as opposed to directly molding it. NAM-GPP differs from POPLA in the method of presurgical alveolar molding and surgical technique as well as in more rigorous selection criteria by which patients are deemed appropriate candidates for a GPP. This is partly because its advocates think NAM is a more accurate means of molding the leading edges of the alveolar cleft into a close parallel relationship to optimize flap design and osteogenesis.

PATIENT SELECTION AND EVALUATION

Prerequisites for a GPP are: (1) an informed consenting family, (2) appropriate cleft anatomy to allow alveolar bony approximation, (3) an optimally molded alveolar cleft and intact mucosa, and (4) no dental eruption. Once these criteria are met, the GPP can be scheduled at the time of the primary lip repair.

Before undergoing a GPP, the infant with a cleft must be evaluated by the practitioner administering the NAM, as well as the surgeon who will be performing the GPP. Before initiating NAM, the guardians of the infant should be introduced to the concept of a GPP, the risks and benefits reported in earlier GPP techniques, and the current data on NAM-GPP.

Variations in cleft anatomy may exclude some patients from NAM-GPP. For example, patients with isolated clefts of the primary palate are usually not good candidates for NAM-GPP. Due to the bony fusion of the secondary palate, the alveolar segments of the primary palate are more resistant to parallel presurgical molding and, in many cases, cannot be adequately aligned for a successful GPP. Another group of patients who are not candidates for a NAM-GPP are the "mesenchymal deficient" infants, with such wide unilateral clefts that the alveolar arch form would be excessively constricted should the segments be presurgically approximated. Finally, in bilateral complete clefts, it is not always possible to align both sides of the premaxilla with the lateral alveolar segments to allow for bilateral GPP. In these cases, the one aligned alveolar cleft can undergo a GPP to convert the arch form to a lesser and greater segment instead of a three-piece upper jaw. Although the contralateral cleft will need to be secondarily grafted, the premaxilla will be stabilized by the GPP to facilitate incisor mastication during early childhood.

PREOPERATIVE MANAGEMENT

During the preoperative management (NAM) process, the preoperative assessment of alveolar ridge alignment and parallel alveolar molding is subjective. Alveolar segment alignment is assessed based on the underlying bone, not on the visible gingival mucosa (**Fig. 1**). In some cases, the gingival tissue can be hypertrophied in the area of the cleft, mimicking close approximation, while the underlying bone gap is wide. In other cases, the alveolar cleft is compressed, but the bony arch forms are not in alignment, with the premaxilla wedged anterior to collapsed lesser segments or the lesser segment posterior to the greater (**Fig. 2**). If a GPP is performed in these situations, the mucoperiosteal tunnel between the exposed bone edges will be "kinked," creating a soft tissue barrier instead of GTR.

PERFORMING GPP

It is technically easiest to perform the GPP after all the primary cleft lip dissection had been completed but before repair of the lip elements is started. To understand GPP flap design, the alveolar cleft should be visualized as a pyramid on its

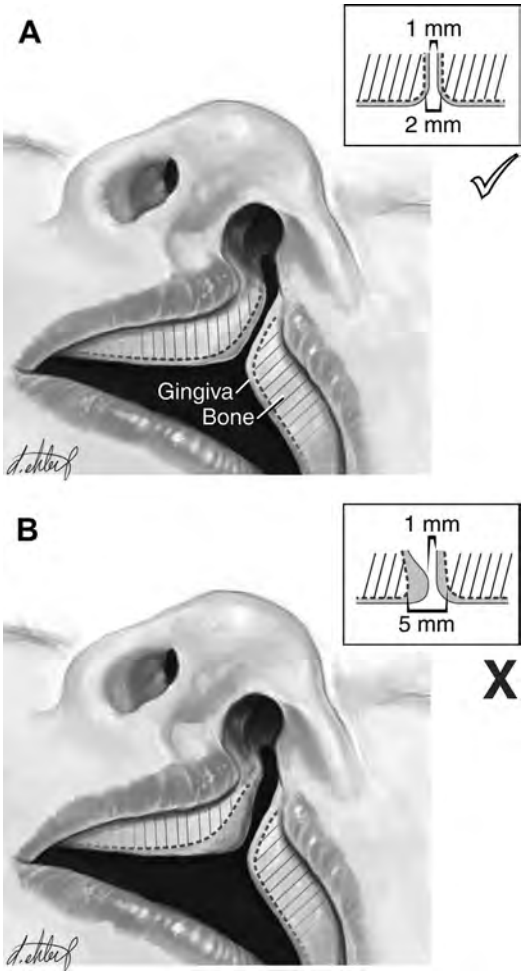

Fig. 1. (*A*) An appropriately molded unilateral complete cleft for GPP. There is parallel alignment the alveolar cleft edges with a 2 mm bone gap. (*B*) A molded unilateral complete cleft with gingival hypertrophy masking a deceptively wider cleft. The hypertrophy creates a nonparallel soft tissue gap of 1 mm but the true bony gap is 5 mm. This cleft would not be an appropriate candidate for a GPP. (*From* Losee J, Kirschner R. Comprehensive cleft care. New York: McGraw-Hill; 2008. p. 832; with permission.)

side, with a labial base and an apex located at the incisive foramen where the cephalad and caudad incisions converge (**Fig. 3**).

GPP is performed as follows:

- The goal is to seal the cleft nasally, orally, and labially by mucoperiosteal flaps, with two opposing bone surfaces on the mesial and distal walls.
- To promote GTR in this sealed tunnel, all soft tissue interference must be removed from the alveolar cleft during creation of the flaps.

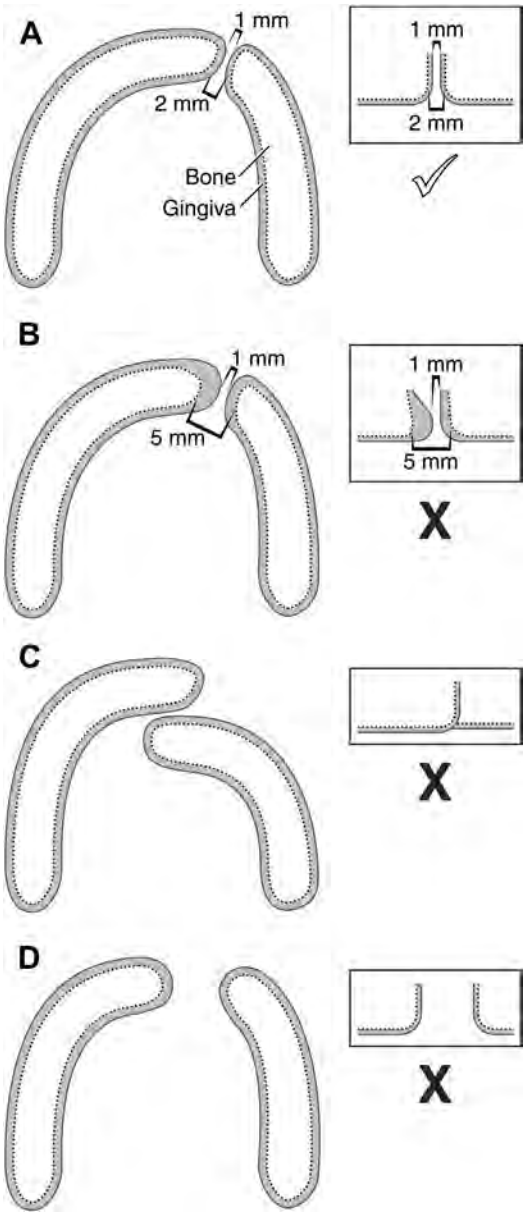

Fig. 2. (*A*) An appropriately molded unilateral complete cleft for GPP. There is parallel alignment the alveolar cleft edges with a smooth arch form. (*B*) Gingival hypertrophy in the alveolar cleft can mask a bone gap that is too wide for a GPP. (*C*) A collapsed arch form is not a candidate for GPP. Although the alveolar segments are touching, the edges of the cleft are not opposing, preventing the formation of a subperiosteal tunnel. (*D*) A mesenchymal deficient cleft arch form. If this cleft was approximated by preoperative molding, it would unnaturally constrict the projection of the alveolar arch due to a deficiency in either the lesser or greater segments. (*From* Losee J, Kirschner R. Comprehensive cleft care. New York: McGraw-Hill; 2008. p. 832; with permission.)

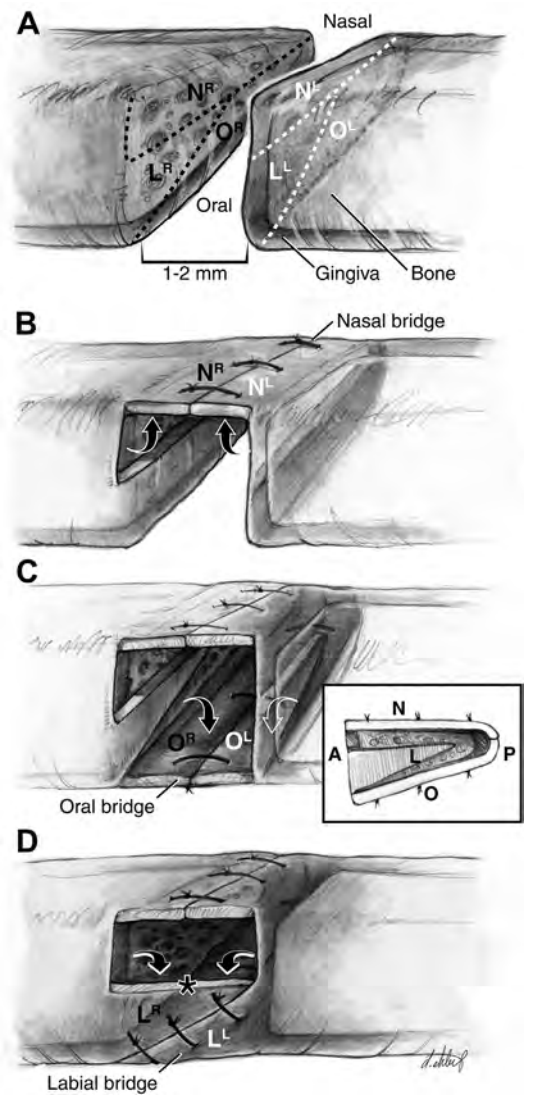

Fig. 3. (*A–D*) Gingivoperiosteal flap design and elevation for the Millard GPP. The dissection is limited to the tissues within the cleft. The flaps are named by the part of the periosteal tunnel they construct. A, anterior; L^L, left labial flap; L^R, right labial flap; N^L, left nasal flap; N^R, right nasal flap; O^L, left oral flap; O^R, right oral flap; P, posterior; *, Marks the mucosal edge repaired to the lip mucosa. (*From* Losee J, Kirschner R. Comprehensive cleft care. New York: McGraw-Hill; 2008. p. 833; with permission.)

- Occasionally, a deciduous tooth follicle is encountered during the flap dissection and careful sharp dissection is required to prevent disruption of dental eruption. If the flaps appear too thin or nonviable during the follicle dissection, the GPP should be aborted and the mucosa replaced.
- The "roof" of the GPP is the repair of the anterior palate, or the nasal floor, from the nasal sill back to the incisive foramen, which is typically done with most modern cleft lip repairs. This is typically achieved by suturing the inferior edge of the reconstructed lateral nasal wall to a superiorly based mucoperiosteal vomer flap.
- The vomer flap vertical dissection is kept to the bare minimum needed to achieve closure of the nasal floor, typically 1 to 2 mm at most, to avoid inadvertent damage to the premaxillary growth suture.
- The floor of the GPP is created by elevating inferiorly based mucoperiosteal flaps from the oral edges of the alveolar cleft. These flaps are raised from within the alveolar cleft itself starting from the labial surface of the alveolus back to the incisive foramen.
- The floor flaps are inferiorly rotated and sutured to each other, effectively closing the floor of the GPP.
- The mucoperiosteum that remains attached within the alveolar cleft between the cephalad roof incision and the caudad floor incision is the tissue used to close the labial (anterior) border of the GPP.
- An anteriorly based triangular flap, raised on either side of the alveolar cleft, is flipped labially and sutured to the contralateral flap.
- In designing these particular flaps, the incisions on one side of the alveolar cleft are shifted slightly superiorly relative to the other side, so that one flap covers the upper half of the anterior labial base of the cleft pyramid, while the contralateral flap covers the lower half of the base.
- To completely close off the anterior border of the GPP tunnel, the inferior edge of the lower labial flap is sutured to the anterior edge of the two oral flaps, and the superior edge of the upper labial flap is sutured to the lip mucosa.

POSTOPERATIVE CARE

The postoperative care of the GPP patient is no different than that of the cleft lip patient. The infant is resumed on normal feeding with no need for arm restraints. Postoperative flap loss may be encountered if flaps are inaccurately designed, traumatized by compressive handling, or elevated in a thin submucosal (rather than subperiosteal) plane.

TRENDS AND CONTROVERSIES

Although the international cleft community agrees that the primary goal of cleft care is to optimize function, appearance, and self-image with a minimum of surgical intervention, the community

continues to debate on how best to attain it. One of the most controversial topics of debate in current cleft care is the indication for primary GPP. Advocates of GPP report the elimination of secondary alveolar bone grafting at the time of mixed dentition, hence minimizing the number of secondary surgeries (and associated morbidity and cost) by optimizing results of primary cleft lip surgery. However, GPP critics voice concerns about iatrogenic facial growth restriction and subsequent malocclusion due to early closure of the alveolar arch. Such restriction could actually increase the need for orthodontic and orthognathic treatment at time of skeletal maturity. The history and evolution of GPP, from Skoog, to Rintala, to Millard, teaches us that it can take up to 20 years before the risks and benefits of a surgical technique, performed on an infant with a cleft, can be fully appreciated. As more centers outside of the originating institution practice the technique, preliminary objective evaluation of NAM-GPP is starting to be available to the cleft care community and, it is hoped, definitive results will soon be available to define the appropriate place of the technique in the arsenal of cleft care.

Compared with studies on earlier variations of GPP, the current data on the benefits of NAM-GPP are so far more favorable. The earliest data became available through the continued work of Grayson,[15] Cutting, and Brecht from New York University (NYU), the developers of the NAM-GPP technique. In addition to their studies on the improved nasal morphology associated with the NAM technique, they have also evaluated their outcomes to date with the NAM-GPP. They have reported bone formation in 80% of unilateral clefts treated with a primary NAM-GPP, with 40% requiring secondary bone grafting (**Fig. 4**).[16] More recently, the Chang Gung Memorial Hospital group reported similar results with a 28% rate of secondary alveolar bone grafting.[17] The also critically evaluated bone quality, noting least bone formation on the palatal apical portion of the previous alveolar cleft following GPP.[17]

For bilateral clefts, unilateral primary GPP is associated with a 63% success rate; however, bilateral primary GPP has a dependent probability of 52% and conditional probability of 82% of bilateral successful dentoalveolar bone formation.[18] Bilateral GPP is recommended by the NYU group as "it results in greater stability of the premaxilla and functional occlusion of the central incisors. Furthermore, secondary bone grafting when required after GPP is technically easier with the presence of bone along the occlusal surface of the dentoalveolar ridge."[18] In support of that argument, the group demonstrated superior radiographic bone levels and alveolar anatomy in patients undergoing GPP alone or combined with secondary alveolar bone grafting as compared with conventional alveolar bone grafting alone.[19] "Failed GPP" that require secondary bone grafting have more favorable outcomes than non-GPP secondary grafting cases at time of mixed dentition, presumably due to a bone bridge formation in 80% of GPP cases (**Fig. 5**).

In a cost comparison between the nonmolded, secondary bone graft, control group and the GPP group, a savings of $2999 was seen when a primary GPP was performed.[20] This cost analysis,

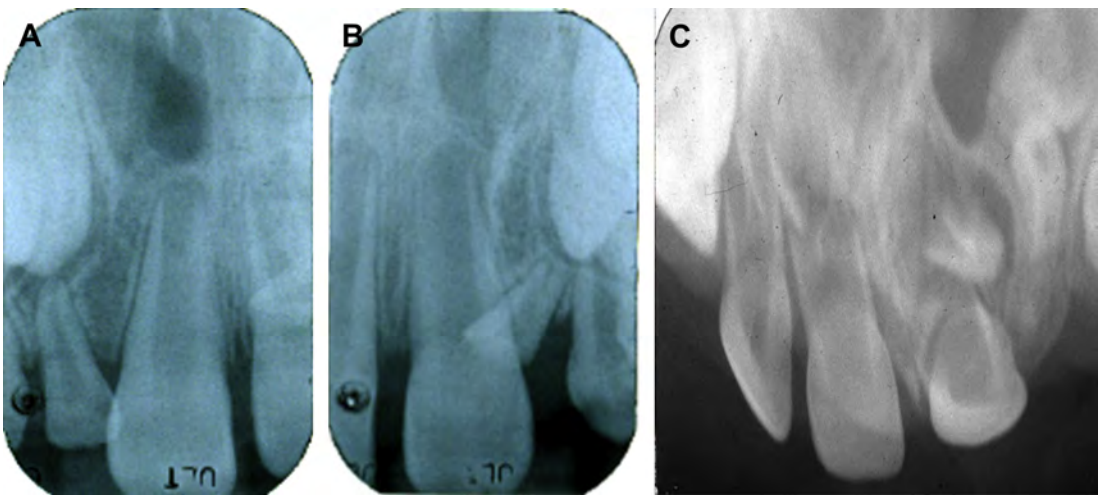

Fig. 4. (*A–C*) Periapical radiographs of a bilateral cleft lip and palate patient in mixed dentition with a successful previous bilateral GPP. There is adequate vertical bone height within the alveolar cleft to support tooth eruption. (*Courtesy of* Barry Grayson, DDS, New York University Medical Center.)

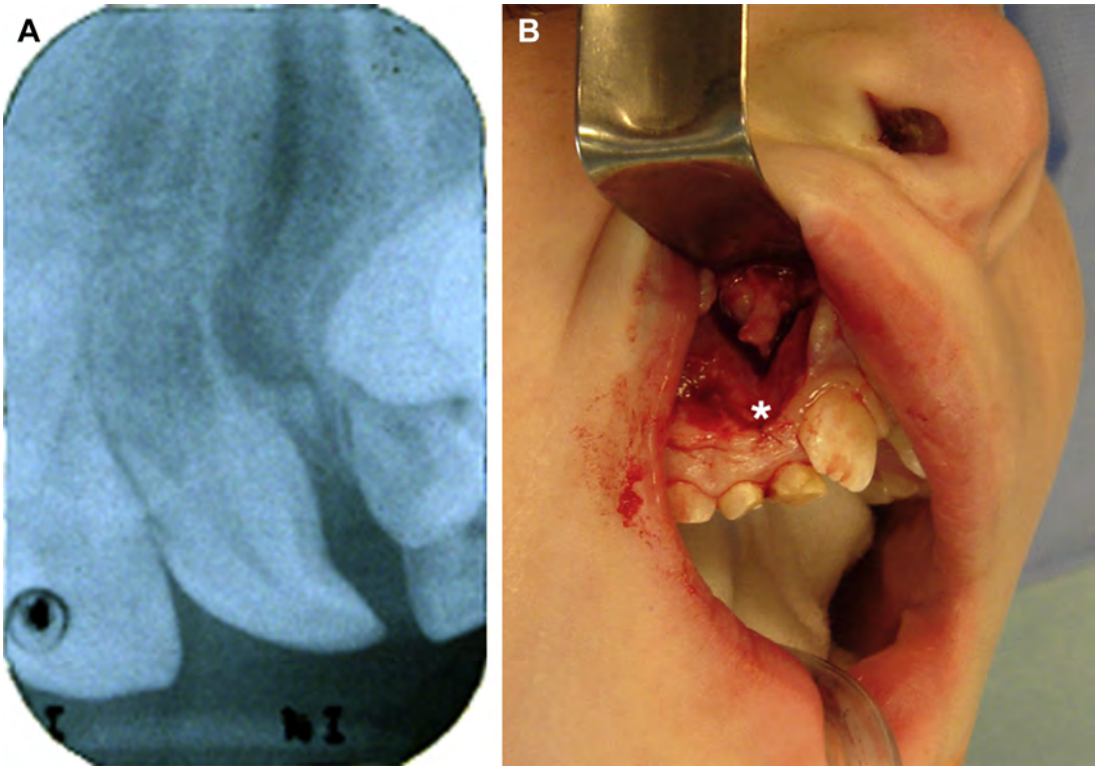

Fig. 5. A right unilateral alveolar cleft following a "failed GPP." A periapical radiograph at mixed dentition (*A*). There is formation of a bone bridge across the cleft but insufficient vertical height to support tooth eruption. At the time of secondary bone grafting, the bone bridge formation (*asterisk*) from the previous GPP provides a stable platform for the graft augmentation (*B*). (*Courtesy of* Barry Grayson, DDS, New York University Medical Center.)

however, did not take into account the potential added cost of increased orthodontic and orthognathic treatments in patients undergoing GPP. Another potential advantage of GPP is early closure of fistula. However, studies have reported fistula rates as high as 36% with GPP.[21]

In evaluating facial growth, the NYU group found no adverse effects on midface growth during mixed dentition,[22] or just before pubertal growth spurt,[23] when compared with an unmolded control group. However, Hsieh and colleagues[24] demonstrated a 2.1 and 2.9 mm deficiency in sagittal maxillary and maxillary alveolar lengths with GPP at the age of 5 years. Other investigators have also reported GPP as a stronger predictor of poor dental arch relationship outcome (Goslon 5) than initial cleft size regardless of type of presurgical orthopedics (odds ratios 2.8 and 1.3, respectively).[25] Whether delaying GPP until the time of palatal surgery improves maxillary outcome is yet to be seen.[26] Interestingly, salvage bone grafting after a failed GPP can improve vertical maxillary height compared with successful GPP. This may be due to improvement in tooth eruption at the GPP site.[27] GPP may also result in compensatory mandibular growth disruption with decreased anteroposterior measurements. Although not studied with NAM, this has been demonstrated with patients with bilateral clefts undergoing alveolar molding with the Latham device before GPP[21] and patients undergoing primary bone grafting of unilateral clefts.[28,29]

Although early results may confirm the benefits of NAM-GPP, the effects of the technique on facial growth through skeletal maturity remain unclear. Rodent models of GPP are now available to facilitate bench-side investigations of methods to improve osteogenesis in alveolar defects while limiting adverse effects on maxillary growth.[30,31] The role of recombinant human bone morphogenic protein-2 in primary alveolar reconstruction is also under investigation.[32,33]

SUMMARY

GPP plays a central and controversial part in the cleft surgeon's mission to provide the best results in the least number of surgeries. Although it may normalize form and function at infancy and obviate secondary alveolar bone grafting at the age of

mixed dentition, the technique has historically been associated with iatrogenic dentofacial restriction requiring more extensive orthodontic and orthognathic treatment at the age of skeletal maturity. Compared with previous protocols, the most recent evolution of the GPP technique associated with NAM uses passive, guided, presurgical molding of the alveolar cleft with strict patient selection criteria. At this time, the data available of the benefits of NAM-GPP are favorable but further long-term outcome studies are required before its final role in cleft care can be determined.

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Review Article

Wharton's Jelly Derived Mesenchymal Stem Cells: Future of Regenerative Medicine? Recent Findings and Clinical Significance

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Around 5 million annual births in EU and 131 million worldwide give a unique opportunity to collect lifesaving Wharton's jelly derived mesenchymal stem cells (WJ-MSC). Evidences that these cells possess therapeutic properties are constantly accumulating. Collection of WJ-MSC is done at the time of delivery and it is easy and devoid of side effects associated with collection of adult stem cells from bone marrow or adipose tissue. Likewise, their rate of proliferation, immune privileged status, lack of ethical concerns, nontumorigenic properties make them ideal for both autologous and allogeneic use in regenerative medicine applications. This review provides an outline of the recent findings related to WJ-MSC therapeutic effects and possible advantage they possess over MSC from other sources. Results of first clinical trials conducted to treat immune disorders are highlighted.

1. Introduction

Interest in mesenchymal stem cells has been kindled in 1960s as the result of Friedenstein's observations who reported that the bone marrow stroma can generate bone [1]. It was later shown that bone marrow stromal cells have chondrogenic and adipogenic properties and a high ability for self-renewal [2]. Even though there is debate on the technical name (mesenchymal or multipotent stem cells), there is an agreement to the acronym "MSC". Since their original description, presence of MSC has been proven in many adult and embryonic tissues such as adipose tissue [3], muscle [4], peripheral blood [5], lung [6], heart [7], corneal stroma [8], dental pulp [9], placenta [10], endometrium [11], amniotic membrane [12], and Wharton's jelly [13]. MSC have the capability to differentiate into wide range of specialized cells of mesodermal origin: bone cells, cartilage, fat, cardiomyocytes, muscle fibers, renal tubular cells, and break germ layer commitment and differentiate into cells of ectodermal origin, for example, neurons, and endodermal origin, such as hepatocytes and pancreatic islets cells. Due to the above properties, MSC are considered as a new emerging treatment option and therapeutic agent in regenerative medicine. MSC therapeutic potential can be executed by direct replacement of injured tissue cells or by paracrine effect on surrounding environment, indirectly supporting revascularisation, protecting tissue from stress-induced apoptosis, and appropriately modulating inflammatory reaction. Results of MSC-based cell therapies are very promising in various clinical fields based on *in vitro* and *in vivo* research results and more than 400 clinical trials registered.

2. Are All MSC Phenotypically and Functionally Equivalent? Age Does Matter

The lifelong perseverance of adult MSC in the body makes them particularly susceptible to the accumulation of cellular

damage, which can lead to cell death, senescence, or loss of regenerative function and in extreme cases to neoplastic transformation. In contrast, neonatal MSC such as Wharton's jelly derived MSC, in their short, prenatal life are spared from proaging factors. Decreased repair capacity and increased susceptibility to degenerative diseases may stem from the fact that the function of stem cells declines with age. The e is increasing evidence that the age of the donor tissue affects several properties of mesenchymal stem cells [14–16]. By means of single cell transcriptional analysis, it was shown that aged adipose tissue derived MSC (ADSC) are signific ntly compromised in their ability to support the vascular network formation and are unable to rescue age-associated impairments in cutaneous wound healing [17]. Further, bone marrow derived MSC have lesser myogenic potential and engraftment properties than developmentally early MSC [18]. As recently shown, one of the mechanisms implicated in MSC aging involves Akt/mTOR pathway and its inhibition prevents the development of age-related phenotype and maintains MSC morphology, self-renewal, and differentiation capacity [19]. Further studies demonstrate that the expression levels of inflammatory response genes change with age and that the age-dependent decrease in expression of several cytokine and chemokine receptors is important for the migration and activation of BMSC. By adoptive transfer of aged BMSC to young endotoxemic mice, authors showed that aged cells lacked the anti-inflammatory, protective eff ct of their young counterparts, which indicate that BMSC undergo an age-related decline in their immunomodulatory activity [20].

A growing body of evidence suggests that elevated activity of certain proteins can have benefici l effects on aging and aging-related diseases. Among them is SITR1, NAD+-dependent protein deacetylase, which is downregulated in rodent bone marrow derived MSC with aging [21] or human MSC with increasing passages [22]. It is also shown that expression of genes related to senescence such as CHEK1, $p16^{ink4a}$ increases in ADSC with age where at the same time proapoptotic regulators levels, ATR, TNFα, and NFκB decreased [23]. Aging alters the availability of $CD45^{-}/CD34^{+}/CD133^{+}$ ADSC and their angiogenic properties [24].

Healthspan of mesenchymal stem cells also depends on maintaining physiological level of reactive oxygen species (ROS). However, during lifetime and exposure to environmental stress, ROS levels can increase dramatically. This may result in significant damage to cell structures and promote MSC aging. In support of the hypotheses, increased levels of ROS have been reported in aging BMSC [14]. Furthermore, exposure of adult ADSC isolated from old rat donors to H_2O_2 resulted in decreased expression of integrin and reduced phosphorylation of focal adhesion kinase Src and FAK. In consequence, intramyocardial transplantation of aged ADSC into acute myocardial infarction model rats resulted in a decreased survival rate of old MSC in the infarct region. Th authors conclude that the old ADSC are more sensitive to the microenvironmental ROS and their therapeutic effectiveness is impaired [25]. In another study, in a rat myocardial infarction model, authors evaluated regenerative capacity of human MSC derived from young versus older patients (1–5 versus 50–70 years old). "Young" MSC outperformed "older" MSC in cardiac parameters: ejection fraction, fractional shortening, and left ventricular end-diastolic and end-systolic volumes. Increase in vascular density and decrease in metalloproteinases levels and activity were observed in recipients of "young" BMSC [26]. Similarly, MSC obtained from young individuals have been induced to undergo neuroectodermal differentiation *in vitro*, but this effect could not be reproduced in BMSC from elderly individuals [27]. Thi proves adult BMSC unsuitable for successful cell replacement strategies for neurologic diseases in elderly patients in autologous setting.

During normal aging cells divide and telomeres that are essential to maintain the stability of genomes shorten. Even though MSC in their niche are relatively quiescent, adult MSC during their lifespan undergo signific ntly more divisions shortening their telomeres than neonatal cells. Thus, in comparison to MSC from adult tissues, WJ-MSC at such an early embryonic state retain telomere at highest possible length, which protects them from premature loss of viability.

A very important issue, which does not apply to Wharton's jelly derived MSC, is an exposure of adult MSC during the lifetime to intrinsic (e.g., infl mmatory mediators) and extrinsic factors, for example, nonsteroidal anti-infl mmatory drugs (NSAIDs) commonly used in patients to treat inflammation, pain, and fever. These factors may greatly infle t MSC viability or plasticity. Effects of NSAIDs on the MSC potential for proliferation and differentiation towards the osteogenic and chondrogenic lineages were investigated [28]. It was shown that type X collagen, a marker of late stage chondrocyte hypertrophy, is constitutively expressed by mesenchymal stem cells (MSC) from osteoarthritis patients treated with NSAID, Naproxen [29]. Similarly, osteogenic diff rentiation of MSC was aff cted, and downregulation of mineral deposition in the extracellular matrix was observed [30]. The e results contradicted previous findi gs, demonstrating no effect of several types of NSAIDs on osteogenic differentiation. However, *in vitro* chondrogenesis, shown by glycosaminoglycans production, was signific ntly inhibited. The e findi gs suggest that NSAIDs may inhibit MSC chondrogenic differentiation and disrupt endochondral bone formation [31]. Despite discrepancies, it is evident that NSAID can alter certain essential processes involved in the MSC performance as therapeutic agent.

Th therapeutic potential of adult MSC can be also affected by donors lifestyle. Although high-fat diet induced type 2 diabetes did not affect the number of cells per gram of adipose tissue, analysis of diff rentiation potential of ADSC derived from high-fat diets fed mice showed a higher adipogenic potential and a lower endothelial differentiation potential *in vitro* compared to control group [32]. Impaired response to osteogenic stimuli was also shown for ADSC from obese patients. *Ranx2* expression was 6–9 times lower than in control cells and mineralization nodules were fewer and smaller [33]. Altered properties of ADSC and BMSC were also demonstrated by others. Surprisingly, in obese mice, increased frequency of BMSC and subcutaneous ADSC was shown. However, adipogenic, osteogenic, and chondrogenic

potential of BMSC from obese mice was diminished. ADSC showed increased adipogenic and osteogenic differentiation but decreased CD105 expression consistent with inefficient chondrogenic potential [34]. Observed phenotype might be associated with increased levels of free fatty acids (FFA) in plasma of obese patients. Consistent with this notion, palmitate (most abundant FFA in plasma of obese patients) treated BMSC showed induced expressions of adipogenic transcription factors, namely, CCAAT enhancer-binding protein, C/EBPβ, C/EBPα, and PPARγ, and in consequence increased adipogenic differentiation [35]. The elevated level of FFA in obese individuals may initiate events leading to irreversible changes in MSC from bone marrow and adipose tissue. Consistently, another study confirmed upregulation of adipocyte lineage commitment genes, such as *Tcf 21*, *Pitx2*, and *Lif*. At the same time, the expression of "stemness" genes (*Sdf1*, *Tbx15*) was downregulated [36].

Obesity is one of the factors increasing the risk of developing type 2 diabetes [37]. Metabolic diseases such as diabetes may influence stem cell niche and endogenous MSC properties. Therefore feasibility of autologous stem cell therapy in diabetic patients may not be possible or at least significantly hampered. Indeed, it was shown that BMSC from diabetic patients, although phenotypically similar to healthy human BMSC, expressed insulin, C-peptide, and other pancreatic markers not observed in control healthy cells [38]. Furthermore, in a recent study, investigators demonstrated that diabetes alters ADSC milieu and diminishes the cells' ability to establish a vascular network both *in vitro* and *in vivo* in wound healing mouse model [39]. It could be expected, since significant decrease of major angiogenic genes (*Vegf-a, Fgf-2*, and *Pdfg-a*) and their associated receptors (*Cxcr-4*, *Fgfr-2*, and *Pdgfr-a*) expression was observed.

Collectively, this observation indicates that the microenvironment in disease influences the stem cells. Therefore, tissues from patients with various metabolic diseases may not be satisfactory as an autologous source of mesenchymal stem cells for therapeutic purposes [40].

According to WHO statistics, 35% of adults aged 20 and over are overweight and 11% are obese (as of 2008), while 8% are living with diabetes. Taking into account the fact that the passing of time and changes in MSC microenvironment due to disease translate into reduced effectiveness of tissue regeneration, MSC derived from Wharton's jelly offer a good clinical alternative to adult MSC. In the near future, autologous use of these cells will be possible due to growing interest in Wharton's jelly banking.

3. More Differences between Adult and Wharton's Jelly Derived MSC

The superiority of WJ-MSC is based not only on adult MSC limitations but on its own prominent capacity.

5 million annual births in EU and 131 million worldwide give a unique opportunity to collect umbilical cord (UC), isolate lifesaving mesenchymal stem cells, and cryopreserve them for allogeneic or autologous application as soon as the need arises. The unlimited availability of tissue source is not the only advantage of WJ-MSC.

3.1. Isolation Efficiency: Number Does Matter. Most of clinical applications of MSC require a large number of cells for transplantation. Therefore, abundance, easiness of isolation, and proliferative potential may be deciding factors while choosing a source of MSC. The amount of mesenchymal stem cells, which can be obtained from bone marrow, is very limiting. Only 0.001 to 0.01% of mononuclear cells were reported [41], while 1 g of adipose tissue yields approximately 5×10^3 stem cells, which is 500-fold greater than in the bone marrow [42]. The isolation efficiency from Wharton's jelly is high and ranges from 1 to 5×10^4 cells/cm of umbilical cord [43]. Side-by-side comparison of MSC from bone marrow adipose tissue and Wharton's jelly demonstrated that WJ-MSC have highest proliferative capacity among tested cell types [44]. MSC from the umbilical cord can be isolated either by enzymatic digestion or by explant culture of 1–3 mm pieces of the UC [45–48]. However, at p0 explant culture method yielded 2.8 times more cells per gram of UC than enzymatic digestion [46]. Of great importance for large-scale MSC production is the fact that population doubling time of WJ-MSC isolated by enzymatic method is significantly longer [49]. Furthermore, enzymatic digestion may induce cellular damage, as MSC isolated by explant method demonstrated increased viability. Another advantage of explant method is growth factors release from tissue pieces during *in vitro* culture. Large amounts of different growth factors were reported in Wharton's jelly [46, 50]. Among them, bFGF is noteworthy, as it regulates self-renewal and positively affects osteogenic and chondrogenic differentiation of MSC while added to the growth medium [51–54]. Wharton's jelly released bFGF mediates stimulation of WJ-MSC growth in a way external supplementation provides.

To further increase isolation and culture efficiency, several modifications of explant culture methods and dedicated devices were proposed [55, 56]. Interestingly, a device designed for repeated explant culture at the same time prevented floating of Wharton's jelly pieces [55]. By sequential transfer of device with fragments of tissue strung on the steel rings, investigators reported 15–20 times higher number of cells derived by this method. However, mentioned method seems to be labor-intensive, especially for fast and large-scale production of WJ-MSC. Another approach, proposed by others in order to optimize method of MSC isolation, is based on isolation of WJ-MSC from large pieces or the entire cord piece [56–58]. The only concern posed by this method is possible heterogeneity of derived cells. However, no differences in cell surface antigen expression, population doubling time, or pattern of adipogenic, osteogenic, and chondrogenic differentiation were observed [56]. Therefore, explant culture methods of Wharton's jelly only or entire umbilical cord are worth of consideration for labor-, time-, and cost-effective WJ-MSC isolation for clinical purposes.

3.2. Properties of WJ-MSC Crucial for Clinical Application. Phenotypic analysis performed by many groups proved that

WJ-MSC fit the minimal criteria outlined for MSC by the International Society for Cellular Ther py [59]. WJ-MSC express mesenchymal markers such as CD73, CD90, and CD105 and are negative for endothelial, CD31, and hematopoietic, CD45, CD34, markers [13, 60, 61]. What sets WJ-MSC apart and makes them more unique and useful for therapeutic applications from adult MSC is their more primitive characteristics [62]. It is already well known that WJ-MSC display several features of embryonic stem cells (ESC), especially regarding the expression of ESC-like stem cell markers and wide spectrum of differentiation beyond mesodermal origin. Expression of pluripotency genes, Oct-4, Nanog, and SOX-2, was reported for WJ-MSC [46, 63, 64], although much lower than in ESC [65]. Modest expression of pluripotency genes might explain why WJ-MSC are not tumorigenic (do not form teratomas) as demonstrated in numerous preclinical studies in immunocompetent and immunodeficie t animals [66, 67]. Furthermore, as recently shown by the comprehensive analysis of WJ-MSC and ESC transcriptome, the high expression level of several tumor suppressor genes may explain the lack of *in vivo* teratoma induction [65]. Th same mechanism might be one of many responsible for attenuation of tumor growth by WJ-MSC. Moreover, large amounts of various cytokines and growth factors are secreted by WJ-MSC which result in cancer cells *in vitro* and tumor *in vivo* growth inhibition. WJ-MSC cell lysates or conditioned medium inhibited growth of breast adenocarcinoma, ovarian carcinoma, osteosarcoma [68], benign neoplastic keloid cells [69], bladder tumor [70], or lymphoma cells [71] *in vitro*. Similarly, intratumorally administered cell lysates and WJ-MSC conditioned medium inhibited mammary carcinoma, osteosarcoma, and pancreatic and lung tumor growth and resulted in decreased tumor sizes and weights *in vivo* [72–75]. Th antitumor effect of WJ-MSC was shown to be accomplished through multiple mechanisms. Antiproliferative properties of WJ-MSC were demonstrated by cell counting, MTT, BrdU or [^{3}H]-thymidine incorporation assays, cell cycle regulators, and fl w cytometric analysis. In lung or bladder tumor cells, cell cycle progression was blocked in G0/G1 phase and resulted in the downregulation of cyclin A2 and its associated kinase, cdk2, downregulation of Akt, and upregulation of tumor suppressor p53 phosphorylation, as well as cyclin dependent kinases inhibitor, p21 protein level [70, 73, 76]. In the breast cancer cells, synthesis of DNA was inhibited and arrest of cells in G2 phase of the cell cycle observed [74]. By cleaved caspase 3/9 upregulation in cancer cells, WJ-MSC were executing its proapoptotic effect [70, 77]. Consistently, increase in tumor cell death driven by WJ-MSC was due to an inhibitory effect on cancer "survival genes," such as Bcl-2, Bcl-xL, Survivin, Mcl-1, and cIAP-1 [78]. Autophagy was also indicated as one of the mechanisms responsible for anticancer effect of WJ-MSC. Upregulation of autophagy-related BAX, ATG5, ATG7, and BECLIN-1 genes was observed in osteosarcoma [68] and keloid cells [69] upon treatment with WJ-MSC conditioned medium or lysates.

Still, we must undertake far-reaching precautions and moderate enthusiasm in the implementation of WJ-MSC as anticancer therapy, since reports of tumor supporting function were recently published in regard to esophageal carcinoma [79] and renal cancer [80].

3.3. Immunoprivileged Status of WJ-MSC. Th ability to modulate immunological responses ranks WJ-MSC as an important compatible stem cell type for therapeutic applications in allogeneic setting. Th mechanisms of immunoprivilege are still investigated; however, low MHC-I level and absence of MHC-II expression protect them from NK-mediated lysis [81, 82]. Despite the fact that they synthesize, though low, amounts of MHC class I, WJ-MSC do not demonstrate immunogenicity. It can be attributed to the lack of co-stimulatory molecules-CD 40, CD80, CD86 expression, and high levels of inhibitors of immune response: indoleamine-2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2). Of particular importance is the fact that WJ-MSC express high levels of leukocyte antigen G6 (HLA-G6), the same which is produced by trophoblast and protects the embryo from immune-based destruction [83]. Notably, in such a nonchallenging to allogeneic immune cells setup, immunorejection of WJ-MSC seems not to pose a threat and HLA matching may not be required before MSC transplantation. Therefore, administration of immunosuppressive drugs is not required, thereby protecting the patient against their side effects. Besides mechanisms described above, immunoprivilege of WJ-MSC depends on immunosuppressive functions mediated by the wealth of paracrine factors as well as cell-cell contact (reviewed in detail in Jyothi Prasanna and Jahnavi [84] and Ma et al. [85]).

The question remains if immunoprivilege of allogeneic WJ-MSC upon differentiation is maintained. Although use of allogeneic MSC in clinic is considered safe, reports of limited survival and long-term engraftme t of MSC in such a setting are published. For instance, increased immunogenicity of BMCS was shown upon endothelial and myogenic differentiation [86]. A shift in the expression of immune antigens MHC-I and MHC-II made BMSC susceptible to immune rejection in a rat model of myocardial infarction. In another case, when the composite of hydroxyapatite and allogeneic BMSC was implanted, none of the allograft survived or showed osteogenic differentiation. Treatment with FK506 immunosuppressant prevented rejection and stimulated allogeneic BMSC osteogenic differentiation *in vivo* [87]. So far, such discouraging results were not reported for WJ-MSC. Results published so far demonstrated that the chondrogenic differentiation of human WJ-MSC did not change the level of expression of the aforementioned genes except for a very minor increase in the level of MHC class I. Costimulatory factors were not expressed and could not activate T lymphocytes. Moreover, high levels of potent inhibitors of immune response (IDO, HLA-G, and PGE2) were detected in differentiated WJ-MSC [88]. *In vivo* analysis of pig WJ-MSC injected into damaged rat brain revealed successful engraftme t, proliferation, and differentiation into tyrosine hydroxylase positive neuronal cells without requiring immune suppression [89]. So far, it seems that state of immunoprivilege is stable in WJ-MSC upon multidirectional diff rentiation. Further studies are

required in order to prove sustained immunoprivilege status of WJ-MSC upon differentiation which may depend on the species or stimulating factor.

4. Clinical Applications of WJ-MSC

Th fi st clinical trial to test the feasibility and effic y of WJ-MSC therapy was registered in 2008. By November 2014, the public clinical trials database http://www.clinicaltrials.gov/ has shown 51 clinical trials using WJ-MSC for a very wide range of therapeutic applications (Table 1, keywords used: Wharton's jelly mesenchymal stem cells or umbilical cord mesenchymal stem cells). Most of these trials are safety studies (Phase I) and proof of concept (Phase II) with very few in Phase III (comparison of a new treatment to the standard treatment).

To date, the results of studies listed are not published yet. However, rapidly increasing interest in WJ-MSC clinical application has resulted already in several published observations.

4.1. Type 1 Diabetes Mellitus. In a double blind study 15 patients with newly onset type 1 diabetes mellitus received 2 doses of $1.5–3.2 \times 10^7$ of WJ-MSC at 4-week interval by intravenous delivery [90]. Strikingly, within a period of 24 months, in 3/15 patients insulin supplementation was discontinued and in 8/15 and 3/15 the daily dosage was reduced by more than 50% and 15–50%, respectively. Only 1 patient did not benefit from WJ-MSC treatment. In the control group, not subjected to WJ-MSC treatment, the dose of insulin increased gradually. No adverse reactions, chronic side effects were reported during the follow-up study.

4.2. Type 2 Diabetes Mellitus. In a nonplacebo controlled study, 22 patients (17 on insulin therapy) received WJ-MSC [91]. A first dose of 10^6/kg was infused intravenously. Five days later, another dose was delivered to the pancreas via the splenic artery. Within 6 months after treatment, from 17 patients receiving insulin, 7 became insulin free and 5 had a reduction in insulin requirement by ≥50%, in the rest ≤50%, with only 1 patient who did not respond to MSC therapy. Interestingly, WJ-MSC treatment resulted in a significant decrease in proinflammatory IL-1β and IL-6 plasma level. This may have *in vivo* implications because IL-6 is an osteoclastogenic stimulus. Therefore, treatment of diabetic patients may also protect them from osteoporosis. Such effect may not be achieved by bone marrow derived MSC from aged patients, since BMSC from a mouse model of early aging secrete higher levels of IL-6 and have higher osteoclastogenesis-inducing activity [92]. Moreover, adult aged BMSC cocultured with activated T-cells were found to secrete more IL-6 than younger cells [93].

In both studies, parameters such as levels of glycated hemoglobin, C-peptide, and fasting plasma glucose were monitored. All parameters improved, HbA1c level gradually decreased, and progressive increase of C-peptide and C-peptide/glucose ratio was observed.

4.3. Systemic Lupus Erythematosus (SLE). SLE is common and potentially fatal autoimmune disease resulting in renal, neural, cardiovascular, musculoskeletal, or cutaneous injury. In a nonplacebo controlled study, 40 patients received 2 doses of 10^6/kg of WJ-MSC at 1-week interval by intravenous delivery [94]. No transplantation related side effects were observed. During 12 months of follow-up study 13/40 and 11/40 achieved major or partial clinical response manifested by signific nt improvement in renal function, decrease in SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) and BILAG (British Isles Lupus Assessment Group) scoring. 16/40 patients did not respond to MSC therapy. At 9 months after treatment, 7 patients experienced disease relapse; therefore, the authors concluded that repeated infusion with WJ-MSC is necessary to avoid disease relapse.

4.4. Late-Onset Hemorrhagic Cystitis (HC). HC is a common complication after allogeneic hematopoietic stem cell transplantation, characterized by hemorrhagic inflammation of the bladder. Late-onset of HC is frequently associated with ongoing graft- ersus-host disease (GVHD). Seven patients received 1–3 doses of $0.8–1.6 \times 10^6$/kg WJ-MSC by injection through a central line. As a result of stem cell treatment, gross hematuria dramatically resolved in 2–12days, while the time to remission for patients not treated with WJ-MSC was signific ntly longer [95].

Reported results confi m that WJ-MSC are viable option as an adjuvant treatment for late-onset hemorrhagic cystitis.

The above results of pioneering studies demonstrated the effectiveness of WJ-MSC infusion for immune disorders.

5. Conclusions

Taken together, the clinical implication of oxidative stress, telomere length, DNA damage and disease is impaired therapeutic potential of MSC isolated from aged patients. The e changes in MSC biology indicate that aged patients may require an alternative source of stem cells for treatment. Th high efficie y of WJ-MSC recovery, the minimal ethical concerns associated with its acquirement and use, low immunogenicity, and the fact that they are from healthy, young donors make them an ideal source of MSC for autologous and allogeneic applications. Private and public banking of perinatal tissues gains popularity. During MSC preparation for clinical applications, observance of national and international regulations regarding standards and procedures is required. Quality management systems already in place in functioning tissue/cell banks guarantee high standards for the donation, procurement, testing, processing, storage, and ptdistribution of the WJ-MSC. Therefore, as the off-the-shelf product, WJ-MSC can be applied safely, immediately, and on demand. Th next several years should abound in results of clinical applications of WJ-MSC and hopefully prove their invaluable properties.

Table 1: A summary of clinical trials of WJ-MSC registered on http://www.clinicaltrials.gov/ as of December 2014. ?: not mentioned.

Application	ClinicalTrials.gov identifie	Registered/phase	Number of cells	Regimen	Delivery route	Estimated enrollment
GvHD	NCT01754454	2012/1, 2	10^6/kg	4× at 1-week intervals	Intravenous	30
	NCT00749164	2008/1, 2	1-2 × 10^6/kg	?	Intravenous	20
Autism	NCT02192749	2014/1, 2	?	4× at 3-month intervals	Intravenous	20
Multiple sclerosis	NCT02034188	2014/1, 2	?	7× once per day	Intravenous	20
	NCT01364246	2011/12	?	?	?	20
Hereditary cerebellar ataxia	NCT01489267	2011/2	10^7/2 mL	4× at 3–5-day intervals	Lumbar puncture	20
	NCT01360164	2011/12	?	?	?	20
Amyotrophic lateral sclerosis	NCT01494480	2011/2	?	4× at 3–5-day intervals	Lumbar puncture	30
Hypoxic ischemic encephalopathy	NCT01962233	2013/1	1–8 × 10^8	single dose	Intravenous	10
Alzheimer's	NCT02054208	2014/1, 2	Low dose: 1× 10^7/2 mL High dose: 7.5 × 10^7/15mL	3× at 4-week intervals	Intraventricular	40
	NCT01547689	2012/1, 2	0.5 × 10^6/kg	8× at 2-week interval	Intravenous	30
Spinal cord injury	NCT02237547	2014/1, 2	?	Multiple times over the course of one month	Intravenous and intrathecal	20
	NCT01393977	2011/2	?	?	Lumbar puncture	40
	NCT01873547	2013/3	?	?	Lumbar puncture	300
Cerebral palsy	NCT01929434	2013/3			Lumbar puncture	300
Severe aplastic anemia	NCT02218437	2014/4	0.5–1 × 10^6/kg	3× at 1-week intervals	?	20
	NCT01182662	2010/2	10^6/kg	2× at 3-month intervals	Intravenous	30
Myelodysplastic syndromes	NCT01129739	2010/2	10^6/kg	2× at 3-month intervals	Intravenous	30
Cardiopathy	NCT01739777	2014/1, 2	10^6/kg	Single dose	Intravenous	30
Dilated cardiomyopathy	NCT01219452	2010/1, 2	?	?	Intramuscular	30
Myocardial infarction	NCT01291329	2011/2	?	?	Intracoronary	160
Autoimmune hepatitis	NCT01661842	2012/1, 2	10^6/kg	3× at 4-week intervals	Intravenous	100
Lupus nephritis	NCT01539902	2012/2	?	?	Intravenous	25
Systemic lupus erythematosus	NCT01741857	2012/1, 2	?	?	?	40
Epidermolysis bullosa	NCT01033552	2009/2	?	?	Intravenous	75
Burns	NCT01443689	2011/12	?	?	?	20
Diabetic foot ischemia	NCT01216865	2010/1, 2	5 × 10^7	?	Intramuscular	50
Osteoarthritis	NCT02237846	2014/1, 2	?	3× once daily or single dose	Intravenous or intra-articular	40
Type 1 diabetes	NCT01219465	2010/1, 2	2 × 10^7	Single dose	Intravenous	50
Type 2 diabetes	NCT01954147	2013/1,2	?	?	Intravenous	100
	NCT01413035	2011/12	10^6/kg	2× at 90-day intervals	Intravenous	30
Ulcerative colitis	NCT01221428	2010/1, 2	2 × 10^7 + 10^7	One week apart	Intravenous + mesenteric artery	50
Duchenne muscular dystrophy	NCT01610440	2012/1, 2	?	?	?	15
	NCT02235844	2014/1	?	?	?	1
Liver failure	NCT01724398	2012/1, 2	10^5/kg	4× at 1-week interval	Intravenous	120
	NCT01218464	2010/1, 2	5 × 10^5/kg	3× at 4-week interval	Intravenous	70
	NCT01844063	2013/1, 2	10^5, 10^6, or 10^7/kg	8× at 1-week intervals	Intravenous	210

Table 1: Continued.

Application	ClinicalTrials.gov identifie	Registered/phase	Number of cells	Regimen	Delivery route	Estimated enrollment
Liver cirrhosis	NCT01224327	2010/1,2	?	Single dose	Via hepatic artery	50
	NCT01233102	2010/1, 2	?	Single dose	Intravenous or via hepatic artery	200
	NCT01220492	2010/1, 2	5×10^5/kg	2× at 4-week intervals	Intravenous	45
	NCT01662973	2012/1 2	10^6/kg	3× at 4-week intervals	Intravenous	100
	NCT01877759	2013/1, 2	?	6× at 1-week intervals	Intravenous	20
	NCT01342250	2011/12	?	?	?	20
	NCT01728727	2012/1 2	10^6/kg	Single dose	Via hepatic artery	240
Liver transplantation	NCT01690247	2012/1	10^6/kg	3× at 4-week intervals	Intravenous	50
Ischemic-type biliary lesions	NCT02223897	2014/2, 3	10^6/kg	4× at 1-week intervals 5× at 4-week intervals	Intravenous	66
HIV infection	NCT01213186	2010/2	Low dose: 5×10^5/kg High dose: 1.5×10^6/kg	At weeks 0, 4, 12, 24, 36, and 48	Intravenous	72
Rheumatoid arthritis	NCT01547091	2012/1 2	4×10^7	4× at 3-month intervals	Intravenous	200
	NCT01985464	2013/1, 2	?	5× daily	Intravenous	20
Ankylosing spondylitis	NCT01420432	2011/1	10^6/kg	2× at 3-month intervals	Intravenous	10
Bronchopulmonary dysplasia	NCT01207869	2010/1	3×10^6/kg	Single dose	Via endotracheal tube	10

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Regenerative medicine in the treatment of alveolar cleft defect: A systematic review of the literature

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ABSTRACT

Despite a possible risk of donor site morbidity, autogenous bone grafting is considered the gold standard treatment for human alveolar cleft defect. Tissue engineering methods have recently been investigated with the aim of minimizing donor site morbidities. Here we systematically review the various tissue engineering methods applied to human alveolar cleft defects. An electronic search was conducted in the PubMed database up to March 2014. Tissue engineering studies on human alveolar subjects were included, and experiments that did not report quality or quantity of new regenerated bone were excluded. Twenty human experiments were included in our review. Regenerative techniques for alveolar cleft bone reconstruction were divided into cell therapy, growth factor application, and a combination of both cell therapy and growth factor. Using these three regenerative methods, a wide range of new bone formation percentages were reported. Due to insufficient evidence and controlled clinical trials, the treatment efficacy of tissue engineering in alveolar cleft bone defects could not be determined. Well-designed controlled studies are needed so that detailed outcomes can be properly compared.

1. Introduction

Cleft lip and palate, a congenital craniofacial malformation, occurs as a result of fusion failure of nasal process and oropalatal shelves in 0.36–0.83 of 1000 live-born infants (Pradel et al., 2008; Vecchiatini et al., 2009; Luaces-Rey et al., 2010). In addition to facial deformity, alveolar bone defect, missing teeth, and maxillary deformity can be seen in cleft patients (Le and Woo, 2009). Canine physiologic eruption, maxillary dental arch stabilization, orthodontic treatment, and implant placement should be considered in cleft patients (Pradel et al., 2008; Luaces-Rey et al., 2010). Autologous bone harvested from the anterior iliac crest, tibia, rib, or cranium have all been reported as preferable choices for alveolar cleft reconstruction, with success rates of greater than 88% due to sufficient osteogenic factor (Hibi et al., 2006; Le and Woo, 2009; Luaces-Rey et al., 2010; Marukawa et al., 2011; Behnia et al., 2012a; Janssen et al., 2014). Using iliac crest bone, 43.1% bone resorption has been reported 1 year after alveolar cleft repair (Le and Woo, 2009). Postoperative pain, donor site morbidity, inadequate bone regeneration, additional cost, and long surgical time are additional factors supporting the application of regenerative medicine in the treatment of alveolar cleft bone defects (Hibi et al., 2006; Herford et al., 2007; Le and Woo, 2009; Lee et al., 2009; Luaces-Rey et al., 2010).

In recent years, regenerative medicine has established its place as an alternative method for the treatment of bone defects, including the treatment of alveolar cleft using cell therapy, growth factor application, and scaffolds (Pradel et al., 2008; Luaces-Rey et al., 2010; Behnia et al., 2012a; Morad et al., 2013). Mesenchymal stem cells (MSCs) and differentiated osteoblasts are two groups of cells that have been used in bone engineering (Luaces-Rey et al., 2010; Behnia et al., 2012a; Morad et al., 2013).

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Recombinant human bone morphogenetic protein-2 (RhBMP-2), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and platelet-rich plasma (PRP) are the principal growth factors used for stimulating proliferation, chemotaxis, and differentiation of osteogenic essential cells to promote alveolar bone reconstruction (Kawata et al., 2005; Lee et al., 2009; Luaces-Rey et al., 2010; Marukawa et al., 2011; Behnia et al., 2012a; Khojasteh et al., 2013a; Janssen et al., 2014). The source of the cells, laboratory procedures for differentiating and culturing of cells, use of differentiated or undifferentiated cells, incubation period with scaffold, dose and time of application of growth factor, type of scaffold (synthetic vs. natural), concomitant use of cells plus growth factors, and the presence of vectors are the important factors that could affect treatment outcome (Khojasteh et al., 2013a). These factors have not been adequately considered in most tissue engineering studies (Khojasteh et al., 2013a). The notable exception is the recent review by Janssen et al., focused solely on growth factors (Janssen et al., 2014). In this systematic review, we aimed to investigate in fine detail the cell therapy methods, types of growth factors, and scaffolds used in regenerative treatment of human alveolar cleft defects.

2. Material and methods

2.1. Study design

Studies that used tissue engineering approaches for treatment of alveolar bone cleft in humans were included in this review. Treatments that involved application of cell transplantation,

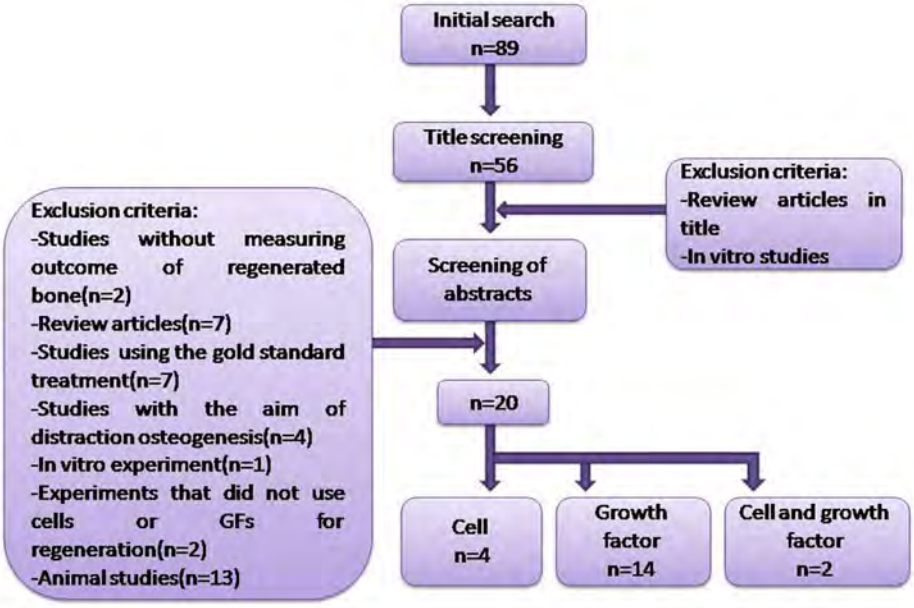

Fig. 1. Literature search strategy.

Table 1
Quality assessment.

Study	Assessment criteria					Estimated risk of bias
	Randomization	Control	Surgeon blinded to treatment	Blindness to outcome	Follow-up	
Pradel and Lauer (2012)	No	Yes	No	No	Yes	High
Pradel et al. (2008)	No	No	No	No	Yes	High
Behnia et al. (2009)	No	No	No	No	Yes	High
Hibi et al. (2006)	No	No	No	No	Yes	High
González-Sánchez and Jiménez-Barragán, (2011)	No	Yes	No	No	Yes	High
Kumar et al. (2013)	No	Yes	No	No	Yes	High
Luaces-Rey et al. (2010)	No	Yes	Unclear	Yes	Yes	High
Oyama et al. (2004)	No	Yes	No	Unclear	Yes	High
Marukawa et al. (2011)	Yes	Yes	No	Yes	Yes	High
Rullo et al. (2007)	No	Yes	No	No	Yes	High
Lee et al. (2009)	No	Yes	No	No	Yes	High
Dickinson et al. (2008)	Yes	Yes	Unclear	Yes	Yes	High
Alonso et al. (2010)	Yes	Yes	Unclear	Yes	No	High
Chin et al. (2005)	No	No	No	No	Yes	High
Canan et al. (2012)	Yes	Yes	Unclear	Unclear	Yes	High
Herford et al. (2007)	No	Yes	Yes	Yes	Yes	High
Neovius et al. (2013)	Yes	Yes	No	Unclear	Yes	High
Balaji (2009)	No	Yes	No	No	No	High
Stanko et al. (2013)	No	No	No	No	Yes	High
Behnia et al. (2012)	No	No	No	No	Yes	High

Table 2
Comparison of cell studies.

Author/year	Study type	Number of patients	Control	Carrier	Cell type	Result
Pradel and Lauer (2012)	CS[a]	8 (4C[b],4T[c])	IBG[d]	DBM[e] (Osteovit)	Autog[f] osteoblasts	Cell group = 40.9%BF[g] IBG group = 36.6%BF
Pradel et al. (2008)	CR[h]	1	–	Resorbable bovine Coll[i] matrix(osteovit)	Autog osteoblasts from maxilla	Complete bony closure of cleft and spontaneous eruption of canine in right place happened.
Behnia et al. (2009)	CR	2	–	Osteoset(calcium sulphate) + DBM	MSC[j] from BM[k] aspirate of post.[l] Iliac crest	BF = 34.5%
Hibi et al. (2006)	CR	1	–	–	MSCs (marrow aspirate)	BF = 79.1%

[a] Case series.
[b] Control.
[c] Test.
[d] Iliac bone graft.
[e] Demineralized bone matrix.
[f] Autogenous.
[g] Bone formation.
[h] Case report.
[i] Collagen.
[j] Mesenchymal stem cells.
[k] Bone marrow.
[l] Posterior.

growth factor delivery, or a combination of these, with or without bone scaffold, were considered as tissue engineering approaches. Only experiments that reported quality or quantity of new bone formation were included, and reports on complications were excluded. In addition, animal studies and review articles were also excluded.

2.2. Search strategy

An electronic search of the literature in PubMed was carried out in March 2014, limited to English-language and human studies using a combination of following key words: alveolar cleft, tissue engineering, bone regeneration, regenerative medicine, stem cells, and cell therapy. No publication year limitation was applied. A total of 89 search results were returned (Fig. 1). Primary selection of titles and abstracts were based on the inclusion criteria, and full texts of all eligible studies were obtained and reviewed by authors.

Type of study, number of treated patients, methods, and results regarding new bone formation were extracted. Data were divided in to following groups: cell group; growth factor group; and both cell and growth factor groups.

2.3. Quality assessment

During data extraction, the quality assessment of the included articles was undertaken by the authors according to the following parameters (Table 1): proper randomization (yes/no); presence of both control and test groups (yes/no); surgeon blinded to treatment (yes/no/unclear); blindness to outcome (yes/no/unclear); follow-up completion (yes [withdrawal or dropout explanation]/no).

The experiments were then grouped as either low risk of bias (bias unlikely to influence the outcomes) if all criteria were met; or high risk of bias (bias that might weakens confidence in results) if one or more criteria were not met.

3. Results

Of the 89 studies evaluated, 20 met the inclusion criteria after title-, abstract-, and full text-screening steps (Fig. 1). Regenerative medicine as a replacement for autogenous bone graft in human cleft defects was investigated in these studies. Four studies applied stem cells alone, 14 were designed to assess only growth factor application, and two used a combination of both items. All included studies were judged to be at high risk for bias (Table 1), showing low quality of evidence on the effectiveness of tissue engineering treatments for alveolar cleft defects.

3.1. Cell group

Of the cell-only experiments, three case reports (Hibi et al., 2006; Pradel et al., 2008; Behnia et al., 2009) and one case series (Pradel and Lauer, 2012) were reported. The case reports did not use control groups (Hibi et al., 2006; Pradel et al., 2008; Behnia et al., 2009). Whereas two studies by Pradel et al., used autogenous osteoblasts as differentiated cells (Pradel et al., 2008; Pradel and Lauer, 2012), Behnia et al. harvested mesenchymal stem cells from posterior iliac crest bone marrow and loaded these onto a composite of calcium sulfate with demineralised bone mineral (DBM) (Table 2) (Behnia et al., 2009).

Application of stem cells in alveolar cleft patients resulted in less than 50% of new bone formation (Pradel et al., 2008; Behnia et al., 2009; Pradel and Lauer, 2012), except in one case report, which was remarkable for 79.1% bone formation (BF) (Hibi et al., 2006). Pradel et al. used differentiated osteoblast cells and showed 40.9% new BF plus complete defect closure (Pradel et al., 2008; Pradel and Lauer, 2012). MSCs seeded on DBM with calcium sulfate achieved 34.5% of new BF (Behnia et al., 2009).

3.2. Growth factor group

Five of the14 growth factor studies were clinical trials with control groups (Herford et al., 2007; Dickinson et al., 2008; Alonso et al., 2010; Marukawa et al., 2011; Canan et al., 2012). BMP-2 and PRP were the most studied growth factors (seven times for BMP-2 (Chin et al., 2005; Herford et al., 2007; Dickinson et al., 2008; Balaji, 2009; Alonso et al., 2010; Canan et al., 2012; Neovius et al., 2013) and six times for PRP (Oyama et al., 2004; Rullo et al., 2007; Lee et al., 2009; Luaces-Rey et al., 2010; Marukawa et al., 2011; Kumar et al., 2013). Application of BMP-2 with collagen led to 71.7% new BF (Herford et al., 2007). In one study, 90.9% of fistula closure was reported in cleft patients after use of PRGF (González-Sánchez and Jiménez-Barragán, 2011). PRP showed 71.27% BF in comparison to 47.47% in the control group, with 26.5% of secondary bone loss in comparison to 35.5% in the control group (Oyama et al., 2004;

Table 3
Comparison of growth factor studies.

Author/year	Study type	Number of patients	Control	Carrier	Growth factor	Result
González-Sánchez et al. (2011)	PR[a]	6	ABG[b] (palate)	–	PRGF[c]	Complete closure of palate fistula in 90.9%
Kumar et al. (2013)	PR	3	IBG[d]	–	PRP[e]	More mature bone with same opacity and hardness to native bone obtained.
Luaces-Rey et al. (2010)	RR[f]	20 (10C[g], 10T[h])	ABG (iliac, mandsymphis and tibia)	–	PRP	PRP group = 2.43±0.6BF[i] IBG group = 3.17 ± 0.866BF
Oyama et al. (2004)	Pre[j]	7 (5C,2T)	IBG	–	PRP	PRP group = 71.27–87.32%BF IBG group = 47.47–77.97%BF
Marukawa et al. (2011)	CT[k]	20 (6C,14T)	IBG	–	PRP	PRP group = 26.5 ± 0.71%bone loss IBG group = 35.5 ± 2.12%bone loss
Rullo et al. (2007)	CR[l]	1	ABG (Chin)	–	PRP	After 6 m, the site was sufficient for applying fixture.
Lee et al. (2009)	Long[m]	60 (30C,30T)	ABG	–	PRP	PRP group = 0.94 to 2.54 cm^3 IBG group = 0.59 to 2.16 cm^3
Dickinson et al. (2008)	CT	21 (12C, 9T)	IBG	–	BMP-2[n]	BMP group = 95%BF IBG group = 63%BF
Alonso et al. (2010)	CT	16 (8C, 8T)	IBG	–	rhBMP-2[o]	BMP group = 65.0% mean BH[p], 247.1 mm^3 BFV[q] IBG group = 83.8% mean BH, 207.8mm3 BFV
Chin et al. (2005)	CT	43	–	–	rh-BMP2	Alveolus performed normal vital bone & responded to natural tooth eruption.
Canan et al. (2012)	CT	18 (6C, 6T, 6PP)	IBG	Coll[r] sponge	rh-BMP2	IBG group: 520.5 mm3 BFV BMP group: 354.4 mm^3 BFV PP[s] group: 105.7 mm3 BFV
Herford et al. (2007)	CT	12 (2C,10T)	IBG	Type 1 bovine Coll sponge	rh-BMP2	BMP group = 71.7% mean BF(24.1–90.6%) IBG group = 78.1% mean BF(71.3–84.9%)
Neovius et al. (2013)	PR	5 (3C,2T)	IBG	Hyaluronan-based hydrogel	BMP2	BMP group = 59% and 33%BF IBG group = 29%, 48%, and 69%BF
Balaji (2009)	RR	60 (30C, 30T)	IBG	–	rhBMP2	BMP group = 91.74%BF IBG group = 87.96%BF

[a] Prospective.
[b] Autogenous bone graft.
[c] Platelet rich growth factor.
[d] Iliac bone graft.
[e] Platelet rich plasma.
[f] Retrospective.
[g] Control.
[h] Test.
[i] Bone formation.
[j] Preliminary.
[k] Clinical trial.
[l] Case report.
[m] Longitudinal.
[n] Bone morphogenetic protein.
[o] Recombinant human bone morphogenetic protein.
[p] Bone height.
[q] Bone formation volume.
[r] Collagen.
[s] Periosteoplasty.

Marukawa et al., 2011). Absorbable collagen sponge (two studies) (Herford et al., 2007; Canan et al., 2012) and hyaluronan-based hydrogel (one study) (Neovius et al., 2013) were the most commonly used carriers for delivering rh-BMP-2 (recombinant human bone morphogenetic protein) (Table 3).

3.3. Cell and growth factor group

Mesenchymal stem cells were harvested mostly from bone marrow aspirate and posterior iliac bone aspirate (Behnia et al., 2012a; Stanko et al., 2013). PRP and PRGF were the growth factors that were used concomitantly with cell therapy (Behnia et al., 2012a; Stanko et al., 2013). Quantitative measurement showed 51.3% of new bone formation induced by simultaneous application of MSCs with growth factors (Behnia et al., 2012a). Collagen membrane, hydroxyapatite, biphasic hydroxyapatite/tricalcium phosphate were used as a carriers (Table 3) (Behnia et al., 2012a; Stanko et al., 2013). MSCs with PRP-loaded hydroxyapatite granules showed complete closure of the oral nasal fistula in one patient (Table 4) (Stanko et al., 2013).

4. Discussion

Here we reviewed regenerative treatments that were used for closure of alveolar cleft defects. Carriers, cell type, growth factor, and adhesive agents were analyzed in these patients. Physiologic canine eruption can occur sooner in new tissue-engineered bone, and although this prohibited histomorphometric analysis in these studies, new bone formation could still be measured by serial tomography (Hibi et al., 2006). Cells or growth factors were mixed

Table 4
Comparison of cell and growth factor studies.

Author/year	Study type	Number of patients	Carrier	Cell type	Growth factor	Result
Stanko et al. (2013)	CR[a]	1	Coll[b] mem[c]/HA[d] particles	MSC[e] (pelvic BM[f] aspirate)	PRP[g]	Initial bone formation in oronasal fistula after 10 w[h]
Behnia et al. (2012a)	Pre[i]	3	Biphasic scaffold	MSC (post[j] iliac bone aspirate)	PDGF[k]	BF[l] = 51.3%

[a] Case report.
[b] Collagen.
[c] Membrane.
[d] Hydroxyapatite.
[e] Mesenchymal stem cell.
[f] Bone marrow.
[g] Platelet rich plasma.
[h] Weeks.
[i] Preliminary.
[j] Posterior.
[k] Platelet derived growth factor.
[l] Bone formation.

with scaffolds to be delivered from the *in vitro* to the *in vivo* side. In some of the studies, entrapment of the cells in the pores of the substitutes was demonstrated by the use of scanning electron microscopy (SEM), after 24 h of cell/scaffold mixing (Jafarian et al., 2008; Soleymani Shayesteh et al., 2008; Behnia et al., 2009, 2013; Khojasteh et al., 2013b). Most of the cell–scaffold mixtures in the studies included in this review were delivered to the patients after 24–48 h (Behnia et al., 2009, 2012a; Khojasteh et al., 2013b; Stanko et al., 2013). Recent evidence suggested that increasing the loading time from 24 h to 2 weeks increased the number of entrapped cells that could be delivered (Motamedian et al., article in press). Adding an adhesive agent to the culture medium resulted in a higher rate of cell delivery (Baghaban Eslaminejad et al., 2008; Jafarian et al., 2008; Khojasteh et al., 2013c). Type I collagen gel can solidify the medium on the day of delivery and might increase the amount of new BF (Baghaban Eslaminejad et al., 2008; Jafarian et al., 2008). Fibrin glue was also used as an adhesive agent during the delivery procedure and showed promising results (Khojasteh et al., 2013c). Transferring of cells after 24 h' incubation without adhesives resulted in 34% of new BF, whereas adding PRGF with the same method of incubation achieved 51.3% new BF (Behnia et al., 2009; Behnia et al., 2012a). Animal models showed a higher amount of bone formation when MSCs were used in combination with PRP (Khojasteh et al., 2008), PRGF (Behnia et al., 2012b; Behnia et al., 2013), or rh-PDGF (Khojasteh et al., 2014). Each of these growth factors showed less BF when used alone in bone defects. PRP was not always prepared in a similar way in regenerative studies, which might have affected the results (Oyama et al., 2004; Rullo et al., 2007; Lee et al., 2009; Luaces-Rey et al., 2010; Marukawa et al., 2011; Kumar et al., 2013). Human serum was used as a nutrient for MSCs in human trials despite fetal calf serum during the culturing period (Khojasteh et al., 2012; Tabatabaei et al., 2012; Houshmand et al., 2013). This likely resulted in a decrease in the cellular proliferation rate and a lessened capability of multi-lineage differentiation (Khojasteh et al., 2012; Tabatabaei et al., 2012; Houshmand et al., 2013).

Differentiation staining, reverse transcription-polymerase chain reaction (RT-PCR), and flow-cytometric analyses were used for *in vitro* detection of MSCs (Baghaban Eslaminejad et al., 2008; Jafarian et al., 2008; Behnia et al., 2012a). CD34 was reported as a diagnostic marker for MSCs (Behnia et al., 2012a). With the except of Behnia et al. (Behnia et al., 2009; Behnia et al., 2012a), no studies reported any tests in this regard.

Collagen was the most commonly used carrier for growth factors in treating cleft patients (Herford et al., 2007; Canan et al., 2012). However, cells might be transferred better by HA-based scaffolds (Behnia et al., 2012a; Stanko et al., 2013). Application of a new generation of scaffold may help to promote bone engineering in cleft patients (Khojasteh et al., 2013b). The best result within the regenerative groups was reported with the use of rh-BMP-2 (95% BF) in an adult patient (Dickinson et al., 2008). Mesenchymal stem cells from bone marrow aspirate loaded on DBM resulted the least amount of bone formation (34.5% BF) (Behnia et al., 2009).

The absence of control groups is the most important criticism of all studies that have used cells for regenerative treatment in cleft patients (Tables 2 and 4), which were therefore limited to case reports and case series. This makes it as yet impossible to reach a consensus regarding the use of MSCs in cleft patients.

Although a huge amount of effort has been devoted to the use of tissue engineering as a solution for treating bone defects, a higher level of evidence is still needed, including more controlled trials using the same methods for all *in vitro* and *in vivo* steps.

Given that the lack of control groups and diversity in *in vitro* procedures is likely to reduce the accuracy of results, further clinical studies, preferably including control and test groups, are required to identify the best approaches for applying regenerative medicine in alveolar cleft defects with minimal donor site morbidity. We recommend that researchers recognize better the appropriate age for surgery and design a standard method for this procedure.

5. Conclusion

Pending the presence of standard well-designed studies and proper evidences, the researchers are not able to compare the results of tissue engineering efficacy in alveolar cleft bone defects treatment studies.

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Review

Wharton's Jelly-Derived Mesenchymal Stem Cells: Phenotypic Characterization and Optimizing Their Therapeutic Potential for Clinical Applications

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Abstract: Wharton's jelly (WJ) is a gelatinous tissue within the umbilical cord that contains myofibroblast-like stromal cells. A unique cell population of WJ that has been suggested as displaying the stemness phenotype is the mesenchymal stromal cells (MSCs). Because MSCs' stemness and immune properties appear to be more robustly expressed and functional which are more comparable with fetal than adult-derived MSCs, MSCs harvested from the "young" WJ are considered much more proliferative, immunosuppressive, and even therapeutically active stem cells than those isolated from older, adult tissue sources such as the bone marrow or adipose. The present review discusses the phenotypic characteristics, therapeutic applications, and optimization of experimental protocols for WJ-derived stem cells. MSCs derived from WJ display promising transplantable features, including ease of sourcing, *in vitro* expandability, differentiation abilities, immune-evasion and immune-regulation capacities. Accumulating evidence demonstrates that WJ-derived stem cells possess many potential advantages as transplantable cells for treatment of various diseases (e.g., cancer, chronic liver disease, cardiovascular diseases, nerve, cartilage and

tendon injury). Additional studies are warranted to translate the use of WJ-derived stem cells for clinical applications.

Keywords: umbilical cord; wharton's jelly; mesenchymal stem cells; phenotypic characteristics; therapeutic applications; experimental protocol

1. Introduction

The advent of stem cells as a tool to decipher the cell's biology and as a source of transplant therapy to correct aging and diseases has become a core research arena for tissue engineering and regenerative medicine. A pivotal source of stem cells is the umbilical cord's Wharton's jelly (WJ) [1]. A unique cell population of WJ that has been suggested as displaying the stemness phenotype is the mesenchymal stromal cells or MSCs. The prototypical feature of MSCs is their plastic adherence expressing a phenotypically defined set of surface markers including CD90, CD73 and CD105. Although MSCs have been harvested from many different tissues, novel considerations of tissue specificity may dictate the eventual fate of MSCs. In particular, MSCs' stemness and immune properties appear to be more robustly expressed and functional with fetal than adult-derived MSCs. To this end, the young age of WJ suggests that MSCs harvested from this fetal origin will exhibit a much more proliferative, immunosuppressive, and even therapeutically active stem cells than those isolated from older, adult tissue sources such as the bone marrow or adipose. This alternative source of MSCs became feasible with the report by McElreavey *et al.* [2] of the culture of cells from WJ, which is the primitive connective tissue of the human umbilical cord (UC), first described by Thomas Wharton in 1656 [3]. Thereafter, research efforts have attempted to optimize the isolation and differentiation of these cells derived from WJ [4–11]. The present compilation of milestone discoveries on WJ-derived stem cells should aid in further moving the field of cell biology and therapy towards clinical applications.

2. Anatomical Relationship of Various UC Structures and WJ as Sources of MSCs

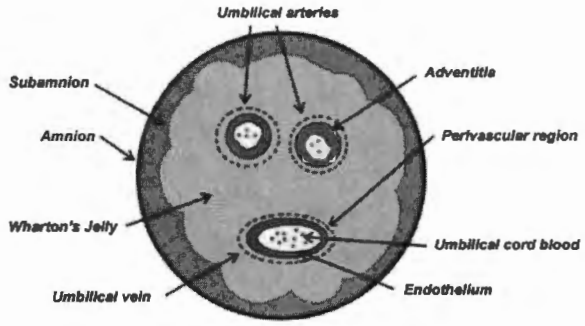
During pregnancy, the fetus and placenta is connected by an elastic UC which prevents umbilical vessels from compression, torsion, and bending while providing a good blood circulation. Anatomically, the UC consists of two umbilical arteries and one umbilical vein, both embedded within a specific mucous proteoglycan-rich matrix, known as WJ, which is then covered by amniotic epithelium (Figure 1).

WJ which contains a multipotent fibroblast-like MSC population were first obtained more than 10 years ago [12]. Previously, WJ-MSCs were termed as "umbilical cord matrix stem cells (UCMSCs)" to distinguish them from endothelial cells isolated from umbilical vein (HUVEC) as well as MSCs isolated from UC blood (UCB-MSCs) [13,14].

There are two possible theories on how stem cells existed in the WJ. First, there were two waves of migration of fetal MSCs in early human development. During these waves of migration, some of MSCs got trapped and resided in the gelatinous WJ of the UC [15]. Second, the cells in the WJ are actually primitive MSCs originating from mesenchyme that were already there within the UC matrix. The function of these cells may be to secrete the various glycoproteins, mucopolysaccharides,

glycosaminoglycans and extracellular matrix proteins to form a gelatinous ground substance to prevent strangulation of the UC vessels during gestation [16].

Figure 1. Cross-sectional diagram of human umbilical cord shows anatomical compartments, including Wharton's jelly, as a source of stem cells.



Stem cells have been derived in the amniotic compartment (outer epithelial layer and inner subamniotic mesenchymal layer), the WJ compartment, the perivascular compartment surrounding the vessels, the media and adventitia compartment of the walls of UC blood vessels, the endothelial compartment (inner lining of the vein) and the vascular compartment (blood lying within the UC blood vessels) [16]. All these compartments have been described as distinct regions [17] and the nomenclature has not been standardized, with terms such as "subamnion", "cord lining (sub-amnio)", "intervascular", "perivascular" and "hUVEC" being used. Also, isolation methods and region of interest for WJ-MSCs have not been standardized. Indeed, it is not known whether the stem cell populations within WJ-MSCs between compartments are one and the same as there is no clear demarcation histologically between these compartments. At the same time the various individual derivation protocols are ambiguous and further compound the differences in stem cell populations between compartments [16]. WJ-MSCs can be isolated from two regions, namely, intervascular and sub-amnion [18], while others have isolated WJ-MSCs from three regions, namely, the perivascular zone, the inter-vascular zone, and the sub-amnion [19]. Structural, immunohistochemical, and functional analysis performed *in vitro* show significant differences in the number and nature of cells among these three regions and they have different properties [20,21]. These findings led to the hypothesis that these regions might be originating from different pre-existing structures [22]. A stem cell population has been isolated from around the umbilical vessels, termed human umbilical cord perivascular cells (HUCPVCs) [23,24] while equally potent stem cell-like cells have been harvested from sub-amnion (cord lining; CL) [17,25]. Of note, WJ-MSCs located close to amniotic surface display enhanced ability to proliferate, whereas WJ-MSCs with more differentiated were found in closer proximity to the umbilical vessels [20,21].

3. Characteristic Features of WJ-MSCs for Cell Therapy

3.1. Sources of Stem Cells

Various types of stem cells have been isolated to date in the human from a variety of tissues including preimplantation embryos, fetuses, birth-associated tissues and adult organs. Based on

biochemical and genomic markers, they can be broadly classified into embryonic stem cells (ESC), mesenchymal stem cells (MSC), and hematopoietic stem cells (HPS).

ESCs are pluripotent stem cells which theoretically can be differentiated into almost all tissues in the human body. However, ESCs have limitation for use. The principal limitation is an ethical problem. Because ESCs are derived from the inner cell mass of a blastocyst, an early-stage embryo [26], isolating the embryoblast or inner cell mass results in destruction of the fertilized human embryo, which raises ethical issues. Although the source of the blastocyst was generally discarded material from *in vitro* fertilization clinics there is no consensus whether or not a human life at the embryonic stage should be granted the moral status of a human being [27]. Other limitations are the risks of immunorejection and tumorigenesis. To overcome the problem of immunorejection, protocols were developed where tissue could be personalized to patients by transfecting the patient's somatic cells with pluripotent genes to produce human induced pluripotent stem cells (hiPSCs); unfortunately, epigenetic changes in the form of chromosomal duplications and deletions have been reported in the ensuing hiPSCs [28,29]. Additionally, hiPSCs induce tumorigenesis in immunodeficient mice and such teratoma formation is faster and more efficient than their ESCs counterpart [30]. The risk of tumorigenesis is of particular importance when using pluripotent cells, since these are characterized by the ability to form teratomas in animal models [26,29]. Thus, the differentiation state of transplanted cells will need to be defined with high precision to avoid delivery of residual pluripotent cells that may differentiate aberrantly *in vivo*.

HSCs have limited plasticity in that they can differentiate only into blood and blood-related lineages. In addition, the HSC numbers from bone marrow and UC are low and require *ex vivo* expansion for the treatment hematologic diseases in adult humans. However, a recent study showed there is strong evidence that HSCs are pluripotent and are the source for the majority, if not all, of the cell types in our body [31].

Fetal MSCs are controversial as they are derived from human abortuses. Since Pittenger and colleagues demonstrated the successful isolation of multipotent MSCs from bone marrow, it has become the primary source from which to obtain MSCs [32]. Although BM-MSCs are the most studied and well-documented, BM-MSCs have limitation in terms of cell numbers and as such require expansion *in vitro* running the risk of loss of stemness properties, induction of artifactual chromosomal changes, and problems of contamination [16,32]. Adipose tissue has recently emerged as an alternative source of MSCs. Despite its plentiful nature, an invasive procedure is still required to collect the tissue [33].

Extra-embryonic perinatal MSCs harvested from placenta, fetal membrane (amnion and chorion), UC, UC blood, and amniotic fluid represent an intermediate stem cell type that partially combines some pluripotent properties of adult MSCs [34–37]. Because they have close ontogenetic relationship with embryonic stem cells, extra-embryonic tissue-derived MSCs have immunoprivileged characteristics, possess a broader multipotent plasticity, and proliferate faster than adult MSCs [37,38]. Moreover, these cells could be isolated and used without ethical problem, because extra-embryonic tissues are normally discarded after birth [38].

3.2. Immunomodulatory Property of WJ

The practical utility of WJ-MSCs would be in allogeneic transplantation. One important requisite for allogeneic transplantation is low immunogenicity. The therapeutic utility of the WJ-derived stem cells can be ascribed to their regenerative and immunomodulatory potential of these cells. A review paper discusses immunomodulatory molecules expressed by WJ-MSC and also analyzes the *in vitro* and *in vivo* data on their immune-modulating activities [18]. WJ-MSCs are also capable of immune suppression and immune avoidance similar to other types of MSCs. They express MHC class I (HLA-ABC) at low levels but not class II (HLA-DR) and co-stimulatory antigens such as CD80, CD86 implicated in activation of both T and B cell responses [18,39–42]. Low levels of MHC class I antigens could be a mechanism to protect them from Natural killer cell-mediated lysis [18]. Even though the overall expression of immune-stimulatory ligands on WJ-MSCs remains similar to that of bone marrow-derived MSCs (BM-MSCs), their induction with pro-inflammatory cytokines might differ. HLA-DR is induced substantially in BM-MSCs with IFN-γ treatment but the induction is very negligible in WJ-MSCs [39,43]. In addition, WJ-MSCs produce large amounts of tolerogenic IL-10, higher levels of TGF-β than BM-MSCs, and express HLA-G, which is not expressed in BM-MSCs [39,40,42,43]. HLA-G appears to play a role in the immune tolerance during pregnancy by evading a maternal immune response against the fetus and inducing the expansion of regulatory T cells, which would contribute to the suppression of effectors responses to alloantigens [44,45]. Compelling evidence has shown that the low rate of rejection seems to be associated to the expression of these antigens in blood, heart and liver/kidney grafts [46]. Furthermore, WJ-MSCs express IL-6 and VEGF, which have recently been shown to be pivotal in the immunosuppressive capability of MSCs [42,47]. WJ-MSCs are less immunogenic than BMMSCs as well as fetal MSCs making them more amenable for allogeneic as well as xenogeneic transplantation. However, under certain circumstances, UCMSCs can elicit an immune response. A single injection of MHC mismatched inactivated UCMSCs did not induce a detectable immune response. When injected in an inflamed region, injected repeatedly in the same region, or stimulated with IFN-γ prior to injection, UCMSCs can be immunogenic [48]. Therefore, care must be taken to avoid sensitization against the cell therapy, especially if these cells are used for repairing damaged, inflamed tissue that needs repeated administration into the same location.

WJ-MSCs also afford robust immunomodulatory properties compared to BM-MSCs. BM-MSCs have been widely reported to attenuate mitogen driven as well as alloantigen or specific antigen driven T cell response in a dose dependent manner *in vitro* [49]. MSCs have been shown to equally inhibit CD4(+), CD8(+), CD2(+) and CD3(+) subsets [50]. However, WJ-MSCs exhibit a prominent suppression even at very low dose range as compared to BM-MSCs in terms of mitogen induced CD3(+) T cell responses [39,51]. In addition, WJ-MSCs suppress allogeneically-stimulated T cells to a greater extent than either BM-MSCs or adipose-derived MSCs [18]. Fetal liver-derived MSCs suppress lympho-proliferative responses to mitogens, but do not attenuate allo-proliferative responses [52]. In this context, peri-natal MSCs, like that of WJ-MSCs, not only seem to attenuate lymphoproliferation more robustly than BM-MSCs, but also the regulation is stimuli-independent unlike fetal MSCs [18]. Additionally, WJ-MSCs can affect the maturation and activation of dendritic cell (DC) precursors. WJ-MSCs, when cultured with CD14(+) monocytes, inhibited their differentiation into mature DCs in

a contact-dependent manner. WJ-MSCs co-cultured monocytes were shown to be locked in an immature DC phenotype and the up-regulation of co-stimulatory ligands was blocked in the co-cultures [53]. Thus, WJ-MSCs might indirectly affect T cell allogeneic responses through attenuation of DC functions. There are a limited number of studies with purified populations of immune cells tracing their activation and effector functions closely in presence of WJ-MSCs. Prasanna *et al.* have tracked the pro-inflammatory cytokine secretion patterns kinetically in co-cultures of WJ-MSCs/BM-MSCs with PHA-activated lymphocytes [39]. A change in the threshold and kinetics of IL-2 secretion was observed only with BM-MSCs and not with WJ-MSCs. Additionally, an early activation of negative co-stimulatory ligands on peripheral blood lymphocytes was observed more evidently with WJ-MSCs co-cultures [39]. Although the major secretary profiles of different tissue derived MSCs are similar, WJ-MSCs and cord blood MSCs only secrete IL-12, IL-15 and Platelet-derived growth factor (PDGF). In summary, the putative mechanisms of immunomodulatory properties of WJ-MSCs include upregulation of negative co-stimulatory ligands, secretion of immunosuppressive soluble factors, generation of memory cells, cell fusion to escape recognition, immune avoidance mechanisms specific to fetal-maternal interface, attenuation of antigen-presenting cell functions, altered migration of immune cells, and T cell anergy apoptosis tolerance [18].

3.3. Phenotypic Characterization of WJ

In 2011, Conconi *et al.*, laid out the groundwork on the WJ's characterization by providing an overview on the human UC [54]. In this review, a panoramic view of phenotypic characteristics of human UC cells derived from various UC parts are described. The high heterogeneity of extraction, culture, and analysis procedures hinder the ability to precisely identify UC stromal cells. Overall, cells from WJ fit with the minimal criteria for MSCs. The mesenchymal features of WJ cells have been confirmed by the expression of specific lineage cytoskeletal markers, such as SMA and vimentin. Furthermore, ESC markers, such as Oct-4, SSEA4, nucleostemin, SOX-2 and Nanog, have also been revealed, though HUCPV cells do not express Oct-4, SSEA4. Other cell surface molecules are CD59 and CD146 which are not expressed in HUCPV cells. CD59 is involved in the complement system regulation thus preventing cell lysis. CD146 is a cell adhesion molecule expressed not only on endothelial cells but also on MSCs[54]. Furthermore, the HUCPV cells stain for pan-cytokeratin more strongly than WJ-MSCs [20]. This group suggested that HUCPV cells are more differentiated than WJ-MSCs and this explains why the HUCPV cells may not differentiate to neuronal cells. The most outstanding feature of CL-MSCs is the expression of CD14 which is not expressed in WJ-MSCs [25]. CD14 is widely recognized as a common marker for marcrophages. The function and significance of CD14 expression on CL-MSCs has not to be determined yet, but it is interesting to note that the soluble form of CD14 can down regulate T cell activation [55]. The most striking feature of WJ-MSCs is their unique ability to express the HLA-G6 isoform. As mentioned previously, HLA-G6 is implicated in immune-modulation. Thus, WJ-MSCs are particularly suitable for cell-based therapy. As a result, different phenotypic profiles are detectable not only among the cells obtained from the various parts of cord, but also inside the same UC regions, suggesting that UCMSCs may represent an unique cell family whose components present various degree of stemness. However, *in vitro* and *in vivo* evidence indicates WJ as an excellent source of MSCs because its cells present a wide range of

potential therapeutic applications. In addition, Conconi and co-workers [56] first reported that CD105(+)/CD31(−)/KDR(−) cells from WJ are able not only to differentiate *in vivo* towards the myogenic lineage, but also to contribute to the muscle regenerative process. Such myogenic differentiation potential of CD105(+) cells from WJ was further confirmed using *in vitro* assays.

Subsequently, Jeschke and colleagues identified the specific region of the UC lining (sub-amnion) and WJ enriched with stem cell niches [17]. Before this report, Kita and co-workers [25] previously attempted to isolate MSCs from sub-amnion of the UC and they reported that sub-amniotic MSCs are distinct from ESCs and do not show tumorigenicity *in vitro*. The CL-MSCs isolated by their method maintain typical characteristics of MSCs *in vitro*, but also showed several specific features [25]. Because of several anatomically distinct zones found in the UC, isolated multipotent cells sometimes show heterogeneity. In addition, differences in isolation technique may lead to further variation. Of note, CL-MSCs have excellent potential in terms of their proliferative capacity and possibly multipotency [17]. However, the main disadvantage of CL-MSC is the extremely time-consuming nature of the isolation process. In contrast, WJ provides an ample supply of MSCs. Although WJ–MSCs show more variation in terms of quality of cells, WJ is still a very useful depot of MSCs. Accordingly, the choice of MSC source should consider the quality and quantity of stem cells required for each specific application.

Interestingly, biological characteristics of MSCs can be influenced by perinatal environment. There is increasing evidence that intrauterine metabolic disturbances produced by hyperglycemia during pregnancy appear to increase the risk in offspring for obesity and diabetes [57–59]. In addition, studies in animal models suggest that the MSC commitment into pre-adipocytes begins during fetal development and perinatal life [60]. Since the number of pre-adipocytes and mature adipocytes is lower in normal subjects than in obese subjects [61], changes in the prenatal maturational process may play a role in the pathogenesis of obesity and metabolic-associated diseases. For this reason, it would be useful to investigate how the perinatal environment may affect fetus-derived MSCs, especially in unregulated gestational diabetes. Recently, Pierdomenico *et al.*, have compared WJ-MSCs obtained from UC of both healthy and diabetic mothers, in order to better understand the mechanisms involved in metabolic diseases in offspring of diabetic mothers [62]. Although the same markers were expressed in WJ-MSCs obtained from both healthy and diabetic mothers, their expression levels differed, possibly due to a difference in functional characteristics of the two WJ-MSCs groups. Lower levels of CD90 were observed in WJ-MSCs from diabetic mothers, which could be to the result of a plasticity decrease of these cells. It was also shown that WJ-MSCs from diabetic mothers presented higher adipocyte differentiation efficiency, compared to WJMSCs obtained from healthy mothers, suggesting, therefore, a possible pre-commitment of these cells to the adipogenic lineage. In addition, the up-regulation of CD44, CD29, CD73, CD166, SSEA4 and TERT in WJ-MSCs obtained from diabetic mothers might be related to the slight increase of proliferative ability of these cells. Results indicate that in contrast to cells from healthy mothers, WJ-MSC from diabetic mothers display a higher ability to differentiate towards the adipogenic lineage. This suggests that the diabetic uterine environment may be responsible for a "pre-commitment" that could give rise in the post natal life to an alteration of adipocyte production upon an incorrect diet style, which in turn would produce obesity.

4. Clinical Applications of WJ-Derived Stem Cells

4.1. Cancer Therapy

Stem cell based therapy has significant potential to treat various diseases including primary and metastatic cancers. Tamura and co-workers reported previously showed that un-engineered human and rat UCMSC significantly attenuated the growth of multiple cancer cell lines *in vivo* and *in vitro* through multiple mechanisms [63,64]. Intrinsic stem cell-dependent regulation of cancer growth, potential mechanisms involved in this unique biological function, delivery of exogenous anti-cancer agents, and the potential for clinical applications were discussed in a previous paper [65]. Since naive UCMSC have the intrinsic ability to secrete factors that can result in cancer cell growth inhibition and/or apoptosis *in vitro* and *in vivo*, they have many advantages for cell-directed cancer therapy. The mechanisms by which naïve UCMSC attenuate tumor growth have yet to be fully clarified, however, two potential mechanisms have been suggested [65]. The first potential mechanism is production of multiple secretory proteins that induce cell death of cancer cells and cell cycle arrest. This suggests that UCMSC stimulate caspase activities and arrest the cell cycle even in the absence of direct contact with cancer cells [43,66]. In addition, microarray analysis of rat UCMSC revealed over-expression of multiple tumor suppressor gene [65]. The second potential mechanism is the enhancement of an immune reaction to cancer cells. Immunohistochemistry revealed that the majority of infiltrating lymphocytes in rat UCMSC-treated tumors were T cells. The treatment of rat UCMSC apparently increased CD8(+) T cell infiltration throughout the tumor tissue [64]. Although these results contradict results described above which show the low immunogenicity of human UCMSC, the immunogenicity of UCMSC in tumor bearing animals may be dependent upon the microenvironment of UCMSC and tumor cells.

The homing ability of stem cells seems to be mediated by the interaction of cytokines/growth factors and their receptors. Large amounts of various cytokines and growth factors are secreted by tumor cells. Since UCMSC and other MSCs express various cytokine and growth factor receptors on their surface, they are likely to migrate towards cytokine/growth factor production sites by sensing these cytokine gradients [65]. Due to the over-expression of IL-8 receptor and CXCR, UCMSCs have a greater capacity to migrate towards tumor than BM-MSCs. It has also been demonstrated that these cells can be engineered to express cytotoxic cytokines before being delivered to the tumor and can be preloaded with nanoparticle payloads and attenuate tumors after homing to them [67,68]. Human UCMSC engineered to express INF-β produced sufficient amounts of INF-β to induce death of human breast adenocarcinoma cells and bronchioloalveolar carcinoma cells *in vitro* and *in vivo* [41,68]. Thus, the INF-β-human UCMSC could also be a new therapeutic modality for the treatment of various cancers. Among many tissue-originated multipotent stem cells, UCMSC may be suitable for allogenic transplantation as a therapeutic tool due to their abundance, low immunogenicity, lack of CD34 and CD45 expression, and simplicity of the methods for harvest and *in vitro* expansion. The homing ability to inflammatory tissues, including cancer tissues, and tumoricidal ability of UCMSC further confers upon these cells the potential for targeted cancer therapy.

4.2. Liver Disease

Cell therapy has also emerged as an attractive alternative to orthotopic liver transplantation for the treatment of liver disease. WJ-MSCs have demonstrated a potential to differentiate into endodermal lineage, including hepatocyte-like cells. The *in vitro* and *in vivo* use of UCMSCs for liver cell therapy has been described [69]. UCMSCs represent a very attractive cell source for treatment of liver disease as they display several hepatic markers characterizing the sequential steps of liver development. Moreover, *in vivo* experiments showed that after transplantation of undifferentiated UCMSCs in the liver of SCID mice with partial hepatectomy, the engrafted cells expressed human hepatic markers such as albumin and AFP, after 2, 4, and 6 weeks following transplantation. This strongly suggests that UCMSCs could be of great interest for the regenerative medicine approaches in liver disease [70]. Interestingly, a different study suggests a supportive role of undifferentiated UCMSCs in rescuing injured liver functions and reducing fibrosis *in vivo*. This study supports the hypothesis that, even in the absence of an actual transdifferentiation process, UCMSCs could exert a supportive action in increasing the functional recovery of recipient livers, perhaps stimulating the differentiation of endogenous parenchymal cells and promoting degradation of fibrous matrix [71]. In addition, their differentiation ability to hepatic lineage can be enhanced *in vivo* and *in vitro* after culture with hepatogenic factors. In treating liver cirrhosis, UCMSCs have properties of anti-inflammatory and anti-fibrosis by endogenous secreted factors such as metalloproteinases. This ability of UCMSCs to differentiate into hepatocyte-like cell warrant further investigations designed to better understand that cells can repopulate and rescue the liver function.

4.3. Cardiovascular Diseases

The therapeutic potential of WJ for cardiovascular tissue engineering has been suggested [72]. Because surgical treatment using non-autologous valves or conduits have distinct disadvantages including obstructive tissue ingrowths and calcification of the implant [73,74], cardiovascular fetal tissue engineering focuses on the *in vitro* fabrication of autologous, living tissue with the potential for regeneration of heart muscle. The general concept of WJ-MSCs based cardiovascular tissue engineering has been validated in large animal studies [75]. In brief, completely autologous, living trileaflet heart valves generated using human WJ-MSCs have been successfully implanted in growing sheep models for up to 20 weeks. These valves showed good functional performance as well as structural and biomechanical characteristics strongly resembling those of native semilunar heart valves. In comparative studies of various cell sources for cardiovascular tissue engineering, UC stem cell represent an attractive, readily available autologous cell source for cardiovascular tissue engineering offering the additional benefits of utilizing juvenile cells and avoiding the invasive harvesting of intact vascular structures [6]. Recently, a 3D aligned microfibrous myocardial tissue construct cultured under transient perfusion was introduced [76]. The goal of this study was to design and develop a myocardial patch to use in the repair of myocardial infarctions or to slow down tissue damage and improve long-term heart function. The basic 3D construct design involved two biodegradable macroporous tubes, to allow transport of growth media to the cells within the construct, and cell seeded, aligned fiber mats wrapped around them. The microfibrous mat housed WJ-MSCs

aligned in parallel to each other in a similar way to cell organization in native myocardium. The 3D construct was cultured in a microbioreactor by perfusing the growth media transiently through the macroporous tubing for 14 days. Experimental data confirmed that 3D constructs from static and perfused cultures enhanced cell viability, uniform cell distribution and alignment due to nutrient provision from inside the 3D structure. Experimental results during the last decade have shown that WJ-MSCs have great potential in tissue engineering, in which one of most promising directions is cardiovascular tissue engineering [72]. Despite knowledge of their advanced characteristics and first reports of successful pre-clinical and clinical applications, WJ-MSCs require further study to determine their clinical limitations and establish realistic clinical protocols. For example, replacements currently applicable in scaffold-based tissue engineering are mostly based on foreign materials, such as natural, synthetic or hybrid polymers. This results in a lack of growth and remodelling and carries the risk for thrombo-embolic complications and infections. Possible problems concerning these systems are systemic toxicity, growth limitation, differentiation and function restraints, incorporation barriers and cell or tissue delivery difficulties. Thus, the development of compatible biomaterials that do not mitigate WJMSC regenerative- and immuno-modulatory-potential is necessary [72]. In addition, because long term survival of the stem cells in the host tissue and establishment of treatment regimen are critical issues which still hamper broad clinical applications of WJ-MSCs, the establishment of relevant clinical criteria for isolation, characterization, long-term cultivation, and maintenance of human MSCs is necessary for the successful use of WJ-MSCs in regenerative medicine.

4.4. Cartilage Regeneration

Cartilage is a specialized connective tissue which has poor regeneration and self-repair capacity *in vivo*. Traumatic injury or autoimmune processes are among the main causes of cartilage damage and degeneration, for which new hope comes from tissue engineering using stem cells which have undergone chondrocyte-like differentiation. To this end, *in vitro* and *in vivo* data on the use of perinatal stem cells, in particular WJ-MSC, for regenerative medicine aimed at cartilage repair and regeneration have been reported [77]. UCMSCs are able to differentiate into chondrocyte-like cells if cultured in a supplemented medium. Analysis of the chondrogenic potential of WJ-MSCs showed they have the multipotential capacity and their chondrogenic capacity could be useful for future cell therapy in articular diseases [78]. Wang *et al.* demonstrated that seeding density of WJ-MSCs in poly-glycolic acid (PGA) scaffolds, in the presence of chondrogenic medium, had important effects on their chondrogenic potential [79]. This study demonstrated the potential for chondrogenic differentiation of WJ-MSCs in three-dimensional tissue engineering; higher seeding densities better promoted biosynthesis and mechanical integrity, and thus a seeding density of at least 25 million cells/mL is recommended for fibrocartilage tissue engineering with umbilical cord mesenchymal stromal cells [79]. Chondrogenic differentiation of WJ-MSCs can also be enhanced when cultured on nanofibrous substrates with a sequential two cultures medium environment. Moreover, WJ-MSCs are able to upregulate the production of hyaluronic acid and GAGs, as well as the expression of key genes as SOX9, COMP, Collagen type II and FMOD [80]. Because osteochondral tissue consists of cartilage and bone, cell sources and tissue integration between cartilage and bone regions are critical to successful osteochondral regeneration. Recently, Wang *et al.* developed a supportive structure which

mimics native osteochondral tissue [81]. In this study, WJ-MSCs were introduced to the field of osteochondral tissue engineering and a new strategy for osteochondral integration was developed by sandwiching a layer of cells between chondrogenic and osteogenic constructs before suturing them together. Two groups of WJ-MSCs were seeded in different poly-L-lactic-acid (PLLA) scaffolds with chondrogenic and osteogenic medium respectively for 3 weeks. After this period of time, chondrogenic and osteogenic constructs were sutured together surgically to create four different osteochondral assemblies. Histological and immunohistochemical staining, such as for glycosaminoglycans, type I collagen and calcium, revealed better integration and transition of the matrices between two layers in the composite group containing sandwiched cells as compared to other control composites. These results suggest that hUCMSCs may be a suitable cell source for osteochondral regeneration, and the strategy of sandwiching cells between two layers may facilitate scaffold and tissue integration [81]. In short, WJ-derived cells are promising cellular source for cartilage repair due to both their differentiation and immunomodulatory properties. WJ-MSCs have been demonstrated to successfully differentiate into cells resembling mature chondrocytes. Moreover, their peculiar features of low innunogenicity and their potential to induce immune tolerance in the host justify the efforts for their use in osteoarthritis, rheumatoid arthritis and other disease settings. The high variability of cell sources, the need for scaffolds and matrixes, and the administration of several combinations of growth factors necessitates further research to optimize this cellular therapy approach and translate the results obtained from bench to clinic for cartilage repair.

4.5. Peripheral Nerve Repair

Many therapeutic approaches have been used in an attempt to restore neural function after PNS injury. Recent tissue engineering studies have focused on the development of bioartificial nerve conduits to guide axonal regrowth [82,83]. In this system, the bioartificial nerve conduit is placed between the nerve ends to enclose intervening gap, thereby allowing axons to regrow into the distal nerve segment. However, artificial nerve conduits are limited when the nerve gap is long. Schwann cells, one of the most important components of the peripheral glia that forms myelin, serve as a favorable microenvironment for the repair of damaged nerve fibers in the peripheral nervous system (PNS) [84]. As a rule, Schwann cells are crucial for PNS regeneration, even when artificial nerve conduits are used. Because isolation and expansion of Schwann cells from other peripheral nerve have limitations, many researchers have focused on MSCs from various types of tissues. The induction system for differentiating Schwann cells from BM-MSCs was first reported by Dezawa *et al.* in 2001 [85]. Recently, UCMSCs were shown to differentiate into Schwann cells capable of supporting neural regeneration and constructing myelin [86,87]. Transplantation into rat transected sciatic nerve showed that the human UC-Schwann cells maintained their differentiated phenotype *in vivo* after transplantation and contributed to axonal regeneration and functional recovery. Another group demonstrated that UC-Schwann cells differentiated from WJ produced neurotrophic factors such as NGF and BDNF [88,89]. These findings indicated that UC-Schwann cells are a viable alternative to native Schwann cells and may be applied to cell-based therapy for nerve injuries. Given the intrinsic ability of activated Schwann cells to promote axonal regeneration *in vivo*, UCMSC can be used to successfully derive mature Schwann cells for the regeneration of peripheral nerve. Schwann cells also support axonal

regeneration, construct myelin, and contribute to functional recovery in a spinal cord injury model. In addition to WJ, Schwann cells can be differentiated from MSCs harvested from other sources, such as BMSCs, UC-MSCs, and ADSCs. In the end, a vis-à-vis comparison among these many MSC sources can reveal the potential of WJ-derived MSCs for therapeutic application to spinal cord injury [87].

Along this line of investigations, efforts to maximize the isolation and differentiation of stem cells derived from WJ have utilized studies designed to optimize cell harvest protocols, such as the use of oxygen concentration and plating density [90]. Such standardized isolation protocols would permit the expansion and maintenance of colony forming unit-fibroblast (CFU-F). Previous work reported that low plating density and/or exposure to 5% oxygen *vs.* 21% oxygen increased proliferation rate and enhanced expansion of MSCs. Recently, the effects of both plating density and oxygen concentration on MSCs derived from WJ have been evaluated [90]. Reducing oxygen concentration from 21% (room air) to 5% during expansion increased cell yield and maintained CFU-F, without affecting the expression of surface markers or the differentiation capacity of WJ-MSCs. The proposed mechanism is that reducing oxygen concentration in culture up-regulates hypoxia inducible factors (HIFs) and downstream effects from HIF activation include increased cell proliferation and maintenance of CFU-F, perhaps by affecting telomerase. In addition, reducing plating density from 100 to 10 cells/cm^2 increased CFU-F frequency. Therefore, plating density and oxygen concentration are two important variables that affect the expansion rate and frequency of CFU-F of WJ-MSCs. These results suggest that these two variables are key stem cell isolation factors to produce different input populations for tissue engineering or cellular therapy.

4.6. Cardiac Differentiation of Human WJ-Derived Stem Cells

Since undifferentiated MSC tend to spontaneously differentiate into multiple lineages when transplanted *in vivo*, the developmental fate of transplanted BM-MSCs is not restricted by the surrounding tissue after myocardial infarction. It is possible that such uncommitted stem cells undergo maldifferentiation within the infracted myocardium with potentially life-threatening consequences [91]. Therefore, it was postulated that a certain cardiac differentiation of stem cells prior to transplantation would result in enhanced myocardial regeneration and recovery of heart function [92,93]. In this context, initiating the transformation of stem cells into a cardiomyogenic lineage is accomplished by culturing them in defined culture conditions. WJ-MSCs can be induced toward heart cells; after 5-azacytidine treatment for 3 weeks, WJCs expressed the cardiomyocyte markers, cardiac troponin I, connexin 43, and desmin, and exhibited cardiac myocyte morphology [94]. In addition, oxytocin, embryo-like aggregates and several growth factors like transforming growth factor-β1 (TGF-β1), PDGF and basic fibroblast growth factor (bFGF) are used to induce myocyte differentiation of various stem cell types [95–97]. The expression levels of oxytocin are higher in developing hearts than in adult hearts suggesting that oxytocin may be involved in cardiomyocyte differentiation [98]. A variety of protocols of cardiac differentiation designed for different stem cell types have been published [97]. One such study showed that cardiac differentiation of UCMSC was driven by cell treatment with 5-azacytidine, oxytocin as well as by forming of "embryoid bodies" [97]. The morphological and immunocytochemical analysis of cardiac differentiated UCMSC (cUCMSC) with an extensive panel of cardiac markers showed that oxytocin is a more potent inducer of cardiac differentiation than

5-azacytidine and the forming of "embryoid bodies". In conclusion, comparative immunocytochemical analyses revealed that WJ-MSCs can be differentiated into cardiomyocyte-like cells with oxytocin being the most efficient differentiation agent. Very recently, a comparison study reported the long-term therapeutic effect of MSC from two different sources (adult bone marrow or Wharton's jelly from umbilical cord) following MI in a rat model [99]. A significant improvement in ejection fraction was seen in animals that received MSCs in time points 25 to 31 wks after treatment. In addition, Wharton's jelly MSCs were co-cultured with fetal or adult bone-derived marrow MSCs to investigate MSCs' cardiac differentiation potential. When Wharton's jelly MSCs were co-cultured with fetal MSCs, and not with adult MSCs, myotube structures were observed in two-three days and spontaneous contractions (beating) cells were observed in five-seven days. Taken together, these results suggest that MSCs administered 24–48 h after MI have a significant and a strong beneficial effect lasting longer than 25 weeks after MI; additionally, WJCs may be a useful source for off-the-shelf cellular therapy for MI.

The easy accessibility and the ability of UCMSC to differentiate into cells with characteristics of cardiomyocytes render UCMSC an attractive candidate for cell based therapies and cardiac tissue engineering. The next step is to show whether UCMSC, as well as WJ-derived stem cells, possess functional properties of cardiomyocytes in order to fully assess their utility for cardiac repair.

5. The New Research Frontiers in WJ Research

5.1. Clonal MSCs

A rich source of human MSCs was found in the perivascular region of the human UC which called HUCPVCs [24,100,101] which has enabled the first robust single cell clonal confirmation of a hierarchy of MCS differentiation [102]. The isolation of a nonhematopoietic (CD45−, CD34−, SH2+, Thy-1+, CD44+) HUCPVC population [24] may represent a significant source of cells for allogeneic MSC-based therapies due to their rapid doubling time, high frequencies of CFU-F and CFU-osteogenic subpopulation, and high MHC−/− phenotype. HUCPVCs show a similar immunological phenotype to bone marrow-derived MSCs (BM-MSCs) and present a non-hematopoietic myofibroblastic MSC phenotype (CD45−, CD34−, CD105+, CD73+, CD90+, CD44+, CD106+, 3G5+, CD146+) [103]. In addition to robust quinti-potential differentiation capacity *in vitro*, HUCPVCs have been shown to contribute to both musculo-skeletal and dermal wound healing *in vivo* [103]. Similar clonal expansions of WJ-derived stem cells will provide a well-defined set of stem cells allowing consistent validation and replication of studies that could enhance successful translation of laboratory studies of WJ for therapeutic applications.

5.2. Use of Magnetic Resonance Imaging in Contrast Labeled-UC Stem Cells

A recent study reported the isolation of cells from the intervascular and perivascular portion of UCM and compared these cell lineages by characterization of their specific marker expression patterns, capacity for self-renewal and potential to differentiate into multiple lineages [104]. The cells isolated from the intervascular portion showed faster doubling times than cells from the perivascular portion (which are probably more highly differentiated). Cells from both portions expressed MSC mRNA markers (CD29, CD105, CD44, CD166) and were negative for CD34 and MHC-II. Osteogenic,

adipogenic, chondrogenic and neurogenic differentiation were confirmed by specific staining and gene expression. Another aim of this study was to investigate their labeling efficiency of MSC with magnetic resonance contrast agents. To investigate this, pre-clinical experiments involving labeling of cells with magnetic resonance contrast agents (superparamagnetic iron oxide particles-SPIO-and manganese chloride) and the subsequent *in vitro* study of these were conducted. Both contrast agents were found to provide simple, robust and safe methods to label cells; nevertheless, SPIO-labeling method has higher sensitivity. The SPIO labeling procedure proved to be an efficient and non-toxic tool that merits further investigation and the possible development of *in vivo* studies for clinical applications. Such studies will not only provide evidence of stem cell migration and deposition to injured and non-injured tissues, but will also offer insights on mechanisms of action of cell therapy.

6. Conclusions

Altogether, these studies offer authoritative views on phenotypic markers and therapeutic potential of WJ-derived stem cells. We provide insights on gaps in knowledge for the cells' biological properties and translational applications. Cognizant of the many tissue sources of stem cells, further investigations on the advantages and limitations of WJ will reveal their optimal transplant regimens that are tailored for specific diseases.

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Conflict of Interest

The authors declare no conflict of interest.

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Prevalence, demographics, and complications of cleft palate surgery☆

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ABSTRACT

Introduction: Current published data on the demographics of cleft lip and palate is sparse and differs intranationally in reported incidence, demographics, and complication rates, making accurate local data both valuable and useful. We investigate the prevalence, demographics, and complications of cleft palate correction surgery in the inpatient setting over a 15-year period.
Methods: A retrospective review of The California Hospital Discharge Data sets of all pediatric patients who underwent cleft palate repair or cleft palate revision from 1997 to 2011. Children's hospitals (CHs) were analyzed as a separate group. For each record, age, gender, ethnicity, length of stay, total charges, principal payer, complications, and disposition were analyzed.
Results: 10,450 correction surgeries were performed during 1997–2011. This was an annual case-volume of 697 and annual population-adjusted rate of 2.0, neither of which changed over time ($p = 0.9$ and 0.06, respectively). Of all surgeries, 21.5% were revisions, 48.3% were performed in CHs, 56.2% were performed on males, and 65.5% were performed on Caucasians. The median length of stay was 1 day, which did not change over time ($p = 1.0$). The median total charges increased from $9.074 to $35,643 over the studied period ($p < 0.001$). Admission to CHs was associated with shorter stay (1–3 days vs. 1–4 days) and higher total charges ($15,560 vs. $13,242; both $p < 0.001$). Complications occurred in 393 (3.8%) of the surgeries. This percentage did not change over time ($p = 0.2$). The most common complication was fistula/abscess/infection, which occurred in 159 cases (1.5%). Respiratory complications requiring ventilation occurred 66 cases (0.6%). Complications were more common in CHs (4.8% vs. 2.8%; $p < 0.001$). Mortality rate was <0.1%.
Conclusions: Our study constitutes the entire surgical cohort within a state, allowing for an accurate representation of the true perioperative complication rate of these procedures. The prevalence, demographics, and outcomes of the cleft palate correction surgery have remained unchanged during 1997–2011. Collectively, our data suggest that primary and secondary palatoplasty present low perioperative risk.

1. Introduction

Cleft palate (CP) and cleft lip (CL) both result from the failures of frontonasal and maxillary prominence fusion during development and can occur together (CL + P) or in isolation. Although these two processes are often continuations of the same process, joint CL + P is considered epidemiologically and etiologically different from isolated CP [1]. Globally, the incidence of CL + P varies between different ethnicities: from 0.3 per 1000 in African American populations to 2.1 per 1000 in Japanese populations [2]. In contrast, the incidence of isolated CP is racially homogenous with a reported incidence of 0.5 per 1000 births [1,3]. Currently, the number of children born with congenital orofacial defects is now greater than neural tube defects or Down syndrome and the average lifetime medical cost per child has increased over time to an estimated $100,000 [3,4]. We focus our investigation on cleft palate repair and revision.

Previously published data in regards to the demographic data and complications associated with cleft palate surgery have historically consisted of single center case series or national surveys [5,6]. The largest study has been a quarter million surgeries by the Smile Train organization providing surgical cleft

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lip/palate treatment in developing countries [7]. There is a paucity of data on the trends and complication rates of CP surgeries in the recent literature. Even within countries, collected reports differ in reported incidence, demographics, surgical techniques, and complication rates, making accurate CL/CP data from large datasets both valuable and useful [3,5]. Recently, new access to hospital databases has allowed in-depth analysis of multicenter regional trends. By analyzing information from the California Hospital Discharge Database, we were able to obtain a broad based, multicenter assessment of demographic, prevalence, and inpatient complication rates of primary CP surgery and CP revision surgery.

Palate surgeons have long been wary of the complications related to performing palatoplasty. The surgery itself is very invasive, often involving the elevation of large mucoperiosteal flaps pedicled only on the greater palatine arteries. Meanwhile, the surgical wound is bathed in saliva and the oral flora. In addition, the airway becomes compromised after surgery due to swelling from oral retractors, and the physical narrowing of the palate itself after surgery. The techniques used for palatoplasty have changed within the past 15 years including the adoption of the Sommerlad-type radical intravelar veloplasty and the Furlow double opposing Z-plasty techniques, a trend toward one-stage palatoplasty repair, and the debated use of lateral releasing incisions to minimize the rate of fistulae formation [1,8–11]. Additionally, there is still much disagreement on the ideal timing of intervention, and ideal CP treatment strategy including pre and post-surgical care [12,13].

Post-operatively, children are usually observed as inpatients for 24 h after surgery to ensure adequate oral intake, pain control and a stable airway. Published data regarding the perioperative complications of cleft palate surgery have been from single center case series [6]. Meanwhile, other studies that look at cleft palate complications focus on the long term risk for oronasal fistula and velopharyngeal insufficiency, and not on the immediate perioperative risks [14]. Using data from the California Hospital Discharge Data set, our goal is to provide comprehensive data in regards to the risk of perioperative complications from multiple centers within the California region, as well as gain insight on the demographic trends of palate surgery.

2. Methods

2.1. Data source

The California Hospital Discharge Data sets are de-identified records of all inpatient hospital stays annually in the state of California. These data are gathered from all licensed hospitals including general acute care, acute psychiatric, chemical dependency recovery, and psychiatric health facilities. By definition, outpatient surgeries or surgeries performed in ambulatory surgery centers were not included. Data from the latest available data sets, 1997–2011, were obtained from the California Office of Statewide Health Planning and Development (OSHPD). Patient-level data such as demographics and diagnoses and procedures coded through *International Classification of Diseases, 9th edition, Clinical Modification* (ICD-9-CM). The data collection instruments and methodology for collection of data is available on the website of OSHPD [15]. The data set does not contain identifiable patient information thus approval for this study by our institutional review board was not required.

Pediatric records affiliated with ICD-9-CM procedural codes "27.62 Correction of cleft palate" and "27.63 Revision of cleft palate repair" were extracted. A record was considered pediatric if the associated age was less than 18 years old. The records with unknown age were excluded (8.1% of the records). Patients with Pierre Robin sequence were not excluded.

2.2. Variables and definitions

For each record, age, gender, ethnicity, length of stay, total charges, principal payer, complications, and disposition were analyzed. Institution type was classified as children's hospitals (CHs) versus non-children's hospitals (non-CHs) according to OSHPD list of licensed hospitals. Trends in total number of surgeries, population-adjusted surgery rates (number of surgeries per 100,000 California residents), percentage of revision surgeries, demographics, and complications were analyzed as well as compared between CHs and non-CHs. Data for the population of California were obtained from the Census Bureau [16].

Ethnicity was re-coded as Caucasian versus non-Caucasian (representing African-Americans, Native-Americans/Eskimo/Aleut, Asian/Pacific Islanders, and others). Length of stay by definition was counted as the number of days from admission to discharge. Total charges were calculated using all charges for services provided during hospital stay except for hospital-based physician fees. These charges were calculated and reported by the OSHPD and included, but were not limited to, daily hospital services, ancillary services and any patient care services. Prepayments (e.g. deposits and prepaid admissions) were not deducted from total charges. Principal payer was defined as the type of entity or organization expected to pay the greatest share of the patient's bill. The principal payer were re-coded as: (1) Medicare and Medi-Cal (Medicaid in California), (2) private insurance coverage (i.e. health maintenance organizations), and (3) other (this included self-pay, worker's compensation, county indigent programs, other government and indigent programs, research or courtesy patients where no payment was required, or unreported). Perioperative complications were identified using a series ICD-9-CM diagnosis and procedural codes (Table 1). Disposition status was re-categorized as: (1) routine discharge (patient's home or residence), (2) death, and (3) other-than-routine. Other-than-routine included any of the following: acute care within admitting hospital, other care within admitting hospital, long-term care within admitting hospital, acute care at another hospital, other care (not including long-term care) at another hospital, long-term care at another facility, residential care facility, prison/jail, left against medical advice, and home health service.

2.3. Statistical analysis

All variables were examined for whether they had a normal distribution. Mean ± standard deviation (SD) was calculated wherever appropriate. Nonparametric tests were used and median was calculated for age, total charges, and length of stay, which had positively skewed distributions. Linear regression analysis was used to evaluate the changes over time. Chi square test was used for analyzing categorical variables. Fisher's exact test was used where Chi

Table 1
List of ICD-9-CM diagnosis and procedural codes used to define complications.

Complications	ICD-9-CM codes
Venous thrombosis	453.4–453.42, 453.8, 453.9
Transfusion of packed erythrocytes	99.04
Hematoma/seroma	998.1–998.13
Seizure	436, 780.3–780.39
Wound infection/fistula/abscess	478.2–478.29, 478.79, 682.1, 686.9, 998.5–998.6
Pulmonary embolism	415.1–415.19
Pneumonia	480–486
Acute myocardial infarction	410–410.92
Sepsis	038–038.9, 995.9–995.94, 999.3–999.39
Postoperative shock	998.0
Airway compromise/ reintubation	96.7–96.72

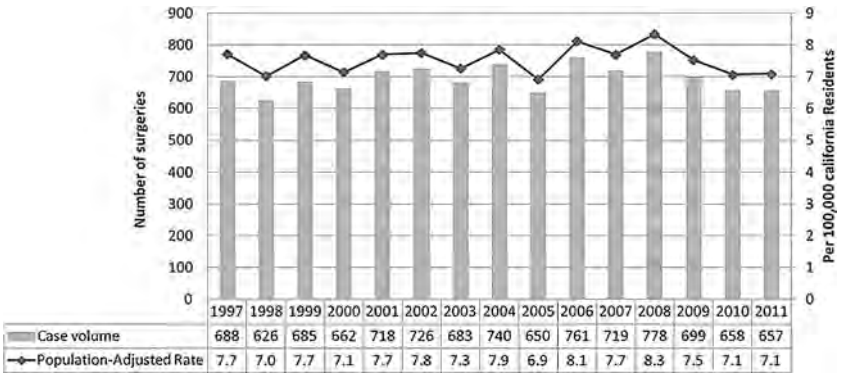

Fig. 1. Title: Annual case-volumes and population-adjusted rates for cleft palate surgeries in California. Description: the average annual case-volume/year = 696.7 surgeries, unchanged over time ($p = 0.9$). The average population-adjusted rate = 7.5 surgeries/100,000 California residents aged 0–17 years, also unchanged over time ($p = 0.06$).

square assumptions were not met. Mann–Whitney U Test was used to compare total charges and length of stay between the institution types. The PASW Statistics 18.0 (SPSS, Inc., Chicago, IL) was used for all data analyses. A p-value of less than 0.05 was considered significant.

3. Results

Overall, 10,450 surgeries for correction of cleft palate were performed during 1997–2011 across 107 hospitals in the state of California (Fig. 1). This translated into an average annual case-volume of 696.7 surgeries, which did not change significantly over time ($p = 0.9$). The average population-adjusted rate was 7.5 surgeries per 100,000 California residents aged 0–17 years. This rate also did not change over time ($p = 0.06$). The demographics of patients undergoing surgery also remained relatively unchanged; 56.2% on males and 43.8% on females ($p = 0.2$); 36.3% on <1 year olds, 35.6% 1–4 year olds, 15.8% on 5–9 year olds, 9.8% on 10–14 year olds, and 2.5% on 15–17 year olds ($p = 0.4$); 65.5% on Caucasians and 34.5% on non-Caucasians ($p = 0.9$).

On average, 21.5% were revision surgeries and 48.3% were performed in CHs (Table 2). Neither of these proportions changed over time ($p = 0.6$ and 0.1 respectively). The median length of stay was 1 day, which did not change over time ($p = 1.0$). The mean length of stay was 1.67 day $\pm$ 2.83 ($p = 0.6$). The median total charges increased from $7679 in 1997 to $31,645 in 2011 over the studied period ($p < 0.001$). The mean also increased from $9073 $\pm$ 7742 to $35,642 $\pm$ $27,831 during 1997–2011 ($p < 0.001$). Admission to CHs was associated with shorter stay (1–3 days vs. 1–4 days) and higher total charges ($15,560 vs. $13,242; both $p < 0.001$). Complications occurred in 393 (3.8%) of the surgeries (Table 3). This percentage did not change over time ($p = 0.2$). The most common complication was wound infection/fistula/abscess, which occurred in 159 cases (1.5%). Respiratory complications requiring ventilation occurred in 66 cases (0.6%). Complications were more common in CHs (4.8% vs. 2.8%; $p < 0.001$). Patient disposition profile of patients did not change over time ($p = 0.4$). Overall, 99.5% were routinely discharged to their home residence and 0.4% had other-than-routine disposition status. Mortality rate was <0.1% (4 cases).

4. Discussion

This data set provides an overview of the demographic, incidence, and risk of perioperative complications for cleft palate surgery. The incidence of cleft palate surgery remained stable throughout the study period. The gender distribution within our data was approximately 1:1 male-to-female and remained unchanged throughout the time course. Our data does not support the traditional 1:2 male-to-female ratio of cleft palate incidence

Table 2
Location, characteristics, and outcomes for cleft palate correction surgeries performed in California 1997–2011.

	Surgery type		Location		Complications	Median length of stay (days)	Median total charges	Expected source of payment		
	Primary	Revision	CHs	Non-CHs				Medicare or Medi-Cal	Private	Other
Overall	78.5%	21.5%	51.7%	48.3%	3.8%	1	$19,346	44.2%	41.8%	14.0%
1997	75.4%	24.6%	61.9%	38.1%	4.1%	1	$7679	49.1%	40.8%	10.0%
1998	79.7%	20.3%	59.7%	40.3%	3.4%	1	$8146	46.6%	43.5%	9.9%
1999	80.1%	19.9%	48.5%	51.5%	4.5%	1	$8558	43.6%	45.8%	10.5%
2000	80.8%	19.2%	54.1%	45.9%	5.6%	1	$8628	43.7%	47.0%	9.4%
2001	78.0%	22.0%	49.7%	50.3%	4.7%	1	$9662	41.4%	46.1%	12.5%
2002	77.3%	22.7%	45.5%	54.5%	3.7%	1	$10,961	43.0%	46.1%	10.9%
2003	77.2%	22.8%	49.5%	50.5%	4.0%	1	$12,830	40.0%	44.1%	16.0%
2004	77.3%	22.7%	50.3%	49.7%	3.6%	1	$15,817	43.9%	37.7%	18.4%
2005	78.9%	21.1%	49.5%	50.5%	2.8%	1	$16,881	41.8%	40.0%	18.2%
2006	77.9%	22.1%	51.6%	48.4%	2.8%	1	$19,874	47.0%	40.2%	12.7%
2007	79.4%	20.6%	53.1%	46.9%	2.5%	1	$21,642	48.0%	39.2%	12.8%
2008	78.8%	21.2%	50.5%	49.5%	4.0%	1	$24,175	43.8%	40.9%	15.3%
2009	79.1%	20.9%	51.5%	48.5%	3.6%	1	$27,775	45.2%	38.6%	16.2%
2010	78.0%	22.0%	53.6%	46.4%	3.3%	1	$26,642	42.2%	43.2%	14.6%
2011	79.5%	20.5%	47.6%	52.4%	4.0%	1	$31,645	43.8%	33.8%	22.4%
Adjusted R^2	0.05		0.11		0.13	N/A	0.95	0.05	0.35	0.51
p value	0.6		0.1		0.1	1.0	<0.001	0.6	0.01	0.002

Table 3
Most common complications in correction surgeries for cleft palate performed in California 1997–2011.

	Frequency	Percentage
All complications	393	3.8%
Wound infection/fistula/abscess	159	1.5%
Hematoma/seroma	96	0.9%
Airway compromise/reintubation	66	0.6%
Seizure	63	0.6%
Packed RBC transfusion	27	0.3%
Pneumonia	22	0.2%
Sepsis	5	<0.1%
Venous thrombosis	1	<0.1%
Pulmonary embolism	0	0%
Postoperative shock	0	0%
Acute myocardial infarction	0	0%

similar to other recent national data reviews [3,17]. The distribution between primary and revision cases did not change, suggesting that surgical techniques and indications for revisions remained stable over this time period. Notably, the median total charges increased dramatically representing a fourfold increase in cost over a 14-year span. This is far greater than the national increase in aggregate charges for orofacial cleft repairs of 110% over a ten-year period [3]. Additionally, recent data shows metropolitan areas both consistently charge more and increase cost progressively faster per year than non-metropolitan hospitals with no difference between teaching and non-teaching hospitals.

The complication rate in children's hospitals compared to non-CHs was significantly higher. A growing trend has been the centralization of CP repair from multiple areas to few high-volume centers. This consolidation has produced better clinical outcomes in the United States and other Asian and European countries [10,18,19]. CHs have historically had decreased lengths of stay and fewer complications but increased number of co-morbidities when compared to their non-CH counterparts [20]. In California, CHs function as high-volume centers and receive the bulk of complex palate repairs. While our data shows no change in case distribution between CHs and non-CHs from 1997–2011, the increased complication rate at CHs vs non-CHs may be explained by a bias toward operating on more complex cases at CHs. While our data is the first American study to show an increased complication rate in high-volume centers, further studies are needed to better describe complication rates of both CH and non-CHs in detail.

The perioperative risks of cleft palate surgeries have historically been difficult to assess. Previously published in the literature are case series from single institutions without assessment of overall risk from a large population group. The specific rate of perioperative complications, especially early complications, has been lacking. Deshpande et al. [10] recently reported early complications after cleft palate repair in a single center cohort of 6 surgeons, however their analysis of perioperative complications was limited to fistula formation and wound dehiscence. California with its large and diverse population can be considered a representative sample of the United States and the trends and rates reported in this study are more likely to provide accurate estimates for CP surgeries. However, the findings of this study are subject to several limitations. The complications reported here include only those that occurred during the same admission and therefore, emergency department visits or re-admissions in the perioperative period were not captured. This may mean that the true complication is higher than that which we found in our study. These datasets were dedicated to inpatient stays and thus do not include ambulatory surgeries. In addition, the diagnoses, procedures, and complications were defined using ICD-9-CM codes and therefore, were subject to entry bias. Finally, the complication rate calculated in this study is based on the definitions outlined in the methods section, which may differ from the criteria used in other studies.

The rate of perioperative complications in our study was an aggregate 3.8%, which was higher than the 2.8% rate of the 750 patient 2012 NSQIP data, but remains lower than numerous other rates quoted in the literature [10,21]. The highest single complication rate in our study was 1.5% from patients developing either a wound infection, fistula, or abscess. In comparison, other studies have shown much higher complication rates exclusively by fistulas [7,22]. Fistulas revealing themselves later in the postoperative period may explain this result. The relatively low rate of respiratory complications provides valuable incidence data for this feared complication. The low rate also allows the physician to provide a more accurate informed consent to parents prior to surgery. Additionally, the median length of stay of only one day remained stable throughout the time period studies. Combined, the data suggest primary and secondary palatoplasty present low perioperative risk.

5. Conclusion

This data set provides a thorough overview of the demographic, incidence, and risk of perioperative complications for cleft palate surgery in California, the most populous state in the United States. The incidence of cleft palate surgery remained stable throughout the study period with a gender distribution of approximately 1:1 male-to-female. The complication rate in children's hospitals compared to non-children's hospitals was significantly higher, likely due to a bias toward the higher volume of complex cases at children's hospitals. Collectively, our data suggest that primary and secondary palatoplasty present low perioperative risk.

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Conflict of interest

None.

Author contributions

Dr. Pham had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Dr. Mahboubi and Adam Truong contributed equally to this work.

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ORIGINAL ARTICLE

Improving the Evaluation of Alveolar Bone Grafts With Cone Beam Computerized Tomography

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Objective: **Cone Beam computed tomography (CBCT) is used increasingly as a replacement for periapical x-rays when evaluating alveolar bone grafting. The manufacturer's standard settings for dental imaging may not, however, represent the optimal settings for evaluating postoperative alveolar bone grafts. We examined the influence of exposure parameters on CBCT image quality to optimize the quality of CBCT images while reducing the radiation dose to the minimum level necessary to obtain adequate images.**

Methods: **A defect was created in a cadaver head to simulate an alveolar cleft, and the area was filled with a synthetic material to simulate an alveolar bone graft. Serial CBCT scans were acquired, systematically varying tube voltage and tube current settings from 72 to 96kV and 3 to 12mA. Region of interest analysis was undertaken, and image quality was evaluated by comparing the ratios of native alveolar bone to soft tissue and the ratios of synthetic bone graft to soft tissue and by assessing image noise.**

Results: **Twenty-one CBCT data sets were obtained. Reducing tube voltage (kV) resulted in increased contrast ratio between bone and soft tissue and between synthetic bone graft and soft tissue, with maximal contrast at values of 76 kV/11 mA, 72 kV/12 mA, and 72 kV/11 mA. Of these, the setting with lowest image noise was 76 kV/11 mA. This setting also resulted in a radiation dose of less than half of the manufacturer's recommended settings for the same scan volume.**

Conclusions: **There is potential to improve CBCT image quality significantly while dramatically reducing the radiation dose during postoperative examinations for alveolar bone grafting in patients with cleft lip and palate.**

KEY WORDS: *alveolar bone graft, cleft lip and palate, cone-beam computed tomography, radiation dose*

Alveolar bone grafting has been the standard of cleft care for the vast majority of cleft teams since the 1970s (Boyne and Sands, 1972). The procedure stabilizes the maxillary dental arch and periodontium and allows for the eruption of teeth into the grafted area. Moreover, it promotes the closure of oronasal fistulas, improves nasal symmetry, and permits orthodontic movement and placement of osseointegrated implants into the cleft area when indicated (Trindade et al., 2005). It is generally accepted that autogenous bone is the gold standard among the many different types of bone graft materials for hard tissue defect restoration in patients with cleft palate (Sivak et al., 2014). In order to overcome shortcomings of autogenous bone, however, allograft bone grafting (Macisaac et al., 2012; Sivak et al., 2014) and human bone morphogenic protein (Chin et al., 2005; Dickinson et al., 2008) have been advocated to treat patients with cleft.

The clinical use of noninvasive methods is crucial in assessing bone volume and quality after alveolar bone grafting. While conventional radiographs have been shown to have a number of limiting factors, such as distortion, a limited number of reliable landmarks, and superimposing structures (Van der Meij et al., 2001), the effectiveness of dental cone-beam computed tomography (CBCT) capability in assessing the trabecular bone parameters remains unclear (Quereshy et al., 2012).

Previous studies have shown that CBCT images of the upper jaw suffer from intensity inhomogeneity and truncated view artifact (Schulze et al., 2004; Loubele et al., 2006; Loubele et al., 2008; Hassan et al., 2010). However, CBCT images have been advocated for outcome assessment and follow-up, especially because they allow the visualization of cross-sectional images and the measure-

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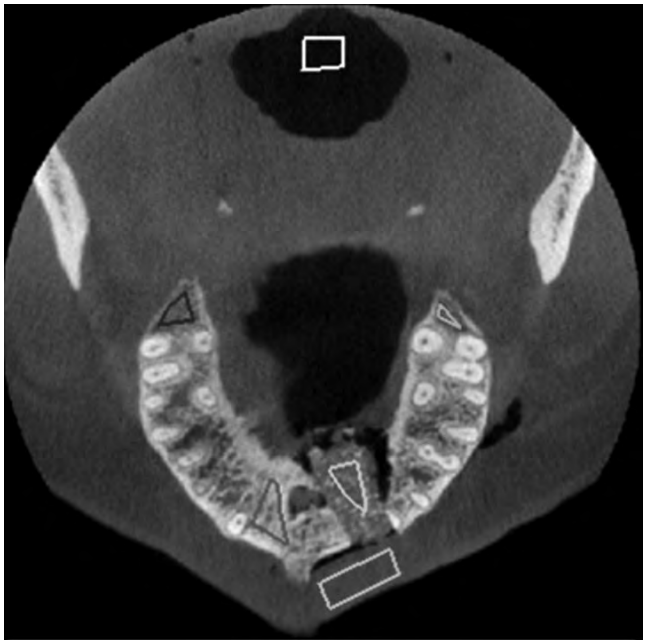

FIGURE 1 Illustration of the six regions of interest.

ment of depth and volume of the grafted bone (Oberoi et al., 2009; Trindade-Suedam et al., 2012; Wangsrimongkol et al., 2013).

In order to interpret hard and soft tissue density on CBCT images or to perform the image segmentations necessary for volumetric rendering, the computed tomography (CT) numbers (Hounsfield units [HUs]) should ideally represent the absolute physical radiodensity of the tissue. The x-ray attenuation represented by each voxel in a volume of interest is expressed by the CT number. However, unlike true HUs, which are based on an absolute scale, the CT numbers in a particular image vary between CBCT devices and with exposure factors (Molteni, 2013). Furthermore, in CBCT, the accuracy of gray levels is affected by scattering and inherent artifacts of the system (Parsa et al., 2013). Studies comparing the reliability, consistency, and accuracy of CT numbers generated by CBCT to HUs demonstrate concerns about the quantitative use of CT numbers in CBCT (Hua et al., 2009; Mah et al., 2010; Hohlweg-Majert et al., 2011; Molteni, 2013; Pauwels et al., 2013).

To date, the manufacturers of CBCT devices use different approaches for setting exposure parameters. As there is a balance between the diagnostic value of the scanned volume and the radiation exposure risk to the patient, there is a real need to select the ideal exposure settings in order to optimize image quality and to minimize radiation dose. This study aims to investigate how variations in CBCT scanning parameters affect measured CT numbers of different tissue densities in order to make the acquisition protocol as effective as possible to assess alveolar bone grafting in patients with cleft in our center.

Materials and Methods

Sample Preparation and Image Acquisition

A human cadaver head was obtained from the University of Texas Southwestern Medical Center Willed Body Program. An alveolar defect, similar to that seen in patients with cleft, was cut into the anterior part of the maxilla using osteotomes and an oral surgical burr drill. This area was then filled with a synthetic bone graft produced using a sponge impregnated by a resilient denture liner material (COE-SOFT, GC America Inc., Chicago, IL). In order to generate radiopacity in the synthetic bone, 2 mL of barium hydroxide was added to the dental material mixture, 8 mL liquid to 11 g powder. A CBCT scan was acquired using the manufacturer's recommended exposure settings of 96 kV and 11 mA for a female adult (ProMax 3D Max, Planmeca, Helsinki, Finland). Further CBCT scans were then acquired, initially decreasing the kV settings in 4-kV steps from 96 kV to 72 kV while maintaining a tube current of 11 mA, and then by varying the tube current in steps of 3 mA from 12 mA to 3 mA at 96 kV, 84 kV, 80 kV, and 76 kV. The acquisition volume was that of the manufacturer's recommended settings for maxilla and mandible acquisition and represented a cylinder 10 cm in diameter and 9 cm high with a 0.2 mm voxel size. Other settings, for example the number of frames and the gantry rotation, were the same for each acquisition. This gave 466 axial slices of 501×501 pixels for each scan.

Image Analysis

All data sets derived from CBCT were processed as Digital Imaging and Communications in Medicine (DICOM) 3.0 files and were analyzed using ImageJ v 1.39s (National Institute of Health, available at http://rsb.info.nih.gov/ij) and Matlab (The MathWorks, Inc., Natick, MA). The CBCT volumes were realigned to a reference volume (the manufacturer's settings). The CT numbers were compared by assessing the mean CT numbers inside six regions of interest (ROIs), defined within: the synthetic graft, the maxillary alveolus contralateral to the side of the synthetic graft placement, the posterior maxillary alveolus (right and left side), soft tissue of the upper lip, and the airspace of the nasopharynx. Each ROI, shown in Figure 1, was assigned a number (1 to 6) for the purpose of identification, the details of which can be found in Table 1. Images were normalized to the mean CT number of the soft tissue (ROI 1), and the mean CT numbers for the four regions of interest (ROIs 2 through 5) were compared across the multiple acquisition settings. The noise within the acquired images was evaluated by measuring the mean

TABLE 1 Mean CT Number for Each Region of Interest (ROI) and Acquisition Parameter

kV/mA	*ROI 1 Soft Tissue*	*ROI 2 Anterior Alveolar Bone*	*ROI 3 Right Posterior Alveolar Bone*	*ROI 4 Left Posterior Alveolar Bone*	*ROI 5 Synthetic Bone*	*ROI 6 Air Space*
96/12	855	1627	1048	1057	1114	168
96/11	895	1579	1051	1010	1099	180
96/9	898	1545	1035	1010	1099	210
96/6	891	1550	1055	1036	1107	214
96/3	897	1555	1065	1037	1115	229
92/11	899	1632	1074	1049	1135	182
88/11	911	1678	1098	1073	1164	194
84/12	918	1708	1115	1093	1181	214
84/11	922	1731	1114	1080	1197	206
84/9	917	1692	1110	1075	1182	232
84/6	923	1683	1102	1083	1188	259
80/12	939	1764	1165	1151	1225	224
80/11	945	1777	1151	1133	1235	230
80/9	939	1744	1151	1106	1220	243
80/6	942	1745	1140	1093	1219	271
80/3	950	1745	1189	1160	1215	290
76/12	955	1832	1193	1140	1264	246
76/11	945	1837	1186	1174	1272	250
76/9	955	1822	1183	1173	1269	262
76/6	948	1803	1189	1129	1255	263
72/12	975	1912	1212	1219	1315	273
72/11	976	1913	1234	1194	1314	272

signal within the ROI of the airspace of the nasopharynx (ROI 6).

Results

By varying acquisition parameters (kV and mA), 21 CBCT data sets were obtained. The radiation dose for each acquisition setting is detailed in Figure 2. Table 1 contains the pre-normalized CT numbers for each ROI at each acquisition parameter. ROI 6 in Table 1 demonstrates that the lowest tube current (3 mA) was associated with the highest image noise and that 72 kV was associated with the highest levels of CT number for the alveolar bone ROIs, likely because of the reduced bone penetrance at lower beam energies. The change in CT values within the ROIs were nonlinearly related to scan dose in that relatively large changes in radiation dose resulted in relatively small changes in gray value.

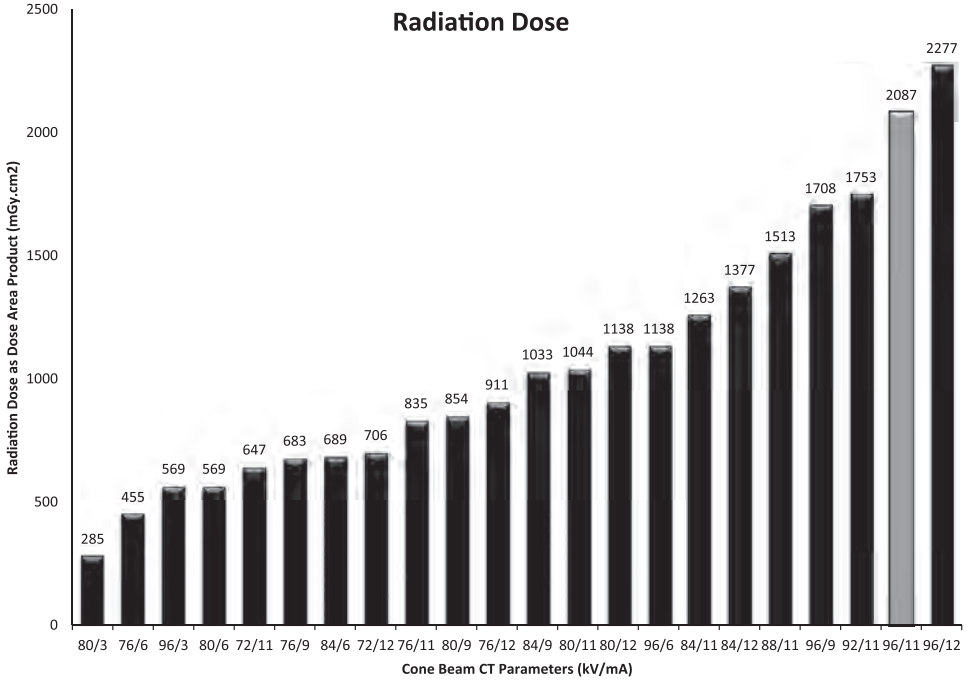

FIGURE 2 Graphical representation of the exposure dose (mGy.cm^2) for each of the tube parameters (kV/mA). The manufacturer's recommended settings are emphasized (96/11).

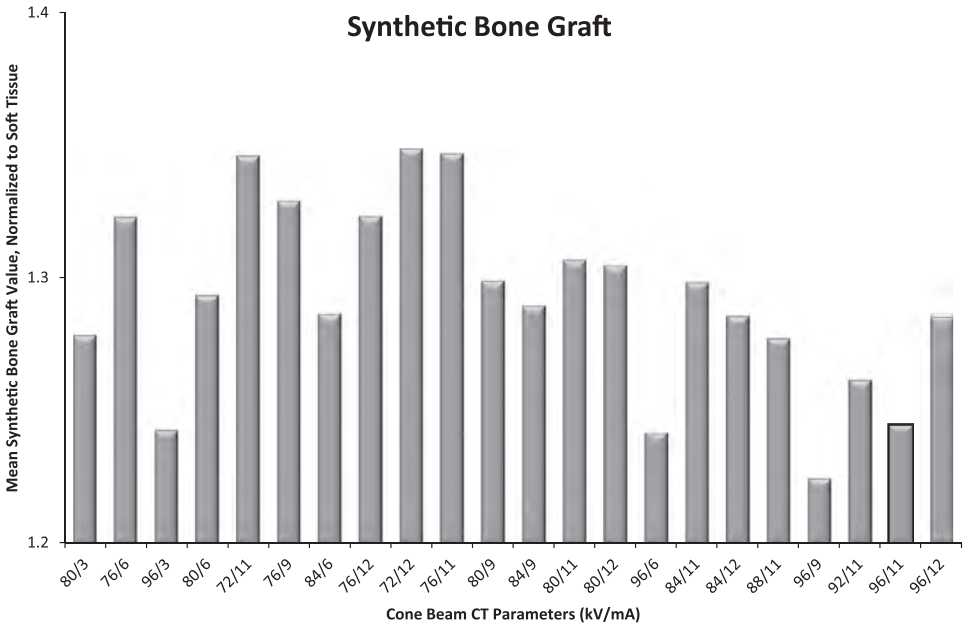

FIGURE 3 Graphical representation of the normalized CT numbers for ROI 5 (synthetic bone graft). The manufacturer's recommended settings are emphasized (96/11).

The normalized CT numbers for the synthetic bone graft in Figure 3 and natural anterior alveolar bone in Figure 4 are presented graphically, arranged by increasing radiation dose. This figure demonstrates that for both of these ROIs a reduction in the kV setting resulted in an increased contrast between the bone within the ROI and soft tissue, with maximal contrast at values of 76 kV/11 mA, 72 kV/12 mA, and 72 kV/11 mA. Of these three, there is the lowest noise at 76 kV/11 mA. This suggests that 76 kV/11 mA represents the optimal acquisition parameters to evaluate postoperative alveolar bone grafting, and it also results in a radiation dose decrease from 2087 to 835 mGy.cm^2.

Discussion

In this study, a human cadaver head containing a simulated alveolar cleft and a synthetic alveolar bone graft construct underwent multiple examinations of the maxil-

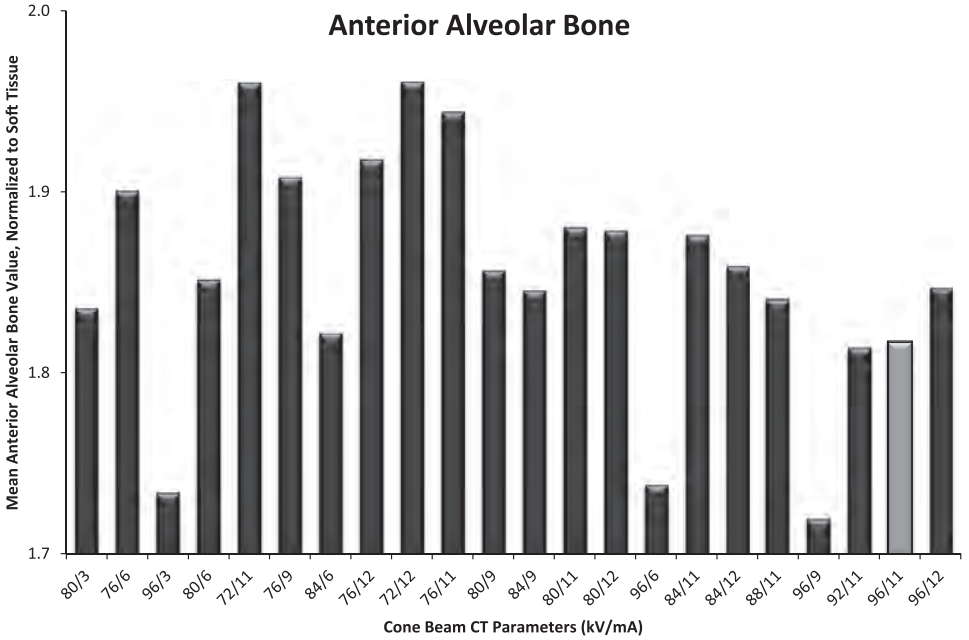

FIGURE 4 Graphical representation of the normalized CT numbers for ROI 2 (natural alveolar bone). The manufacturer's recommended settings are emphasized (96/11).

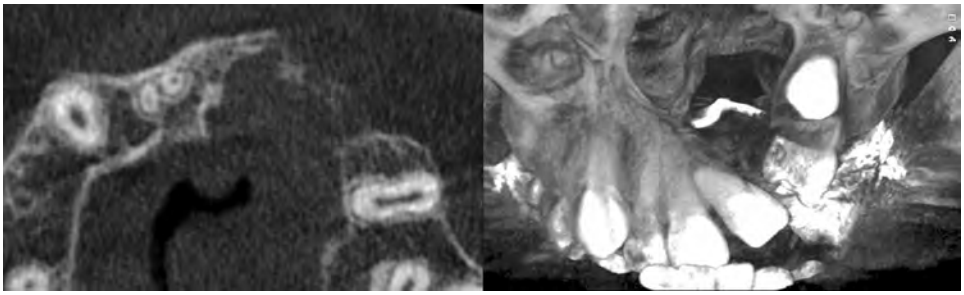

FIGURE 5 Example of a cone beam investigation at the manufacturer's recommended settings (96kV/11 mA).

lary region with variation of CBCT acquisition parameters in order to ascertain the optimal settings for evaluating alveolar bone grafting while delivering the lowest practical patient dose.

The synthetic bone graft was designed to be a model, as close as practical to an alveolar bone graft after 3 to 6 months of incorporation. Previous studies have used synthetic bone to assess the performance of CBCT devices. Amirlak et al. (2013) used polyvinyl siloxane to fill defects in dry skulls made by a burr drill in order to assess CBCT volumetric measurement. We considered using this material but concluded that as a solid, radiopaque material, its radiodensity would not effectively model an alveolar bone graft. Ho et al. (2013) used four densities of a cellular synthetic bone, mimicking the trabecular structure of cancellous bone (Sawbones, Vashon, WA), which were scanned in air with CBCT and micro-CT. We attempted to use this model but discovered that it was too radiolucent to be an effective model for our study, being considerably more radiolucent than the soft tissue of our cadaver. We concluded that we would need to create our own synthetic bone to achieve a model with a pseudo-trabecular structure and an appropriate level of radiopacity on CBCT: a radiodensity lower than that of native maxillary alveolar bone but higher than that of soft tissue. We used a sponge to represent the trabecular structure of cancellous bone and, to increase its density and reduce its compressibility; impregnated the sponge in a soft dental liner material while the material was wet and not yet hardened. We added barium hydroxide at differing concentrations and compared the models we had created to the native cadaveric bone and soft tissue, choosing a model that most accurately met our criteria for structure and radiodensity.

To better understand how our cone-beam unit works in a specified field of view (FOV), only one cadaver head was used to reduce bias from biological variation. The selected FOV (Ø10 × 9 cm) allows evaluation of the full lower and upper arches simultaneously and meets surgeons' and orthodontists' needs to evaluate bone grafts postoperatively and to plan any further orthodontic treatment.

It is well established that there is a proportional relationship between dose and tube current (mA), exposure duration, and degree of rotation of the gantry; in addition, there is a greater than proportional relationship between dose and tube voltage (kV). Reducing any of these parameters alone or in combination has the potential to reduce patient exposure (Lofthag-Hansen et al., 2008; Sur et al., 2010). Careful reflection on the characteristics of the technology used and individual patient imaging requirements can achieve significant dose reductions without compromising image quality. This principle of dose optimization is advocated in guidelines on use of CBCT by the European Academy of Dentomaxillofacial Radiology, SEDENTEXCT (Pauwels et al., 2012). The ProMax 3D Max allows changes in the acquisition parameters, varying kV from 96 to 66 and mA from 1 to 12. We started the image acquisition at the manufacturer's recommendations and then the kV/mA was progressively reduced. The lowest tube parameter was defined according to our subjective assessment of the presence of unacceptable noise to evaluate two-dimensional slices (3 mA). It is interesting that the highest level of contrast between soft tissue and synthetic bone graft, as well as between soft tissue and natural maxillary alveolar bone, was not found using the standard settings or the highest radiation dose. This illustrates how a better understanding of an individual CBCT unit can yield higher-quality images while also reducing the radiation exposure to the child during postoperative assessment of alveolar bone grafting and potentially for other craniofacial applications.

As suggested by Dawood et al. (2012), it is possible that the standard exposure parameters defined by manufacturers of CBCT apparatus are optimized for exporting data for three-dimensinal planning software, where low-noise data is the priority in order to construct a meaningful virtual model. While the human eye appears to be able to intelligently distinguish the cortical boundary of the jaw in a two-dimensional image, this process cannot be so easily achieved by three-dimensional modeling software.

About 10 years ago, CBCT was introduced for imaging the head and neck region and, until now, research has focused on applications of CBCT for several diseases. A future goal should be to find ways to optimize disease-related image quality while minimizing radiation dose. This current study aims to start closing this gap by improving the

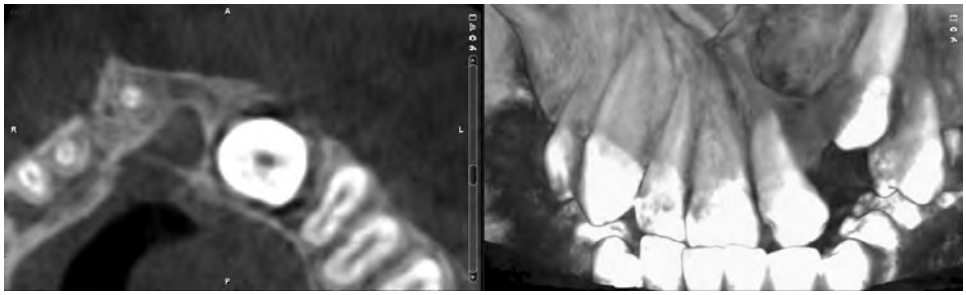

FIGURE 6 Example of a cone beam Investigation at our optimized settings (76kV/11mA).

imaging of alveolar bone grafting in the management of patients with cleft lip and palate.

While the settings of 76kV and 11mA represent the optimal image quality for the postoperative assessment of alveolar bone grafts on our CBCT device, these results may not be appropriate or optimal for other CBCT devices, or even to other imaging applications on our CBCT device. It is also unclear whether our synthetic bone graft construct truly represents the characteristics of an alveolar bone graft and whether a female human adult cadaver model is representative of a child undergoing alveolar bone grafting. To demonstrate a clinical improvement in the postoperative assessment of alveolar bone grafting, CBCT imaging of two patients is presented in Figures 5 and 6. Both patients were born with complete unilateral clefts of the lip and palate, underwent alveolar bone grafting by the same surgeon, and were under the same orthodontist's care. Figure 5 is a CBCT volume of a 12-year-old female patient 9 months after alveolar bone grafting using the manufacturer's recommended CBCT settings of 96 kV and 11 mA. Figure 6 is an 11-year-old male patient 8 months after alveolar bone grafting using our optimized settings of 76 kV and 11 mA. The improvement in contrast between soft tissue and alveolar bone, and especially between soft tissue and alveolar bone graft, is clearly visible in these figures.

In conclusion, there is potential to improve CBCT image quality while reducing the radiation dose to the patient very significantly during postoperative examinations for alveolar bone grafting in patients with cleft lip and palate.

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ORIGINAL ARTICLE

Cleft Palate Surgery: An Evaluation of Length of Stay, Complications, and Costs by Hospital Type

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Objective: **The purpose of this study was to assess length of stay (LOS), complication rates, costs, and charges of cleft palate repair by various hospital types. We hypothesized that pediatric hospitals would have shorter LOS, fewer complications, and lower costs and charges.**

Methods: **Patients were identified by ICD-9-CM code for cleft palate repair (27.62) using databases from the Agency for Health Research and Quality Healthcare Cost and Utilization Project Kids' Inpatient Database from 1997, 2000, 2003, and 2006. Patient characteristics (age, race, gender, insurer, comorbidities) and facility resources (hospital beds, cleft palate surgery volume, nurse-to-bed ratio, pediatric intensive care unit [PICU], PICU intensivist, burn unit) were examined. Hospitals types included pediatric hospitals, general hospitals, and nonaccredited children's hospital. For each hospital type, mean LOS, extended LOS (LOS $>$ 2), and complications were assessed.**

Results: **A total of 14,153 patients had cleft repair with a mean LOS of 2 days (SD, 0.04), mortality 0.01%, transfusion 0.3%, and complication $<$3%. Pediatric hospitals had fewer patients with extended hospital stays. Patients with an LOS $>$2 days were associated with fourfold higher complications. Comorbidities increased the relative rate of LOS $>$2 days by 90%. Pediatric hospitals had the highest comorbidities, yet 35% decreased the relative rate of LOS $>$2 days. Median total charges of $10,835 increased to $15,104 with LOS $>$2 days; median total costs of $4367 increased to $6148 with a LOS $>$2 days.**

Conclusion: **Pediatric hospitals had higher comorbidities yet shorter LOS. Pediatric resources significantly decreased the relative rate of LOS $>$2 days. Median costs and charges increased by 41% with LOS $>$2 days. Further research is needed to understand additional aspects of pediatric hospitals associated with lower LOS.**

KEY WORDS: *cleft palate surgery, complications, costs, hospital type, length of stay, safety*

Introduction

Cleft palate is a relatively common congenital anomaly in which the palatal shelves do not fuse during embryonic development (Wilkins-Haug et al., 2010). It occurs in 6.35/10,000 births in the United States, and 2651 children are born with cleft palate each year

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(Centers for Disease Control and Prevention, 2012). As with any surgery, palatoplasty has some inherent risks, and careful preoperative counseling about associated surgical risks is required (Stone et al., 2010). However it is difficult to quantify the average risks of primary palate repair because most of the literature is based on case series at academic institutions (DeMey et al., 1997). Yet in actual practice in the United States, cleft palate repair is performed in a variety of hospital settings.

Pediatric hospitals generally have more pediatric resources than do nonpediatric hospitals because they must meet children-centered service requirements to receive pediatric hospital accreditation by the National Association of Children Hospitals and Related Institutions (National Association of Children's Hospitals and Related Institutions, 2006). These additional resources provide both structural and process support to pediatric patients, and as a result, the American Pediatric Surgical Association recommends high-intensity infant surgery be performed at full-service children's facilities whenever possible (Birkmeyer & Dimick, 2009; Raval et al., 2010). It is not clear that there is also a need for increased pediatric resources for less intensive procedures such as palatoplasty.

This study examined a nationally representative sample of patients who have undergone palatoplasty to assess LOS together with charges and costs, as well as perioperative morbidity and mortality of cleft palate repair associated with this procedure. Hospital type and facility resources were included as variables that might influence the outcome metrics studied. We hypothesized that morbidity, mortality, length of stay, costs, and charges of palatoplasty would be lower in pediatric hospitals relative to nonpediatric hospital.

Methods

Data Source

Data were extracted from the Kids' Inpatient Database (KID) for 1997, 2000, 2003, and 2006. The KID is produced by the Healthcare Cost and Utilization Project (HCUP) with support from the Agency for Healthcare Research and Quality (AHRQ). For each year, this data set contained two to three million pediatric inpatient discharges from more than 2500 to 4000 U.S. community hospitals. The KID sample was weighted to give national estimates and compare hospital types on a national scale. We also used the American Hospital Association (AHA) Annual Survey Data for 2008. The AHA data set contained 1000 data fields on facility resources from 6500 hospitals published annually. These facility resources included organizational structure, personnel, hospital facilities and services, and financial performance.

Cohort

The cohort was identified using primary and secondary ICD-9-CM procedure codes for cleft palate surgery (27.62) accompanied by an ICD-9-CM diagnosis code for cleft palate (749.0). Cleft palate surgery revisions were excluded in the cohort by an additional code (27.63).

Study Variables

Patient Characteristics

Patient factors examined included age (in months and days), race, gender, estimated socioeconomic status using zip code income, and examined expected primary payor. These variables were defined in the KID data set. Significant comorbidities expected in this population were also identified: anomalies of skull and face bones (744.9, 756.0), congenital heart defects (745.0 to 745.9, 746.0 to 746.9), chromosomal abnormalities (758.0), and low birth weight (765.0 to 765.2).

Hospital Characteristics

The KID categorized hospitals as one of four types based on the National Association of Children's Hospitals and Related Institutions (NACHRI) classification: accredited pediatric hospitals (pediatric hospitals), children's specialty hospital (CSH), children's unit in a general hospital (general hospitals), and nonaccredited pediatric hospital (NAPH). Nonaccredited pediatric hospitals identified themselves as pediatric hospitals but were not accredited as a pediatric hospital by NACHRI whereas, pediatric hospitals were accredited. More than 99% of patients underwent cleft palate surgery in pediatric hospitals, general hospitals, or NAPH. As a result, CSH was not included in our analyses. For each hospital type, we assessed number of hospitals, mean cleft palate surgeries performed per hospital per year, percentage of pediatric discharges, teaching status, location, size, and region. The KID was merged with the AHA Annual Survey Data for 2008 using unique hospital identifiers.

For all hospitals, we evaluated facility-level factors. Specific hospital characteristics were identified on previous studies to be associated with improved surgical outcomes using the 2008 AHA Annual Survey Database (Hartz et al., 1989; Rosenthal et al., 1997; Aiken et al., 2002; Ghaferi et al., 2010). These hospital characteristics included high hospital technology defined as a hospital performing open heart surgery or organ transplantation (yes/no), membership in the Council of Teaching Hospitals of the American Association of American Medical Colleges (yes/no), nurse-to-bed ratio, hospital size, and average daily census >50% capacity (Ghaferi

TABLE 1 ICD-9-CM Codes for Complications Adapted from Agency for Healthcare Research & Quality Pediatric Quality Indicators

Complication	*ICD-9-CM Diagnosis Code*
Airway/respiratory failure	518.81, 96.7, 96.71, 96.72
Hemorrhage/hematoma	998.11, 998.12
Pneumonia	481, 482, 482.1, 482.9, 483.8, 482.81, 486, 997.31, 518.0
Postoperative infection	998.59, 998.51, 730.08, 730.09, 730.98, 730.99, 682.0, 528.3
Transfusion	99.00, 99.01, 99.03, 99.04, 99.05, 99.06, 99.07
Wound disruption	998.3, 998.31, 998.32

et al., 2010). These hospital characteristics were adapted for our procedure of interest through consultation with clinical and research experts. The adapted hospital characteristics included number of hospital beds, volume of cleft palate surgeries, nurse-to-bed ratio, presence of pediatric intensive care unit (PICU), PICU intensivist, and burn unit. For every hospital, the volume of cleft palate surgeries was determined by summing the number of surgeries performed. The nurse-to-bed ratio was calculated using a well-defined formula for determining the number of nurse hours per patient day: full-time equivalent nurses $\times$ 1768/adjusted patient days (Ghaferi et al., 2010). These two variables, full-time equivalent nurses and adjusted patient days, were present in the AHA database.

Outcomes

Our primary outcome of interest was average length of stay (LOS) because it is an important metric of efficient care (Ruttimann et al., 1998). Shorter hospital stay reflected uncomplicated postoperative care as well as reduced hospital-acquired infection risks, costs to the patient and health system, and psychosocial stress. Length of stay was a proxy for a routine (short) versus complicated (long) hospital course (Horn et al., 1991; Thomas et al., 1997; Ruttimann et al., 1998).

Additional measures included the AHRQ Pediatric Quality Indicators (PDI; Table 1). These measures were constructed and standardized by the AHRQ and represent safety events that may be encountered in an inpatient stay (AHRQ, 2006). The PDIs were adapted when necessary to identify complications in the cohort of interest through consultation with clinical and coding experts. Complications were based on the presence of any ICD-9-CM secondary diagnosis code as follows: postoperative infection (998.59, 998.51, 730.08, 730.09, 730.98, 730.99, 682.0, 526.4, 528.5), hemorrhage/hematoma (998.11, 998.12), wound disruption (998.3, 998.31, 998.32), pneumonia (481, 482, 482.1, 482.9, 483.8, 482.81, 486, 997.31, 518.0), airway/respiratory failure (518.81, 96.7, 96.71, 96.72), and transfusion (99.00, 99.01, 99.03, 99.04, 99.05, 99.06, 99.07). The Stanford University Research Compliance Office reviewed this study.

Charges and Cost

The KID and KID Cost-to-Charge Ratio data files provided total charges and costs for the hospital stay irrespective of expected payor. Total charges were the amount hospitals charge per hospital stay; it did not include physician fees or reflect payments received by the hospital for services. Total costs was the amount hospitals pay to provide care (i.e., the amount paid by the hospital for the personnel and technology). For example, a hospital may charge \$10,000 for an appendectomy that costs them \$5000 to provide care; charges may be inflated to offset total costs or to compensate for unreimbursed services. Hospital charges were converted to cost estimates by merging data elements from the KID and KID Cost-to-Charge Ratio for 2003 and 2006 using a unique hospital identifier. For each year, total charges were multiplied by the appropriate cost-to-charge ratio using the following formula: costs = total charge $\times$ APICC for each year. These standard guidelines were provided by HCUP (2008). Costs for 2003 and 2006 were saved under the "common costs" variable using the following formula: common costs = (costs 2003 + costs 2006)/2. Charges for 2003 and 2006 were saved under the "common charges" variable using a similar formula: common charges = (total charges 2003 + total charges 2006)/2.

Statistical Analyses

Length of stay was dichotomized (greater than the mean LOS of 2 days and equal to or less than 2 days), hospital type (pediatric hospital, general hospital, NAPH), gender, race (white and other), insurance status (private and other), comorbidity (present yes/no), PICU hospital (present yes/no), PICU intensivist (present yes/no), teaching status (present American Medical Association residency program yes/no), and burn unit (present yes/no). The Kruskal-Wallis test was used to test differences in mean LOS. Poisson regression was performed with our dependent variable being those with a longer average hospital LOS >2 days and accounted for confounders, including race, comorbidity, and year of surgery. The institution type (e.g., pediatric hospitals, general hospitals, or NAPH) was a categorical variable and was included in our model as a series of indicator variables: 1 indicated pediatric hospitals, 3 indicated general hospitals, and 4 indicated NAPH. Hospital beds, cleft palate surgeries, and nurse-to-bed ratio were continuous variables. All other variables in our models were binary response variables (i.e., female, white race, privately insured, comorbidities, teaching status, PICU hospital, PICU intensivist, and burn unit). Length of stay

TABLE 2 Characteristics of U.S. Cleft Palate Surgery in 1997, 2000, 2003, and 2006*

	Pediatric Hospitals	*General Hospitals*	*NAPH*	*Standard Errors*	*P Value*
Patient factors					
Patients, n (%)	4174 (36)	4646 (39)	2972 (25)	—	—
Mean age, days	281.4	267.2	267.5	1.197	.00
Mean age if LOS >2 days	263	287	272	3.177	.00
Female, %	48	49	49	0.514	0.75
Race, %					
White	57	64	63	0.495	.02
Insurance, %					
Medicaid	37	42	39	0.443	.00
Private	57	50	55	0.453	.00
Other	6	8	6	0.211	.27
Comorbidities, %					
Yes	17	13	11	0.314	.00
Congenital heart defects	3	2	2	0.141	.55
Anomalies of skull and face bones	12	10	8	0.281	.00
Chromosomal abnormalities	2	2	1	0.112	.69
Premature or low birth weight	0	0	0	—	1.00
Hospital factors					
Hospitals, n (%)	27 (9)	92 (30)	202 (66)	—	—
Procedures per hospital per year, n	50	18	7	—	—
Pediatric discharges, %	97.0	27.0	23.6	0.3639	.00
LOS					
Overall mean	1.8	2.1	1.9	0.0372	.00
Overall median	1	2	2	—	.01
Mean LOS >2 days	4.4	5.3	4.3	0.2074	.00
Median LOS >2 days	3	3	3	—	.32
LOS >2 days, %	16	21	22	—	.00
Teaching status, %					
Teaching	98.1	98.2	73.9	0.0026	.00
Location, %					
Urban	100	100	93	0.0016	.00
Census region, %					
Northeast	11.8	24.4	23.3	0.0038	.00
Midwest	19.2	18.0	21.6	0.0048	.00
South	21.7	38.4	24.4	0.0044	.00
West	47.3	19.2	30.7	0.0046	.00
Year of surgery, %					
1997	1256 (26)	1347 (25)	1075 (30)	0.0043	.00
2000	1227 (25)	1278 (24)	1050 (30)	0.0042	.00
2003	1188 (25)	1231 (23)	844 (24)	0.0036	.52
2006	1159 (24)	1448 (27)	591 (17)	0.0036	.00

* *P* value compares sample numbers. NAPH = nonaccredited pediatric hospital; LOS = length of stay.

was dichotomized to $\leq$2 days and >2 days in the two separate multivariate models used to estimate the association between identified risk factors and extended LOS. The correlation value provided by the Poisson regression is the pseudo R^2 value. Both models were hierarchical, accounting for the correlation between patients nested within the same hospitals. A *P* value of .05 was considered significant. Statistical analyses were performed using Stata software, version 12.1.

Results

Of the 21,964 patients with cleft palate identified, a total of 14,153 patients underwent cleft palate surgery in 1997, 2000, 2003, and 2006. One outlier with a LOS greater than 365 days was removed from the analysis because of concerns about miscoding and that this extraordinary stay would skew our results. Table 2 reports the characteristics of the cohort and the hospitals at which procedures were performed. Nearly all patients underwent the surgery in a teaching hospital (93%), and nearly all hospitals were located in urban centers (98%).

The volume of cleft palate surgeries between 1997 and 2006 decreased by 2%. The average volume of cleft palate surgical procedures performed per pediatric hospitals, general hospitals, and NAPH were 102 procedures/year, 36 procedures/year, and 10 procedures/year, respectively. Pediatric hospitals and NAPH each decreased the volume of cleft palate surgeries by 2% and 14%, respectively, but

TABLE 3 Median Total Charges and Costs Associated With Mean Length of Stay Less Than or Equal to 2 Days (LOS ≤2) As Well As Extended Length of Stay Greater Than 2 Days (LOS >2)

	Overall	*LOS ≤2*	*LOS >2*
Patients, n (%)	14,153	11,789 (84)	2364 (16)
Total charges			
Median	$10,835	$10,194	$15,104
Total costs			
Median	$4367	$4056	$6148

general hospitals had a slight 2% increase during the same time period.

Overall, the mean LOS was 2 days (median 1 day). The total number of patients with a LOS >2 days was 2250; the total number of patients with a LOS ≤2 days was 11,792. The overall mortality rate was 0.01%, the transfusion rate was 0.3%, and less than 3% of children experienced a complication during their hospital stay versus 12% of patients with extended an LOS >2 days (Table 2). Airway/respiratory failure was the most common complication, as high as 2% overall and 9% with a LOS >2 days. Less than 1% had an infection, hemorrhage/hematoma, or wound disruption. There were no significant differences in rates of complications across the hospital types.

Children who underwent cleft palate surgery in pediatric hospitals had the shortest LOS with a mean of 1.8 days and median of 1 day relative to patients who underwent the surgery in general hospitals with the longest LOS of mean 2.1 days (median 2 days). This pattern for LOS for each hospital type was conserved in patients who had a LOS >2 days. About 16% of patients from pediatric hospitals had an extended LOS >2 days compared with approximately 21% of patients from general hospitals and NAPH (Table 2).

Longer LOS was associated with higher total charges and costs. The total charges for cleft palate repair was a median of $10,835 but increased to a median of $15,104 with LOS >2 days. Similarly, total costs per discharge was a median of $4367 but increased to a median of $6148 with a LOS >2 days (Table 3).

TABLE 4 Poisson Regression for Factors Associated With Extended Length of Stay (LOS >2)*

Characteristic	*IRR*	*95% CI*		*P Value*
NAPH	1	—	—	—
Pediatric hospitals	**0.71077**	**0.50770**	**0.99506**	**.04**
General hospitals	0.98927	0.70966	1.37904	.95
Age	**0.95962**	**0.93381**	**0.98615**	**.00**
Female	1.03979	0.89240	1.21153	.62
White	1.05524	0.85429	1.30345	.62
Private	0.88804	0.74406	1.05988	.19
Comorbidity	**1.65971**	**1.31877**	**2.08880**	**.00**
Year	0.97717	0.94188	1.01378	.22

* The model compares pediatric hospitals and general hospitals to nonaccredited pediatric hospitals. IRR = incidence rate ratio; CI = confidence interval. The model also compares older age to younger age, female to male, white to other races, private insurance to other insurance, and having comorbidities to not having comorbidities.

TABLE 5 Poisson Regression for Hospital Factors Associated With Extended Length of Stay (LOS >2)*

LOS >2	*IRR*	*95% CI*		*P Value*
Hospital beds	0.99989	0.99949	1.00029	.59
Cleft palate surgeries	**0.99725**	**0.99558**	**0.99892**	**.00**
Nurse:bed ratio	1.04898	0.98769	1.11407	.12
PICU hospital	1.51894	0.86310	2.67312	.15
PICU intensivist	**0.58182**	**0.37917**	**0.89278**	**.01**
Teaching	1.37775	0.98365	1.92975	.06
Burn unit	1.00725	0.71085	1.42724	.97

* The model compares higher volume of hospital beds, cleft palate surgeries, and nurse-to-bed ratio to lower volume of these factors. The model also compares presence of pediatric intensive care unit (PICU), PICU intensivist, teaching residency program, and burn unit to the absence of these factors. IRR = incidence rate ratio; CI = confidence interval.

The multivariate model yielded associations with LOS >2 days, which included hospital type, age, race, insurance status, and presence of comorbidities. Socioeconomic status based on zip code income was not significantly associated with LOS >2 days. Hospitals factors associated with LOS >2 days included hospital beds, cleft palate surgeries, nurse-to-bed ratio, teaching status, presence of PICU, PICU intensivist, and burn unit. Poisson regression showed that pediatric hospitals had a decreased rate of LOS >2 days by 35% relative to NAPH. The volume of cleft surgeries marginally decreased the relative rate of LOS >2 days by 1%, but having a PICU intensivist significantly decreased this rate by 40%. Values from the Poisson regression models can be found in Tables 4 and 5. The pseudo R^2 values were .01 for factors associated with extended LOS (Table 4) and .02 for hospital factors associated with extended LOS (Table 5).

Discussion

This study found that cleft palate surgery has low rates of mortality and acute complications (0.01% and 3%, respectively). Hospital type was associated with LOS after surgery, with the shortest average LOS in pediatric hospitals. Twenty-one percent of patients undergoing cleft palate repair in general hospitals had an extended hospital stay, as defined as LOS >2 days, relative to 16% of patients in pediatric hospitals. While hospital type was skewed towards NAPH, patients were equally distributed across all hospital types. Therefore, we did not control for this skew in our statistical models.

Length of stay was chosen as the main measure because it captures a more complex hospital course; patients who have postoperative complications require additional time in the hospital (Thomas et al., 1997). Shortened LOS is an important metric and is reflected in the strong push to minimize the time patients stay in the hospital (Bellet & Whitaker, 2000; Shapiro & Bhattacharyya, 2003). Minimizing LOS can be beneficial to the patient with decreased

costs of hospitalization and reduced risks of iatrogenic complications such as nosocomial infections (World Health Organization, 2002). In this study, several factors associated with increased LOS were expected; for example, children with comorbidities had 90% increased relative rate of LOS >2 days, and patients of younger age had 5% increased relative rate of LOS >2 days. The strength of the association of the hospital type with LOS was surprising. Pediatric hospitals had a 35% decreased rate of LOS >2 days relative to nonpediatric hospitals, despite having more patients with comorbidities. These results show the strength of facility-level factors on pediatric patient care.

What factors at the facility level could affect care? It is possible that pediatric hospitals have more pediatric-specific resources (e.g., pediatric airway carts, pediatric anesthesiology team, dedicated feeding specialist), and these could translate into higher quality of care and therefore shorter LOS. For example, a pediatric hospitalist service is a pediatric-specific resource that has been shown to decrease LOS (Bellet & Whitaker, 2000, pp. 478–484). Particularly for children with associated disorders who require more complicated anesthesia, higher quality and shorter LOS are attributed to specialized pediatric equipment and an anesthesiology team who are apt to carry out these procedures (Arul & Spicer, 1998). This theory of pediatric resources being an important component to decreasing LOS correlates with our finding that hospitals with a pediatric intensivist had a 40% decreased rate for LOS >2 days compared with hospitals without a pediatric intensivist. This association suggests that the presence of pediatric specialists is related to LOS and perhaps to complication rates; however, the cause of this association requires further investigation.

Another hospital factor potentially associated with surgical quality is hospital volume (Halm et al., 2002; Hernandez-Boussard et al., 2011). It has been suggested that greater volume of certain surgical procedures results in improved clinical and technical skills across the entire care team (Birkmeyer, 2000). For example, pediatric patients undergoing heart surgery at low-volume hospitals were 42% more likely to die than those undergoing the same procedure at high-volume hospitals (Birkmeyer, 2000). However, other common procedures such as colon resection have not seen surgical volume associated with mortality (Billeter et al., 2011). Cleft palate repair is a fairly straightforward surgical procedure but does require specialized perioperative care (e.g., pediatric anesthesia, feeding specialist). We found that although the volume of cleft palate surgeries was associated with a shorter LOS, its association was small when compared with pediatric resources. Volume was different in our hospital types: pediatric hospitals and general hospitals were nearly 20% higher than NAPH. We also found that pediatric hospitals averaged more than three times the number of procedures than general hospitals and 10 times that of NAPH. This volume does play a role in pediatric care because children younger than 1 year have a 10-fold increase in anesthesia risks compared with older children (Arul & Spicer, 1998). The mean age at the time of palatoplasty is 9 months, which necessitates an experienced, specialized pediatric team. Increased volume and facility characteristics entwined in this study likely contributed to the decreased LOS seen in the pediatric hospitals.

The differences in LOS in this study may seem quite small, but even these small changes had large economic impact. In this study, the median total charge for LOS >2 days was $13,404, which is 41% more than the overall median charge for LOS less than or equal to 2 days of $9503. Similarly, the median total cost for LOS >2 was $6148, which was also 41% more than the overall median cost for LOS less than or equal to 2 days of $4367. In a time when health care resources are scarce and health care costs continue to rise, minimizing costs is crucial to both the sustainability and improvement of caring for patients who need cleft palate surgery.

Reducing LOS for pediatric surgery, through the reduction of complications or other practice pattern changes, may be an opportunity to develop and implement new models for better quality care. Patients who underwent palatoplasty in pediatric hospitals had shorter extended stay, thereby reducing exposure to iatrogenic risks. These findings align with previous studies that showed pediatric procedures performed in pediatric hospitals compared with general hospitals had fewer complications and resulted in both high-quality and cost-effective care (Brain & Roberts, 1996; Snow et al., 1996; Kokoska et al., 2001; Allan, 2006; Whisker et al., 2009; Raval et al., 2010; Evans & Van Woerden, 2011).

Given the decreased LOS of pediatric hospitals, it might seem appealing to regionalize cleft palate care to pediatric hospitals. However, pediatric hospitals represent only 9% of the hospitals, and regionalization might present a geographical barrier to care. Another suggestion has been that surgeons from general hospitals undergo training in pediatric hospitals to strengthen diagnostic and surgical skills for common surgeries such as appendectomies (Whisker et al., 2009). However, in addition to a surgeon's skills, other pediatric resources such as pediatric anesthesia team, technology, and bed availability likely also affect surgical outcomes. In adult patient populations, standard surgical procedures such as colon resection have not had different mortalities based on hospital type and surgeon volume (Billeter et al., 2011). This suggests that pediatric care is unique and that facility resources may take on increasing importance in the care of children. An alternative option is to strengthen structure and process in non-pediatric hospitals. To achieve this aim, we must first understand what facility-level factors are driving reduced hospital stay. Then nonpediatric hospitals could develop a pediatric medical team, appropriate facilities, and clinical pathways to achieve the efficiency of care seen in pediatric hospitals.

Limitations

Administrative data sets, such as the KID, are subject to several limitations including coding errors and variation in systematic coding methods in hospitals participating in HCUP. These differences in coding may vary systematically by hospital type. The scope of our research aimed to provide a broad national perspective of cleft palate surgery limits the extent of our understanding events from an individual patient level. The study did not capture the clinical severity of a complication or the clinical complexity of each individual cleft because factors, such as the width of the cleft palate, are not captured in the data set. This study included only complications that occur during the perioperative hospitalization. Thus, complications that occurred after the patient discharge in the subacute or long-term periods are not captured. There have been concerns that shortened lengths of stays may adversely affect outcomes with increased readmissions; we did not track readmissions in this study but will include it in future research. However, several recent studies have found that reduced LOS was associated with stable or even reduced readmission rates (Baker et al., 2004; Kaboli et al., 2012).

Patients may stay in the hospital longer because of either medical or social complications. For example, patients who do not have access to high-quality home care may require longer LOS. However, our analysis showed a high association between complication rates and LOS. Although we were unable to capture all contributors to the variance in this analysis (i.e., width of cleft palate), our models have a goodness-of-fit test to confirm that they are appropriate.

Conclusion

This nationwide study examined whether hospital type affects outcomes after pediatric cleft palate repair. Patients undergoing palatoplasty in pediatric hospitals had higher comorbidities but shorter LOS, they were discharged more quickly when they had an extended stay, and they had 35% decreased relative rate for LOS >2 days. Median total costs and charges were 41% greater with a LOS >2 days. Hospitals with a pediatric intensivist had 40% decreased relative rate for LOS >2 days. Further research is needed to understand which additional aspects of pediatric hospitals are associated with lower LOS and thereby lower complications and costs.

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Management of the Alveolar Cleft

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KEYWORDS

• Alveolar cleft • Nasoalveolar molding • Presurgical infant orthopedics • Alveolar bone grafting

KEY POINTS

- The interdisciplinary approach to primary and secondary surgical procedures has been proven to be beneficial for patients.
- In the neonate, nasoalveolar molding has been found to optimize the aesthetic outcome of the nasal and labial repair while minimizing the extent of surgery and formation of scar tissue.
- During the mixed dentition stage, orthopedic arch preparation before the secondary alveolar bone-grafting procedure improves arch morphology, restores the functional interarch relationship, and facilitates surgical access.
- The cooperative effort between the surgeon and craniofacial orthodontist in designing strategies customized to the patient's specific needs has resulted in better esthetic and functional outcomes, minimizing surgical interventions.

INTRODUCTION

An alveolar cleft refers to the space between the maxillary segments anterior to the incisive foramen, and therefore presents a discontinuity in the dental arch. Routine cleft lip repair and subsequent cleft palate repair do not specifically address the bony deficiency at this site. Consequently, strategies specifically designed to manage the alveolar cleft must be incorporated into the complete treatment itinerary, and require a cooperative effort of the craniofacial orthodontist and surgeon. Orthopedic and orthodontic management of patients born with clefts of the lip, alveolus, and palate is based on the application of basic biomechanical principles adapted to the individualized cleft anatomy. This article focuses on orthopedic and orthodontic preparation for 2 stages of interdisciplinary orthodontic/surgical cleft care: (1) presurgical infant orthopedics for primary lip/alveolus/nasal surgical repair; and (2) maxillary arch preparation for secondary alveolar bone grafting. These preparatory stages of orthopedic/orthodontic therapy are undertaken with the goal of restoring normal anatomic relationships to assist the surgeon in providing the best possible surgical care.

PRESURGICAL INFANT ORTHOPEDICS

The history of presurgical infant orthopedics (PSIO) dates back to 1686 when Hoffman described the use of an extraorally anchored headcap to place a retraction force on the premaxilla.[1] A similar method is described in more contemporary literature, such as in Berkowitz's 1996 article that described "a head bonnet with an external elastic that is sometimes used before surgical lip closure to ventroflex the premaxilla, thereby reducing tension at the surgical sites."[2] McNeil[3] introduced

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intraorally anchored appliances in 1950, using a progressively modified feeding plate to align maxillary alveolar segments. Another popular variant of an intraoral device to mold the alveolar segments was introduced in 1980 by Latham[4]; this acrylic appliance was retained by pins to the alveolar segments and used a system of elastomeric material to move the alveolar segments in proximity. A more recent technique, nasoalveolar molding (NAM), was introduced in 1993 by Grayson and colleagues.[5] This technique not only molds the cleft alveolus as in previous PSIO protocols but also shapes and forms the nasal cartilages in preparation for the primary surgical repair.

PSIO appliances have been described as either active or passive devices. Active appliances are fixed intraorally and apply traction through mechanical means such as elastic chains, screws, and plates (eg, the Latham appliance). A passive appliance maintains the distance between the 2 maxillary segments while external force is applied to the system.[6] NAM is an example of a passive PSIO technique and is the focus of this article.

A recent study by Sischo and colleagues[7] examined the prevalence of NAM among cleft centers in the United States. Among teams interviewed via telephone survey, 37% offered NAM therapy. It was speculated that the high prevalence of NAM therapy might be due to increased insurance coverage and growing adoption of this treatment approach.

Grayson and Santiago[8] described the NAM protocol for cleft patients in 1997. NAM takes advantage of the high degree of plasticity of neonatal cartilages, molding the deformed cleft nose by incorporating a nasal stent to an alveolar molding plate. The overall goal of this presurgical orthopedic therapy is to restore the correct skeletal, cartilaginous, and soft-tissue anatomic relationships, providing the most optimal conditions for the primary lip, alveolus, and nose surgery. The intent is to create a lasting aesthetic outcome and reduce the need for, or minimize the extent of, secondary surgical revision procedures.

NAM objectives include:

1) Molding and repositioning of the alveolar processes
2) Molding and reposition of the nasal cartilages, and
3) Lengthening of the deficient columella.

Short-term (4 months to 1 year)[9–11] and long-term (4.5–9 years) studies[11–13] indicate that NAM significantly improves nasal symmetry over surgery alone.

An adjunctive surgical option, gingivoperiosteoplasty (GPP) (closure of the soft-tissue alveolar segments), is possible if there is close approximation of the cleft alveolar segments. Santiago and colleagues[14] have shown a 60% reduction in the need for a secondary alveolar bone-graft procedure in cases where a combined approach of PSIO and a GPP was performed. This combination was less expensive than a traditional protocol (lip repair, primary nasal repair, and secondary alveolar bone graft).[15] It is important to note that GPP is a separate therapeutic option from NAM, associated with its own risks and benefits.

Timing of Cleft Surgical Preparation

Referral to the cleft team before birth is now commonplace if there is diagnosis of the cleft on ultrasonography. This timing affords an opportunity for the family to become acquainted with the concept of team care and learn the generalities of the treatment options. Early consultation with the treating surgeon and NAM provider to discuss the logistics of NAM therapy in preparation for the primary lip and nose surgical repair is an important first step toward the parents' informed decisions regarding the collaborative care options.

Ideally, the interdisciplinary cleft team evaluates the neonate 1 week after birth. The cleft surgeon and craniofacial orthodontist perform a clinical examination, photos are taken, and an impression of the intraoral cleft defect is taken in a hospital setting with the surgeon as part of the impression team. This noninvasive procedure, during which the infant is fully awake and not anesthetized, is done using a premade acrylic impression tray and an elastomeric material (**Fig. 1**).

Fig. 1. Intraoral impression of a unilateral cleft lip and palate infant taken with polyvinylsiloxane material to capture the alveolar and palatal anatomy. The impression is used to create a precise stone cast model.

The impression is then used to create a stone cast and a conventional acrylic molding plate. The molding plate is made of clear orthodontic resin, and 1 or 2 (depending of the deformity) buttons or extensions are added to the anterior part of the appliance. These buttons are made of stainless-steel orthodontic wire covered with clear resin material, and grooved to hold orthodontic elastics and surgical tapes used to control the force applied to the alveolar segments (**Fig. 2**). The intraoral acrylic plate is examined for rough areas, polished, and inserted within a week of the initial visit. The molding plate is then modified on a weekly basis to approximate the intraoral segments and reduce the alveolar cleft. Once the segments are in close proximity, a nasal stent is added to mold the distorted nasal cartilages.

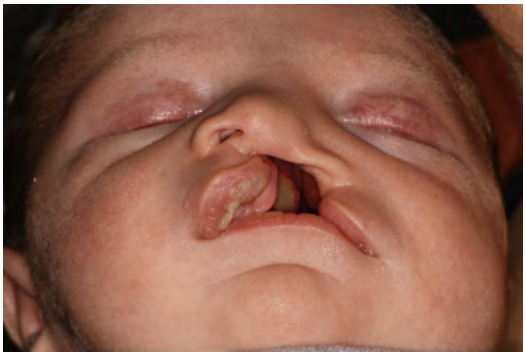

Fig. 3. The unilateral cleft lip and palate anomaly presents a significant nasal deformity. Note the depression and displacement of the nasal tip, with the columella and nasal septum inclined over the cleft with the base deviated toward the noncleft side.

CORRECTING THE UNILATERAL DEFORMITY

The unilateral cleft lip and palate anomaly presents a significant nasal deformity; a detailed description of the cleft nasal deformity at infancy is provided in the article "Correction of the Cleft Nasal Deformity: From Infancy to Maturity" elsewhere in this issue by Marcus and colleagues. In brief, the lower lateral cartilage is depressed and concave on the cleft side and is separated from the contralateral cartilage located high in the nasal tip.[16] This configuration results in depression and displacement of the nasal tip and lateralization of the nostril apex. The columella and nasal septum are inclined over the cleft with the base deviated toward the noncleft side (**Fig. 3**).[17]

The goals of NAM in patients with unilateral cleft lip and palate are to reduce the severity of the cleft deformity by approximating the alveolar and lip segments and correcting malposition of the nasal cartilages, nasal tip, philtrum, and columella. These corrections are achieved through periodic modifications of the intraoral molding plate and adjustments of the extraoral nasal stent.

At the NAM appliance insertion visit, the intraoral molding plate is seated, adjusted, and activated to provide alveolar movement. Intraorally the plate is activated by the selective addition and/or removal of hard and soft acrylic. Correction of the alveolar malposition and associated soft-tissue structures is achieved by weekly modifications of the molding plate, which allows for gradual and controlled approximation of the alveolar segments.

The extraoral activation system consists of 2 surgical tapes individually folded inside the lumen of 2 orthodontic elastics, which are placed on the channel of the acrylic button. The 2 strips are then stretched and taped over a wider facial tape used to protect the neonate from skin irritation, which helps to secure the molding plate to the palate and alveolar processes. Therefore, the tape and elastic construct is usually more active on the side toward the intended movement. As the effectiveness of the NAM therapy depends on the

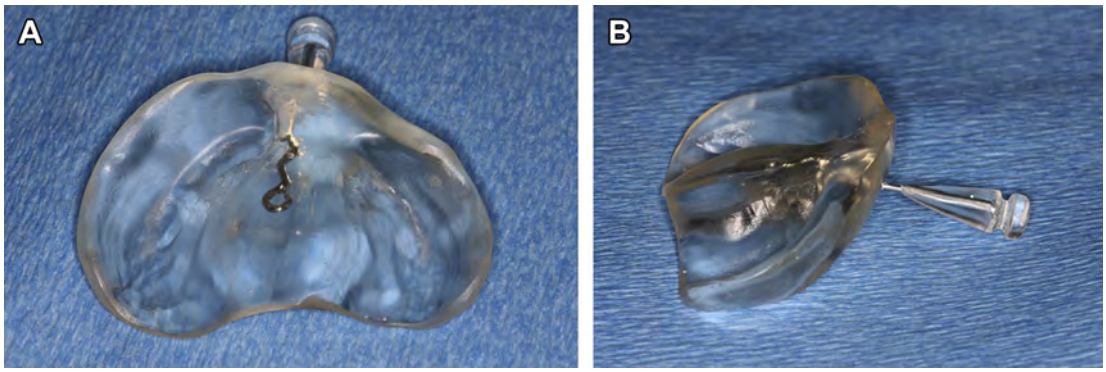

Fig. 2. (*A*) Acrylic molding plate made from a stone cast model. The plate is polished, rough surfaces removed, and a layer of soft acrylic added to the borders to avoid soft-tissue irritation. (*B*) An anterior wire and acrylic extension (button) is added and channeled to support the elastic bands attached to the tape system.

stability of the plate on the transported segment, some clinicians prefer to use a small amount of denture adhesive only on the segment to be mobilized (**Fig. 4**).

Once the alveolar segments are in close approximation (around 4 mm), lip taping and a nasal stent are added to the system (**Fig. 5**). Taping of the cleft lip segments in tight apposition assists the elastic orthopedic forces in achieving controlled approximation of the alveolar cleft segments. In addition, it improves alignment of the nasal base region by bringing the columella toward the midsagittal plane and improving symmetry of the nostril apertures. In select patients, lip taping can be started before the nasal stent is incorporated into the molding plate.

The nasal stent is constructed from an orthodontic wire carefully bent into a gooseneck shape, which is incorporated into the anterior flange of the oral molding plate. The most superior part of the wire is covered with hard and soft acrylic to ensure that tissue breakdown does not occur when positive pressure is applied to the nasal lining. The stent is positioned inside the nose underneath the apex of the alar cartilage on the cleft side. The alar dome cartilage on the cleft side is lifted by the stent to achieve normal elevation and symmetry.

The nasal stent is gradually modified on a weekly basis. The shape of the cartilaginous septum, dome, and medial and lateral crura are carefully molded to resemble the normal shape of these structures.

At the conclusion of nasal and alveolar molding, the nasal cartilages, columella, philtrum, and alveolar segments should be properly aligned to facilitate the surgical restoration of normal anatomic relationships (**Fig. 6**).

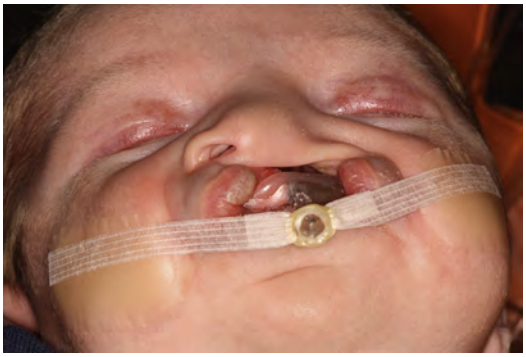

Fig. 4. During the first stage of nasoalveolar molding (NAM) therapy, the intraoral molding plate and elastics–surgical tapes system are used to differentially mobilize the alveolar segments and reduce the cleft gap.

CORRECTING THE BILATERAL DEFORMITY

The bilateral deformity presents a different set of challenges. In the bilateral cleft, the alar cartilages have failed to migrate up into the nasal tip and elongate the columella. The alar cartilages are positioned along the alar margins and are stretched over the cleft as flaring alae. In addition, the premaxilla is suspended from the tip of the nasal septum, whereas the lateral alveolar segments remain behind.[18,19] Disparate growth may occur, causing overprojection, flexion, or rotation of the premaxillary segment (**Fig. 7**).

The goal of presurgical NAM in patients born with bilateral cleft lip and palate is to lengthen the columella, reposition the nasal cartilages toward the tip, and align the alveolar segments.

As in the unilateral deformity, an intraoral impression of the neonate is taken and a bilateral acrylic molding plate is fabricated. In contrast to the unilateral plate, the bilateral plate has 2 anterior wire and acrylic extensions or buttons. The first stage of treatment consists of retracting and uprighting the everted premaxilla into the space between the 2 lateral alveolar segments (**Fig. 8**). This goal is achieved by weekly modifications of the intraoral molding plate and the use of orthodontic elastics and surgical tapes, as described for unilateral deformity. In the second stage of treatment, as the alveolar segments gradually approximate one another, 2 nasal stents are incorporated into the anterior rim of the molding plate and enter the nasal apertures (**Fig. 9**). The nasal stents support the nasal tip and create tissue-expanding forces that are directed to the columella and domes internally. A surgical tape is applied to the prolabium, pulled down, and adhered to an orthodontic elastic attached to the 2 anterior buttons (**Fig. 10**). A horizontal prolabial band made of soft acrylic may also be attached across the 2 nasal stents to depress the columella base in the region normally represented by the lip-columella junction, providing countertraction force to the columellar tissue. Each functional component of the bilateral system is gradually modified to mold the hard and soft tissues and achieve the desired goals (**Fig. 11**).

Presurgical NAM has proved to be a valuable adjuvant therapy to the primary surgical repair, offering 4 major benefits:

1. The controlled presurgical alignment of the cleft alveolar and lip segments into normalized anatomic relationships and the correction of the nasal deformity reduces tension on the repair and may reduce the extent of the primary lip and nasal surgery required. Reduction of

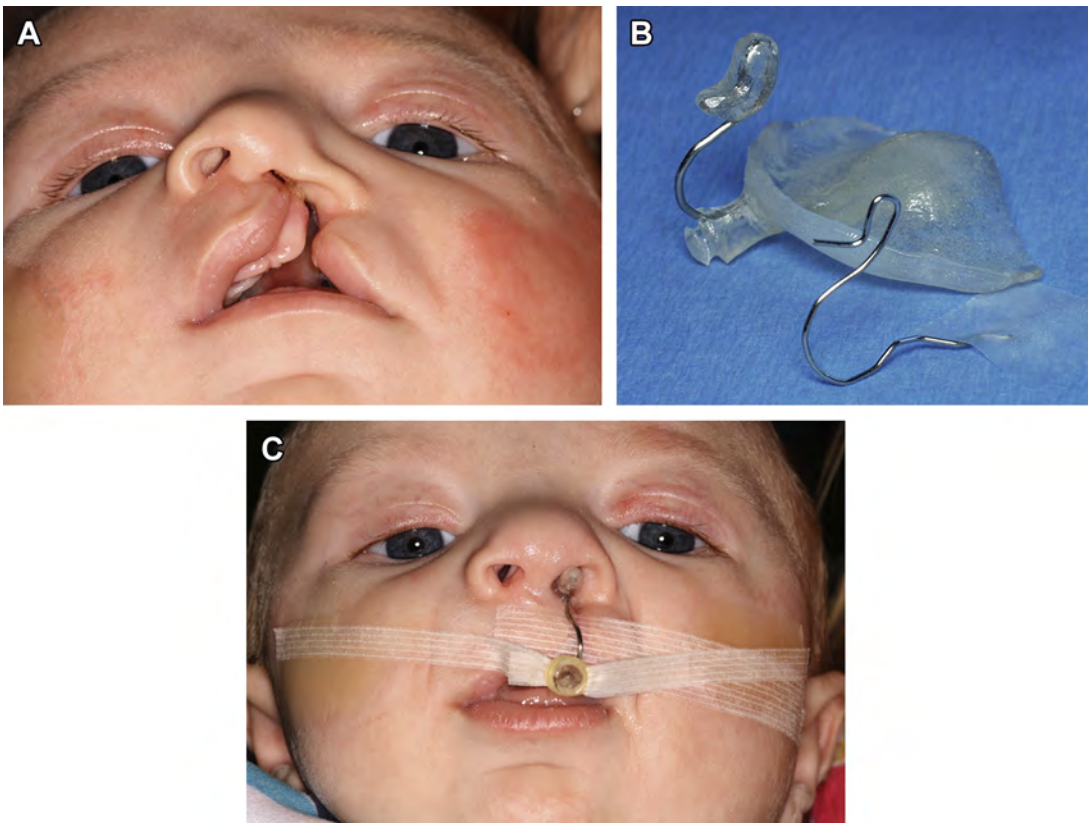

Fig. 5. (*A*) Alveolar segments in close approximation after 5 weeks of NAM therapy. (*B*) The nasal stent is constructed from an orthodontic wire carefully bent into a gooseneck shape and incorporated into the anterior flange of the oral molding plate. The most superior part of the wire is covered with hard and soft acrylic to ensure that tissue breakdown does not occur when positive pressure is applied to the nasal lining. (*C*) Taping of the cleft lip segments in tight apposition assists the elastic orthopedic forces to achieve controlled approximation of the alveolar cleft segments, and also improves alignment of the nasal base. The nasal stent molds the distorted nasal structures to resemble their normal shape.

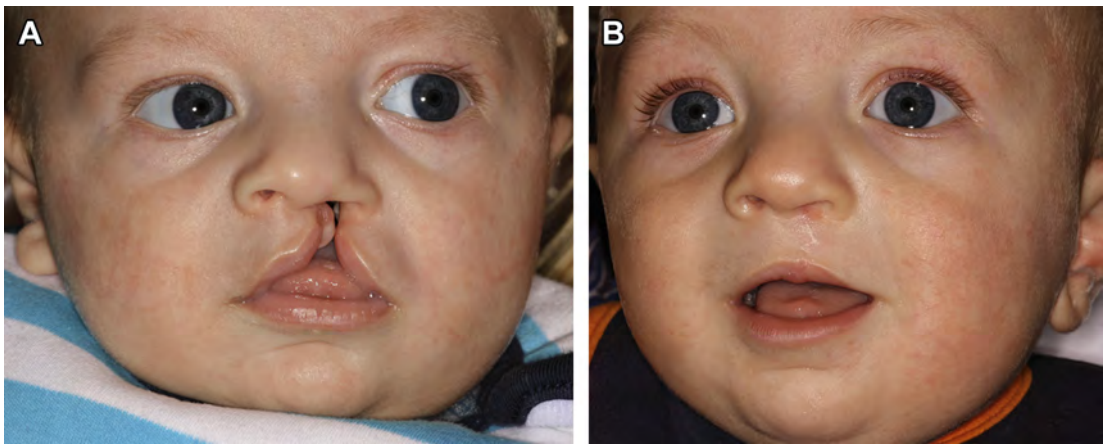

Fig. 6. (*A*) At the conclusion of nasal and alveolar molding (11 weeks), the nasal cartilages, columella, philtrum, and alveolar segments are properly aligned to facilitate the surgical restoration of normal anatomic relationships. (*B*) The goal of the combined presurgical NAM therapy and primary lip/nose surgery is to create a lasting aesthetic outcome and reduce the need or minimize the extent of secondary surgical revision procedures.

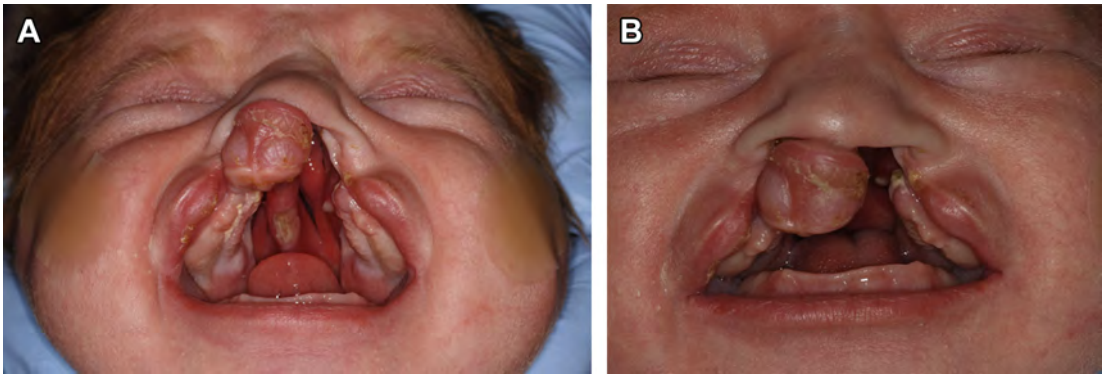

Fig. 7. (*A*, *B*) The bilateral cleft lip and palate deformity presents a displaced and protruded premaxilla, wide alar bases, and a deficient columella.

the primary deformity creates a more favorable and uniform starting point, which may facilitate more consistent results and diminish the residual deformity.

2. The presurgical closure of the alveolar gap provides the surgeon with the option to perform a GPP at the time of lip closure. Santiago and colleagues[14] have reported that this procedure reduces the need of a secondary bone grafting in more than 60% of the cases studied.
3. In the bilateral cleft deformity, nonsurgical columella lengthening eliminates the need for secondary surgical columella elongation and the accompanying scars at the lip-columella junction (**Fig. 12**).

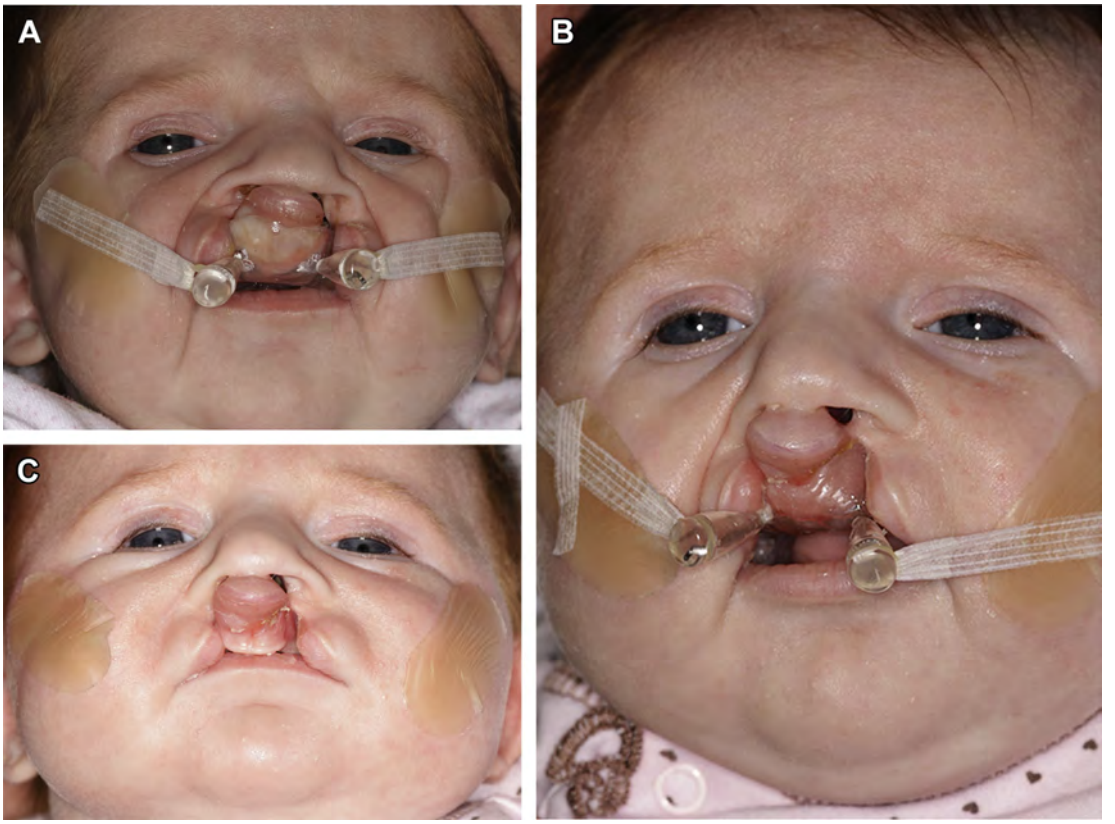

Fig. 8. (*A*) In the bilateral NAM therapy the first stage of treatment consists of retracting and uprighting the everted premaxilla into the space between the 2 lateral alveolar segments. (*B*, *C*) At approximately 8 weeks of NAM therapy, the premaxilla has been retracted and positioned between the lateral alveolar segments. The prolabium has been uprighted.

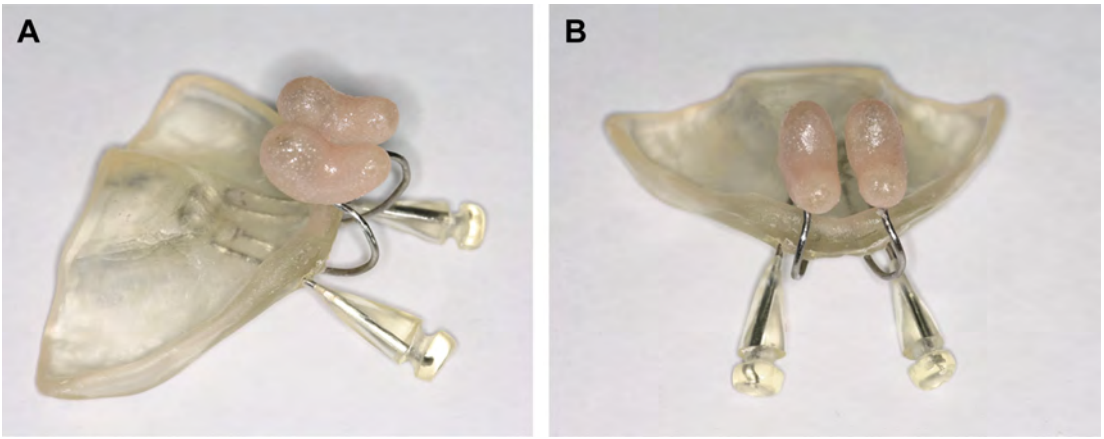

Fig. 9. (*A, B*) In the second stage of treatment, as the alveolar segments gradually approximate one another, 2 nasal stents are incorporated into the anterior rim of the molding plate to enter the nasal apertures.

4. NAM, when used in conjunction with a modified surgical approach, allows for a single initial surgical procedure to address the lip-nose-alveolus complex and its deformity. By minimizing the residual deformity, revisionary surgery may be avoided, thereby reducing the number and extent of surgeries that a cleft patient will undergo during a lifetime.

SECONDARY ALVEOLAR BONE GRAFTING IN MIXED DENTITION

In patients born with a cleft lip and palate, lip/nasal and palatal surgery are commonly performed during the first 14 months of life. If a GPP is not included at the primary surgery, the remaining bony defect in the alveolar region is addressed with a secondary alveolar bone-graft procedure during the stage of mixed dentition.

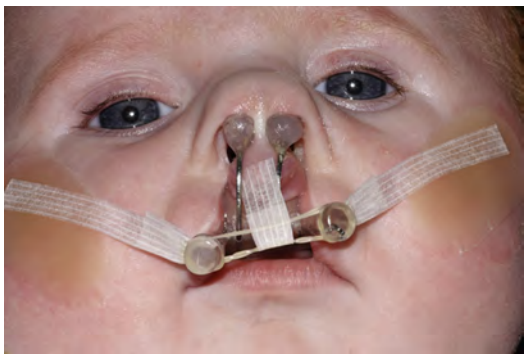

Fig. 10. A surgical tape is applied to the prolabium, pulled down, and adhered to orthodontic elastics attached to the 2 anterior buttons. This downward pull, combined with the upward and anterior force applied by the nasal stents at the tip of the nose, results in a stretching and lengthening of the columella.

Bone grafting was introduced at the beginning of the twentieth century when Von Eiselberg used a small finger as a pedicle graft in 1901. Bone grafting using tibia and periosteum was introduced in 1914 by Drachter.[20] Secondary bone grafting using the iliac crest was introduced by Boyne in 1970. Before the advent of osseous grafting techniques to restore the bony cleft defect, prosthodontic rehabilitation was the common method of reconciling the alveolar cleft. After World War II, with the advent of antibiotic therapy, the use of bone-graft procedures for the correction of the alveolar cleft increased significantly.[21] Today, bone grafting of the alveolus has become widely accepted as the standard of care in patients with cleft lip and palate.[22–24]

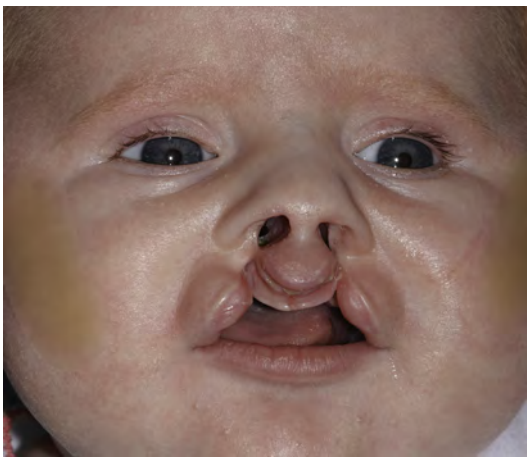

Fig. 11. At the conclusion of the bilateral NAM therapy, the alveolar and lip segments should be approximated and aligned and the columellar tissue expanded to normal anatomic values, facilitating primary lip/nose/alveolar surgery.

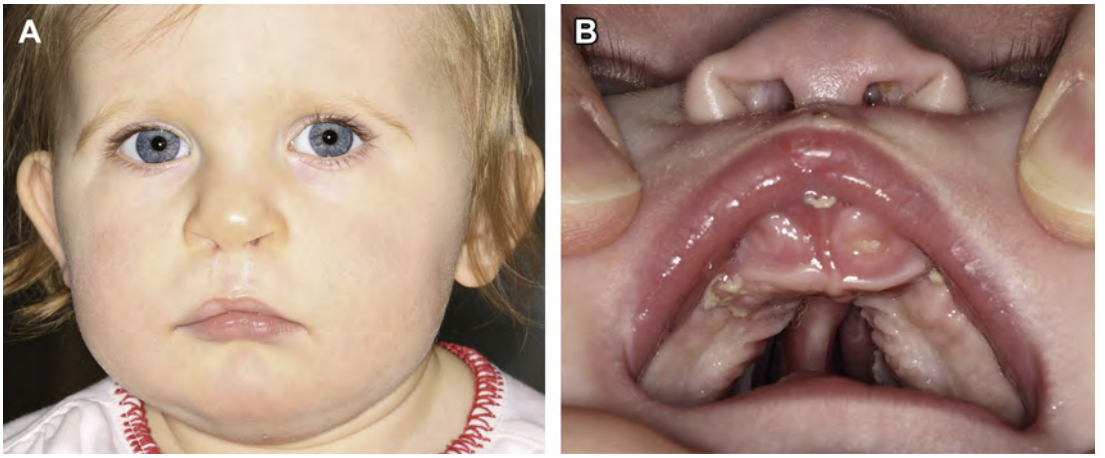

Fig. 12. (*A*) NAM with nonsurgical columella elongation may eliminate the need for surgical lengthening of the columella, minimizing the extent of scarring in the nasolabial complex. (*B*) In this patient a gingivoperiosteoplasty procedure was performed at the time of the primary surgery, providing alveolar arch continuity and a possible elimination of a secondary bone-grafting procedure.

Secondary bone grafting requires collaborative treatment planning between the members of the craniofacial team, especially the orthodontist and the surgeon. It is performed in patients born with a cleft alveolus to restore the normal architecture of the maxilla. Secondary bone grafting allows for eruption of the permanent teeth (specifically the permanent lateral incisor and the canine), closure of an oronasal fistula if present, and the stabilization of the maxillary arch by providing bone continuity between the segments. In addition, such bone grafting improves the aesthetic outcome by providing support and elevation to the alar base. Finally, it allows adequate bone support in cases where an endosteal implant is needed for final restorative rehabilitation.[22,23,25]

TIMING OF BONE GRAFTING ON THE CLEFT MAXILLA

Bone grafting in patients with cleft lip and palate can be divided into 2 main categories, primary and secondary. Primary bone grafting is performed in patients younger than 2 years with the intent to improve arch form, preserve the lateral incisor, decrease the need for orthognathic surgery, and stabilize the premaxilla in bilateral patients.[26] However, primary bone grafting has declined in popularity because of negative reports regarding compromised midfacial growth, high incidence in malocclusion, inadequate bone formation, and need of an additional bone-graft procedure later in the patient's life.[22,23,27]

Secondary bone grafting can also be classified as early, intermediate, or late. Early secondary bone grafting is performed between ages of 2 and 5 years, or at a primary dentition stage. Some advantages of this procedure have been reported, such as good quality of bone formation, allowing for the eruption or movement of the central incisor adjacent to cleft or the future eruption of the lateral incisor.[21,22,28] However, midfacial growth can be affected, as in primary bone grafting.[23] Intermediate bone grafting is carried out between 5 and 12 years old during the mixed dentition stage, with the goal of having bony support for the eruption of the permanent lateral incisor and canine without negative consequences in terms of midfacial growth.[29] Finally, late secondary bone grafting is performed in late adolescents or adult patients in the state of permanent dentition, in whom the growth of the maxilla has been completed. At this stage, the main goal is the provision of maxillary continuity in cases where orthognathic surgery is indicated, to enable a one-piece Lefort advancement and/or have adequate bone available for a future implant restoration.[22,30,31] Late bone grafting may place the teeth adjacent to the cleft at risk because of lack of bone support, and complications can occur at this stage, such as progressive root resorption caused by the direct contact of the grafted bone and the exposed root surface.[32]

In summary, timing is critical relative to the age of the patient and the stage of eruption of the teeth adjacent to the cleft.[33] The ideal timing for bone-graft surgery is more dependent on dental development than on chronologic age. Grafting during the state of primary dentition or at full dental maturity carries risks to maxillary growth and dental support, respectively. Ideally the procedure should be timed to minimize growth disturbance and

detrimental effects on the adjacent dentition. The root of the permanent canine provides a guide to treatment timing; it should be formed at least to one-half or two-thirds of its definitive length at the time of the graft placement.[23,25] Numerous studies have demonstrated high success rates when the graft procedure is undertaken before the canine eruption, in comparison with delayed grafting.[28]

SOURCE OF BONE-GRAFT MATERIAL

Materials of various origins have been used to correct the maxillary alveolar cleft, including autogenous, allogeneic, and xenogeneic bone materials and, more recently, growth factors such as bone morphogenetic protein (BMP). There is a general consensus that fresh autogenous cancellous bone is the ideal bone-grafting source, because it supplies living, immunocompatible bony cells that integrate fully with the maxilla, and are indispensable for osteogenesis.[20] For autotransplantation several donor sites have been used: iliac crest, cranial bone, tibia, and mandibular symphysis.

Iliac Crest for Secondary Bone Graft

Iliac crest is used in most craniofacial centers as a source of bone for secondary bone graft.[30,34] It is easy to access, and contains a large volume of cancellous bone and a larger stem cell population that support osteogenesis after grafting.[20,21,23,28] The main concern when using iliac crest is postoperative discomfort, which can effect gait and result in a prolonged recovery. These complications can be minimized with a careful surgical approach involving limited incision, minimal elevation of the musculature on the crest, meticulous hemostasis, carefully layered wound closure with reapproximation of the cartilage cap, adequate postoperative pain control, and early ambulation.[20,28] Most surgeons favor iliac crest bone graft because it provides an adequate amount of bone and an unlimited supply of marrow rich in cellular matrix for grafting.[24,30,35]

Cranial Bone for Bone Graft

Cranial bone has been used for the last 2 decades. In comparison with iliac crest, it provides a convenient source of cortical and cancellous bone with minimal postoperative pain and a hidden scar. Grafting with calvarial bone varies with respect to the method of harvest, the area of the cranium from where it is taken, the size of the defect, and the proportion of cortical to cancellous bone available.[24,36] Potential complications include hematoma, seroma, dural tear, dural exposure, subdural hemorrhage, cerebrospinal fluid leak, and brain injury or neurologic sequelae.[20,22,23]

Tibia Bone for Bone Graft

Tibia has been a common donor site among orthopedic surgeons, and has recently gained popularity among maxillofacial surgeons for grafting in patients with cleft lip and palate and for preprosthetic rehabilitations. Most studies using tibial bone have been conducted in adult patients, and a relatively limited quantity of bone has been obtained. For this reason, the technique is not indicated for every cleft deformity, and it is important to inform the patient about the possibility of harvesting bone from both legs. Concerns exist regarding disturbances to the epiphyseal cartilage affecting the growth of the individual, which is a disincentive to application in young patients. Some advantages have been reported, such as reduced operating time, minimal scarring, early ambulation, and reduced stay in hospital.[20,22,34]

Mandibular Symphysis for Bone Graft

The mandibular symphysis is an attractive donor site, as it involves the same operative field and has an embryonic origin analogous to that of the maxilla. Absence of visible scar, reduction of postoperative discomfort, reduced stay in hospital, faster revascularization, and an improved preservation of the graft volume have been reported as advantages of this source area.[20,34,37] As in other bone donor sites, the mandibular symphysis involves a specific set of potential complications, including the risk of damage to the canine and incisor roots and injury to the mental nerve. In addition, the quantity of bone available may be limited by the state of mandibular development.[23,34]

Rib for Bone Graft

For primary bone grafting, the Rosenstein protocol[24] uses rib as the donor source. Specific potential complications reported using rib as donor include bone resorption, atelectasis, pneumothorax, wound breakdown, scarring, and pain.[34] Other investigators have found difficulty with tooth movement during the orthodontic treatment and inconsistent tooth eruption through the graft.[21,22]

Bone Substitutes for Bone Graft

Bone substitutes such as allogeneic materials and bovine-derived hydroxyapatite have been used for secondary alveolar bone grafting. These materials share the common advantage of eliminating the morbidity of a second operative site.[22] Studies

using allogeneic bone graft (freeze-dried bone) have shown the potential to achieve orthodontic movement of the canine into the grafted region, eruption of the canine, and stabilization of the segments.[38–40] Complications of infection, disease transmission from donor to host, and host incompatibility have been reported in the literature.[23,28] The consolidation of allogeneic graft seems to require a longer period of time, thereby delaying the beginning of the orthodontic treatment.[41]

BMPs are a group of growth factors that play an important role in osteogenesis and chondrogenesis. Specifically, BMP-2 and BMP-7 are known for their osteoinductive properties.[42,43] BMP has been used for several maxillofacial surgical purposes, including maxillary sinus floor for augmentation before endosteal implant placement for prosthodontic rehabilitation.[44] Several studies have shown encouraging results in alveolar cleft repair using recombinant human BMP (rhBMP). In one study performed in skeletally mature patients with cleft lip and palate, rhBMP-2 in a collagen matrix demonstrated better results when compared with autogenous iliac crest bone graft. The repaired defect had greater volume in the rhBMP-2 group; there were also reductions in hospital stay, cost, healing problems, and pain.[45] Conversely, in another study performed in skeletally immature patients, rhBMP-2 in a collagen matrix showed less bone volume measured by computed tomography (CT) when compared with the iliac crest group; however, acceptable results were obtained with both materials.[46] Despite clinical studies showing the benefits in using the rhBMP-2 for the correction of craniomaxillofacial defects, this material has been related to its own set of complications, most prominently in the orthopedic literature; significant operative-site edema has been noted, as well as resorption of vertebral bodies in spinal column repair.[43] The use of rhBMP-2 among growing patients remains off-label; the safety associated with application in this population remains uncertain. In animal models, the use of rhBMP-2 has resulted in premature fusion of sutures and growth restriction, which could be detrimental in a clinical setting.[47]

Platelet-rich Plasma Injections

Finally, among emerging experimental techniques, platelet-rich plasma injections in an animal model of the alveolar cleft has shown promise in permitting orthodontic tooth movement into the alveolar defect. The enriched blood plasma is thought to enhance new bone formation and reduce the dimensions of the alveolar bone defect.[48]

MAXILLARY ARCH PREPARATION BEFORE AN ALVEOLAR BONE-GRAFT PROCEDURE

Orthodontic Planning

Orthodontic records in patients born with a cleft lip and palate include thorough evaluation and documentation of the cleft region. Conventional records in orthodontics include facial and intraoral photographs, study models, and panoramic and cephalometric radiographs. As the cleft deformity usually presents both skeletal and dental abnormalities, it is recommended to include a 3-dimensional cone-beam CT (CBCT) of the cleft maxilla to evaluate dental development and bony architecture. CBCT facilitates the timing of decisions relative to dental eruption, and evaluation of the cleft osseous gap and adjacent dentition including supernumeraries, as well as tooth size and shape abnormalities. Moreover, CBCT may facilitate detailed and accurate postoperative outcomes analysis.[31]

There are 3 important issues to consider when performing presurgical orthodontic preparation for a secondary bone-grafting procedure: the dentition around the cleft area, the eruption of the permanent teeth through the graft, and the anteroposterior and transverse maxillary deficiencies. Supernumerary teeth, malformed permanent teeth (normally lateral incisor with poor root development), or remaining primary dentition on the cleft region should be removed before the secondary bone graft. Usually the extraction of these teeth should be performed 2 to 3 months before the surgical intervention to maintain the integrity of the palatal mucosa, cover the entire graft, and eliminate any portal for potential infection during the postoperative period.[49]

The maxillary incisors normally erupt rotated, retroclined, or in anterior cross-bite.[29] The alignment of the teeth adjacent to the cleft should not be corrected before the graft procedure, or in such a case the orthodontic movement should be limited to the available bone into which the roots of the teeth can be moved. These 2 aspects are essential in avoiding dehiscences and fenestrations.[50]

The bony and soft-tissue defects, and the presence of anomalies as well as supernumerary, missing, or malformed teeth, create an abnormal eruption pattern and increase the risk of impaction of the canine.[51] One of the most important benefits of the secondary bone graft is that the new grafted bone acts as alveolar bone, allowing the natural migration and eruption of the permanent canine. The maxillary canine can erupt spontaneously after the bone-graft procedure, with a success rate between 72% and 80%. Surgical exposure of the

canine for orthodontic traction is performed in 6% to 18% of cases.[52,53] Moving the canine into the graft allows space closure and mesial movement of the posterior teeth on the affected side, eliminating the need for prosthetic replacement of the absent lateral incisor. In cases where the canine erupts before the bone-graft surgery, close evaluation of the area is necessary to reduce the possibility of a periodontal defect. Occasionally the graft can cause resorption on the cervical third of roots of the teeth adjacent to the cleft, specifically the canines. This root resorption is caused by direct contact between the grafted bone and the exposed root surface.[54]

Maxillary Expansion

Management of maxillary constriction is a ubiquitous challenge in the treatment of cleft alveolus. Patients born with a cleft of the alveolus and palate usually undergo palatal closure between 10 and 14 months of age. The surgical procedure and scar-tissue retraction often produce a collapse of maxillary alveolar segments, resulting in unilateral or bilateral posterior cross-bites. This transverse deficiency must eventually be addressed by expanding the maxillary arch to provide a functional dental occlusion. A decision has to be made on whether to expand the collapsed segments before or after the secondary bone-grafting procedure. Each case needs to be evaluated individually.

The benefits of expanding the maxillary arch before the graft procedure include improvement of the upper arch morphology, correction of posterior cross-bite, avoidance of resistance to the expansion, and increase of the upper arch perimeter in cases where dental crowding is present. Expansion of the maxilla facilitates surgery by permitting improved access for meticulous repair of the nasal floor mucosa. Presurgical expansion also increases the space for graft placement, resulting in the need for insertion of greater bone-graft volume.[23,48,55]

When bone grafting is performed before expansion, the size of the defect is smaller, which facilitates tension-free closure of the gingiva and limits the volume needed for graft material. However, access for meticulous nasal mucosal repair is limited, and the morphology postoperatively becomes stabilized in the constricted state, with less bone volume in the cleft site. Despite these shortcomings, some clinicians advocate expansion after the bone-graft procedure. Advocates report that expansion and correction of posterior cross-bite is permitted despite bone continuity at the alveolus via permission of expansile forces along the midpalatal suture in the premaxillary region, demonstrated by the presence of a diastema between the central incisors. No radiographic alteration was observed in the grafted area during the expansion process.[56] In another study using rapid maxillary expansion after the bone graft, the midpalatal suture was opened, but the results were unpredictable.[53]

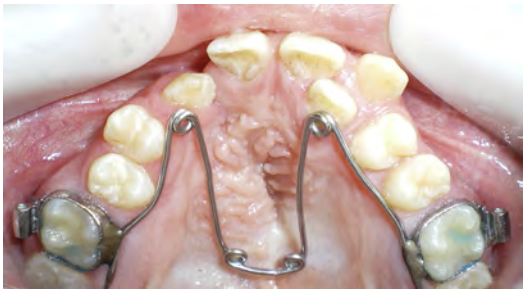

Fig. 13. Reverse quad-helix appliances provide for differential expansion in cases where more anterior than posterior expansion is needed.

The authors' approach to maxillary constriction involves expansion before bone grafting, performed during the mixed dentition stage. The appliances may be either banded or bonded, and include the quad helix, reverse quad helix (**Fig. 13**), hyrax, fan (**Fig. 14**), and Hass expander.[30,31] In some cases, cross-bite is not observed because of the lingual tipping observed in the lower arch as a compensatory effect of the maxillary atresia.[49] Rapid maxillary expansion presents a different pattern of expansion depending on the type of the cleft. In unilateral cases the minor segment moves apart from the major segment, and in bilateral patients both segments move away symmetrically. This pattern could be affected by the moment of the graft placement,

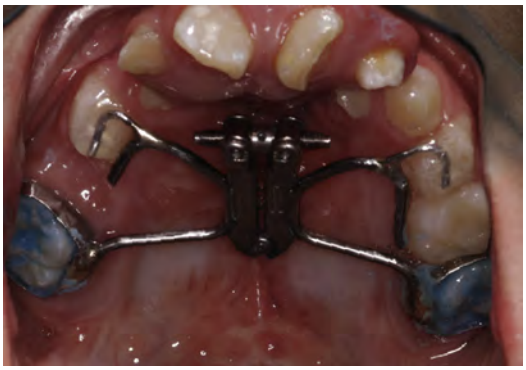

Fig. 14. A fan expander with expansion screw is a more rigid appliance by which the amount of expansion is controlled by the number of turns or activations per day. It also provides differential expansion, maintaining a stable posterior width while expanding the anterior region.

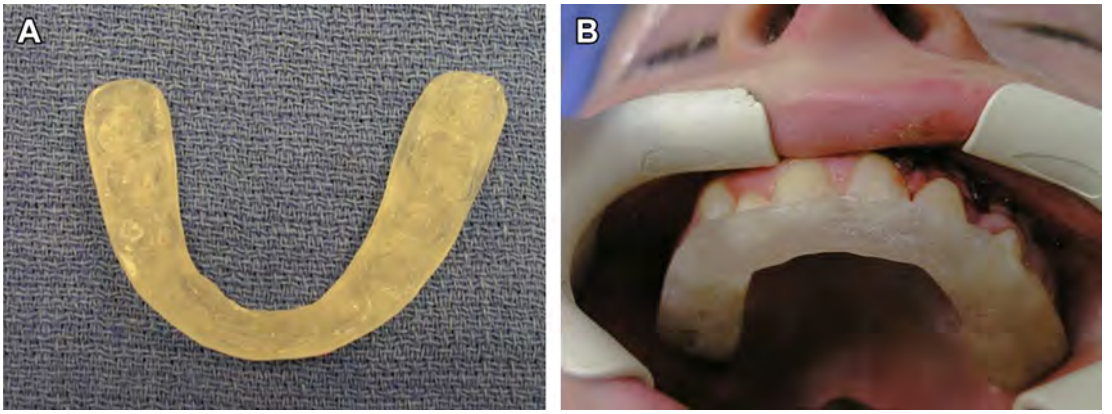

Fig. 15. (*A*) A customized acrylic surgical splint is fashioned to be placed immediately after the secondary alveolar bone-graft procedure. (*B*) The craniofacial orthodontist inserts the surgical splint while in the operating room after the surgery is performed. The splint is an integral part of the authors' protocol, as it preserves the arch width achieved during the expansion phase, protects the delicate graft area from food impaction, and reminds the patient to take proper care of the site.

because the maxillary segments move individually before the graft, and after it the palatal processes behave as a single piece.[53] Retention of the corrected cross-bite using orthodontic appliances such as the transpalatal bar with lateral projections is indicated immediately before and after the graft. The transpalatal bar can be used also as an anchorage component during comprehensive orthodontic treatment.[49]

Management Protocol

The authors' orthopedic and orthodontic protocol sequence during the bone-grafting phase includes the following.

1. A low-dose maxillary CBCT scan is ordered to evaluate dental development and skeletal architecture at the cleft site.
2. Maxillary expansion is advised if a posterior cross-bite is present.
3. After adequate expansion is achieved and a few days before surgery, the expander is removed and a maxillary retainer (Hawley) is delivered the same day to preserve arch width. An impression for a surgical splint is taken.
4. The craniofacial orthodontist inserts the surgical splint, in the operating room, after the alveolar bone graft surgery is performed (**Fig. 15**). The splint is an integral part of the protocol, as it preserves the arch width achieved during the expansion phase, protects the delicate graft area from food impaction, and reminds the patient to take proper care of the site. The splint is kept in the mouth and is evaluated by the orthodontist biweekly for 8 to 12 weeks until radiographic evidence of bone formation is present.
5. The orthodontist removes the splint, fits bands, and makes a new impression for a transpalatal arch retainer with arms to hold the expansion and arch integrity.
6. If indicated, limited orthodontic appliances (braces) are applied to the anterior teeth to correct dental rotations or malpositions. If a maxillary hypoplasia causing a sagittal skeletal discrepancy or anterior cross-bite is present, maxillary protraction (face mask) therapy could also be incorporated into the treatment plan.

SUMMARY

The interdisciplinary approach to primary and secondary surgical procedures has proven to be beneficial for patients. In the neonate, NAM has been found to optimize the aesthetic outcome of the nasal and labial repair while minimizing the extent of surgery and scar-tissue formation. During the mixed dentition stage, orthopedic arch preparation before the secondary alveolar bone-grafting procedure improves arch morphology, restores the functional interarch relationship, and facilitates surgical access. The cooperative effort between the surgeon and craniofacial orthodontist in designing strategies customized to the patient's specific needs has resulted in better aesthetic and functional outcomes, minimizing surgical interventions.

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ORIGINAL ARTICLE

Early Surgical Complications After Primary Cleft Lip Repair: A Report of 3108 Consecutive Cases

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Objective: **To analyze short term surgical complications after primary cleft lip repair.**

Patients and Design: **A total of 3108 consecutive lip repairs with 2062 follow-ups were reviewed retrospectively through medical records. Patients were aged 3 months to 75 years at the time of surgery, with a median of 7 years.**

Setting: **Guwahati Comprehensive Cleft Care Center, Assam, India.**

Intervention: **Primary cleft lip repair.**

Main Outcome Measures: **Documented complications in terms of dehiscence, necrosis, infection, and suture granuloma were compiled. Logistic regression was used with dehiscence (yes/no) or infection (yes/no) as binary dependant variables. Age, cleft type, and surgeon (visiting/long term) were used as covariates.**

Results: **Among the 2062 patients who returned for early follow-up, 90 (4.4%) had one or more complications. Dehiscence (3.2%) and infection (1.1%) were the most common types of complication. Visiting surgeon, complete cleft, and bilateral cleft were significantly associated with wound dehiscence, and complete cleft was associated with wound infection according to the logistic regression analysis. Of patients with bilateral complete clefts, 6.9% suffered from some degree of wound dehiscence.**

Conclusion: **In a setting where presurgical molding is unavailable and patients present at all ages, lip wound dehiscence is a relatively common complication in patients with bilateral complete clefts. The risk of dehiscence, however, is reduced when these cases are assigned to surgeons with experience with these types of clefts. We also found that the incidence of wound infection can be kept relatively low, even without the use of postoperative antibiotics.**

KEY WORDS: *dehiscense*, *developing world*, *infection*, *late cleft lip repair*, *lipplasty*

Cleft lip repair is a common surgical procedure all over the world. However, conflicting data regarding the incidence and definition of surgical complications following lip repair can be found in the literature. Complication rates range from 1.7% to 8.2%, and definitions include dehiscence, infection, stitch granuloma, hypertrophic scarring, and notching (Wilhelmsen and Musgrave, 1966; Bromley et al., 1983; Weatherley-White et al., 1987; Lees and Pigott, 1992; Eaton et al., 1994; Al-Thunyan et al., 2009; Aziz et al., 2009; Nagy and Mommaerts, 2011; Halli et al., 2012; Abdurrazaq et al., 2013). Some report higher complication rates, but no distinction is then made of cleft lip and palate cases (Schettler, 1973; Orkar et al., 2002; Jones et al., 2010). Moreover, in the existing studies, series are often small and follow-up rates are not always accounted for, making the combined results inconsistent and inconclusive. Most previous studies have only included around a hundred patients or fewer (Bromley et al., 1983; Weatherley-White et al., 1987; Lees and Pigott, 1992; Orkar et al., 2002; Al-Thunyan et al., 2009; Aziz et al., 2009; Jones et al., 2010; Halli et al., 2012; Abdurrazaq et al., 2013), but nonetheless, a few larger series have been published. Wilhelmsen and Musgrave (1966) presented a series of 565 patients with a 4.6% incidence of suture line breakdown. However, their study was based on surgeries performed from 1950 to 1964, and given that surgical protocols and materials have evolved over the years, a more current report would be of interest. Schettler (1973) presented a study of 1565 cleft surgeries in 1973, but unfortunately, no distinction was made of cleft lip and palate cases. More recently, Nagy and Mommaerts (2011) reported a 2.6% incidence of wound infection and/or dehiscence in a series of 302 cases. At their center in Bruges, Belgium,

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TABLE 1 Background of 3108 Primary Cleft Lip Repairs

Age	Mean = 11.9 y Median = 7 y Range, 3 mo–75 y
Sex	Male: 1825 (58.7%) Female: 1283 (41.3%)
Diagnosis*	UCL incomplete: 1434 (46.1%) UCL complete: 1248 (40.2%) BCL incomplete: 138 (4.4%) BCL complete: 288 (9.3%)†
Surgeon	Long term: 2360 (75.9)‡ Visiting: 746 (24.0%) Missing: 2 (0.1%)

* UCL = unilateral cleft lip; BCL = bilateral cleft lip.
† Complete on one or two sides.
‡ 6 months of service or more.

patients follow an extensive postoperative wound care protocol including a Logan's bow, arm restraints, postoperative antibiotics, specific wound ointment, and dressings as well as a wound cleaning protocol after feeding. Therefore, the results from Bruges might not be applicable in a wider perspective. Most cleft surgeries are performed in less developed regions of the world, where resources are limited, hygiene standards are lower, and patient compliance and follow-ups are less predictable (World Health Organization, 2000).

In this study we report of the surgical complications in 3108 consecutive primary cleft lip repairs performed at the Guwahati Comprehensive Cleft Care Center in Assam, India. This is, to our knowledge, the largest study of its kind and could serve as a benchmark in terms of incidence of lip complications in this setting.

Patients and Methods

Retrospective data was collected from medical records. A total of 3108 consecutive patients underwent primary cleft lip repair at Guwahati Comprehensive Cleft Care Center in Assam, India, between February 2011 and October 2013. Background data of the patients are listed in Table 1. Malnourished patients were enrolled in a nutrition program and were not operated upon until they were considered fit for surgery. The majority of the patients with unilateral cleft lip were operated upon with a rotation advancement technique (Mohler), and in general, a Millard-type repair was used for those with bilateral cleft lip. Surgeons were labeled as either visitors or long-term staff (>6 months of service at the center). The visitors consisted of surgeons with limited or extensive experience with cleft surgery. The patients typically received a single intraoperative dose of cefuroxime (30 mg/kg). After surgery, patients and their families were involved in an education program for postoperative care. The wound care protocol included gently washing the wound area with soap and water two to three times daily. Patients were advised to resume a regular diet immediately, with breast-feeding for infants, and instructions were given for oral hygiene including brushing teeth twice a day. No arm restraints were used. Patients were discharged the day after surgery with postoperative instructions written in Assamese (the predominant local language), including pictographs for illiterate patients.

TABLE 2 Complications Based on 2062 Follow-Ups

Type of Complication	*Incidence, n (%)*
Dehiscence	61 (3.0)
Infection	17 (0.8)
Dehiscence and infection	5 (0.2)
Stitch granuloma	5 (0.2)
Philtral flap necrosis and dehiscence	1 (0.05)
Other (pressure necrosis)	1 (0.05)

Of the 3108 patients, 2062 (66.3%) returned for follow-up within 4 weeks. At the time of follow-up the patients were examined by a cleft surgeon, and complications in terms of dehiscence, necrosis, infection, and suture granuloma were recorded in a standardized manner. *Dehiscence* was defined as any disruption in the suture line in the vermillion or in the skin of the lip, columella, or nasal sill.

Statistics

Statistical analyses were preformed using IBM SPSS version 21 for Windows (SPSS Inc., Chicago, IL). Logistic regression was used with dehiscence (with or without infection) or infection (with or without dehiscence) as binary dependent variables in two separate models. Age, visiting surgeon versus long-term surgeon (>6 months of service), incomplete versus complete clefts, and unilateral versus bilateral clefts were used in each model as covariates.

The Hosmer-Lemeshow goodness-of-fit test was used to confirm the reliability of the model.

P values less than .05 were considered statistical significant.

Ethics

This study was reviewed and approved by Operation Smile India Institutional Ethics Committee.

Results

Among the 2062 patients who returned for early follow-up, 90 (4.4%) had one or more complications (Table 2). The majority of the complications consisted of wound dehiscence (3.2%) and/or wound infection (1.1%). In the majority of the patients with wound dehiscence, the complication involved less than 25% of the skin/vermillion (Table 3). Ten complications (0.5%)

TABLE 3 Degree of Dehiscence

Degree of Dehischence	*Incidence, n (%)*
≤25%	42 (62.7)
26%–74%	13 (19.4)
≥75%	11 (16.4)
Missing value	1 (1.5)

were severe enough for the patients to be scheduled for revision surgery.

The logistic regression analysis showed that the incidence of dehiscence (with or without infection) was significantly associated with visiting surgeons ($P < .001$, odds ratio [OR] = 2.64, 95% CI, 1.61 to 4.33), complete clefts ($P < .05$, OR = 1.830, 95% CI, 1.07 to 3.11), and bilateral clefts ($P < .05$, OR = 2.01, 95% CI, 1.14 to 3.57). The incidence of dehiscence according to surgeon and cleft type can be seen in Figure 1.

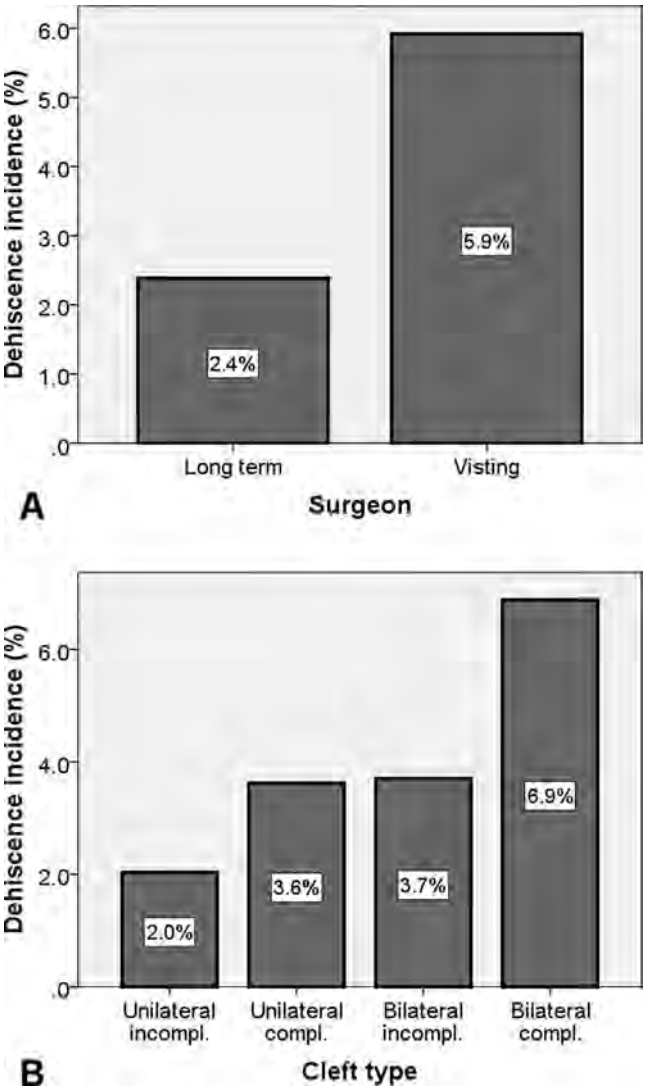

FIGURE 1 Incidence of wound dehiscence after primary lip repair, depending on A: surgeon (visiting or permanent) and B: cleft type.

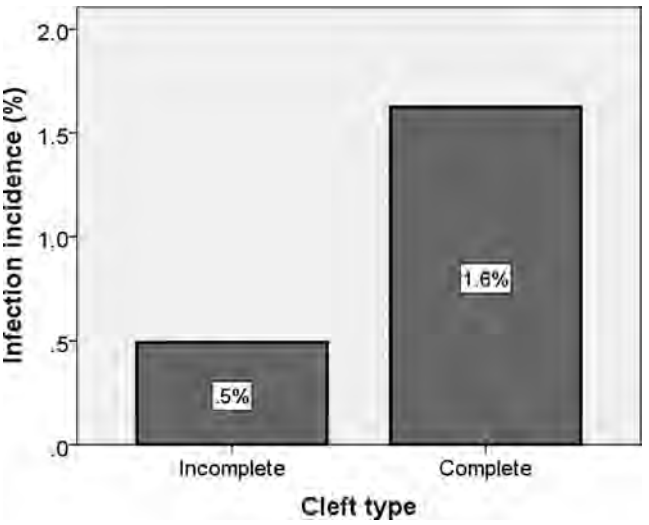

FIGURE 2 Incidence of wound infection after primary lip repair, comparing complete and incomplete clefts.

We also found that wound infection (with or without dehiscence) was significantly associated with complete clefts ($P < .05$, OR = 3.34, 95% CI, 1.23 to 9.10). The incidence of wound infection depending on complete or incomplete clefts can be seen in Figure 2.

The age of the patient was not found to be associated with dehiscence or infection in a statistically significant manner.

Discussion

In our study, based on 2062 patients with follow-ups, we found an overall complication rate of 4.4%, consisting mainly of skin dehiscence (3.2%) and wound infection (1.1%).

This rate compares well with Wilhelmsen and Musgrave's study from 1966. They reported a 4.6% incidence of minor or major suture line breakdown. No distinction, however, was made between wound infection and dehiscence. Wound infection can lead to wound dehiscence and vice versa, but our study suggests that these two complications represent different entities. Among the 83 patients who suffered from infection and/or dehiscence in our series, only five patients presented with both these complications. Furthermore, our logistic regression analysis suggests that wound infection and dehiscence have different etiologies.

We found a statistically significant relationship between lip dehiscence and visiting surgeon, complete cleft, and bilateral cleft. High dehiscence rates among visiting surgeons could perhaps be due to the different preconditions compared with a familiar working environment back home. The cleft panorama and the patient clientele in a less developed environment can differ greatly from a Western setting where patients are treated with presurgical molding and are operated upon at standardized ages. As a

consequence, the clefts may be wider and less aligned than one is used to, which may require adjustments of one's standard surgical technique or wound closure under greater tension.

The association between dehiscence and complete and bilateral clefts is not surprising and supports the assumption that excessive skin tension can cause dehiscence. In line with this, the bilateral complete clefts were distinctly overrepresented among the cases of dehiscence. Bromley et al. (1983) made a similar observation, where 5 (16%) of 31 patients with bilateral cleft lip suffered from dehiscence. We believe that extra precautions should be considered when treating patients with bilateral complete lip, and our regression analysis suggests that experienced surgeons should be assigned to these patients. Part of the permanent staff at our center uses a de-epithelialized strip of flanking dermis on both sides of the philtral flap, according to Mulliken (2001), to strengthen the suture line and prevent dehiscence. Presurgical molding, lip adhesion, or staged lip repair could also be considered, but for many patients in the less developed part of the world this is not a realistic option. Whether the setting is a mission or a center, many patients will have the opportunity to receive treatment only a limited number of times due to accessibility, loss of income, and so forth (Schwarz and Bhai Khadka, 2004; Adeyemo et al., 2009).

According to our logistic regression analysis, wound infections were associated with complete clefts. An explanation for this could be that complete clefts need more extensive surgery, and the surgery involves the nose and oral cavity to a greater extent. Our findings are in support of Schettler (1973), who found a 13.3% infection rate if surgery was more than 2 hours compared with 5.1% if surgery time was less than 2 hours. Even though Schettler (1973) did not differentiate between lip and palate surgeries, our infection rate of 1.1% can be regarded as relatively low. Nagy and Mommaerts (2011) reported 8 patients (2.6%) of 302 primary cleft lip repairs with dehiscence and/or infection. The article did not distinguish between the two types of complications, but it was stated that five patients (1.6%) were treated with additional oral antibiotics, indicating an infectious component in these cases. Nagy's postoperative wound care protocol included arm restraints, wound ointment, and dressings as well as postoperative intravenous and oral antibiotics. The use of these routines to decrease wound infections can perhaps be questioned, considering that our center seems to have a comparable incidence of wound infections without using any of these preventive measures. We do, however, feel that our patient education program, including basic instructions for post–op wound care, has brought our infection rates down. We have previously compared cleft missions, with or without this program, and found that infection rates were reduced from 3.7% to 0.4% (Schönmeyr et al., 2014).

Conclusion

In our series of 3108 primary lip repairs, we found a complication rate of 4.4% based on 2062 follow-ups. The most common complication was skin dehiscence, and this complication was significantly overrepresented among patients who had bilateral complete clefts. We therefore advise that extra precautions should be considered when operating upon bilateral complete clefts in this setting. Furthermore, these patients should be assigned to surgeons with experience with this type of clefts.

We also found a low incidence of wound infection among our patients without the routine use of oral or local antibiotics postoperatively. Instead, we recommend educating patients in simple routines of hygiene and wound care before discharge.

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Update on outcomes research for cleft lip and palate

David Shaye

Purpose of review
The purpose of this review is to summarize the recent evidence-based literature focusing on cleft lip and palate outcomes research.

Recent findings
The findings of recently published literature focus on cleft lip and palate outcomes research, patient-based outcomes measurement tools, nasoalveolar molding, and how speech outcomes relate to palatoplasty timing, technique, and intravelar veloplasty. Studies have investigated the relationship between palatoplasty timing and facial development.

Summary
The literature lacks any evidence-based consensus to support a superior method of cleft lip repair. A majority of North American surgeons, however, utilize a rotation-advancement technique and perform cleft rhinoplasty at the time of primary lip repair, with the idea that this could decrease the number of revision surgeries needed over the long term. Most cleft surgeons perform a single-stage palatoplasty at 9–12 months of age for improved early speech outcomes. There is insufficient evidence to support a two-stage palatoplasty with the intention of improved maxillary growth. Controversy persists on the relationship between early palatal surgery and its deleterious effects on facial development. A shift toward patient-reported outcomes is called for; however, this remains difficult, as there are few validated, cleft-specific outcome measurement tools.

Keywords
cleft lip, cleft palate, outcomes, palatoplasty, speech

INTRODUCTION

Orofacial clefting is the most common congenital facial anomaly [1]. A cleft of the lip or palate is not only a physical deformity, but affects the development, psychology, and social well being of the patient and their family. As the impact of an orofacial cleft extends beyond a physical deformity, so too should research extend beyond surgical repair. To improve upon existing treatment strategies, a shift toward outcomes-based research is taking place.

This article reviews the recent, pertinent literature that focuses on outcomes-based research in the cleft lip and palate population. This includes outcomes-based research for cleft lip repair, cleft palate repair, midface growth, speech, intravelar veloplasty, and other treatment modalities.

CLEFT LIP

Attempts to objectively compare various cleft lip repair techniques are wrought with difficulty because of the heterogeneous nature of the cleft population. This makes it challenging to draw comparative conclusions. As each cleft varies in type and severity, so too do the treatment protocols from different cleft teams. Some of these differences include variations in repair technique, the use of presurgical infant orthopedics, minor variations of surgical technique, surgeon preferences, timing of surgery, and gingivoperiosteoplasty, to name a few. Finally, the child with a cleft matures and the final surgical results are not seen for decades, making multicenter, longitudinal data collection challenging at best. Studies that have attempted to examine various cleft lip repair

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KEY POINTS

- The literature lacks any evidence-based consensus to support a superior method of cleft lip repair; however, a recent survey of North American surgeons shows that a majority utilize a rotation-advancement technique.
- A majority of North American surgeons perform primary cleft rhinoplasty at the time of primary lip repair, with the idea that this could decrease the number of revision nasal surgeries needed over the long term. There is some evidence that NAM improves nasal symmetry.
- Most cleft surgeons perform a single-stage palatoplasty at 9–12 months of age for improved early speech outcomes. There is insufficient evidence to support a two-stage palatoplasty to improve maxillary growth.
- A shift toward patient-reported outcomes is needed; however, this remains difficult, as there are few validated, cleft-specific outcome measurement tools. VELO is one cleft-specific tool that has been validated to serially test quality of life related to VPI.

techniques (rotation-advancement versus triangle repair) have suggested no significant aesthetic advantage of one over the other [2,3]. Once repair has been performed, any asymmetry in lip height improves with time, studied recently in the case of an extended Mohler repair [4].

Primary cleft rhinoplasty

Historically, it was hypothesized that the scar caused by a rhinoplasty dissection inhibited further nasal growth. Although controversy exists on the subject, the majority of North American cleft surgeons do perform a limited cleft rhinoplasty at the time of primary lip repair [5]. Once facial growth has ceased or tapered in the adult years, a definitive rhinoplasty is sometimes performed based on the severity of the cleft nasal deformity. Although there are no randomized controlled trials looking at the benefits of primary cleft rhinoplasty, many studies suggest that primary rhinoplasty improves nasal appearance and possibly decreases the number of revision surgeries in the future [6–10].

Nasoalveolar molding

The use of nasoalveolar molding (NAM) has grown considerably over the last two decades, with at least 71% of cleft surgeons of the American Cleft Palate Craniofacial Association using NAM occasionally [5]. Nasoalveolar molding takes advantage of a custom-made mold of the nasal and alveolar cleft. The mold undergoes periodic adjustments to narrow the cleft, improve the nasal deformity, and bring together the lateral lip segments prior to surgery (which is delayed to accommodate NAM). Abbott and Meara [11▪▪] performed an evidence-based review of clinical studies that looked at NAM and an objective measurement of nasal symmetry. Several factors made direct statistical comparison difficult, which include variations between studies in NAM treatment length, use of stents, and lip repair technique. The results of the six included studies were presented, and overall there is some evidence that NAM improved nasal form and symmetry. However, in each study, additional nasal interventions were completed, which precludes one from attributing the final results to NAM alone.

Timing of cleft lip repair

The timing of cleft lip repair can range from the neonatal period to 6 months depending on a variety of factors. Proponents of lip repair in the neonatal period feel this increases maternal bonding and has improved aesthetic outcomes. However, evidence has shown that repair in the neonatal period results in increased scarring [12,13] and no improvement in maternal–child bonding [14,15]. Repair at 2–3 months of age permits better intraoperative visualization of the muscular anatomy and decreases anesthetic complications. If presurgical orthopedics are used, the lip repair is delayed up to 5–6 months of age. Intrauterine repair has been contemplated based on evidence for improved fetal healing [16,17]; however, significant fetal and maternal risks have not made this a realistic avenue of research.

CLEFT PALATE

The two most commonly used techniques for repair of the cleft palate are the two-flap palatoplasty and the Furlow double opposing Z-plasty [18]. Furlow introduced the double opposing Z-plasty in 1978 as a new technique to reapproximate a cleft of the soft palate. Using two layers of Z-plasties, Furlow realigned the tissue to recreate the muscular sling of the soft palate. The result improved anatomic orientation of the muscle, which was thought to improve function. Furlow reported superior results in comparison to the two-flap technique, his previous procedure of choice [19]. Others reported similar, superior results with the Furlow technique [20–23]. However, these studies are mostly single-center or single-surgeon studies with results measured retrospectively.

In addition, studies focusing on anesthesia complications in palatoplasty patients have shown that

surgeons should be aware of an increased risk of perioperative airway compromise (particularly hypoxemia after extubation) [24–26]. In the palatoplasty postoperative setting, 39 and 43% of surgeons discharged their palate patients within 24 and 48 h after surgery, respectively [27].

Intravelar veloplasty

The intravelar veloplasty has been described to maximize anatomic alignment of the muscle [28]. An intravelar veloplasty involves removing the abnormally attached levator veli palatini muscle from the posterior edge of the hard palate, and then realigning the levator muscles to form a palatal muscular sling. Marsh *et al.* [29] performed a prospective study of 51 consecutive patients that were randomized to either undergo or not undergo an intravelar veloplasty during the repair of their cleft palate. The group that underwent intravelar veloplasty showed significantly increased surgical time, increased cost, no increased morbidity, but most importantly, no significantly improved speech, which was measured by a single, blinded speech pathologist when the child reached 3 years of age.

In contrast, other studies have shown improvements with the intravelar veloplasty. Andrades *et al.* [30] performed a retrospective review of 213 consecutive two-flap palatoplasty patients before and after the introduction of the intravelar veloplasty. Speech evaluation performed by two speech pathologists, looking at nasal emission, articulation, intelligibility, and velopharyngeal competence, showed significantly improved speech and a lower rate of secondary velopharyngeal insufficiency (VPI) surgery for the group that underwent intravelar veloplasty. No differences were found between the two groups in terms of postoperative complications. Although the study design has experience bias, a strong correlation is shown between intravelar veloplasty and improved speech outcomes, without any increase in postoperative complications.

Facial development

There has long been concern that surgery on the developing facial skeleton inhibits facial growth. Chen *et al.* [31] compared sagittal maxillary growth in adult patients who had undergone palatal repair with adult patients that had unoperated, isolated cleft palates. Nonclefted individuals served as controls. The unoperated cleft palate patients showed similar maxillary retrusion to those that had undergone palatoplasty. This study showed no correlation between surgical trauma and maxillary retrusion, as the unoperated cleft group showed maxillary retrusion as well.

Ye *et al.* [32] looked at adult dental arch length and width using computed tomography, comparing operated cleft palate patients, unoperated cleft palate patients, and nonclefted individuals with normal occlusion as controls. The operated group showed significantly smaller widths and lengths of the anterior dental arch in comparison with unoperated cleft palate patients and controls. They concluded that although the existence of a cleft palate has a limited effect on the dental arch (mostly anterior), a palatoplasty results in a constricted dental arch.

These reports have informed the heuristic to limit dissection over the hard palate during palatoplasty. They feel that if the periosteum is disturbed and results in scarring, then facial growth will be inhibited. Some advocate for a two-stage approach, where initial repair of the soft palate is followed by a delayed repair of the hard palate, to lessen the negative effects on facial growth [33–35]. Liao *et al.* [33] looked at the 20-year maxillary growth of 72 patients that had been randomized to either a one-stage or two-stage palatal repair. There was no significant difference in maxillary growth rate, but the group undergoing single-stage repair had less maxillary length and protrusion measured at 20 years.

Furthermore, the size of the cleft has been shown to correlate with maxillary retrusion, whereby clefts comprising a larger percentage of the total surface area of the palate correlated with less maxillary protrusion and length measured at 9 years of age [36]. These findings are in contrast to Chen *et al.* [31], who found that maxillary growth did not correlate to severity of the cleft.

Speech

One of the goals of the multidisciplinary cleft team is to maximize speech and language outcomes. Proponents of single-stage palatoplasty argue that early repair improves speech, and has no significant deleterious effects on maxillary growth [37]. Pradel *et al.* [37] examined 24 children, half of which underwent staged repair of the soft palate at age 9–12 months and hard palate at age 24–36 months; the other 12 children underwent a single-staged procedure at 9–12 months with the same technique as what was done in two stages for the first group. Children in the early, single-stage palatal repairs showed significantly improved speech at 4 and 6 years of age, and improved maxillary growth. This

study showed no significant deleterious effects of early palatal cleft repair, and overall improved speech outcomes.

VPI has been shown to be more severe if palatoplasty was performed at a later age [38]. The authors postulate that if palatoplasty is delayed to accommodate facial growth, VPI may not be able to be overcome with compensatory speech or revision surgery. They caution a balance between obtaining good speech outcomes, but limiting inhibition of facial growth.

Considering the mixed evidence that staged palatal repair permits improved facial growth against the strong evidence for improved speech outcomes with early palatal repair, most surgeons perform a single-staged palatal repair between 9 and 12 months. Maxillary hypoplasia can be repaired surgically, whereas overcoming VPI speech at a later age remains a much more challenging endeavor.

Williams *et al.* [39▪] compared the speech of two cohorts of cleft palate patients randomized to undergo either a Furlow palatoplasty or a two-flap palatoplasty with intravelar veloplasty. VPI was measured with a cul-de-sac test and mirror test for nasal air emission by a speech pathologist. Results showed that the Furlow palatoplasty had better velopharyngeal function.

PATIENT-REPORTED OUTCOMES

Success in the treatment of cleft lip and palate deformities is traditionally measured through objective physician-centered data such as VPI severity, photographs, and complications, to name a few. Although these data are important, no relationship between objective physician-reported outcomes and patient-reported outcomes has been found [40]. Therefore, it is imperative to independently measure patient-reported quality of life (QOL) outcomes as an integral determinant of success. In fact, this was one of the goals of the National Center of Birth Defects and Developmental Disabilities at the Centers for Disease Control and Prevention's 2006 workshop to prioritize a research agenda for orofacial clefts [41]. QOL for families and children with orofacial clefts was identified as a key research gap requiring additional public health research.

Patient-reported outcomes include self-image, integration into society, functional status, speech and aesthetic perceptions, to name a few. In a 2011 literature review, Eckstein *et al.* [42▪▪] identified validated questionnaires to serve the cleft population. The authors found only three validated instruments, none of which were specific to the cleft lip and palate population. One was a craniofacial-specific measure (The Youth Quality of Life-Facial Differences), whereas the other two (Child Oral Health Quality of Life and the Child Oral Health Impact Profile) focused on oral health. Klassen *et al.* [43] also performed a literature review with similar findings of a complete lack of cleft-specific outcomes measurement tools.

In 2013, however, Skirko *et al.* [44▪▪] further investigated the VPI Effects on Life Outcomes (VELO) instrument. The VELO instrument was prospectively administered to 59 children and 84 parents of children that had undergone treatment for VPI (i.e., Furlow palatoplasty, pharyngoplasty, or obturator). A baseline was established and then the VELO instrument was administered 3 months later, with 81% of the patients completing it. VPI severity and speech intelligibility were also analyzed by pediatric speech language pathologists using perceptual speech analysis. The VELO instrument demonstrated concurrent and anatomic construct validity with excellent test–retest reliability. The VELO instrument was found to provide a VPI-specific quality-of-life instrument that demonstrates concurrent validity and responsiveness to change (i.e., surgical treatment). VELO also showed test–retest reliability meaning it is an accurate instrument for serial measurements of VPI. Validated, cleft-specific instruments such as this must be available to begin assembling patient-reported outcomes in a meaningful way.

CONCLUSION

The repair of a cleft lip or palate deformity is rewarding for both patient and surgeon alike. The medical community strives to improve upon this with innovation and measuring results. Traditionally, this has been physician-centered data; however, a shift is occurring to patient-reported outcomes. It is logical that a patient's perspective is critical to determining success.

This review article summarizes the recent cleft lip and palate literature, with special focus on lip repair, primary rhinoplasty, the utility of NAM, facial growth, speech outcomes, and patient-reported outcome measurement tools. Evidence-based research as discussed in this article will further develop this very rewarding field. This will be driven by validating outcomes with cleft-specific instruments, followed by high-quality, evidence-based research.

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None.

Conflicts of interest

There are no conflicts of interest.

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The VELO instrument was found to provide a VPI-specific quality-of-life instrument that demonstrates concurrent validity and responsiveness to change (i.e., surgical treatment). This is an accurate instrument for serial measurements of VPI.

Cleft Lip and Palate
An Evidence-Based Review

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KEYWORDS

• Cleft lip • Cleft palate • Evidence-based medicine • Outcomes

KEY POINTS

- The repair of unilateral cleft lip is performed using a rotation-advancement, geometric, straight-line, or hybrid technique.
- For bilateral cleft lip repair, most surgeons use either the Millard or Mulliken technique, and their variations.
- Most cleft centers perform cleft lip repair at the age of 3 to 5 months.
- Presurgical infant orthopedics, which can include nasoalveolar molding, is used before definitive cleft lip repair.
- For cleft palate repair, the 2-flap palatoplasty and Furlow double-opposing Z-plasty are most commonly used.

INTRODUCTION

At an estimated prevalence of 16.86 cases per 10,000 live births, isolated cleft palate, as well as cleft lip with or without cleft palate, is the most common congenital orofacial malformation in the United States.[1] Children with cleft anomalies may experience a multitude of physical and developmental challenges. There also may be psychosocial and emotional concerns for the patients and their families. As such, comprehensive care for the patient with cleft lip and/or palate requires an interdisciplinary team. The guidelines for team care outlined by the American Cleft Palate Association recommend team members that may include anesthesiology, audiology, genetics, neurosurgery, nursing, ophthalmology, oral maxillofacial surgery, orthodontics, otolaryngology–head and neck surgery, pediatrics, pediatric dentistry, physical anthropology, plastic surgery, prosthodontics, psychiatry, psychology, social work, and speech-language pathology.[2] Although every specialty may not be represented, the quality of care is augmented through collaborative discussion and coordination of care.

Broadly speaking, orofacial cleft anomalies may be unilateral or bilateral and involve the lip, the palate, or both. Although there have been considerable publications on this topic, most are single-surgeon/center experience papers or are retrospective in nature. As a result, the cleft lip–cleft palate literature regarding the clinical and surgical decision points lacks consensus. This review article seeks to define the typical management plans, describe the various viewpoints, and suggest recommendations based on the levels of evidence (**Table 1**) on the management of cleft lip

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Table 1
Levels of evidence

Level I	High-quality, properly powered and conducted randomized controlled trial, systematic review, or meta-analysis of these studies
Level II	Well-designed controlled trial without randomization; prospective comparative cohort trial
Level III	Retrospective cohort study, case-control study, or systematic review of these studies
Level IV	Case series with or without intervention; cross-sectional study
Level V	Expert opinion, case reports, or bench research

Adapted from Oxford Centre for Evidence-Based Medicine. Available at: http://www.cebm.net/index.aspx?o51001. Accessed April 16, 2015.

and palate. The article is organized to address management of the techniques, timing, outcomes, and complications starting with cleft lip, and then addressing the same in cleft palate management.

CLEFT LIP

Overview

A typical orofacial cleft can be classified by *laterality*, *extent*, and *severity*. The *laterality* (left, right, asymmetric/symmetric bilateral) is noted with the unilateral deformity being more common than the bilateral. The *extent* of the cleft lip is variable and can include the cleft alveolus, which can be complete or notched. Independent of the cleft lip type, the cleft palate is described as unilateral (one palatal shelf is attached to the nasal septum) or bilateral. The extent of the cleft is classified as complete (**Fig. 1**), incomplete (**Fig. 2**), or microform (**Fig. 3**). In the complete cleft, there is disruption of the lip's mucosal up to the nasal floor with the associated nasal deformity. There is a spectrum of incomplete clefting, ranging from vermilion notching to near-complete disruption of the lip with a remaining Simonart band.[3] An incomplete bilateral cleft lip can be quite asymmetric (**Fig. 4**). The *severity* of the cleft lip width can make the repair more difficult because of wound tension. Management of the more severe cleft lip often requires a more prolonged presurgical preparation period (eg, presurgical infant orthopedics [PSIO]).

In the complete unilateral cleft lip, there is an external and upward rotation of the medial segment of the premaxilla and an internal and posterior rotation of the lateral segment.[2] Fibers of the orbicularis oris muscle attach medially to the base of the columella and laterally to the alar base. The nasal septum is dislocated from the vomerian groove with a shortening of the columella. The alar cartilage of the cleft side is deformed such that the medial crus is displaced posteriorly and the lateral crus is flattened over the cleft.[2]

In the complete bilateral cleft lip deformity, the premaxilla and prolabium are entirely separate from the lateral lip and maxillary segments. As a result, the premaxilla protrudes past the lateral segments. The prolabium can vary in size and lacks the normal philtral structure of a central groove and philtral ridges. The vermilion cutaneous junction and cutaneous (white) roll are often

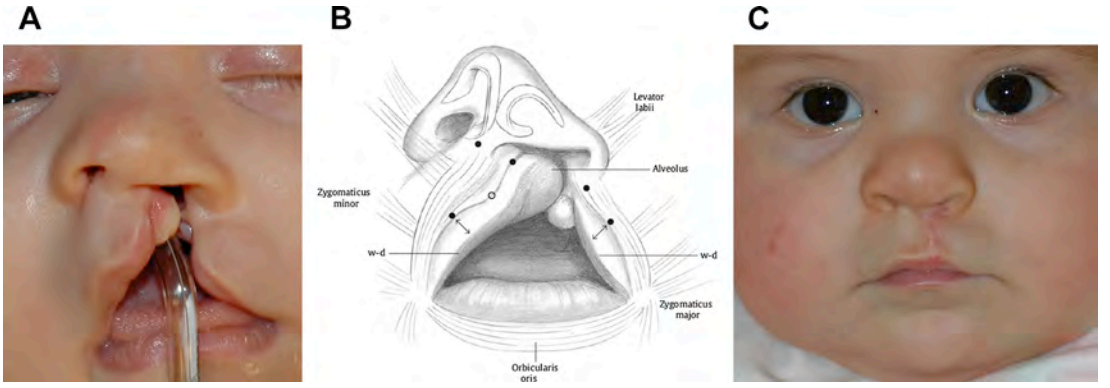

Fig. 1. Infant with unilateral complete cleft lip and palate. (*A*) Preoperative. (*B*) Illustration depicting the alveolus of the premaxilla, perioral muscles, and typical cleft nasal deformity. The arrows show the vermilion height, which should be made symmetric and the red line of Noordhoff (wet-dry junction) of the lip. (*C*) Postoperative view of same child after modified Mohler rotation-advancement repair and primary rhinoplasty. w-d, wet-dry vermillion. (*From* [*A*, *B*] Tollefson TT, Sykes JM. Unilateral cleft lip. In: Goudy S, Tollefson TT, editors. Complete cleft care. New York: Thieme; 2015. p. 40; with permission.)

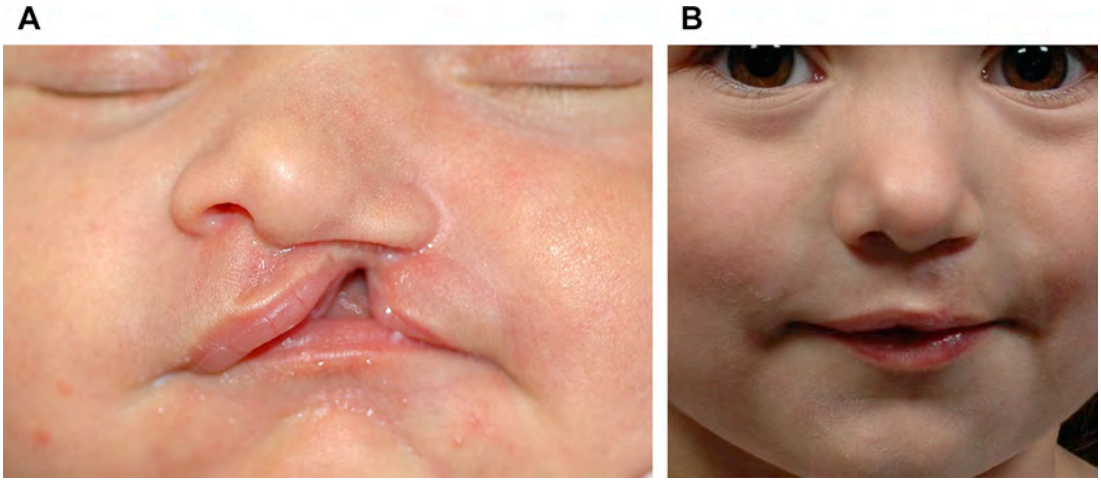

Fig. 2. Infant with incomplete cleft lip. (*A*) Preoperative. (*B*) Postoperative after a Fisher Subunit repair was used.

deficient. In a completed bilateral cleft lip, the prolabium does not contain orbicularis oris muscle. The nasal deformity associated with bilateral cleft lip is a shortened columella, flattened nasal tip, and alar hooding. Flaring of the alar base is common with inadequate alar base repair.[2]

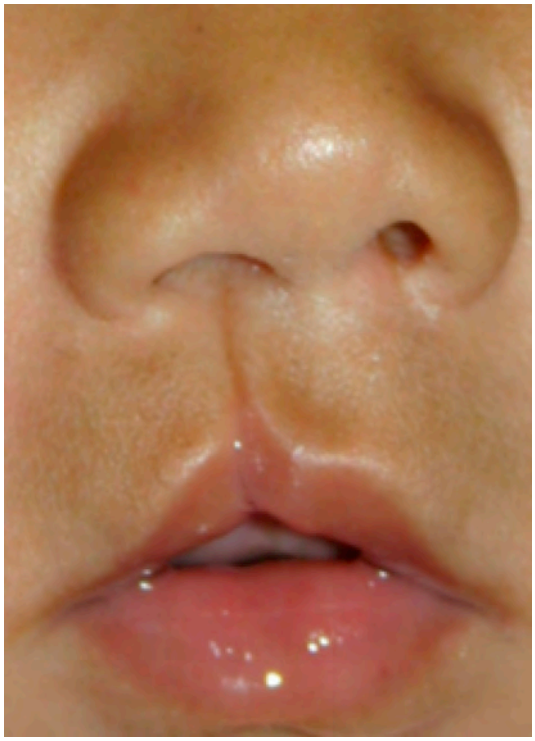

Fig. 3. Infant with microform cleft lip showing the (1) elevated Cupid peak, (2) furrowing of the philtrum, (3) medial dry vermilion deficient, (4) alar base malposition, (5) notched mucosa, and (6) deficient orbicularis oris muscle.

Surgical Techniques

Unilateral cleft lip

The objective of cleft lip repair is to approximate the medial and lateral lip elements with preservation of natural landmarks, align a functional concentric orbicularis, and to establish symmetry and proportionality. Unilateral cleft lip repair designs can be divided into 3 schools, which include (1) straight-line closure, (2) geometric, and (3) rotation-advancement techniques. The most common technique used to repair a unilateral cleft lip is the Millard rotation-advancement flap, as well as its modifications, including the Noordhoff vermilion flap and the Mohler modification.[3] There are few studies that compare the outcomes of various cleft lip repair techniques. Holtmann and Wray[4] (1983) studied patients randomized to receiving either the Millard rotation-advancement repair or the triangular (geometric) cleft lip repair, as described by Randall and colleagues[5] (Level II evidence). They did not find any significant differences in esthetic outcomes between the 2 groups. Chowdri and colleagues[6] (1990) also compared the Millard and Randall techniques in a randomized study (Level I evidence). Similar to Holtmann and Wray,[4] no differences were found in outcomes and both techniques were recommended in the repair of cleft lip.

There has been debate regarding whether the extent that the orbicularis oris muscles should be extensively released from the aberrant insertions on the maxilla to facilitate cleft lip repair. Some have felt that excessive dissection and a tense approximation of the muscular elements will lead to maxillary growth disturbance.[7] However, there is no evidence at present that muscular reconstruction leads to growth disturbance[8] (Level IV

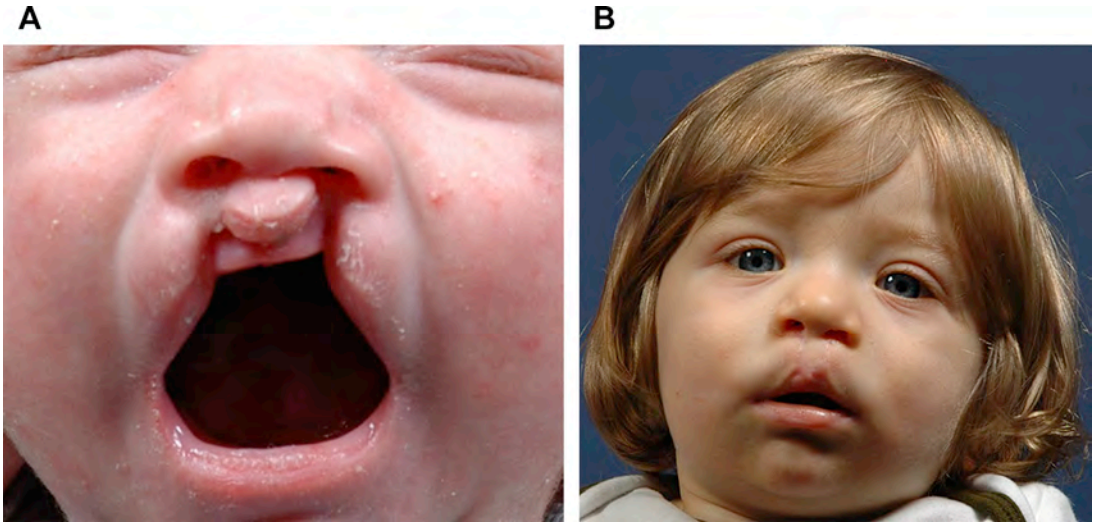

Fig. 4. (*A*) Two-week-old infant with asymmetric bilateral cleft lip and palate (incomplete on right and complete on left). (*B*) Six months postoperative.

evidence). In addition, the prevailing theory is that reconstructed musculature encourages normal and symmetric facial skeletal growth.[9,10] Two studies have suggested that muscular reconstruction leads to improved facial development[10,11] (Level II evidence). Although additional evidence is needed to conclude definitively regarding muscular reconstruction, it does seem to be associated with improved functional and esthetic outcomes.

Bilateral cleft lip

There are a few approaches to the repair of bilateral cleft lip. One approach is a 2-stage repair with columellar elongation as the second procedure between the ages of 1 and 5 years[12] (Level V evidence). Alternatively, a 1-stage approach with primary rhinoplasty at the time of cleft lip repair has been advocated for symmetric cases[13–15] (Level IV–V evidence).

The severely wide bilateral cleft lip with significantly projected premaxilla may necessitate with a staged cleft lip repair, PSIO, delayed repair, or premaxillary setback. In grossly asymmetric clefts or when a prolabium is less than 6 mm in height, a lip adhesion is performed, followed by a delayed definitive cleft lip repair, after the adhesion has successfully brought the soft tissue elements and maxillary arches closer together[16] (Level V evidence). Presurgical infant orthopedics, which includes nasoalveolar molding, is effective at decreasing the severity of the cleft width by applying orthopedic forces to the maxillary arches and premaxilla with an oral appliance.[17] Where presurgical infant orthopedics is ineffective or unavailable, premaxillary setback with vomer osteotomy can be performed with caution. The risks of devascularizing the premaxilla as well as maxillary growth inhibition should be considered[18] (Level IV evidence). Most North American surgeons use the Millard and Mulliken bilateral cleft lip techniques or a variation thereof.[17] Similar to unilateral cleft lip repair, there is insufficient evidence to suggest the superiority of one technique over another.

Timing

There are advocates for cleft lip repair over a range of time frames, from the neonatal period to 5 to 6 months of age[19,20] (Level V evidence). Intrauterine repair has been piloted using animal models based on the potential benefit of no scar formation[21,22] (Level V evidence); however, this has not been seriously pursued in humans, as the theoretic benefits do not outweigh the risks of exposing both the mother and fetus to this procedure. Neonatal repair also has been investigated for the similar reasons of minimizing scar formation and potentially allowing molding of the nasal cartilages due to the intrauterine exposure to maternal hormones.[13] Earlier repair also has the proposed benefits of facilitating maternal-child bonding; however, studies have not been able to substantiate this[23] (Level V evidence).

In the absence of an obvious benefit with earlier repair, most surgeons adhere to the conventional rule of 10's. Specifically, surgery is deferred until the child is 10 pounds in weight, at or after 10 weeks of age, with a hemoglobin concentration of 10 g/dL.[24] This increases the safety of undergoing anesthesia. It also has been argued to improve

esthetic results, as waiting allows for the lip musculature to grow[20,25] (Levels IV and V evidence).

Other Therapeutic Options

Presurgical infant orthopedics and nasoalveolar molding

Evidence supporting the use of PSIO is conflicting. This can likely be attributed to sparse evidence to definitively suggest a presurgical method is superior to another. Existing studies fail to use consistent outcome measures, which have partially driven the development of Eurocleft and Americleft research groups.[26] Two systematic reviews that examine the utility of PSIOs concluded that there is insufficient evidence to suggest an improvement in maxillary arch form/facial growth/occlusion, motherhood satisfaction, infant feeding/nutritional status, or speech[27,28] (Level II evidence).

Nasoalveolar molding (NAM) is a type of PSIO that incorporates the intraoral appliance with nostril prongs to improve the cleft nasal deformity (**Fig. 5**). There is more supportive evidence for PSIO due to the beginning of intraoral devices decades before NAM. Studies have shown that when instituted at 1 week of age and continued for 3 to 4 months, NAM is effective in approximating the cleft as well as improving the nasal deformity. Specifically, patients undergoing NAM treatment experienced improved nasal alar symmetry, columella lengthening, and nasal tip projection[29–32] (Levels II to V evidence). The counter arguments include nasal relapse and maxillary growth constriction. A recent review concluded that there is some evidence for its use in the unilateral cleft population in improving nasal symmetry[33] (Level III evidence). Although randomized controlled trials at multi-institutional levels are lacking, there is evidence that NAM should be incorporated into the routine management of both unilateral and bilateral clefts. In a phone survey that contacted 89% of North American cleft centers, more than one-third of the centers offer NAM as an adjunct to surgical repair of unilateral and bilateral cleft lip.[3,34]

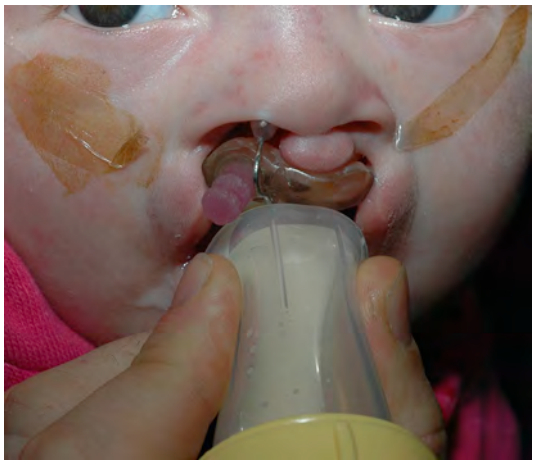

Fig. 5. Infant with left complete cleft lip and palate with NAM appliance. Tape will be secured into place with tape to the cheeks. Note the nasal prong that is expanded over time. This expands the soft tissue and cartilage, molding the nose before cleft lip repair. Also note the Haberman Feeder, allowing the parent to control the flow of formula into the mouth.

Lip adhesion

Lip adhesion surgery can be performed in unilateral and bilateral cleft lip. It is performed before definitive surgery, typically before 3 months of age. The rationale is that it applies orthopedic pressure on the underlying maxilla, thereby narrowing the cleft for the definitive repair[35,36] (Level V evidence); however, the evidence is limited and there is the potential disadvantage of additional scarring[37] (Level IV evidence).

Alveolar bone grafting

Primary alveolar bone grafting is typically performed at approximately 8 to 10 years of age. Some centers graft the alveolar cleft at age of 5 to 7 years, before the eruption of the permanent canines so as to improve bone height, dentofacial esthetics, and function[38] (Level IV evidence). Performing a primary graft in children younger than this is associated with the risk of insufficient alveolar bone volume. Bone grafting in older children may be associated with an increased risk of failure, as healing occurs more slowly and there is increased donor site morbidity[39] (Level II evidence). Iliac crest cancellous bone harvest is the standard, but other donor sites and off-label use of bone-morphogenetic protein have been described. More rarely described is the use of a split-rib technique with minimal maxillary dissection used for primary alveolar bone grafting, but the risks of maxillary growth restriction if performed too early must be considered[40] (Level IV evidence).

Primary rhinoplasty

A paradigm shift to include primary rhinoplasty at the time of cleft lip repair has been noted over the past few decades[41] (Level V evidence). Given the complexity of the nasal deformities associated with cleft lip, definitive rhinoplasty has and still is typically deferred until after adolescence and full skeletal growth[42] (Level V evidence). The rationale for minimal primary rhinoplasty during infancy was concern that significant change would occur during adolescent growth, necessitating repeat surgery.[43] There was also the theoretic risk of excessive scar tissue that would interfere with

nasal growth. Finally, patients with cleft lip often require orthognathic surgery, which should precede definitive rhinoplasty.

Arguments against delaying rhinoplasty until adolescence are that waiting may lead to a worsened nasal deformity as well as symptoms of nasal obstruction and increased rates of revision surgery[44] (Level IV evidence). It also may be associated with psychological stress, given that patients will have to live with the unrepaired deformity until adolescence.[40] Over the past 3 decades, various investigators have published on their experiences with primary cleft rhinoplasty, demonstrating that stable long-term results can be achieved with minimal growth disturbance[45–53] (Level III–IV evidence). Therefore, some evidence does exist to support primary rhinoplasty in improving nasal appearance and function. A recent study showed that more than half of North American cleft surgeons do perform a limited rhinoplasty at the time of primary lip repair.[3]

Postoperative nasal stents

Nasal stents have been used for the goal of preventing secondary deformities with healing and scarring following primary repair (**Fig. 6**).[54] There have been case series, as well as one prospective study, demonstrating improved alar symmetry in those who underwent postoperative internal nostril stenting[54–56] (Level IV evidence). The limitations of using nasal stents include poor patient tolerance, possible airway distress in the case of stent dislodgement, and pressure ulcers.[55] Currently, there are no randomized controlled trials examining the benefits of postoperative nasal stenting.

Fig. 6. Infant shown weeks after cleft lip repair with nasal conformers made of soft silicone secured in the nostrils. The optimal length of stenting the nostrils after primary rhinoplasty has not been established, but the senior author (TT) prefers 6 weeks.

Clinical Outcomes

There is significant variation among studies in measuring and reporting outcomes after cleft lip repair.[57] Some investigators have used clinical photographs with subjective scoring, whereas others use 3-dimensional imaging or anthropometry. The heterogeneity among patient populations, surgical techniques, and outcome assessment strategies make comparisons across studies difficult.

One outcome measure that can be used to gauge the success of cleft lip repairs is the rate of revision surgery. In a review of 50 consecutive patients with bilateral cleft lip with either a cleft palate or cleft alveolus, Mulliken and colleagues[58] found a nasolabial revision rate of 33% in the cleft lip and palate group (Level IV evidence). In the cleft lip and alveolus group, the revision rate was 12.5%. In a review of 750 patients with unilateral cleft lip, secondary reconstruction was performed in approximately 35% of patients[37] (Level IV evidence). The highest revision rates were reported by the Eurocleft study, which assessed the practice patterns and outcomes of 5 cleft centers in Northern Europe[59] (Level II evidence). Four centers provided revision rate data. One center reported a lip revision rate of 4%, and the remaining reported rates from 63% to 69%. For revision rates specific to nasal reconstruction, Mehrotra and Pradhan[60] reported a second rhinoplasty rate of 10% after primary rhinoplasty at the time of cleft lip repair (Level IV evidence).

Although revision rates provide a quantifiable method of gauging outcomes, it must be interpreted with caution. The decision to undertake revision surgery is family and surgeon-dependent. As such, the undertaking of revision surgery may be as reflective of these preferences as it is of the esthetic and functional outcomes from the primary repair. Furthermore, higher revision rates as an indicator of poorer outcome may not be accurate, as a child undergoing multiple revisions may actually have a final result that is more esthetically and functionally pleasing than a child who does not undergo any revisions.

Complications and Concerns

Wound complications

In a recent retrospective review of 3108 cases, Schonmeyr and colleagues[61] reported an overall short-term complication rate of 4.4% (Level IV

evidence). In 0.5% of these cases, the complication was severe enough to warrant revision surgery. The most common early postoperative complications were wound dehiscence and/or infection, which were 4.3% in the previously mentioned study. This was consistent with the rates of 2.6% to 4.6% reported by other studies[26,62] (Level IV evidence). Complete clefts and bilateral clefts were both significantly associated with wound dehiscence[61] (Level IV evidence). Other complications included stitch granuloma (0.2%) and pressure necrosis (0.05%).

Maxillary growth

Concern also has been raised regarding cleft lip repair and effects on maxillary growth. There are various hypotheses for how lip repair can lead to maxillary retrusion. Some postulate that pressure from a repaired lip restricts maxillary growth[63,64] (Level V evidence). Maxillary growth restriction theoretically could be greater in complete cleft lip-palate as the maxillary segments would be less able to withstand the restrictive forces[65,66] (Level IV evidence). In a review of 82 patients with unilateral cleft lip, alveolus, and palate, lip repair was found to be associated with maxillary retrusion[67] (Level IV evidence). Those with more severe defects were found to have greater retrusion. In a prospective study of 22 patients with unilateral cleft lip and palate, lip repair was found to cause transverse narrowing of the maxilla without any effects on sagittal growth[68] (Level IV evidence).

CLEFT PALATE

Overview

A cleft deformity can occur in both the primary and secondary palates. Clefts of the primary palate range from an alveolar notch to those that extend through the hard and soft palates. Clefts of the secondary palate range from a bifid uvula to clefts that extend to the incisive foramen.[2] The soft palate consists of 5 muscles that are responsible for velopharyngeal closure, including the musculus uvulae, the palatoglossus, the palatopharyngeus, the tensor veli palatini, and the levator veli palatini. The levator veli palatini is the primary muscle involved in velopharyngeal closure. Normally, it originates from the Eustachian tube and inserts anteromedially onto the tensor aponeurosis, along with the tensor veli palatine.[69] In the cleft palate, the levator muscles insert aberrantly onto the posterior edge of the hard palate.[2] Contractions of the palatal muscles therefore become ineffective at closing the velopharynx.

Surgical Techniques

The goals of cleft palate repair include closure of the soft palate and reorientation of the levator veli palatini to obtain normal velopharyngeal closure and speech. Closure of the hard palate cleft separates the oral and nasal cavities. There are numerous techniques for cleft palate repair and there is significant variation in treatment protocols across cleft centers.[70]

One of the oldest procedures performed is the von Langenbeck palatoplasty. With this technique, bipedicled mucoperiosteal flaps are raised off of the hard palate. The cleft edges are incised and both nasal and oral mucosa are medialized. The biggest drawback to this technique is that it does not add additional length to the soft palate[20] (Level V evidence). Other techniques have been designed to improve velopharyngeal function by lengthening the velum. One such technique is the Veau-Wardill-Kilner palatoplasty, which is a variation of the V-Y pushback. Mucoperiosteal flaps are raised and retropositioned. This lengthens the velum but leaves a large area of exposed hard palate anteriorly, which heals by secondary intention. Variations of the V-Y pushback technique have fallen out of favor because of poor maxillary growth outcomes[20] (Level V evidence).

Two-flap palatoplasty (**Fig. 7**) was first introduced in 1967 by Bardach.[71] Large mucoperiosteal flaps based on the greater palatine vasculature are raised. Closure is layered to minimize tension, with approximation of the nasal and then oral mucosa. The soft palate musculature is then repaired via an intravelar veloplasty (IVV). IVV involves releasing the levator veli palatini from its aberrant attachment to the posterior hard palate. Among cleft surgeons, consensus is that IVV does improve velopharyngeal function and may reduce rates of secondary speech surgery; the drawbacks include additional operative time and devascularizing the muscle.[20] The muscle fibers are then reapproximated in the transverse direction to establish the palatal muscular sling.[20] Since its introduction, studies on the effectiveness of IVV have had conflicting but overall supportive results. Marsh and Galic[72] prospectively studied 51 patients randomized to receive or not receive IVV during cleft palate repair. In this study, IVV was not associated with improved speech (Level II evidence). On the contrary, a retrospective study of 213 patients showed that IVV improved speech and decreased the rate of secondary velopharyngeal insufficiency[73] (Level IV evidence). Neither study found an increased rate of complications with IVV. Other studies also have found improved

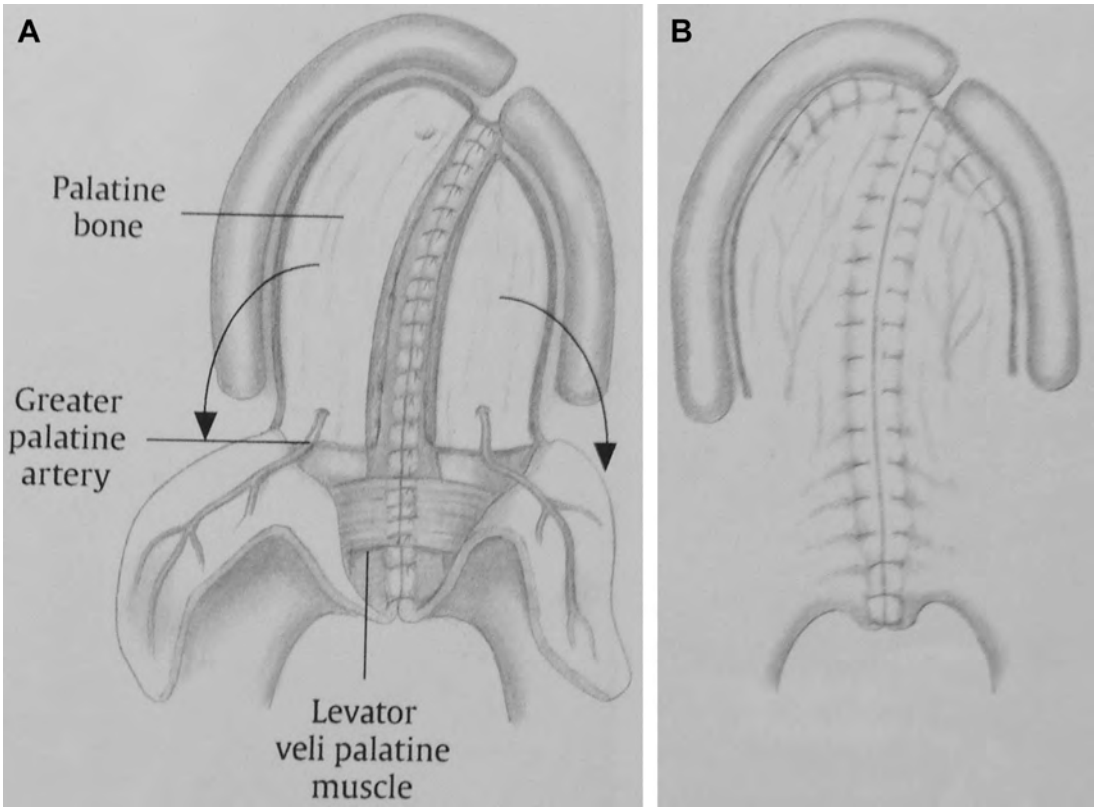

Fig. 7. Two-flap palatoplasty. (*A*) The flaps are elevated off the palatal bones and soft palate is dissected to create 2 flaps based off of the greater palatine neurovascular bundles. The orientation of the levator veli palatini muscles is corrected with or without a more extensive intravelar veloplasty. (*B*) A layered closure of the flap is then performed. (*From* Chiang T, Allen GC. Cleft palate repair. In: Goudy S, Tollefson TT, editors. Complete cleft care. New York: Thieme; 2015. p. 103; with permission.)

speech and velopharyngeal function with IVV[74,75] (Levels I and II evidence).

The Furlow double-opposing Z-plasty technique (**Fig. 8**) has gained popularity since its introduction in 1978. The soft palate is reapproximated in a way that lengthens it and realigns the musculature into a more anatomically correct position.[20] One concern raised with this technique is the increased rates of oronasal fistulas.[76] Only anecdotal evidence is available for the use of acellular dermis placed between the oral and nasal flaps to decrease in fistula rates[77,78] (Level IV evidence).

Studies have compared the various cleft repair techniques. Williams and colleagues[76] randomized patients to receive either a Furlow double-opposing Z-plasty or a von Langenbeck palatoplasty with IVV. Improved velopharyngeal function was found in the group that received the Furlow double-opposing Z-plasty (Level I evidence). Other studies also have found improved speech outcomes with the Furlow technique[79–81] (Level IV evidence). There is insufficient evidence to suggest a difference in outcomes between the Furlow technique and the 2-flap palatoplasty and a need for standardized speech outcomes collection to allow comparisons.

Timing

Evidence of the optimal timing of cleft palate repair remains inconclusive. Earlier repair provides the structural framework for speech development. Delaying repair may avoid potential maxillary growth inhibition. The consensus has leaned toward a timing of 10 and 14 months of age; however, evidence of alternative timing strategies deserve attention, including speech outcomes, maxillary growth, and staged soft palate/hard palate closure.

Speech Cleft palate surgery should occur early enough to facilitate optimal speech development. This means that repair should occur before the development of meaningful speech. Some have argued for palatoplasty no later than 13 months.[82] In a study by Dorf and Curtin,[83] 80 children underwent palate repair. Twenty-one of these children underwent repair earlier than 12 months of age

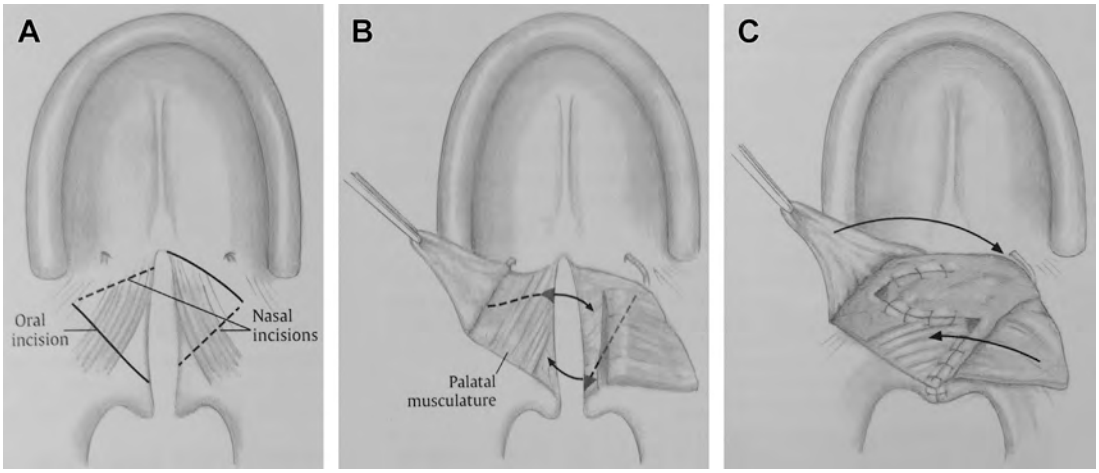

Fig. 8. Double-opposing Z-plasty (Furlow) palatoplasty. (*A*) Note that the left palate posteriorly based oral myomucosal layer is rotated posteriorly, whereas the left nasal mucosal layer is rotated anteriorly. (*B*) Conversely, the right anteriorly based mucosal layer is rotated anteriorly and the nasal myomucosal layer is rotated posteriorly. (C) This allows for the recreation of the levator sling and extends the palate posteriorly. (*From* Chiang T, Allen GC. Cleft palate repair. In: Goudy S, Tollefson TT, editors. Complete cleft care. New York: Thieme; 2015. p. 103; with permission.)

and the remainder underwent "late" repair, between 12 and 27 months. They found that children who underwent repair before 12 months of age exhibited better speech compared with those with late repair (Level IV evidence). In another study, by Pradel and colleagues,[84] 1-stage closure at 9 to 12 months of age was compared with 2-stage closure, with soft palate closure at 9 to 12 months of age and hard palate closure at 24 to 36 months. Again, 1-stage closure at 9 to 12 months was found to yield better speech development (Level IV evidence). Finally, Chapman and colleagues[85] found that children who underwent repair at the average age of 11 months had better speech outcomes compared with those who underwent repair at the average age of 15 months (Level IV evidence). The lack of consistent speech outcomes collection makes direct comparison between studies difficult.

Facial growth Cleft surgeons are concerned that dissection during palatoplasty disrupts the blood supply to the maxilla, leading to inhibited facial skeletal growth[86,87] (Level IV–V evidence). Studies investigating the effect of surgery on maxillary growth have had conflicting results, but often use dental arch models for comparisons and measurements. Chen and colleagues[88] compared sagittal maxillary growth in adults who had undergone palatal repair with those who had unrepaired cleft palates. They concluded that surgical trauma was not associated with more maxillary retrusion due to the similar retrusion between those with and without palatoplasty (Level IV evidence). Alternatively, Ye and colleagues[89] found significant anterior dental arch constriction in those who had undergone a palatoplasty (Level IV evidence).

One-stage versus 2-stage (Schweckendiek) palate repair To mitigate the risk of growth interference, centers have experimented with 2-stage palate repairs with delayed hard palate closure.[90,91] An argument in favor of the 2-stage approach is that by performing a veloplasty first, the hard palate is encouraged to narrow. This allows for the use of smaller flaps at the time of the hard palate repair[92] (Level V evidence). Studies have supported the use of a 2-stage procedure as it facilitates normal midfacial growth[93–96] (Level IV evidence). However, delayed hard palate closure has been associated with a higher incidence of velopharyngeal insufficiency and compensatory misarticulations[97] (Level IV evidence).

With consideration of both speech and facial skeleton growth, most cleft centers perform 1-stage repair. As discussed previously, repair before the age of 15 months is associated with superior speech outcomes[83–85] (Level IV evidence). Kirschner and colleagues[98] investigated whether performing the repair before 7 months improved velopharyngeal function and speech and concluded that there is no benefit (Level IV evidence).

Summary Therefore, the current literature supports timing of the surgery to be between 7 to 15 months of age.[20] Steps taken to optimize maxillary growth include minimizing subperiosteal dissection and reducing exposure of the hard palate[99] (Level IV evidence).

Other Therapeutic Options

Tympanostomy tube placement

Cleft palate can affect the function of the Eustachian tube in part due to aberrant veli palatini muscular attachments and direct exposure of the oral cavity to the nasopharynx. This predisposes the affected child to middle ear dysfunction and subsequent recurrent acute otitis media and chronic otitis media with effusion.[100] The resultant conductive hearing loss carries with it concerns regarding speech and language development.[101] For these reasons, tympanostomy tubes are frequently placed at the time of cleft lip repair or palatoplasty[102] (Level III evidence). The evidence supporting routine versus selective tube placement is conflicting.

Aside from evaluating hearing status and presence or absence of middle ear pathology/effusions, the otolaryngologist must gather the evidence and provide direct clinical correlation. The routine use of tympanostomy tubes may prevent chronic ear effusions and the associated conductive hearing loss, but this is currently a matter of clinical controversy. Ponduri and colleagues[103] completed a systematic review of studies and divided these between *routine* (at palatoplasty) compared with *selective* placement of tympanostomy tubes in children with cleft palate. A paucity of quality randomized controlled trials were available, but routine placement in the neonatal period did not seem to be indicated. (Level II evidence). This is contradicted by the practice patterns of many cleft teams, who tend to place the first set of tympanostomy tubes at the time of the cleft lip repair.[37] Further studies are needed to address this complex clinical dilemma, as the children with cleft palate are an at-risk population regarding speech development. Providing the maximal hearing potential for these children while they develop speech may warrant more aggressive treatment than for children without clefts.

Clinical Outcomes

The outcomes of cleft palate repair can include fistula occurrence, speech outcomes (eg, resonance, nasality, intelligibility), need for secondary speech surgery, and complications. A recent systematic review compared the outcomes of cleft palate repair using the Furlow technique and straight-line repair methods with IVV[103] (Level II evidence). The straight-line techniques include the von Langenbeck, V-Y pushback, and 2-flap palatoplasty. Ponduri and colleagues[103] reviewed data from 11 retrospective studies and 1 prospective randomized trial.

They found an oronasal fistula rate of 7.87% in the group receiving the Furlow repair and 9.81% in the straight-line with IVV group. Children with more severe clefting as determined by the Veau classification were more likely to develop a fistula. The rate of fistula formation in the Furlow and straight-line groups was not significantly different.

Velopharyngeal insufficiency was determined by the need for secondary corrective surgery. The difference in secondary surgery rates between the Furlow and straight-line groups was significantly different only in the unilateral cleft lip and palate population. In the Furlow group, between 0% and 11.4% of patients with an isolated cleft palate and between 0% and 6.7% with unilateral cleft lip and palate underwent secondary surgery. In the straight-line IVV group, between 9.1% and 29.2% of those with an isolated cleft palate and between 6.7% and 19.4% of those with unilateral cleft lip and palate underwent secondary surgery. Overall, the Furlow technique may be the preferred technique, as it leads to a decreased rate of secondary surgery[104] (Level II evidence).

Complications and Concerns

Oronasal fistula

The development of oronasal fistula is a concern following cleft palate repair, especially if the closure is under tension. An overall fistula rate of 4.9% has been reported[105] (Level II evidence). The most common location of occurrence is at the soft and hard palate junction. Using techniques that reduce closure tension, such as the hamular release and relaxing incisions, may decrease fistula occurrence. There are some reports purporting the benefit of placing a layer of decellularized dermis in the palatal closure, as an interpositional graft that may reduce the fistula rate. In a retrospective review of 31 cleft palate cases repaired using the Furlow technique and decellularized dermis, only 1 patient developed a fistula postoperatively[78] (Level IV evidence). This small cohort was not compared with another similar group. In another retrospective review of 7 patients, a 2-flap approach with IVV was used for primary repair[106] (Level IV evidence). Decellularized dermal grafts were used in the repair and there were no fistulas. Prospective studies would be needed to develop the evidence that decellularized dermis has a role in primary palate repairs for decreasing the risk of fistula occurrence. Additional cost and risk of viral transmission are major detractors to the routine use of acellular cadaveric dermis in cleft palate repair.

Velopharyngeal insufficiency

Velopharyngeal dysfunction after primary cleft palate repair may require secondary speech surgery

with rates reported from 5% to 38%.[107] The inability to close the velopharyngeal sphincter leads to nasal air escape during speech. The resulting hypernasality can lead the child to develop compensatory speech errors (eg, glottal stops) and speech quality suffers.[108] Treatment for velopharyngeal insufficiency (VPI) involves secondary speech therapy and correction, either surgical or nonsurgical. Nonsurgical treatment includes an oropharyngeal obturator, prosthetic, or palatal lift; however, their use is limited by poor patient tolerance.

There are 4 components of the velopharynx: the soft palate anteriorly, the lateral pharyngeal walls bilaterally, and the posterior pharyngeal wall posteriorly. Surgery to restore velopharyngeal competence can involve each of these components; however, the most common procedures are the pharyngeal flap and sphincter pharyngoplasty (**Fig. 9**). Retrospective studies have not demonstrated the superiority of one procedure in terms of VPI resolution and postoperative complications[109,110] (Level IV evidence). The speech outcomes (eg, nasal air emissions and resonance scores) of pharyngeal flap surgery were reported in a recent retrospective study of 61 patients. Speech scores increased in all patients with a surgical revision rate of 19.7% (comparable to previously published studies).[111] The difficulty in comparing outcomes from secondary speech surgery lies in the lack of consistent reporting methods, thus supporting evidence that cleft centers should encourage consistent documentation, which would foster interdisciplinary and multi-institutional studies.

Two prospective randomized trials were performed to compare the pharyngeal flap and sphincter pharyngoplasty operations. Neither study found a significant difference between the 2 in terms of VPI outcomes or complications[112,113] (Level I evidence). To optimize outcomes, the width of the pharyngeal flap or the lateral flaps in a sphincter pharyngoplasty can be customized according to the size of the velopharyngeal gap and the quality of palatal and lateral wall motions[114] (Level I evidence).

GENERAL THERAPEUTIC CONSIDERATIONS FOR CLEFT LIP AND PALATE

Airway Concerns

Children who have cleft palate are at a higher risk of upper airway obstruction. Studies have found the incidence of airway obstruction to be up to 18% in nonsyndromic children with an isolated cleft palate[115,116] (Levels II and IV evidence). The risk increases even more when the cleft anomaly occurs as part of a syndrome. In the postoperative period, this risk increases. There are a few contributors to airway obstruction postoperatively. First, closure of the cleft causes a decrease in available airway space. Second, prolonged tongue

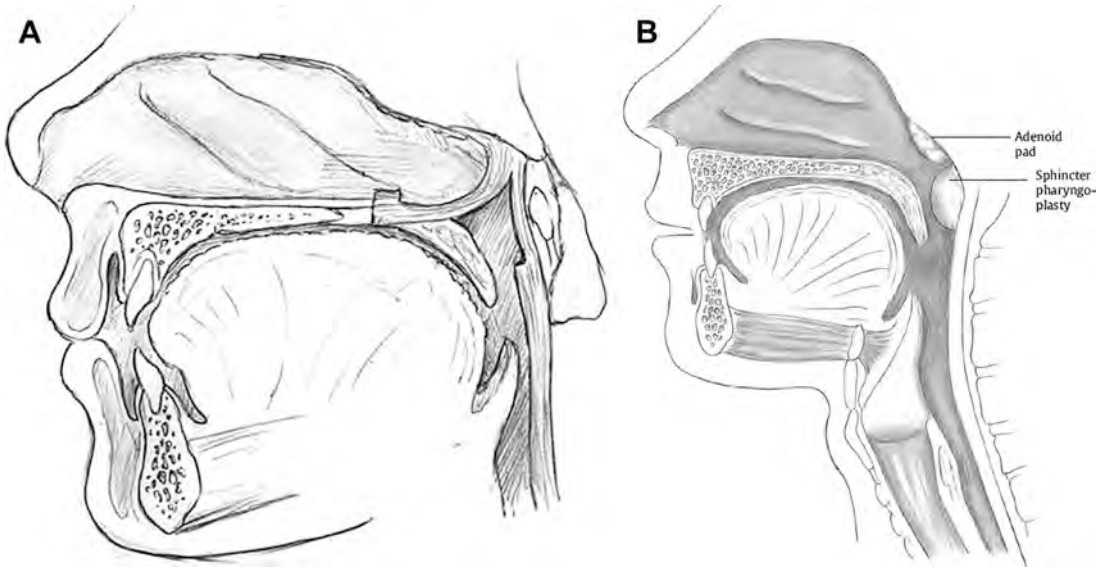

Fig. 9. (*A*) Superiorly based pharyngeal flap. (*B*) Sphincter pharyngoplasty. Along with the Furlow double-opposing Z-plasty (see **Fig. 7**), these represent the most common secondary speech surgeries to address velopharyngeal insufficiency after cleft palate repair. (*From* [*A*] Willging JP, Cohen AP. Pharyngeal flap surgery. In: Goudy S, Tollefson TT, editors. Complete cleft care. New York: Thieme; 2015. p. 173, with permission; and [*B*] Boss EF, Sie K. Sphincter pharyngoplasty. In: Goudy S, Tollefson TT, editors. Complete cleft care. New York: Thieme; 2015. p. 178, with permission.)

retraction during the procedure can cause acute swelling. In anticipation of potential postoperative airway obstruction, a nasal airway can be placed before extubation to decrease the risks of airway compromise.

Feeding

There is no consensus on postoperative feeding protocols following repair of cleft lip and/or palate. The World Health Organization recommends exclusive breastfeeding until 6 months of age, and a recent Cochrane systematic review found a weakly positive effect of breastfeeding on postoperative weight gain compared with spoon feeding in infants with cleft lip[117] (Level I evidence). Mothers should therefore be encouraged to breastfeed when possible, but breast milk pumping and use of a cleft feeder, such as the Haberman, Pigeon, Mead Johnson, or others. In the same review, there was insufficient evidence to conclude whether squeezable bottles are beneficial compared with rigid feeding bottles for improving growth and development.[117] However, a squeezable bottle may be preferred for ease of use in infants with cleft anomalies. Finally, maxillary appliances did not have an adverse effect on growth.[117]

Arm Restraints

Most cleft surgeons in the United States use arm restraints during the postoperative period.[118] The basis for this practice is to prevent children from placing their fingers or objects into their mouth, which can disrupt the surgical site. Two randomized controlled trials failed to show any significant differences in the development of oronasal fistulae in the restrained group compared with the unrestrained[119,120] (Level I evidence). The study designs of these randomized controlled trials were not ideal, and the rate of fistula or complication is rare. There is inadequate evidence to comment on the use of arm restraints in the postoperative period, but a reasonable approach may include situational differences, with parents protecting the surgical sites, and not relying on dogma.

Relevant Pharmacology

Antibiotics

There is evidence to support the use of prophylactic antibiotics in clean contaminated cases, such as in cleft lip and palate repair. Acceptable antibiotics include cefazolin and clindamycin. Antibiotics should be administered before the surgical incision is made. There is no evidence for ongoing antibiotics following surgery[121] (Levels I and IV evidence).

Steroids

Perioperative dexamethasone may decrease the risk of airway swelling and subsequent respiratory distress without detrimental effects on wound healing[115,116] (Levels II and IV evidence).

Analgesia

For immediate postoperative pain control, an infraorbital nerve block with longer-acting local anesthetics, such as bupivacaine or ropivacaine, can be used[122] (Level III evidence). Much of the evidence on post–head and neck surgery analgesia in children is based on the tonsillectomy literature. With the exception of ketorolac, nonsteroidal anti-inflammatory drugs have not been associated with an increased risk of bleeding complications[123–125] (Levels I and IV evidence). Codeine has recently fallen out of favor. Genetic polymorphisms render some individuals unable to metabolize codeine to morphine, whereas others will hypermetabolize it[126,127] (Levels I and II evidence). Overall, codeine has not been found to be more effective at controlling pain compared with plain acetaminophen after tonsillectomy[128] (Level II evidence). Furthermore, hypermetabolism of codeine can lead to toxic levels of morphine and has been associated with postoperative mortality[126] (Level IV evidence). For these reasons, a regimen consisting of acetaminophen and ibuprofen may be the best option, taking into account the potential risk of bleeding with nonsteroidal anti-inflammatory drugs.

SUMMARY

The repair of cleft lip and palate is both challenging and rewarding. Most of the existing literature is practice-centered with retrospective data. There is growing recognition, however, that more level I and II evidence is needed. Furthermore, there is a shift toward patient-reported outcomes with regard to satisfaction and quality of life.

Cleft care has evolved steadily over the past decade and research has advanced our understanding of the sequelae of these anomalies and the implications of various treatment options. This article reviews the pertinent literature on the management of cleft lip and palate. It summarizes the current level of evidence and identifies areas for future study. With ongoing research, this field will continue to grow to one that is firmly rooted in evidence.

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Measuring Outcomes in Cleft Lip and Palate Treatment

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KEYWORDS

• Cleft lip • Cleft palate • Cleft surgery • Evidence base • Outcomes measurement • Outcome data

KEY POINTS

- Outcome measurement is essential to document quality and to facilitate improvement.
- Cleft surgeons should choose outcome measures that are valid, reliable, practical to implement, and broadly adopted.
- New measures are under development, and existing measures will continue to evolve in all aspects of cleft care. Measures should focus on outcomes most relevant to patients and include input from providers and health care purchasers.

If you can not measure it, you can not improve it.

—Lord Kelvin

WHY MEASURE OUTCOMES?

Once the sole purview of clinical and health-services research, *outcome assessment* has become a core component of clinical practice. Generally speaking, outcome measurement may be used for accountability, quality improvement, and health-system design (eg, resource allocation, purchasing decisions, and policy development) (**Box 1**). *Accountability* refers to the demonstration that a particular surgeon's or team's results are within accepted standards. *Quality improvement* is a process of combining domain expertise with knowledge of systems, variation, and psychology to effect meaningful improvement. Originally developed in the manufacturing and service industries, quality improvement is now widely applied to health care delivery systems.[1]

It is intuitive that regularly reviewing one's outcomes is useful and instructive for improving patient care. For some time, the American Board of Medical Specialties' Maintenance of Certification process has required demonstration of quality-improvement practices in an individual's clinical practice.[2] However, it is important to underscore that routine collection and reporting of clinical outcomes are increasingly emphasized in the public sphere. There is a growing movement to tie reimbursement to outcomes, and organizations such as the Leapfrog group and the Agency for Healthcare Research and Quality have advocated public reporting of these data. The American College of Surgeons' National Surgical Quality Improvement Program was conceived as a volunteer program to help hospitals monitor specific clinical outcomes (particularly the so-called never events) that are already being tied to reimbursement. Recently, the Centers for Medicare and Medicaid Services announced its intention to require a proven level of performance to be eligible for payment.[3] In the future, payers will be increasingly

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Box 1
Applications of outcome measurement in health care

- Accountability
  - Accreditation
  - Quality assurance
  - Public reporting
- Quality improvement
  - Improve clinical care
  - Research
  - Board certification
- Health-system design
  - Resource allocation
  - Value-based purchasing
  - Policy development

sensitive to objective data on outcomes when deciding where care should be directed and when negotiating fees.

OUTCOMES ASSESSMENT AND QUALITY IMPROVEMENT REQUIREMENTS IN CLEFT CARE

Specific to cleft care, the American Cleft Palate Association (ACPA) established minimum requirements for accreditation.[4] These requirements include that "the Team has mechanisms to monitor its short-term and long-term treatment outcomes" by documenting "its treatment outcomes, including base-line performance and changes over time" and conducting "periodic retrospective or prospective studies to evaluate treatment outcomes."[4] Similar requirements exist in the United Kingdom.[5] To date, the ACPA offers no specific recommendations regarding *which* outcomes should be assessed, nor *how* these data are to be collected, analyzed, and interpreted. Consequently, the onus is on each cleft team to conceive and develop its own system of outcomes assessment, monitoring, and quality assurance.

OUTCOMES ASSESSMENT IN THE LITERATURE

Cleft lip and palate treatment has been the subject of innumerable studies in the surgical, medical, and allied health literature. Most of the evidence base is level IV and level V evidence—that is, most data derive from case series, experiential reports, and expert opinion. Few papers have been subjected to the rigors of contemporary clinical trial design or systematic review and metanalysis.

Some outcome data do exist. Perhaps the most complete early report was a 1984 study by Bardach and colleagues[6] describing the long-term esthetic, dental, facial growth, and speech outcomes of 45 patients with unilateral cleft lip and palate. In 1987, the Third International Symposium on Early Treatment of Cleft Lip and Palate initiated a collaborative investigation, in which cephalograms and treatment records from 15 international centers were reviewed to evaluate the effects of individual treatment protocols on facial growth.[7] Attendees from the symposium later developed novel measures for objective comparison of treatment outcomes, such as the Great Ormond Street, London, and Oslo (GOSLON) yardstick for assessing dental arch alignment[8] and a validated instrument for rating nasolabial esthetic results.[9]

In the late 1980s and early 1990s, Eurocleft was founded to study treatment outcomes from 6 European cleft centers.[10–14] The Eurocleft study included Caucasian children with nonsyndromic, complete unilateral cleft lip and palate. Initial outcomes of interest were dental arch alignment, midfacial growth and facial profile, and nasolabial esthetics. Follow-up studies also considered orthognathic outcomes at skeletal maturity, speech, burden of care, and patient satisfaction.[15–20] Results are summarized in **Table 1** but highlighted much disparity in protocols and outcomes between centers. Results of the Eurocleft studies kindled a desire for quality improvement in the cleft-care community at large. With funding from the European Union, a registry of European cleft teams was created. It issued a policy statement that delineated practice guidelines for the treatment of children with clefts and that recommended minimum recordkeeping standards for teams. EUROCRAN was conceived to help organize clinical and genetic research and to foster collaboration.[21,22] Many of the Eurocleft researchers also participated in the World Health Organization's (WHO) development of an international strategy to craniofacial research, bringing Eurocleft's quality-improvement aims to a worldwide audience.[23]

In response to poor outcomes obtained by British centers participating in the Eurocleft studies, the Clinical Standards Advisory Group (CSAG) performed an audit of all 5- and 12-year-old children in the United Kingdom with unilateral cleft lip and palate. Results were poor across all measures (see **Table 1**).[24–27] CSAG proposed specific methods for restructuring the cleft-care-delivery process and created specific service specifications for providers. Based on

Table 1
Selected outcomes from intercenter outcomes studies (see text for relevant citations)

Patient Characteristics					
Cleft Type	**Age at Evaluation**	**No. Enrolled**	**No. of Centers**	**Location(s)**	**Measures Reported**
Eurocleft					
cUCLP	8–10 11–14 12, 17	151 131 124	6 6 5	UK, Sweden, Denmark, Netherlands, Norway	Craniofacial form (cephalometry) Dental arch relationships (GOSLON) Nasolabial appearance (Asher-McDade scale) Speech (structured, multilingual) Burden of care Craniofacial form (cephalometry) Dental arch relationships (GOSLON) Nasolabial appearance (Asher-McDade scale) Patient/parent satisfaction questionnaire
CSAG					
cUCLP	5, 12	457	50	UK	Craniofacial form (cephalometry) Dental arch relationships (5-year-old index, GOSLON) Success of alveolar bone grafting (modified Bergland) Nasolabial appearance (Asher-McDade scale) Patient/parent satisfaction questionnaire Speech (modifed CAPS)
Americleft					
cUCLP	6–12	172	5	US, Canada	Craniofacial form (cephalometry) Dental arch relationships (GOSLON) Nasolabial appearance (Asher-McDade scale) Speech (pending)
Scandcleft					
cUCLP	5	445	10	Denmark, Sweden, Finland, Norway, UK	Craniofacial form (cephalometry) Dental arch relationships (5-year-old index) Nasolabial appearance (Asher-McDade scale) Speech (structured, multilingual, nasometry)

Abbreviation: cUCLP, complete unilateral cleft lip and palate.

these findings and recommendations, the National Health Service made reorganization of cleft-care services a national priority in 1999, a process that was completed in 2005.[28] A key principle established when cleft services were centralized in the United Kingdom was that results should be routinely collected and assessed. A national database, CRANE, was set up (https://www.crane-database.org.uk/); use of electronic databases in individual units was made the norm, and the Craniofacial Society of Great Britain and Ireland (CFSGBI) set out an agreed minimum dataset for audit. Recently, a follow-up cross-sectional study, Cleft Care UK, was begun to monitor improvement in outcomes of 5-year-old children treated following the reorganization of services.

Inspired by the success of the Eurocleft project, the ACPA convened a taskforce in 2006 to

establish a similar multicenter collaborative effort in North America. Named Americleft, the 5 participating centers compared outcomes using existing clinical records and the methodology described in the Eurocleft studies.[29–33] Results are summarized in **Table 1**.

The rich history of collaborative research in cleft care underscores the complexity and considerable difficulty inherent in retrospective comparisons of outcomes from diverse institutions. These studies also demonstrate the great potential of multicenter collaborations. Presently, new collaborations are underway in Scandinavia, Japan, India, Brazil, and Australia. In particular, the 10-center Scandcleft collaborative has progressed beyond retrospective observational studies to use randomized controlled trials for the evaluation of surgical technique, timing of individual interventions, and sequence of interventions (see **Table 1**).[34] The planning and execution of prospective research methods mark a significant advance in cleft research.

CONCEPTUALIZING OUTCOMES IN CLEFT CARE

What to Measure in Cleft Outcomes?

From a *holistic* perspective, the patient should be conceptualized as the center of the health care delivery process (**Fig. 1**). Three principal domains for outcomes are clinical, psychosocial, and systems-based parameters. The *clinical* domain includes aspects common to traditional clinical outcomes studies and includes many subdomains such as general pediatrics (eg, physical and cognitive development), surgery, dental/orthodontic, speech, and audiology. The *psychosocial* domain is arguably equally important and includes aspects related to psychological well-being and social functioning. Some psychosocial elements may be intricately related to traditional clinical outcomes; for example, self-perception of appearance may be related to nasolabial appearance, and academic performance may be related to cognitive development. However, these qualitative elements carry the additional semantic value of "how does this matter to the patient?" missing from traditional

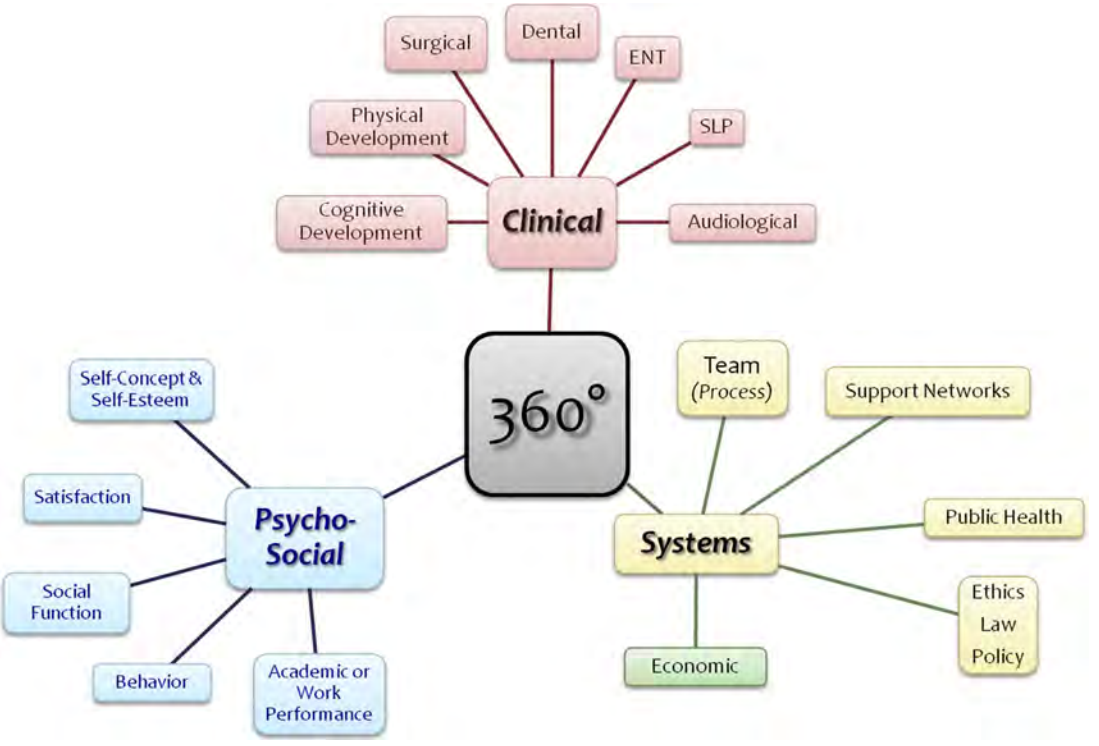

Fig. 1. "Outcomes" viewed from a holistic perspective would conceptualize the patient with cleft lip and palate at the center and at least 3 main domains that directly affect the patient. *Clinical* outcomes include traditional subdomains such as surgery, dental/orthodontics, speech, and audiology. Arguably equally important are *psychosocial* outcomes, which pertain to concepts such as self-esteem, behavior, and social functioning. *Systems*-based outcomes consider economic concepts such as value, team performance, public health, and policy.

quantitative clinical outcomes. A third domain in the conceptualization of outcomes in cleft care is *systems-based parameters*. These elements may pertain to cost, resource allocation, process of care (eg, efficiency), supplemental/ancillary services, and so on and are typically used in value assessments and continuous quality improvement endeavors.

When to Measure Cleft Outcomes?

Compared with many clinical problems, outcomes assessment in cleft care is particularly challenging. First, the child can be considered a "moving target" (**Fig. 2**); that is, treatment of the condition typically requires several sequential steps that include operative and nonoperative interventions delivered over time, all while the child is growing physically, cognitively, and developmentally. The questions then arise: When is the best time to measure an outcome? Is it better to measure outcomes (particularly surgical outcomes) before and after a particular intervention, in the short or long term? Or is it better to look at outcomes at a particular age or developmental stage? Or perhaps at the very end of a treatment protocol?

There is perhaps no single right answer, and arguments can be made for each strategy. Typically, single institutions in their case series tend to look at short- and long-term outcomes surrounding a particular intervention of interest. These types of data tend to generate evidence of efficacy. However, this approach is problematic when attempting to compare results from multiple institutions to generate evidence of effectiveness under different conditions. The Eurocleft collaboration, for example, highlighted that because of diversity of protocols, it was impossible to look at short- and long-term results of a specific intervention. Therefore, it adopted the approach of looking at the long-term results following an entire treatment protocol, which included numerous interventions. Although a practical and reasonable decision, it was impossible to derive conclusions regarding the effectiveness or relative worth of a treatment, the appropriateness of its timing within the protocol, or of the experiences that the child has during this time frame.

How to Measure Cleft Outcomes?

Every domain and each outcome of interest can be viewed from a different vantage point (**Fig. 3**). Consequently, each outcome of interest may be measured in a different way. For example, nasolabial appearance might be measured quantitatively via direct or indirect anthropometry, semi-quantitatively through expert panel assessment of photographs, or qualitatively by way of a patient-reported outcome measure. It is therefore critical to choose an appropriate and practical method of outcome measurement.

At a minimum, measures should be valid and reliable. Measurement validity ensures accurate conclusions are made regarding superior or

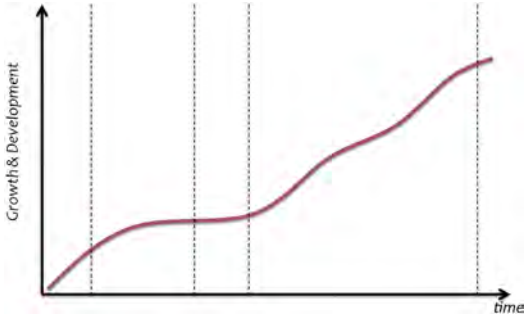

Fig. 2. One challenge to outcomes assessment in cleft care is that the child with cleft lip and palate undergoes several operations and nonsurgical interventions at different stages, all while he is growing and developing physically, cognitively, and psychologically. The question arises: Is it better to measure "outcomes" at a specific biologic age, developmental stage, surrounding an intervention (in the short- or long-term), or at the very end of a treatment protocol?

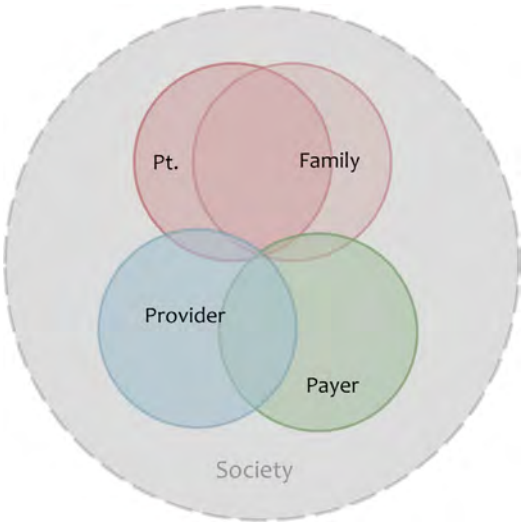

Fig. 3. Each domain and each specific outcome of interest can be viewed from a different vantage point, specifically, from that of the patient, family, clinical care providers, payers, or society as a whole. The perspective(s) chosen will affect which specific outcomes are chosen for assessment and the methods that are used for their assessment (for example, direct quantitative measurement vs qualitative or semi-quantitative patient-reported outcome measures).

inferior treatment methods. Reliability allows comparison between different patients, surgeons, or teams and improves the ability to detect differences in outcome. Ideally measures should also be practical to implement, able to discriminate between different outcomes, and consistently adopted.[35]

Nearly all cleft surgery outcome measures contain a subjective component and can be a limiting factor even for well-validated measures. In the research setting, study methodologies are able to minimize potential bias from this subjective component. However, in clinical practice (audits and public reporting), controlling for bias (particularly selection bias, measurement bias, and unblinded assessment) is more difficult.

When deciding which measures to use in your individual practice, the importance of considering complexity for record collection and analysis cannot be underestimated. Measures that are under development or lack validation, or which require patient involvement beyond routine care, should be approached with caution.

Specific Measures

Several national and international groups have published recommendations for a minimum set of treatment records. The Eurocleft investigators and WHO international collaborative made identical recommendations in 2001 (**Table 2**).[21,23] The CFSGBI adapted these recommendations to include measures of patient and parent satisfaction, psychology evaluation, dental health, and general feeding and growth.[36] All recommendations are based on records that should be collected during the routine treatment of patients.

A comprehensive critical appraisal of all outcome measures existing and under development for children with cleft lip and palate is beyond the scope of this review, but **Table 3** lists available validated measures. Other articles in this issue of *Clinics in Plastic Surgery* expound on specific measures useful to a particular purpose.

PERFORMING OUTCOME MEASUREMENT

After considering which outcomes should be included and when they should be measured, a framework should be built to facilitate the systematic collection of records during routine clinical practice. This is challenging, but fortunately, models exist for the routine assessment of treatment outcomes within the framework of team-based cleft care. The North Thames Regional Cleft Centre uses a specially structured "audit clinic" to collect its outcomes. This clinic is distinct from a clinical care appointment, and its sole purpose is to obtain standardized data for subsequent review. Depending on age and cleft type, a patient may require any or all of the following: Great Ormund Street Speech Assessment (GOS.SP.ASS) speech profile with audiovisual recording for subsequent assessment using the Cleft Audit Protocol for Speech-Augmented (CAPS-S)[37]; clinical photographs and dental impressions for esthetics and 5-year index of facial growth[38]; dental health assessment with the Decayed, Missing, Filled, and Treated (DMFT) index; audiology; psychology; height and weight. The audit review is conducted by the team at a later date, which allows each clinician time to analyze any outcomes between the audit clinic and the review. An important guiding principle is that a surgeon *other than the original operating surgeon* leads the review for a particular child. The team can then highlight any technical issues of the repair, discuss any outstanding issues or learning points, and make a management plan. If specific concerns arise, a normal clinic appointment is booked soon after the review.

Table 2
WHO recommendations for minimum record collection and timing, complete cleft lip, and palate (see text for relevant citation)

Timing	Dental Models	Lateral Skull Radiograph	Photographs	Speech/ Tympanometry	Audiometry	Patient/Parent Satisfaction
Primary surgery	X		X			
3 y				X	X	
5/6 y	X		X	X	X	
10 y	X	X	X	X	X	
18+ y	X	X	X	X		X

From World Health Organization. Global strategies to reduce the health-care burden of craniofacial anomalies: report of WHO meetings on International Collaborative Research on Craniofacial Anomalies, Geneva, Switzerland, 5–8 November 2000 ; Park City, Utah, USA 24–26 May 2001. Geneva: World Health Organization, 2002; with permission.

Table 3
Selected measures for evaluating treatment outcomes (see text for relevant citations)

Type	Measure
Aesthetic	
Passive Active	Asher-McDade
Craniofacial form	Cephalometrics
Dental arch relations	GOSLON 5-year-old index Eurocran index Bilateral index
Speech	CAPS-A Universal parameters (Speech Parameters Group) Nasometry
Dental	DMFS/Dmfs
Oral health	Child Oral Health Impact Profile (COHIP) (under development)
Audiometry	
Psychosocial	Peds-QL Child Health Questionnaire (CHQ) Child Oral Health Quality of Life (COHQOL) Youth Quality of Life-Facial Differences (YQOLFD) CLEFT-Q (under development)
Comprehensive	CLP-360° (under development)

Collated patient data are then presented at an annual meeting with 2 other Cleft Centers. All of the UK Cleft centers are a part of a tricenter or quad-center group. The focus of these meetings is on presenting, discussing, and improving patient outcomes. Finally, certain aspects of the data are submitted for national comparison at the annual meeting of the CFSGBI.

SUMMARY AND FUTURE DIRECTIONS ON OUTCOMES MEASUREMENT

Outcome measurement is an essential component of cleft care that is critical to improving quality and value. Surgeons should lead the way in developing systems for comprehensive appraisal of cleft care—for accountability, quality improvement, and health system design. The system should include systematic methodology built on a sound conceptual framework. Future research should include both development of novel validated measures and effective systems for application of outcome measurement in routine practice, standardization of monitoring methods, and development of automated systems that permit realtime data analysis ("learning health care").

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Review

The Regenerative Medicine in Oral and Maxillofacial Surgery: The Most Important Innovations in the Clinical Application of Mesenchymal Stem Cells

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Abstract

Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, materials science and bioengineering in order to regenerate, repair or replace tissues.

The oral surgery and maxillofacial surgery have a role in the treatment of traumatic or degenerative diseases that lead to a tissue loss: frequently, to rehabilitate these minuses, you should use techniques that have been improved over time. Since 1990, we started with the use of growth factors and platelet concentrates in oral and maxillofacial surgery; in the following period we start to use biomaterials, as well as several type of scaffolds and autologous tissues. The frontier of regenerative medicine nowadays is represented by the mesenchymal stem cells (MSCs): overcoming the ethical problems thanks to the use of mesenchymal stem cells from adult patient, and with the increasingly sophisticated technology to support their manipulation, MSCs are undoubtedly the future of medicine regenerative and they are showing perspectives unimaginable just a few years ago. Most recent studies are aimed to tissues regeneration using MSCs taken from sites that are even more accessible and rich in stem cells: the oral cavity turned out to be an important source of MSCs with the advantage to be easily accessible to the surgeon, thus avoiding to increase the morbidity of the patient.

The future is the regeneration of whole organs or biological systems consisting of many different tissues, starting from an initial stem cell line, perhaps using innovative scaffolds together with the nano-engineering of biological tissues.

Key words: Regenerative medicine; Mesenchymal Stem Cells; Bone regeneration; Dental Pulp Stem Cells; human Periapical Cysts Mesenchymal Stem Cells; hPCy-MSCs.

Introduction

Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, materials science and bioengineering in order to regenerate, repair or replace tissues.

The oral surgery and maxillofacial surgery have a role in the treatment of traumatic or degenerative diseases that lead to a tissue loss: frequently, to rehabilitate these minuses, you should use techniques that have been improved over time. Since 1990, tissue engineering has developed protocols in which it has been proposed the use of platelet concentrates, which showed enormous benefits for the patient: they favored and accelerated the post-surgical and provided

a support for tissue regeneration due to growth factors contained in them. Several authors [1-4] have described the importance of growth factors in tissue repair processes, in fact, they are important elements for new tissue production, moreover, they perform feedback controls on inflammatory processes within the tissue graft, in cases of regenerative surgery.

Whitman[5] and Marx[6] published the first studies on the use of growth factors contained in platelet gel, called Platelet-Rich Plasma (PRP).

Thanks to Marx's studies, it was possible to verify that the platelet concentrate is a very effective tool for the modulation of wound healing and tissue regeneration. However, the PRP showed a number of disadvantages, such as the need of having to run a complex and expensive protocol for its production. To overcome some of these problems, the PRGF (Plasma Rich in Growth Factors) was introduced in the list of platelet concentrates. The PRGF is considered an evolution of the PRP [7,8] and it allows a higher concentration of growth factors in platelet preparation. Among the advantages of the PRGF, we can cite the lesser amount of blood taken for the preparation and a procedure relatively faster, while, among the disadvantages we can mention the rapid clot formation, which require speed in its surgical use.

In 2001, Choukroun *et coll.* have instead proposed an alternative technique: the PRF (Platelet Rich Fibrin). PRF is derived from a simple preparation protocol that does not require alteration of the blood; it is a platelet concentrate rich in GFs that contains a three-dimensional matrix of autologous, elastic and flexible fibrin.

Dohan *et al*. have shown that platelet cytokines (PDGF, TGFbeta1 and IGF-1) are present in three-dimensional fibrin matrix derived from these platelet concentrates; moreover, PRF matrix traps glycosaminoglycans such as heparin and hyaluronic acid, which have considerable affinity with some peptides present in the bloodstream and therefore show strong ability of chemotaxis and diapedesis, useful for the healing of tissue damaged, for example, by trauma [9]. Moreover, it was shown that this matrix can be a valuable support for the transplantation of bone morphogenetic proteins (BMP) issued in a progressive manner to induce osteogenic differentiation, as demonstrated by recent studies on muscle preparations[10,11]; about this, the results of Wiltfang *et al*. are encouraging, in fact, they show an improvement of osteoblast proliferation in cases in which it was used the PRF compared to PRP [12].

Marrelli et al. described a case in which is documented the filling with PRF of a large osteolytic cavity and complete bone reformation [13]. Tatullo *et al*. have suggested that the osteoinductive potential of PRF is related to its neoangiogenic ability and concentration of GFs, in relation to the fibrin content and platelet cytokines present, all suitable for the totipotent cell migration and activation of pre-osteoblastic cells present in the surgical site, fundamental aspects for bone regeneration [14].

Platelets concentrates are, thus, versatile products in surgery, with regard to their biological properties and their easy manipulation in the form of gel or membranes; these features allow the use of PRF as well as other platelet concentrates in cases, for example, of maxillary surgical sites or in the surgery of maxillary sinus [15].

The frontier of regenerative medicine nowadays is represented by the mesenchymal stem cells (MSCs): overcoming the ethical problems thanks to the use of mesenchymal stem cells from adult patient, and with the increasingly sophisticated technology to support their manipulation, MSCs are undoubtedly the future of medicine regenerative and they are showing perspectives unimaginable just a few years ago. Most recent studies are aimed to tissues regeneration using MSCs taken from sites that are even more accessible and rich in stem cells: the oral cavity turned out to be an important source of MSCs with the advantage to be easily accessible to the surgeon, thus avoiding to increase the morbidity of the patient.

Mesenchymal stem cells of oral origin

The aim of the regenerative medicine and tissue engineering is to regenerate and repair the damaged cells and tissues in order to establish the normal functions [16]. The regenerative medicine involves the use of biomaterials, growth factors and stem cells [17]. Regeneration of the tissues exists naturally due to the presence of stem cells with the potential to self-regenerate and differentiate into one of more specialized cell types. However, this regenerative potential decreases with age and regeneration is not sufficient to repair the damages produced by degenerative, inflammatory or tumor based diseases[18]. Stem cells are immature and unspecialized cells with the ability to renew and divide themselves indefinitely through "self-renewal" and able to differentiate into multiple cell lineages [19]. The stem cells use for regenerative medicine should fit the following criteria: they can be: *a)* found in abundant numbers and can be differentiated in multiple cell lineages in a reproducible and controllable manner; *b)* isolated by minimally invasive procedure with minimal morbidity for patients, *c)* produced in accordance with GMP (Good manufacture Practice) and *d)* transplanted safely [20,21]. In the last decade, several improvements have been produced in the comprehension of stem cells properties in view of the fact that these cells have an important role in the repair of

every organ and tissue.

In general, the stem cells are divided into three main types that can be utilized for tissue repair and regeneration: *i)* the embryonic stem cells derived from embryos (ES) [22,23]; *ii)* the adult stem cells that are derived from adult tissue [24]; and *iii)* the induced pluripotent stem (iPS) cells that have been produced artificially via genetic manipulation of the somatic cells [25]. ES and iPS cells are considered pluripotent stem cells because they can develop into all types of cells from all three germinal layers. Both stem cells have technical and moral obstacles, in addition these cells are not easy to control and they can form tumors after injection[22]. On the contrary, adult stem cells are multipotent because they can only differentiate into a restricted number of cell types. Adult stem cells, also termed postnatal stem cells or somatic stem cells, are discovered in a particular area of each tissue named "stem cell niche."

Different type of postnatal stem cells resides in numerous mesenchymal tissues and these cells are at the same time referred to as mesenchymal stem cells (MSCs) [24,26]. MSCs were first isolated and characterized from bone marrow (BMSCs) by Friedenstein *et al.* in 1974 [27]. Subsequently, different studies have showed that MSCs can be isolated from other tissues, such as peripheral blood, umbilical cord blood, amniotic membrane, adult connective, adipose and dental tissues[28-32].

Recently, orofacial and dental tissues have acquired interest as a further accessible source of mesenchymal stem cells [33] due to the fact that the oral area is rich in MSCs (**Table 1**). Today, every cell population which has the following characteristics independently of its tissue source, is usually referred as MSCs: *i)* they adhere to plastic and have a fibroblast-like morphology; *ii)* they have the capacity of self-renewal and could differentiate into cells of the mesenchymal lineage such as osteocytes, chondrocytes and adipocytes. In addition, MSCs also can also differentiate, under appropriate conditions, into cells of the endoderm and ectoderm lineages such as hepatocytes and neurons, respectively [34,35]. Phenotypically, MSCs express the CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146 and STRO-1 surface antigens, and they do not express CD45 (leukocyte marker), CD34 (the primitive hematopoietic progenitor and endothelial cell marker), CD14 and CD11 (the monocyte and macrophage markers), CD79 and CD19 (the B cell markers), or HLA class II [36]. Research related to MSC from oral origin began in 2000 [37] and every year numerous investigations have demonstrated that oral tissues, which are simply available for dentists, are a rich source for mesenchymal stem cells [33,38].

Today numerous types of MSCs have been isolated from teeth: in 2000 MSCs were first isolated by Gronthos *et al.* from dental pulp (DPSCs) [37,38]. These cells possess phenotypic characteristics similar to those of BMSCs [39], and they have definitive stem cell properties such as self-renewal and multi- differentiation capacity, and can form the dentin-pulp structure when transplanted into immunocompromised mice [40]. Moreover, DPSCs participate in the regeneration of non-orofacial tissues, in fact, these cells have been differentiated into hair follicle-, hepatocyte-, neuron-, islet-, myocyte- and cardiomyocyte-like cells [41-46]. Subsequently, MSCs have been also isolated from dental pulp of human exfoliated deciduous teeth (SHEDs). These cells, like DPSCs, have the ability to differentiate *in vitro* in odontoblasts, osteoblasts, adipocytes and neuron-like cells. Also SHEDs were able to form dentin and bone when transplanted with HA/TCP *in vivo*[47].

Table 1: Mesenchymal Stem Cells from dental tissues

Name	Site	Date of discover	Authors	Country	Institution
DPSCs	Dental Pulp	2000	S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi	USA. Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
SHED	human Exfoliated Deciduous Teeth	2003	M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P. G. Robey, S. Shi	USA. Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
PDLSCs	Periodontal Ligament	2004	B. M. Seo, M. Miura, S. Gronthos, P.M. Bartold, S. Batouli, J. Brahim, M. Young, P.G. Robey, C.Y. Wang, S. Shi	USA. Bethesda, Maryland	National Institute on Dental Research, National Institutes of Health
SCAP	Apical Papilla	2006	W. Sonoyama, Y. Liu, D. Fang, T. Yamaza, B.M. Seo, C. Zhang, H. Liu, S. Gronthos, C.Y. Wang, S. Wang, S. Shi	USA. Los Angeles, California JAPAN. Okayama	University of Southern California School of Dentistry; Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
DFSCs	Dental Follicle	2005	C. Morsczeck, W. Götz, J. Schierholz, F. Zeilhofer, U. Kühn, C. Möhl, C. Sippel, K.H. Hoffmann	GERMANY. Bonn	Stiftung Caesar, Center of Advanced European Studies and Research
hPCy-MSCs	human Periapical Cyst	2013	M. Marrelli, F. Paduano, M. Tatullo	ITALY. Crotone	Calabrodental, Unit of Maxillofacial Surgery; Tecnologica Research Institute, Biomedical Section

The periodontal ligament is another adult MSCs source in dental tissue, and periodontal ligament stem cells (PDLSCs) were isolated from extracted teeth [48]. PDLSCs have the ability to regenerate periodontal tissues such as the cementum, periodontal ligament and alveolar bone [49]. Moreover, MSCs have been also isolated from developing dental tissues such as the dental follicle (DFPCs)[50] and apical papilla (SCAPs) [51]. DFPCs have the ability to regenerate periodontal tissues whereas SCAPs demonstrate better proliferation and better regeneration of the dentin matrix when transplanted in immunocompromised mice with compared to DPSCs [50,52,53]. Zhang *et al.* have isolated mesenchymal stem cells from the gingiva, these MSCs exhibited higher clonogenicity, self-renewal and multipotent differentiation capacity similar to that of BMSCs [54]. Moreover, the salivary glands derived MSCs could differentiate into the salivary gland duct cells as well as mucin and amylase producing acinar cells *in vitro* [55]. In addition, De Bari *et al.* demonstrated that single-cell-derived clonal populations of adult human periosteal cells possess mesenchymal multipotency, as they differentiate to osteoblast, chondrocyte, adipocyte and skeletal myocyte lineages *in vitro* and *in vivo*. Therefore, expanded MSCs isolated from periosteum could be useful for functional tissue engineering, especially for bone regeneration [56].

The MSCs contained within the bone marrow aspiration from the iliac crest, and liposuction from extra-oral tissue are not easily-accessible stem cells. On the contrary, the orofacial bone marrow, periosteum, salivary glands and dental tissues are the most accessible stem cell sources. Moreover, the isolation of MSCs from these sources may still not be convenient because it requires surgical methods or tooth or pulp extraction. In addition, even if impacted wisdom teeth could be a mesenchymal stem cell source, these MSCs are present in a low percentage and can, therefore, be difficult to isolate, purify and expand. Furthermore, not all adults need the extraction of the wisdom teeth. To overcome these limitations, recently, Marrelli *et al.* demonstrated that MSCs derived from periapical cysts (hPCy-MSCs) have a mesenchymal stem cell immunophenotype and the ability to differentiate into osteogenic and adipogenic lineages [57]. The periapical cyst, which is a tissue that is easily obtainable and whose cells can be simply expanded from patients with minimal discomfort, seems to be a promising source of adult stem cells in dentistry for regenerative medicine. In fact, a recent study of Marrelli *et al.* showed that hPCy-MSCs similarly to DPSCs have neural progenitor-like properties by expressing spontaneously neuron and astrocyte specific proteins and neural related genes before any differentiation. Furthermore, hPCy-MSCs, under appropriate neural stimulation, acquire neural morphology and significantly over-express several neural markers at both protein and transcriptional level (in press, not yet published research by Marrelli *et al.*).

Mesenchymal stem cells in regenerative medicine

It was reported that MSCs isolated from whole bone marrow aspirates in combination with scaffolds and growth factors are able to repair cranial defects in several animal models [58-60]. These studies demonstrated that MSCs can alleviate the complications of craniofacial surgical procedures that required allogenic tissue grafts or extraction of autologous bone from secondary sites. This approach may alleviate donor site morbidity and allow a virtual unlimited source of cellular material derived from allogenic MSCs [61].

The identification of MSC residing in the oral cavity tissues increases clinical interest in MSCs as a cell source for regeneration of other connective tissues such as cementum, dentin and periodontal ligament (PDL). Many research studies research have been performed to assess the capacity of dental derived MSCs to enhance periodontal regeneration. Seo *et al.* have demonstrated that human PDLSCs were able to generate a cementum/PDL-like structures when transplanted into immunocompromised mice, and consequently transplantation of PDLSCs could be considered as a therapeutic approach for regeneration of tissues damaged by periodontal diseases [48]. Moreover, Kim *et al.* compared the alveolar bone regeneration achieved from implantation of PDLSCs and BMSCs and identified no significant difference in regenerative potential *in vivo* between these MSCs [62].

The three key elements in the field of tissue engineering are stem cells, scaffolds and growth factors [63]. Recently, researchers are trying to identify the ideal scaffold that facilitate growth, cell spreading, adhesion, integration and differentiation of MSCs. This scaffold should be biocompatible and biodegradable, should have optimal physical features and mechanical properties [64]. Different material have been designed and constructed for tissue engineering approaches, using natural or synthetic polymers or inorganic materials, which have been fabricated into porous scaffolds, nanofibrous material, hydrogels and microparticles. Natural materials include collagen, elastin, fibrin, silk, chitosan and glycosaminoglycans [65]. Recently, hydrogels have been investigated for tissue engineering applications because they offer numerous properties including biocompatibility and mechanical characteristics similar to those of native tissue [66,67]. Synthetic poly lactic-co-glycolic acid (PLGA) and titanium provide excellent chemical and mechanical

properties for bone tissue regeneration *in vivo* using DPSCs [68]. Furthermore, recent studies demonstrated that DPSCs loaded onto scaffolds of chitosan formed a dentine-pulp complex *in vivo* [69] whereas DPSCs cultured on hydroxyapatite (HA) and placed subcutaneously in nude mice formed bone [70]. A great number of investigations for evaluating the *in vivo* application of MSCs isolated from the oral cavity were carried out on animal models. A clinical study conducted by Papaccio's group gave evidence of the possibility to utilise DPSCs to repair bone defect in humans. In fact, they showed that DPSCs/collagen biocomplex completely restored human mandible bone defects subsequent to DPSCs transplantation [71].

Conclusions

The future is the regeneration of whole organs and complex biological systems consisting of many different tissues, starting from an initial stem cell line, probably using innovative scaffolds together with the nano-engineering of biological tissues: this approach is already a research topic in several international research institutes, and the best way to merge the numerous skills needed to get a so ambitious result is the multicenter collaboration. The authors are closely collaborating together with high-level international Universities, to develop protocols aimed to control and lead the tissues regeneration. This goal could make born a new generation of stem-cells based therapies, so to open the door to a new highly-performing regenerative medicine.

Starting from 2000, in only fifteen years, researchers have changed the face of the tissues engineering and the expectation of quality of life in more than 2 billions of patients undergone to a regenerative surgery: the challenge is to continue to make the patient's life better, to make the surgery more predictable and to simply replace damaged or degenerated tissues with MSCs from dental and oral sources.

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Competing Interests

The authors have declared that no competing interest exists.

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REVIEW ARTICLE

Stem cells, growth factors and scaffolds in craniofacial regenerative medicine

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Abstract Current reconstructive approaches to large craniofacial skeletal defects are often complicated and challenging. Critical-sized defects are unable to heal via natural regenerative processes and require surgical intervention, traditionally involving autologous bone (mainly in the form of nonvascularized grafts) or alloplasts. Autologous bone grafts remain the gold standard of care in spite of the associated risk of donor site morbidity. Tissue engineering approaches represent a promising alternative that would serve to facilitate bone regeneration even in large craniofacial skeletal defects. This strategy has been tested in a myriad of iterations by utilizing a variety of osteoconductive scaffold materials, osteoblastic stem cells, as well as osteoinductive growth factors and small molecules. One of the major challenges facing tissue engineers is creating a scaffold fulfilling the properties necessary for controlled bone regeneration. These properties include osteoconduction, osteoinduction, biocompatibility, biodegradability, vascularization, and progenitor cell retention. This review will provide an overview of how optimization of the aforementioned scaffold parameters facilitates bone regenerative capabilities as well as a discussion of common osteoconductive scaffold materials.

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Introduction

Large craniofacial skeletal defects secondary to trauma, congenital condition, or cancer resection pose serious challenges to reconstructive surgeons. Extensive defects which prevent spontaneous re-ossification are termed 'critical-sized' and often require complex reconstructive approaches (Fig. 1A).[1] Repair of these defects has traditionally required autologous bone grafts from a variety of sources, including cranium, tibia, rib, and iliac crest (Fig. 1B).[2,3] These procedures, although they have seen success clinically and are currently the gold standard of care, necessitate a second surgical site with a significant risk of morbidity. In particular, undesirable sequelae at the donor site include infection, bleeding, pain, swelling, unanticipated fractures, and injury to adjacent critical structures.[4–6] Additionally, autologous bone graft procedures have been complicated by unpredictable graft resorption rates, limited supply of autologous bone, and rapid bone remodeling in young children.[2,3,7]

Alternatives in the alloplast category, including demineralized bone matrix, bone ceramics, porous polyethylene implants, and various other polymers, have seen variable success. However, they generally carry a greater risk of infection than autologous bone grafts and are more likely to fail over time.[8–12] Permanent methods of rigid fixation utilizing metals or metal alloys suffer similar limitations in addition to integrating poorly with the surrounding tissue.[13] Because craniofacial reconstructive surgeries are often performed on children (Fig. 1) who require repair capable of accommodating natural growth and development, permanent rigid fixation is not the most favorable alternative.

Biocompatible implants that augment natural bone-regenerative capabilities currently represent the most promising and versatile approach to repairing critical-sized craniofacial defects.[14] This tissue engineering-based strategy generally involves three key elements: osteoconductive scaffolding, stem cells, and growth factors (Fig. 2). These three elements allow osteoblastic and endothelial progenitor cell differentiation, bone formation, and integration with surrounding bone tissue even in large defects.[15] Osteoblastic stem cells within an osteoconductive scaffold provide the possibility of a tailored three-dimensional space for bone growth. Osteoblastic differentiation can be induced by a variety of osteoinductive growth factors both *in vivo* and *in vitro*.[16] Finally, efficacious bone regeneration requires integration with surrounding tissue, including vascularization, fusion of the implant with autologous bone without fibrous tissue at the bone-implant interface, and eventual complete replacement of the scaffold with new bone.[17–19]

The goal of achieving these prerequisites has challenged tissue engineers to choose the optimum combination of cell types, scaffold properties, and growth factors. The process is inherently complex and multidisciplinary due to requisite collaboration between molecular biology, materials science, surgery, and mechanical engineering.[20] This review will explore current progress toward achieving reliable repair of craniofacial defects using osteoconductive scaffold and osteogenic stem cell-based tissue engineering.

Stem cells used for bone regeneration

Irrespective of craniofacial bone defect size or complexity, healing is fundamentally dependent on the presence of osteogenic and vasculogenic precursor cells in surrounding tissues.[21] These precursors migrate to the injury site and differentiate into osteoblasts and endothelial cells, promoting bone formation and vascularization.[22] In recent years, clinical reports have suggested that stem cell supplementation may work synergistically with this natural progenitor cell migration and differentiation to produce the best results in healing critical-sized bone defects.[22–31]

Several stem cell types have been used both *in vitro* and *in vivo* to produce new bone (Fig. 3). Bone marrow-derived mesenchymal stromal cells (BMSCs) are increasingly being applied to craniofacial defect repair, and several studies have substantiated their effectiveness as osteoblastic precursors in critical-sized defect reconstruction.[32–34] A recent phase I/II clinical trial determined that $CD90^{+}$ osteoblastic BMSCs and neovascularization-inducing $CD14^{+}$ monocytes and macrophages seeded onto a β-tricalcium phosphate (β-TCP) scaffold provided a viable treatment for patients with severe maxillary bone deficiency.[35,36] When compared with scaffold alone, the progenitor cell-seeded scaffold treatment showed a higher proportion of regenerated viable, highly vascularized, and mineralized bone in addition to a lower proportion of residual β-TCP particles four months postoperatively.[35] Mesenchymal stem cells derived from umbilical cord blood have also been used successfully, in conjunction with poly-lactic co-glycolic acid (PLGA) implants, to heal critical-sized alveolar cleft defects in a swine model. Investigators reported no inflammation and better bone quality than autologous bone graft from the iliac crest by CT volumetric and histological analysis.[37] However, despite its success, the use of BMSCs is limited by finite supply and the morbidity associated with procurement procedures.[38]

Adipose-derived stem cells (ADSCs) represent a promising alternative to BMSCs in that they are more plentiful, less painful to harvest, and easily expandable.[39] ADSCs have showed similar osteogenicity to BMSCs, with certain subpopulations demonstrating enhanced tendency toward osteoblast differentiation and others successfully induced through gene therapy.[34,40] The necessity for invasive procedures during harvesting still constrains ease of access to ADSCs and the scope of their clinical significance.

Urine-derived stem cells (USCs), which can be obtained from voided urine and require no invasive procedures, have recently garnered a great deal of attention in the bone tissue engineering community as a promising, but still poorly studied, alternative stem cell source. Research regarding USCs is still in its infancy, but recent studies by Guan et al have demonstrated their applicability to bone regeneration.[38,41–43] USCs are biologically similar to ADSCs and are capable of osteogenic differentiation *in vitro*.[43] Furthermore, USCs have successfully differentiated into osteoblasts via calcium silicate ion induction of the Wnt/β-catenin signaling pathway.[38] They have also been shown to be compatible with both calcium sulfate/PLGA composite and β-TCP scaffolds.[38,42]

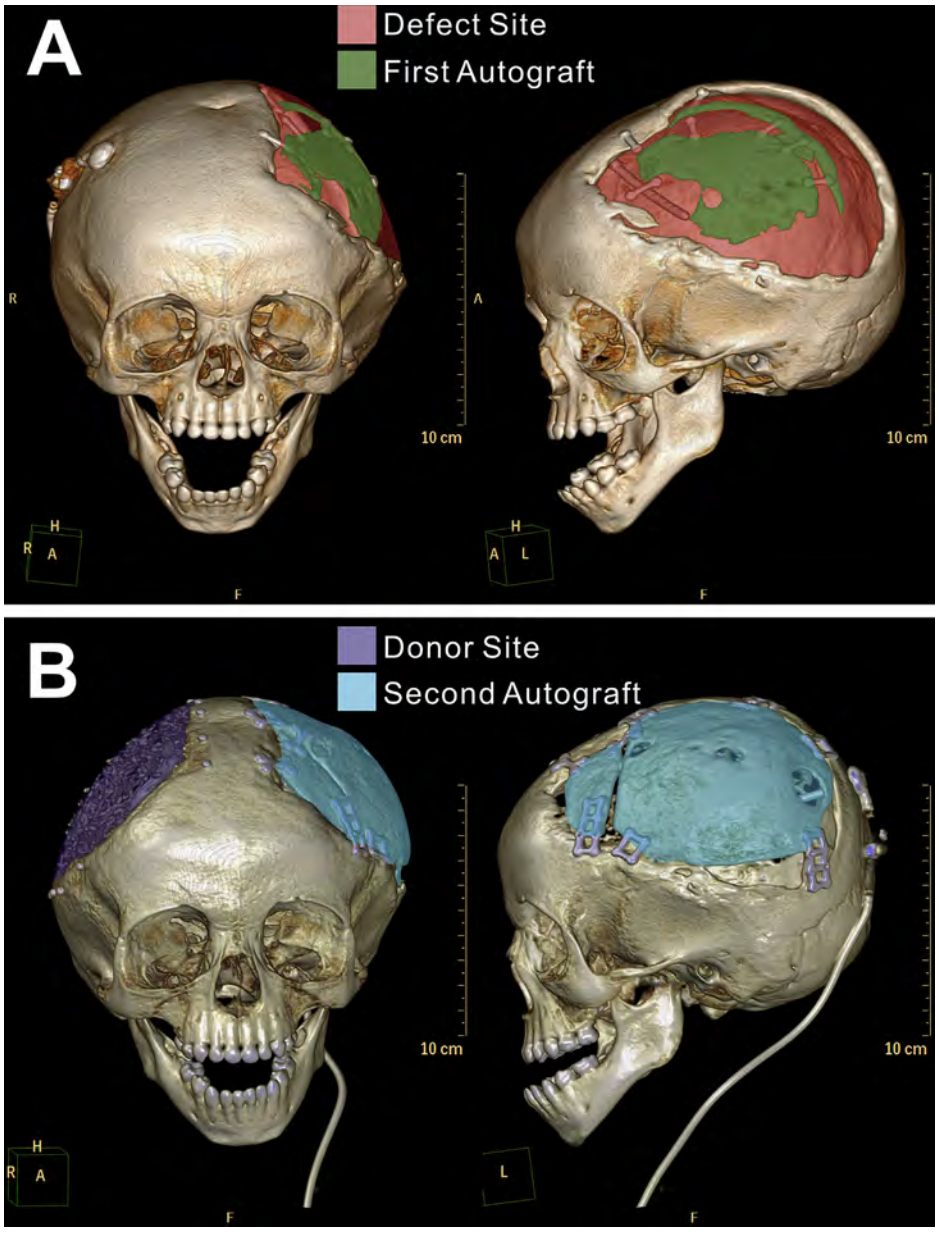

Fig. 1 Case example of a pediatric craniofacial defect. A) Depicted is a large craniofacial skeletal defect resulting from resorption of an autogenous bone graft following emergency craniectomy and delayed replacement of the bone. B) Reconstruction was accomplished through a second autograft involving full-thickness resection of large portions of the frontal and right parietal bones. The donor site was repaired using demineralized bone matrix and particulate bone graft. The use of these CT images follows the guidelines of the University of Chicago Institutional Review Board.

Neovascularization is a critical component of bone tissue engineering, and can be facilitated by incorporation of endothelial progenitor cells (EPCs) in scaffold design. EPCs have been shown to enable neovascularization in response to ischemia.[44,45] This ischemic response is seen in the context of critical-sized craniofacial defects, and EPCs have been used in combination with MSCs and a thermoresponsive porous nano-calcium sulfate/alginate scaffold to repair calvarial defects in rats.[45] EPCs are also compatible with β-TCP scaffolds, in which they have been shown to contribute directly to neovasculogenesis through endothelial cell differentiation and recruitment of additional host EPCs. Exogenous EPCs have also been shown to release pro-angiogenic factors such as vascular endothelial growth factor (VEGF).[46]

Osteoinductive factors

A critical component of osteoblastic progenitor cell differentiation and subsequent bone formation are osteoinductive growth factors (Table 1). Many growth factors are known to enhance bone regeneration, including transforming growth factor β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and platelet derived growth factor (PDGF).[47–50] Several bone

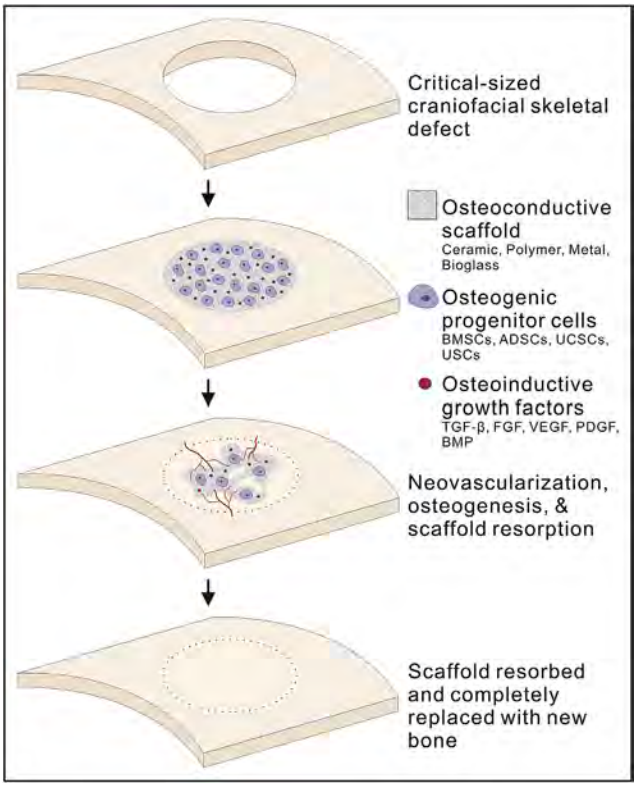

Fig. 2 Tissue engineering paradigm for craniofacial defect repair. Illustration depicting ideal modality for craniofacial defect repair. The strategy involves growth factor-induced osteoblastic differentiation and bone formation within an osteoconductive and biodegradable scaffold.

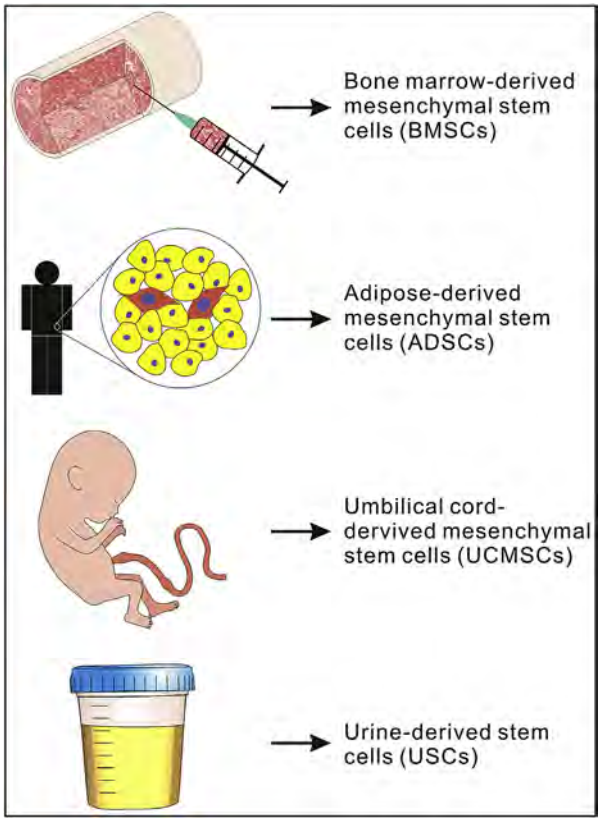

Fig. 3 Osteoblastic stem cell sources. The potential sources of mesenchymal stem cells (MSCs) that can be used for bone tissue engineering and regeneration. The recently described urine-derived stem cells (USCs) may represent one of the most promising and convenient sources of MSCs for tissue engineering and regenerative medicine.

morphogenic proteins (BMPs), members of the TGF-β family, have been used clinically to induce bone regeneration in critical-sized craniofacial defects as well as alveolar ridge and sinus augumentation.[51–53] They bind receptors on multiple stem cell types and induce osteoblastic differentiation through the Smad protein signaling pathway.[14] BMPs, particularly BMP-2 and BMP-7, have been studied extensively in bone healing and produce superior fusion rates with fewer complications than autologous bone grafts.[54–65] Infuse® Bone Graft (Medtronic and Wyeth) and Osigraft® (Stryker Biotech) are two FDA-approved collagen-based scaffolds containing recombinant BMP-2 and BMP-7, respectively. The clinical success of these products demonstrates the importance of growth factors in osteogenesis and underscores the potential of growth factor-infused scaffolds.

Other osteoinductive BMPs include BMP-4, 6, and 9, and previous evidence suggests that BMP-9, a relatively poorly characterized growth factor, is the most potent BMP in promoting *in vitro* and *in vivo* osteogenic differentiation of mesenchymal stem cells.[66–75] Despite such auspicious results, relatively high dose requirements, cases of ectopic bone formation, and paradoxical increase in bone resorption — particularly observed with BMP-2 — have tarnished some of BMPs' initial promise.[76–79] Efforts are ongoing to combine synergistic growth factors and carrier molecules to lower the necessary BMP dose and control its release.[80,81]

Growth factor incorporation into scaffolds may be accomplished in a number of ways, each of which confers unique properties. Soaking a scaffold in growth factor-containing solution results in a loose association with the structural material and, therefore, facilitates quick release of the desired stimulatory molecules. Conversely, growth factors may be incorporated into and even covalently linked to the scaffold microstructure for extended release. Cells modified to express and secrete osteoinductive growth factors may also be seeded in the scaffold, achieving a similar effect.[82] The necessary cell modifications typically involve gene therapy accomplished either by viral or nonviral transduction. Viral transduction is the most effective means of gene transfer and is generally carried out using retroviruses, adenoviruses, or adeno-associated viruses.[83,84] Gene transfer can also be accomplished via direct uptake of gene-containing plasmids from solution or as a conjugate with a nucleus-bound biomolecule.[84]

Issues with growth factor-enriched scaffolds are generally associated with mismatched release profiles — the release of growth factor is often dictated by passive diffusion or degradation rate, and does not appropriately parallel the rate of bone regeneration and healing.[82] It has been shown that covalent linkage of the growth factor to the scaffold may slow and improve its release profile to

Table 1 Osteoinductive growth factors. Growth factors that can be used in bone tissue engineering and their general contribution to osteogenesis.

Growth factor	Osteoblastic differentiation	Osteoblast proliferation	Neovasculogenesis
TGF-B	Promoting	Promoting	
FGF		Promoting	
VEGF			Promoting/Inducing
PDGF	Promoting[a]	Promoting	Promoting
BMP-2	Inducing	Promoting early; Inhibiting late	
BMP-4	Inducing	Promoting early; Inhibiting late	
BMP-6	Inducing	Promoting early; Inhibiting late	
BMP-7	Inducing	Promoting early; Inhibiting late	
BMP-9	Inducing	Promoting early; Inhibiting late	

[a] Only PDGF-AA has been shown to promote osteoblastic differentiation in MSCs.

more closely approximate cellular demands.[85] For example, covalently incorporated VEGF in a fibrin scaffold results in a more tightly controlled release and, subsequently, a more organized vascularization in comparison to scaffold with unlinked VEGF.[86] One risk inherent in covalently incorporated growth factors is altering established mechanical, osteoconductive, or other properties of the scaffold material. Despite this, it has been used in animal models to successfully repair mandibular, zygomatic, and calvarial bone defects.[14,87]

As a supplement to BMPs or other osteoinductive growth factor proteins, small molecules that help induce osteoblast differentiation have been used. Small molecules are generally more cost-effective, easier to synthesize and handle, and diffuse rapidly.[88] Statins, as well as several immunosuppressants, are small molecules that have demonstrated capability to induce osteoblastic differentiation and bone formation.[89–92] Phenamil, an irreversible amiloride analogue, is another small molecule that has been shown to induce osteogenesis in dental pulp cells and BMSCs through robust activation of the BMP signaling pathway.[93–96] Most recently, phenamil has demonstrated synergistic effects with BMP-2 by inducing osteogenic differentiation of ADSCs in calvarial defect repair.[97]

Characteristics of an optimal scaffold

Osteoconduction

In designing scaffolds for bone regeneration, there are several key properties that tissue engineers consider. First is the capacity to deliver exogenous osteoblastic and epithelial progenitor cells to the defect site and/or to facilitate recruitment of host progenitor cells that aid in bone generation and tissue integration. Osteoconduction refers to the ability of the scaffold to not only act as a carrier for these progenitor cells but also to provide a viable template for bone growth.[17] Osteoconductive materials that provide a supportive microenvironment in which exogenous and endogenous progenitor cells can differentiate and produce vascularized bone are a key part of scaffold design.

Natural fracture healing is characterized by the formation of a cartilaginous callus, which undergoes mineralization, resorption, and replacement by new bone.[98] It is this role of the cartilaginous callus as an osteoconductive template that current scaffolds seek to emulate. However, whereas physiologic bone healing is limited to small defects, scaffolds enhance these processes to bridge large segmental defects.[99] Collagen and hydroxyapatite, the primary organic and mineral components of bone, respectively, are prototype osteoconductive materials and will be discussed later in this review.[100]

The concept of mimicking native bone ECM, which serves as a collagenous framework for osteoblasts and a reservoir for growth factors, has played a significant role in scaffold design.[101] Interplay between the scaffold and progenitor cells should closely mimic natural cell surface receptor and ECM interactions.[18] These interactions are critical in bone regeneration processes such as osteoblast adhesion, proliferation, migration, differentiation, and matrix deposition.[18] The importance of biophysical cell/scaffold interactions on cell function has been underscored by studies demonstrating significant differences in cell adhesion and differentiation behavior with changes in scaffold elasticity and surface microstructure.[102,103]

Osteoinduction

In smaller fractures, natural regenerative healing occurs via recruitment of mesenchymal stem cells from adjacent tissues and bone marrow to the site of injury, where they are induced to differentiate into osteoblasts and deposit new bone to bridge the fracture.[98,104] Differentiation of these migratory progenitor cells is accomplished via mechanical, biochemical, and biophysical factors in a process called osteoinduction.[104] Osteoinductive scaffold designs seek to emulate this natural phenomenon through biochemical structure, progenitor cell adhesion properties, and delivery of growth factors.[44,105,106]

Biocompatibility

Biocompatibility is an essential attribute of any scaffold implant, and in order to be clinically successful, it must not elicit a damaging inflammatory response. In the context of

biodegradable scaffolds, the most common way for unwanted inflammatory processes to occur is by production of reactive oxygen species (ROS). Accumulation of degradation products may generate toxic levels of ROS.[107–111] Approaches to minimizing the inflammatory response include incorporation of biomimicking materials as well as conjugate antioxidants in the scaffold itself.[112–115] Utilizing scaffolds that can be delivered through minimally invasive techniques, such as injectable hydrogels or thermoresponsive scaffolds, is also an important tactic to reduce inflammation.[116,117]

Biodegradability

Osteoconductive scaffolds should act only as a temporary framework for bone regeneration.[18] Temporality is critically important, as the ideal scaffold is not meant to be a permanent prosthetic, but rather a provisional support for osteoblastic differentiation, bone regeneration, and vascularization until fully functional tissue has replaced the scaffold and the defect is healed.[18] Full resorption of the original scaffold is necessary for uninterrupted bone remodeling and physiologic responses to mechanical stimuli.[19] Unmatched rates of scaffold material resorption and bone formation may result in incomplete bone regeneration or obstructed remodeling and tissue integration.[118–120] Therefore, degradability of the scaffold into biocompatible byproducts is an essential property that is governed by scaffold chemical composition, micro- and macrostructure, and numerous host factors.[19,121] Clinical factors affecting bone regeneration and scaffold degradation rates, including patient co-morbidities and defect anatomy, must be considered in selecting graft substitutes for repairing craniofacial defects.[19]

Vascularization

An extensive variety of scaffolds and stem cell therapy approaches to healing craniofacial defects have been proposed and tested, but successful treatment ultimately depends on integration with surrounding tissue. That success hinges on two key factors — the ability to recruit local osteoblastic and endothelial progenitor cells to the site of injury and the existence of functioning vasculature near the defect.[13,45] Vasculogenesis, or formation of new blood vessels through differentiation of recruited endothelial progenitor cells (EPCs), is a normal response to traumatic injury and is largely mediated by vascular endothelial growth factor (VEGF).[16,122] Downstream effects of VEGF culminate in proliferation of circulating EPCs, which initiate vasculogenesis at the defect site.[16] Vasculogenesis and angiogenesis, collectively known as neovascularization, are necessary prerequisites for osteogenesis, and it has been shown that bone regenerative capabilities are directly linked to circulating EPC levels.[122]

However, effective delivery of these EPCs is complicated by the vascular deficiency that often exists in the context of critical-sized craniofacial and other bone defects.[45] In order to promote vascularization despite these challenges, scaffolds can be enriched with both growth factors and endothelial progenitor cells. Several strategies have been attempted, including direct integration of neovasculogenic growth factors and cytokines, incorporating cells capable of secreting these growth factors, featuring adhesion proteins conducive to endothelial cell attachment and blood vessel formation, and seeding with endothelial progenitor cells.[46,123–127] Multipotent bone marrow stromal cells enriched for mesenchymal and endothelial phenotypes have also demonstrated capacity for highly vascularized bone generation in mandibular defect repair.[128]

The importance of vascular supply in bone reconstruction is well recognized.[129,130] Osteoprogenitor cells associate with endothelial cells, which supply not only oxygen and nutrients but also growth factors necessary for osteoblastic differentiation.[131] For this reason, neovascularization is an essential step in promoting sustained bone regeneration. Accommodating for endothelial progenitor cell invasion and attachment, delivery of proangiogenic factors, and blood vessel formation necessitates a porous scaffold structure.[132] It is thought that 150–500 μm is a sufficient pore diameter to support neovascularization and blood vessel invasion.[133] However, porosity often relates inversely with material strength. The idea that reduced porosity and higher density confers greater mechanical strength while increased porosity facilitates growth factor delivery, cell migration, and vascularization has been a key principle of scaffold design.[18,134,135] As a result, the ideal scaffold strikes a balance between the two competing properties.[18]

Head and neck cancer treatments involving bone resection and radiation therapy also pose a significant challenge for reconstructive surgeons due to the debilitating nature of radiation toxicity on bone regeneration.[13,136] Radiation therapy severely complicates bone development, remodeling, and fracture healing secondary to progenitor cell loss and compromised vasculature.[137–140] These complicating factors require a combination of neovasculogenic progenitor cells and growth factors to ensure proper vascularization.[141,142]

Biomaterials for osteoconductive scaffold construction

Although autologous bone grafts remain the gold standard for repairing critical-sized craniofacial defects, their use is cost-prohibitive, requires a second surgical site, is associated with significant donor site morbidity, and is limited by the finite supply of autologous bone.[3,4,143] The use of biocompatible scaffolds in healing these defects may provide a more cost-effective and less complicated alternative to autologous bone grafts.[121] Scaffolds provide an osteoconductive and osteoinductive extracellular matrix analog to facilitate cellular migration, proliferation, adhesion, differentiation, and generation of new bone.[105,121] A variety of materials for this purpose have been studied, including ceramics, natural and synthetic polymers, various composite materials, silicon-based bioglass, and metals (Table 2).[13,121,144]

Table 2 Biomaterials for bone tissue engineering. Commonly used biomaterials for bone regeneration in craniofacial defect repair.

Osteoconductive biomaterials for scaffold construction	
Allogenic bone derivative	Demineralized bone matrix (DBM)
Ceramics	Hydroxyapatite (HA)
	Tricalcium phosphate (TCP)
	Biphasic calcium phosphate
	Calcium carbonate
Polymers	Poly(lactic acid) (PLA)
	Poly(glycolic acid) (PGA)
	Poly(lactic-co-glycolic acid) (PLGA)
	Poly(propylene fumarate) (PPF)
	Polycaprolactone (PCL)
	Polyamide (PA)
	Chitosan
Metals	Titanium
	Magnesium Alloy
	Zinc (doping)
Bioglass	Silicon
	Calcium-silicate (CS)
Thermoresponsive	N-isopropylacrylamide (NIPAA)
	Poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN)

Demineralized bone matrix

Demineralized bone matrix (DBM) is produced by acid extraction of allogenic bone, a process that removes the inorganic mineral component of bone and leaves a type I collagen framework.[145] Demineralization also exposes osteoinductive growth factors, including BMPs, making DBM more osteoinductive than complete bone grafts. DBM is currently available as powder, granules, gel, putty, and paste, but an intrinsic limitation of all DBM types is poor mechanical strength and porosity.[146] A recent retrospective study investigating craniofacial defect reconstruction outcomes using bone cement, autologous bone grafts, and DBM revealed the highest rate of residual defect using DBM.[147] Because of such findings, DBM alone is not considered a promising scaffold material. However, recent efforts using poly(lactic acid) (PLA)/DBM composite scaffolds for bone engineering have proven to be more effective.[145]

Ceramics

Some of the most promising initial scaffolds closely mimic the chemistry and structure of native extracellular matrix in bone.[13] Foremost among these are calcium phosphate ceramics, including hydroxyapatite (HA), β-TCP, and biphasic calcium phosphate.[13] Due to their biocompatibility, safety, reliability, availability, ease of sterilization, and long shelf life, calcium phosphate scaffolds have considerable promise as an alternative to bone grafts.[148,149]

Hydroxyapatite bioceramics confer a high degree of osteoconductivity but are brittle and resorbed at a rate much slower than desired, often taking several years. This is in contrast to tricalcium phosphate (TCP) scaffolds, which have been reported to fully resorb within 12 weeks.[18,150] By altering calcium-to-phosphate ratios, internal pore architecture, and other parameters of these TCP scaffolds, engineers have been able to control resorption rates and improve osteogenicity.[4–6] Furthermore, HA-TCP composite scaffolds have demonstrated both osteoconductivity and favorable resorption rates.[151,152] Similarly, it has been shown that HA/collagen composite implants are characterized by improved stiffness and osteointegration in comparison to collagen alone in critical-sized rat calvarial defects.[153] An injectable collagen/calcium phosphate hydrogel has also exhibited efficient umbilical cord-derived mesenchymal stem cell (UCMSC) seeding and ability to support osteoblastic differentiation and osteogenesis.[154]

Although conferring essential osteoconductive, porous, and resorption properties, ceramic scaffolds are relatively brittle and do not have the strength optimally desired. To that end, more recent experiments have found that incorporating hydroxyapatite nanoparticles into more structurally competent polymer scaffolds has resulted in a more favorable combination of strength, protein loading, cell adhesion and migration, and osteogenic properties.[155] In addition, a scaffold comprised of calcium phosphate ceramic tiles set within a titanium framework has recently been described in the context of complex craniofacial defect repair.[3]

Calcium carbonate is another potential ceramic material for osteoconductive scaffold fabrication. It has better natural biodegradation properties than calcium phosphate, and may prove useful in pediatric craniofacial reconstruction, where highly active skeletal remodeling necessitates rapid scaffold resorption.[14,156] As of yet, this material has most significantly been used to repair burr holes from hematoma-related neurosurgery cases.[156] Two studies have tested alveolar bone regenerative capabilities of calcium carbonate scaffolds and concluded that its mechanism of supporting bone growth is primarily through space-provision rather than previously hypothesized osteoconductive properties.[157,158] Since then, little research has been done to further characterize bone tissue engineering applications for calcium carbonate.

Polymers

Natural and synthetic polymers are often used as scaffold materials for bone tissue engineering because of a well-balanced combination of properties, including biodegradability, biocompatibility, porosity, and ease of handling.[159–161] Naturally-derived materials, such as collagen and fibrin proteins, or chitin-derived chitosan polysaccharide, are also an option for bone tissue engineering.[117,162] Such materials may confer greater cell adhesion and functional support properties than synthetic materials, but in most cases, this is offset by several disadvantages. Natural polymers often offer less control over mechanical properties, sometimes exhibit immunogenicity, and frequently exist in finite supply; therefore, they are difficult and expensive to obtain. Synthetic polymers,

however, do not suffer from these shortcomings and have been a more important source of biomaterials for osteoconductive scaffold construction.[162]

Synthetic polymers can be produced on a large scale using reproducible and tunable methods, providing fine control over mechanical and physical properties. They have a well-documented history of clinical application in craniofacial bone reconstruction, especially in children.[163] Synthetic polymers like poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and various iterations of combined poly(lactic-co-glycolic acid) (PLGA) have been used for a range of clinical applications, including critical-sized craniofacial defect repair.[37,164]

PLA is an FDA-approved synthetic biomaterial that has several properties conducive to bone tissue engineering, including controllable biodegradation rate, biocompatibility, and good mechanical strength.[165] It has been applied clinically to fabrication of resorbable sutures, as a drug delivery scaffold, and as resorbable bone fixation devices in fracture healing. However, its application as a scaffold biomaterial for craniofacial bone regeneration is limited by poor osteoinductive properties.[145] PGA is another FDA-approved synthetic biomaterial with a variety of tissue engineering applications, including regeneration of cartilage, bone, tendon, muscle, and skin.[166–168] Despite such adaptability, its mechanical properties are not ideal for the precision bone reconstruction necessary for craniofacial defect repair because of its softness and inability to maintain shape. PGA and PLA alone are not suitable bone tissue engineering scaffold materials, but their respective softness and low osteoinductivity have been partially addressed by combining them to form a PLGA composite scaffold.[169] PLGA has been shown to have a controllable degradation rate (through varying composition of its constituent homopolymers) in addition to supporting osteoblast attachment, growth, and differentiation both *in vitro* and *in vivo*.[162,170–173] Nevertheless, PLGA's mechanical properties and osteoconductivity are suboptimal for bone tissue engineering, and it is most often used as part of a composite material with ceramics, bioglass, or other more osteoconductive materials.[173,174]

Poly(propylene fumarate) (PPF) is a synthetic, unsaturated, linear polyester polymer that is biodegradable, biocompatible, osteoconductive, injectable, and sufficiently strong for craniofacial bone tissue engineering.[175–185] It generally requires a small monomer accelerating agent, such as N-vinylpyrrolidone, in order to crosslink as an injectable polymer.[186] A two-phase PPF cement incorporating cross-linked microparticles to increase strength and lower setting temperature has been developed. This PPF-based system has improved injectability, setting temperature, and setting time over clinically available polymethyl methacrylate (PMMA) bone cement and is believed to be suitable for application in craniofacial bone regeneration.[175] PPF has also been used as a co-polymer with polycaprolactone (PCL) as a scaffold for osteoblastic differentiation and maturation *in vitro*.[187] PCL is a non-aromatic polyester that is highly flexible and has a controllable biodegradation rate owed to alterable substituent molecular weight.[188–190] Similarly, the PPF-PCL co-polymer setting time, setting temperature, mechanical strength, and other physical properties can be tuned through variation of substituent molecular weight as well as relative proportion of PPF and PCL.[186,187] PPF-PCL's chemical structure also allows for HA incorporation, which aids osteoblast progenitor cell adhesion and proliferation.[187]

Polyamide (PA) is a synthetic polymeric collagen analog that provides excellent strength as well as biocompatibility. Those properties have made PA a promising partner for HA or other bioceramics in osteoconductive composite scaffolds. As a BMP-7-transduced MSC-laden composite with HA nanoparticles, PA has been successfully used to repair mandibular defects in rabbits.[191]

Metals

Currently, metals such as titanium are used clinically in craniofacial reconstruction. However, as inert alloplasts, they do not integrate with surrounding tissue and do not stimulate new bone formation.[13] Metals that degrade in a physiological setting have been proposed in order to solve this problem and promote more long-term success. Biodegradable metals, such as magnesium alloys, have generally been shown to possess mechanical properties mimicking that of natural bone while retaining the critical ability to resorb over time.[164,192] Mg-rare earth element compounds, Mg–Ca, pure Fe, Fe–Mn alloys, and Fe foam have all been tested as osteoconductive scaffold materials for bone tissue engineering.[193–204] In particular, Mg and its alloys have been shown to support osteoblastic differentiation of progenitor cells and are degraded *in vivo* to Mg hydroxide and hydrogen gas.[195] Given the importance of porosity for progenitor cell migration and neovascularization, porous Mg scaffolds have been investigated and can be fabricated with preserved mechanical properties.[164,205,206] Their strength, ductility, biodegradability, and osteoconductive properties make Mg alloys, and potentially other metals, possible alternatives to polymer or ceramic scaffolds.[164]

Incorporating metal nanoparticles into polymer scaffold materials has also been an ongoing effort to produce higher strength composite scaffolds that retain their osteoinductivity and osteoconductivity.[144,155,207] Addition of other trace impurities, such as zinc oxide, iron, and silicon dioxide, has been shown to confer a greater degree of control in degradation rates, density, mechanical strength, and biocompatibility.[105] The addition of zinc and silicon has boosted both expression of type I collagen and extracellular signaling promoting angiogenesis as well as osteoblast differentiation.[208,209]

Bioglass

There are two major groups of glass-based osteogenic scaffolds: glass-ceramic and glass-polymer porous composites.[144] It has been demonstrated that silicon found in glass enhances angiogenesis as well as gene expression regulating osteogenesis and growth factor production in osteoblasts.[13] Several studies have confirmed that silicate-based scaffolds are capable of stimulating osteogenesis.[210–212] Accordingly, silicon has been successfully incorporated into bioceramics in order to augment bioactivity and osteostimulatory effects.[211,213–216]

For example, silicon/HA scaffolds have also shown increased bone ingrowth over HA alone, but these hybrids are limited by low mechanical load strength.[13] Alternatives include calcium silicate (CS)-containing scaffolds, which are able to stimulate osteogenic differentiation of several adult stem cell lines, including BMSCs, and have pro-angiogenic properties.[38,215,217–221] Importantly, these scaffolds are able to have these effects without the addition of exogenous growth factors.[217,218] Osteogenic and angiogenic growth factors have previously been utilized in bone tissue engineering, but the prospect of a single scaffold capable of inducing both osteogenesis and angiogenesis without exogenous growth factors has exciting implications.[215,222,223] Silicate bioglass as well as some ceramic scaffolds have been shown to posses this dual-inductive attribute.[211,215,221]

As previously discussed, composite scaffolds combining materials with different desirable properties are a step toward the ideal. Silicate composite scaffolds have been tested, and varying the relative proportion of each component affords some degree of control over mechanical properties, hydrophobicity, and degradation.[217,218,224,225]

Injectable biomaterials

Injectable biomaterials provide two major advantages over traditional solid scaffolds; they can be delivered through minimally invasive means, and they spontaneously mold to the shape of even the most complicated defects. This has important implications for reducing inflammatory side effects and subsequent scar formation stemming from invasive surgery and imprecise scaffold fit. Injectable biomaterials have been tested in the context of tissue engineering and may be appropriate for facilitating osteogenesis in craniofacial defects.[117,226,227] In particular, thermoresponsive biomaterials have been shown to predictably undergo liquid-to-solid phase change at appropriate physiological temperatures and may be a potent delivery mechanism for osteogenic growth factors and progenitor cells.[228–233]

N-isopropylacrylamide (NIPAA) is a particularly well studied thermoresponsive biomaterial, but it is limited by issues including toxicity, nondegradability, and hydrophobicity-driven syneresis with subsequent release of compounds or lysis of cells entrapped within the scaffold.[234–237] Many of these limitations may be overcome with incorporation of poly(polyethylene glycol citrate) acrylate (PPCac) to form a poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN) polymer.[116] This material not only preserves the thermoresponsive properties of NIPAA but also possesses higher protein loading efficiency, supports three-dimensional cell proliferation, retains viable cells for at least 72 days, and has intrinsic antioxidant properties.[116,238,239]

Hydrogels comprise another important class of osteoconductive scaffolds that can be delivered through noninvasive means.[13,240,241] They are water-absorbing matrices composed of cross-linked hydrophilic polymers that are well suited to harboring growth factors and viable stem cells.[241,242] As a result, hydrogels are ideal for stem cell and biofactor delivery that promote bone tissue regeneration.[240–242] For example, a composite hydrogel incorporating BMP-2 and synergistic chitosan (deacetylated chitin) has demonstrated controlled release of BMP-2 with minimal burst phase and shows remarkable bone regenerative capability.[81]

Other injectable scaffolds include hydroxyapatite or calcium sulfate pastes, but are complicated by syneresis and contraction, as well as brittleness following setting.[243,244] Using a combination of these and other materials in injectable composites helps overcome many of the individual materials' limitations and enhances osteoconductivity.[245,246] For example, PLGA microspheres coated with HA form a colloidal gel that can be seeded with osteoblastic progenitor cells and successfully support osteogenesis *in vivo*.[247,248] Furthermore, PLGA-HA microsphere gel is an effective delivery vehicle for the anti-osteoporotic drug alendronate, demonstrating a sustained drug release profile and minimal burst phase.[249] If this can be replicated with osteoinductive small molecules or growth factors, it would greatly enhance the osteogenic potential of PLGA-HA as a biomaterial for bone tissue regeneration. Another composite microgel scaffold composed of chitin, polycaprolactone, and HA has been investigated with ADSCs and has produced promising results for application in bone tissue engineering.[117] As with other composite scaffolds, relative proportions of each component can be tuned to provide optimal degradation rate, viscoelastic and mechanical properties, cell adhesion properties, and osteoconductivity.[117,250,251]

Osteoinductive molecular structure

In addition to the composition of the scaffold, the molecular structure is also a design priority for optimizing osteoconductive and osteoinductive properties. It has been suggested that an optimal approach for bone regeneration should closely mimic that of natural healing, and the design of an osteoinductive scaffold should reflect the basic multicellular unit of corticocancellous bone.[252] This basic structure consists of a long cylindrical unit in line with the bone's long axis and is composed of osteoclasts on the leading end and osteoblasts laying down new bone on the lagging end. Designing scaffolds to initiate this bone remodeling step without the need to first deposit a temporary bone matrix is a novel idea pursued by some investigators.[252] This strategy would utilize osteoinductive geometric cues within the scaffold to initiate bone formation without the need for exogenous osteogenic molecular signals.[252,253]

Conclusions and future directions

Thorough understanding of the physiology and molecular pathways involved in bone formation and remodeling is a prerequisite for making advances in craniofacial bone tissue engineering. Innovations in material science and molecular biology have allowed tissue engineers to augment physiologic bone healing and make bone regeneration via scaffold/stem cell therapy a clinical possibility. Combining biomaterials, often with competing properties, to fabricate optimized scaffolds for use in craniofacial skeletal

regeneration is representative of current research trends and the most promising strategy for tissue engineers and craniofacial surgeons. New advances unlocking the osteogenic potential of several stem cell types, as well as the discovery of more readily available stem cell sources (e.g., urine-derived stem cells), are also providing exciting prospects for craniofacial bone regeneration.

Despite such advances in tissue engineering, craniofacial bone reconstruction is often complicated by scarring, osteomyelitis, osteonecrosis, or previous radiation damage. The combination of stem cells, growth factors, small molecules, and scaffold materials used in reparative bone tissue engineering will largely be guided by these and other complicating factors. Still, relatively little research explores the behavior of tissue engineering approaches in the context of extensive medical comorbidities or compromised wound healing capability. Craniofacial skeletal repair via tissue engineering remains the most promising alternative to autologous bone grafts, and numerous modalities involving a variety of stem cells, growth factors, and osteoconductive scaffold materials have been tested and met with success in animal models. In the future, strategies and materials must be refined to achieve more reliable outcomes and to address the various challenges posed by real clinical scenarios in which craniofacial reconstruction is appropriate.

Conflicts of interest

The authors declare no conflict of interest.

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Comparative Outcomes of Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting in Patients with Unilateral Cleft Lip and Palate

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Background: The role of primary gingivoperiosteoplasty in the repair of alveolar clefts remains controversial. The aim of this study was to compare the outcomes of primary gingivoperiosteoplasty and secondary alveolar bone grafting in patients with unilateral cleft lip and palate.

Methods: In this prospective study, the authors analyzed the postoperative cone-beam computed tomographic scans of 50 children with complete unilateral cleft lip and palate who underwent primary gingivoperiosteoplasty (n = 25) or secondary alveolar bone grafting (n = 25). These two methods of alveolar repair were compared by measuring residual cleft defect and unsupported root ratio of cleft-adjacent central incisors on patient scans.

Results: Patients who underwent repair by primary gingivoperiosteoplasty presented more need for additional bone grafting than those undergoing repair by secondary alveolar bone grafting (28 percent versus 4 percent, respectively; $p < 0.05$). Residual cleft defect was greater in patients who underwent repair by primary gingivoperiosteoplasty than by secondary alveolar bone grafting (305.8 ± 176.5 mm^3 versus 178.6 ± 122.0 mm^3, respectively; $p < 0.05$). Patients who underwent repair by primary gingivoperiosteoplasty showed more residual palatal coronal and palatal apical defects than those who underwent repair by secondary alveolar bone grafting ($p < 0.05$ and $p < 0.001$, respectively).

Conclusions: In patients with unilateral cleft lip and palate, primary gingivoperiosteoplasty can achieve 72 percent success. Primary gingivoperiosteoplasty results in less bone than secondary alveolar bone grafting, particularly on the palatal apical portion of the previous alveolar cleft. Clinical success is lower with primary gingivoperiosteoplasty than with secondary alveolar bone grafting. (*Plast. Reconstr. Surg.* 137: 218, 2016.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, III.

Alveolar repair has become a routine part of treatment protocols for patients with alveolar clefts. Its primary aim is to restore the function and structure of the maxillary arch at the cleft site. The two treatments most commonly used for alveolar repair are secondary alveolar bone grafting performed in patients with mixed dentition,[1] and primary gingivoperiosteoplasty performed in infancy.[2]

Secondary alveolar bone grafting has become the standard therapy for alveolar cleft in most cleft centers. Although many patients have benefited from successful secondary alveolar bone grafting, some disadvantages remain, such as delayed

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timing of alveolar repair, additional surgery, donor-site complications, and graft resorption.[3,4] Primary gingivoperiosteoplasty has been advocated to achieve early union of the maxillary dental arch together with lip repair, thus decreasing the need for secondary alveolar bone grafting.[5,6] Primary gingivoperiosteoplasty was also reported to result in better bone levels than secondary alveolar bone grafting.[6] However, another center found that secondary alveolar bone grafting led to more and better located bone in the previous cleft than primary gingivoperiosteoplasty.[7,8]

Secondary alveolar bone grafting has been performed at the Chang Gung Craniofacial Center, Taoyuan, Taiwan, since 1987. From 1998 to 2002, the center sometimes performed primary gingivoperiosteoplasty described by Millard and Latham[9] together with primary lip repair following presurgical nasoalveolar molding. Only recently, however, after patients who underwent repair with primary gingivoperiosteoplasty reached mixed dentition to decide whether secondary alveolar bone grafting was needed, could the outcomes of primary gingivoperiosteoplasty versus secondary alveolar bone grafting be assessed. This study therefore aimed to assess and compare the effects of primary gingivoperiosteoplasty and secondary alveolar bone grafting in patients with unilateral cleft lip and palate on four outcomes: clinical success, Bergland scale score, residual cleft defect, and periodontal bone support of cleft-adjacent teeth.

PATIENTS AND METHODS

This study was approved by the Chang Gung Memorial Hospital Institutional Ethics Committee. Patients who had been treated at the Chang Gung Craniofacial Center were recruited in the period from 2009 to 2012 according to the following criteria: (1) Taiwanese patients with nonsyndromic complete unilateral cleft lip and palate and born between 1999 and 2002, (2) presurgical nasoalveolar molding (Grayson or Figueroa type) before lip repair, (3) modified rotation-advancement lip repair at age 3 to 6 months, (3) one-stage two-flap palatoplasty at the age of approximately 1 year, (4) alveolar repair by either primary gingivoperiosteoplasty in infancy or secondary alveolar bone grafting by an attending surgeon, (5) no previous craniofacial skeletal surgery, and (6) willingness to have cone-beam computed tomography and occlusal radiographs for postsurgical assessment. The decision of primary gingivoperiosteoplasty or secondary alveolar bone grafting was determined by the size of the alveolar cleft at the time of lip repair. To maintain the integrity of the periosteum, primary gingivoperiosteoplasty was indicated only when the alveolar gap was reduced to 0 to 0.5 mm.

Treatment History

One investigator (Y.C.W.) reviewed each patient's clinical notes. The following information was recorded: type of presurgical nasoalveolar molding (Grayson or Figueroa), use of pre–secondary alveolar bone grafting orthodontic treatment, and details of alveolar repair (i.e., alveolar gap before repair, age at repair, techniques used, and surgeon who performed the repair).

Clinical Evaluation

The eruption of mesial and distal teeth adjacent to the cleft and the presence of nasoalveolar fistulas were recorded. Fistulas were identified either by passing a fine dental probe through to the nasal cavity, or by passing dental floss along a gingival crease to the superior extent of the buccal sulcus. The need for further alveolar bone grafting was evaluated from review of occlusal radiographs by one senior orthodontist (Y.F.L.). The success of alveolar repair was defined by three criteria: the presence of a bony bridge in previous alveolar clefts, and greater than or equal to 75 percent bony height and less than 25 percent residual bony defects on occlusal radiographs.[10]

Bergland Scale Score

The height of the interdental bone was assessed from the postsurgical occlusal radiographs to determine Bergland scale scores: type I, bone height approximately normal; type II, bone height at least three-quarters of normal; type III, bone height less than three-quarters of normal; and type IV, absence of a continuous bony bridge across the cleft.[10]

Cone-Beam Computed Tomographic Evaluation

Cone-beam computed tomography was performed with an iCAT scanner (Imaging Sciences International, Hatfield, Pa.) with settings of 120 kV and 36.9 mA, 40-second exposure time, 14-bit grayscale resolution, and voxel size of 0.4 mm.[3] Field of view was 16 × 16 cm^2. The average effective dose was estimated to be 0.1 μSv. Measurements from cone-beam computed tomographic images were made with Avizo software (Visage Imaging, Carlsbad, Calif.) by one investigator (Y.C.W.). The data were analyzed in two ways: the three-dimensional cleft-defect volume, and the two-dimensional standard unsupported root of central incisors adjacent

to the cleft (i.e., the ratio of unsupported to total root surface).

Three-Dimensional Cleft Defect

At first, the cranium was oriented along the Frankfort horizontal plane. Next, the region of interest was defined by choosing five planes: *x* (the midsagittal plane); *y*-anterior (the plane at the level of the anterior nasal spine); *y*-posterior (the plane at the level of the incisive foramen); *z*-inferior (the plane at the level of the cement-enamel junction of incisor adjacent to the cleft); and *z*-superior (the plane three slices below the anterior nasal spine) (Fig. 1).[11,12] Adjacent axial slices were used to calculate the volumes of the following defects: buccal coronal defect, palatal coronal defect, buccal apical defect, palatal apical defect, and total defect (Fig. 2). The apical defect was defined as that apical to the middle plane of the *z*-superior and *z*-inferior planes. The buccal defect was defined as that buccal to the central line of the alveolar ridge.

Standard Unsupported Root of Central Incisors

At first, the cranium was reoriented along the long axes of the central incisors adjacent to the cleft (i.e., passing from the tips of the crowns to the tips of the roots). Next, the region of interest was defined by choosing four planes: *x*-plane (the plane three slices medial to the central incisor); *y*-anterior (the plane three slices in front of the central incisor); *z*-inferior (the plane at the level of the cement-enamel junction, of the central incisor); and *z*-superior (the plane at the level of the root tips of the central incisor).[12] Adjacent axial slices were used to calculate the surface areas of the following roots: coronal unsupported root, coronal total root, apical unsupported root, apical total root, total unsupported root, and total root (Fig. 3). The apical unsupported root was defined as the unsupported root apical to the middle plane of the *z*-superior and *z*-inferior planes.

Intraobserver reliability (i.e., the consistency of the same observer twice measuring cone-beam computed tomography at least 1 month apart) was analyzed by Pearson correlation ($r = 0.98$ to 0.99, all $p < 0.001$) and paired t test (all $p > 0.5$).

Statistical Analysis

Statistical analyses were performed using the statistical software package SPSS Version 17.0 for Windows (SPSS, Inc., Chicago, Ill.). Descriptive statistics for patient clinical characteristics and cone-beam computed tomographic parameters are presented as mean ± SD. Differences between primary gingivoperiosteoplasty and secondary alveolar bone grafting groups were assessed by Mann-Whitney U or chi-square test if data were nonnormally distributed. Residual cleft defects (buccal coronal, palatal coronal, buccal apical, and palatal apical) and unsupported root ratios (coronal and apical) by site in each group were

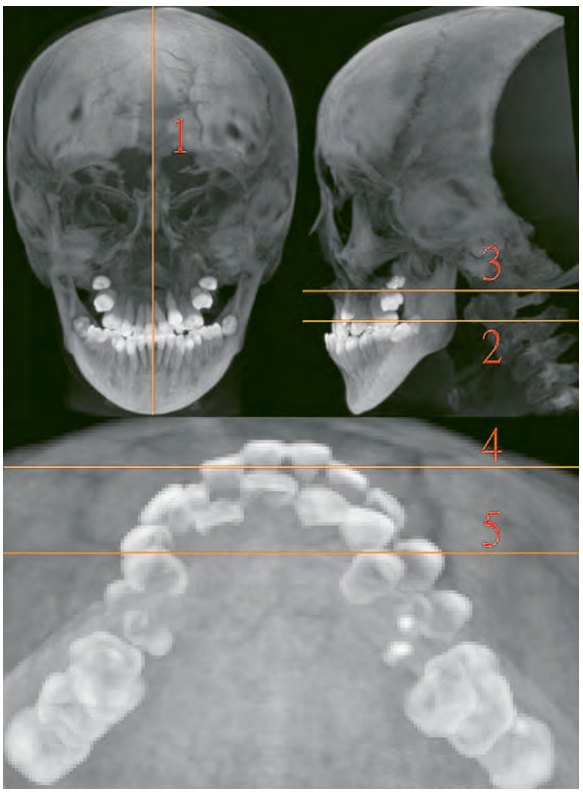

Fig. 1. Five planes for defining the region of interest for a three-dimensional cleft defect. *1*, *x* (midsagittal plane); *2*, *z*-inferior; *3*, *z*-superior; *4*, *y*-anterior; and *5*, *y*-posterior.

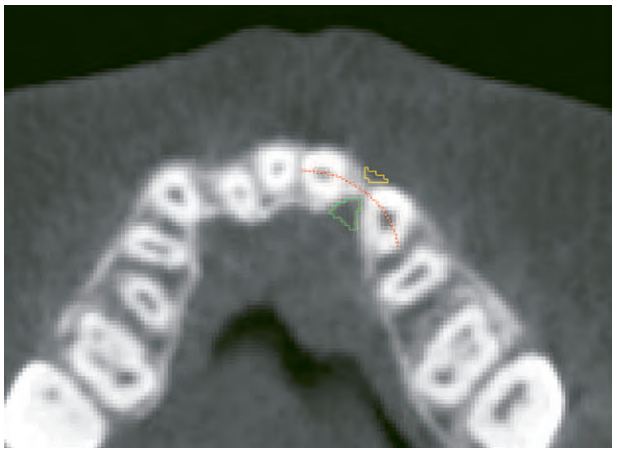

Fig. 2. Tracing of a residual cleft defect on an axial slice of the coronal portion of the previous alveolar cleft (*yellow*, buccal defect; *green*, palatal defect; *red*, central line of the alveolar ridge).

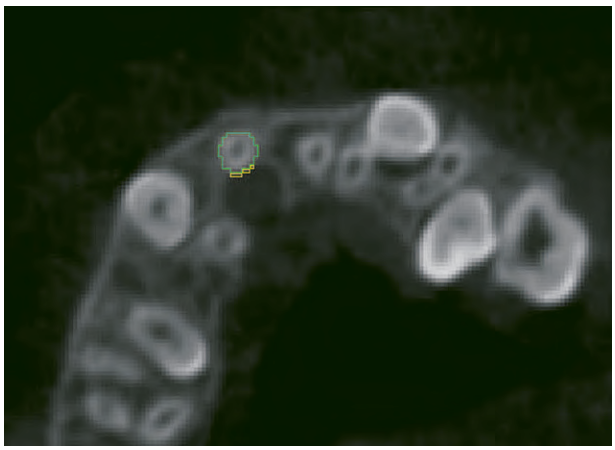

Fig. 3. Tracing of the unsupported and total roots on an axial slice of the apical portion of the central incisor adjacent to the cleft (*yellow*, unsupported root; *green*, total root).

compared using the Friedman and Wilcoxon signed rank tests, respectively. Statistical significance was accepted at $p < 0.05$.

RESULTS

Subjects

Fifty patients who met the selection criteria were recruited for clinical and cone-beam computed tomographic examination. They were divided into two groups according to the technique of alveolar repair: primary gingivoperiosteoplasty ($n = 25$) and secondary alveolar bone grafting ($n = 25$). In the primary gingivoperiosteoplasty group, cleft alveolus repair of 3- to 6-month old patients was performed by a single senior surgeon (P.K.T.C.) using the Millard technique[9] with lip repair. In the secondary alveolar bone grafting group, patients did not undergo repair of the cleft alveolus until late mixed dentition before eruption of the permanent maxillary canines. Secondary alveolar bone grafting repair used bone graft harvested from the anterior iliac crest. Five different surgeons were responsible for the secondary alveolar bone grafting operation (Table 1).

Comparison of Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting on Clinical and Radiographic Outcomes

The clinical success in the secondary alveolar bone grafting group was higher than that in the primary gingivoperiosteoplasty group ($p < 0.05$). The presence of nasoalveolar fistula, the eruption status of cleft-adjacent teeth at the minor segment, and the distribution of Bergland scale score in both groups were comparable (all $p > 0.05$) (Table 2).

Comparison of Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting on Outcomes of Cone-Beam Computed Tomography

The residual cleft defects of total, palatal apical portion, and palatal coronal portion of the previous alveolar cleft were less in the secondary alveolar bone grafting group than in the primary gingivoperiosteoplasty group ($p < 0.05$, $p < 0.001$, and $p < 0.05$, respectively). The unsupported root ratio of central incisors did not differ between the groups ($p > 0.05$) (Table 3).

Comparison of Cone-Beam Computed Tomographic Outcomes by Site in Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting

The residual cleft defect varied by site in both the primary gingivoperiosteoplasty and secondary alveolar bone grafting groups (both $p < 0.001$). The unsupported root ratios for the coronal and apical parts of central incisors were different in the secondary alveolar bone grafting group ($p < 0.05$) but not in the primary gingivoperiosteoplasty group ($p > 0.05$) (Table 3).

Comparison of Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting on Distribution of the Most and Least Residual Cleft Defects by Site

The distribution of the most cleft defect by site was different between the primary gingivoperiosteoplasty and secondary alveolar bone grafting groups ($p < 0.05$). The distribution of the least cleft defect by site did not differ between the groups ($p > 0.05$) (Table 4).

DISCUSSION

Our study is the first to compare the effects of two alveolar repair techniques, secondary alveolar bone grafting and primary gingivoperiosteoplasty, on radiographic outcomes using cone-beam computed tomography. Secondary alveolar bone grafting is more widely used and investigated clinically, whereas primary gingivoperiosteoplasty has received less attention, and the roles of these two techniques in cleft alveolus treatment remain uncertain. Our results indicate differences in their clinical and cone-beam computed tomographic outcomes. Overall, secondary alveolar bone grafting has a more favorable outcome than primary gingivoperiosteoplasty. Primary gingivoperiosteoplasty can achieve 72 percent success.

Table 1. Summary of Patient Characteristics

Characteristic	PGPP (%)	SABG (%)	*p**
Sex			
Male	16 (64)	15 (60)	1.000
Female	9 (36)	10 (40)	
Distribution of cleft			
Right	11 (44)	9 (36)	0.773
Left	14 (56)	16 (64)	
Alveolar gap before alveolar repair, mm			
Mean ± SD	0.2 ± 0.3	0.5 ± 0.4	0.244†
Range	0–1	0–2	
Age at alveolar repair, mo			
Mean ± SD	3.6 ± 1.0	112.8 ± 6.0	<0.001†
Range	2.1–7.5	105.6–126.0	
Age at Bergland scale assessment, yr			
Mean ± SD	10.3 ± 1.4	10.8 ± 0.9	0.190†
Range	8.0–14.0	9.5–14.0	
Age at cone-beam CT assessment, yr			
Mean ± SD	10.0 ± 1.3	10.2 ± 0.5	0.193†
Range	8.0–13.0	9.4–11.0	
Eruption of canine in the cleft side at cone-beam CT assessment			
Yes	10 (40)	8 (32)	0.556
No	15 (60)	17 (68)	
Duration of postsurgical cone-beam CT assessment, yr			
Mean ± SD	9.7 ± 1.3	0.8 ± 0.4	<0.001†
Range	7.6–12.6	0.5–1.9	
Presurgical infant orthopedics (nasoalveolar molding)			
Figueroa (tape type)	10 (40)	22 (88)	0.001
Grayson (elastic/tape type)	15 (60)	3 (12)	
Pre-SABG orthodontic alignment/leveling			
Yes	—	10 (40)	—
No	—	15 (60)	
SABG			
Surgeon grade			
Attending	—	25‡ (100)	—
Fellow	—	0 (0)	
Missing lateral incisor in the cleft side			
Yes	11 (44)	14 (56)	0.396
No	14 (56)	11 (44)	

PGPP, primary gingivoperiosteoplasty; SABG, secondary alveolar bone grafting; CT, computed tomographic.
*χ^2 test except where otherwise indicated.
† Mann-Whitney *U* test.
‡Seventeen of them by the same surgeon as in the PGPP group.

Previous imaging studies of primary gingivoperiosteoplasty versus secondary alveolar bone grafting are limited. Two-dimensional investigations from Canada reported lower clinical success and greater residual cleft defect with primary gingivoperiosteoplasty than with secondary alveolar bone grafting,[7] in accordance with the findings of our study. The lower clinical success and less bone production effects of primary gingivoperiosteoplasty in the previous alveolar cleft may result from the difficulty in managing tiny tissues of infants. We also found that the difference in the bone production effect was evident only in the palatal portion, suggesting that the surgical view and access to the palatal side of alveolar clefts was more limited with primary gingivoperiosteoplasty than with secondary alveolar bone grafting.

In our sample, 28 percent of patients (seven of 25) who had undergone primary gingivoperiosteoplasty still required later secondary alveolar bone grafting. Our findings are similar to those of Sato et al.,[6] who found a 73 percent success rate for primary gingivoperiosteoplasty in 20 children with unilateral cleft lip and palate. However, Matic and Power[7] reported 41 percent primary gingivoperiosteoplasty success in 61 unilateral clefts. This discrepancy might be attributed to different treatment protocols in terms of presurgical infant orthopedics (use of an alveolar molding device) and the experience of the surgeon. For example, we used elastic/tape or tape-type molding devices (Grayson or Figueroa type, respectively) to approximate the alveolar gap to 0 to 0.5 mm before primary gingivoperiosteoplasty, as did Sato et al.[6] However, Matic and Power[7] used pin-type devices (Latham type) to narrow the alveolar gap to 1 to 3 mm. Maintaining the integrity of the periosteum is essential during primary

Table 2. Clinical and Radiographic Outcomes between Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting

Outcome	PGPP (%)	SABG (%)	*p**
Presence of nasoalveolar fistulas			
Yes	2 (8)	1 (4)	0.552
No	23 (92)	24 (96)	
Need for additional alveolar bone grafting			
Yes	7 (28)	1 (4)	0.021
No	18 (72)	24 (96)	
Eruption of cleft-adjacent tooth at minor segment			
Yes	10 (40)	11 (44)	0.774
No	15 (60)	14 (56)	
Bergland scale score			
Type I	23† (92)	19 (76)	0.218
Type II	1 (4)	5 (20)	
Type III	1 (4)	1 (4)	
Type IV	0 (0)	0 (0)	

PGPP, primary gingivoperiosteoplasty; SABG, secondary alveolar bone grafting.
*χ^2 test.
†Six of them had >25% residual bony defects.

Table 3. Outcomes of Cone-Beam Computed Tomography between Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting

Outcome	PGPP	SABG	*p**
Mean residual cleft defect ± SD, mm^3			
Total	305.8 ± 176.5	178.6 ± 122.0	0.035
Buccal coronal	18.3 ± 20.0	14.9 ± 24.2	0.291
Palatal coronal	40.1 ± 29.0	25.7 ± 30.6	0.017
Buccal apical	102.0 ± 76.9	70.6 ± 55.9	0.197
Palatal apical	145.3 ± 73.9	67.4 ± 50.9	<0.001
p†	<0.001[a]	<0.001[b]	
Mean unsupported root ratio ± SD, %			
Total	1.0 ± 2.9	1.7 ± 5.2	0.385
Coronal	0.5 ± 1.4	2.2 ± 6.7	0.033
Apical	1.7 ± 5.9	1.1 ± 3.4	0.560
p‡	0.382	0.020[c]	

PGPP, primary gingivoperiosteoplasty; SABG, secondary alveolar bone grafting.
*Mann-Whitney *U* test.
†Friedman test for comparison of residual cleft defects after PGPP or SABG by site.
‡Wilcoxon signed rank test for comparison of unsupported root ratios of central incisors adjacent to the cleft after PGPP or SABG by site.
[a]Palatal apical > buccal apical > palatal coronal > buccal coronal.
[b]Buccal apical, palatal apical > palatal coronal > buccal coronal.
[c]Coronal > apical.

Table 4. Distribution of the Most and Least Residual Cleft Defects by Site between Primary Gingivoperiosteoplasty and Secondary Alveolar Bone Grafting Groups

	PGPP (%)	SABG (%)	*p**
Site with most cleft defect			
Buccal coronal	0 (0)	0 (0)	
Palatal coronal	0 (0)	1 (4)	0.001
Buccal apical	2 (8)	12 (48)	
Palatal apical	23 (92)	12 (48)	
Site with least cleft defect			
Buccal coronal	24 (96)	19 (76)	
Palatal coronal	1 (4)	3 (12)	0.161
Buccal apical	0 (0)	2 (8)	
Palatal apical	0 (0)	1 (4)	

PGPP, primary gingivoperiosteoplasty; SABG, secondary alveolar bone grafting.
*χ^2 test.

gingivoperiosteoplasty.[5,6] The closer the bones, the more likely that enough bone will form in the cleft alveolus to eliminate the need for later secondary alveolar bone grafting. To achieve this narrower gap, an elastic/tape or tape-type molding device is preferred, as it more closely approximates the cleft gap than a pin-type one.[12] Primary gingivoperiosteoplasty in infants is demanding in terms of technique, and outcomes are expected to be surgeon-dependent. Similar to the primary gingivoperiosteoplasty surgery in the study by Sato et al.,[6] our primary gingivoperiosteoplasty was performed by a single experienced surgeon with more than 20 years' experience in cleft surgery. In contrast, four different surgeons were involved in the primary gingivoperiosteoplasty reported by Matic and Power.[7]

As far as the sites of cleft defects after primary gingivoperiosteoplasty or secondary alveolar bone grafting are concerned, we found that the apical defect was greater than the coronal one (Fig. 4). This finding was expected because alveolar molding devices narrow the coronal portion of the cleft alveolus more effectively than the apical portion. In contrast, Matic and Power[7] showed a larger part of the defect in the coronal portion of the cleft alveolus after primary gingivoperiosteoplasty using Witherow's location scale. Such variations may arise from the variation in measurement tools (three dimensions versus two dimensions) and from the variation in the defined boundary of the cleft alveolus (dental and nasal defects compared with dental defects). We further found that after primary gingivoperiosteoplasty, the palatal defect was greater than the buccal one (Fig. 5). One possible explanation is limited surgical view and access to the palatal side.

This comparative imaging study suggests that both primary gingivoperiosteoplasty and secondary alveolar bone grafting led to similarly good periodontal bone support of central incisors adjacent to the cleft (99.0 percent versus 98.3 percent), in accordance with the findings of a two-dimensional investigation using Long's rating scale (88.6 percent versus 81.6 percent).[6] The greater periodontal effect in our study may be a result of cone-beam computed tomography being more powerful than periapical or occlusal radiography for detecting bone coverage on the dental root. We also found that after secondary alveolar bone grafting, the apical support was greater than the coronal one. This outcome is likely a consequence of the difficulty of grafting bone being maintained in the coronal root of central incisors after surgery.

As primary gingivoperiosteoplasty is technically challenging, a surgeon may injure the underlying tooth buds adjacent to the cleft. In our study, after primary gingivoperiosteoplasty, no patients had missing central incisors and 11 had missing lateral incisors adjacent to the cleft, which is within normal limits for patients with unilateral cleft lip and palate.[13] However, the incidence of missing central incisors was high, up to 33 percent after primary gingivoperiosteoplasty in the study by Matic and Power.[7] This is not completely surprising and may therefore relate to the surgeon's experience.

Although only 28 percent of patients (seven of 25) who had undergone primary

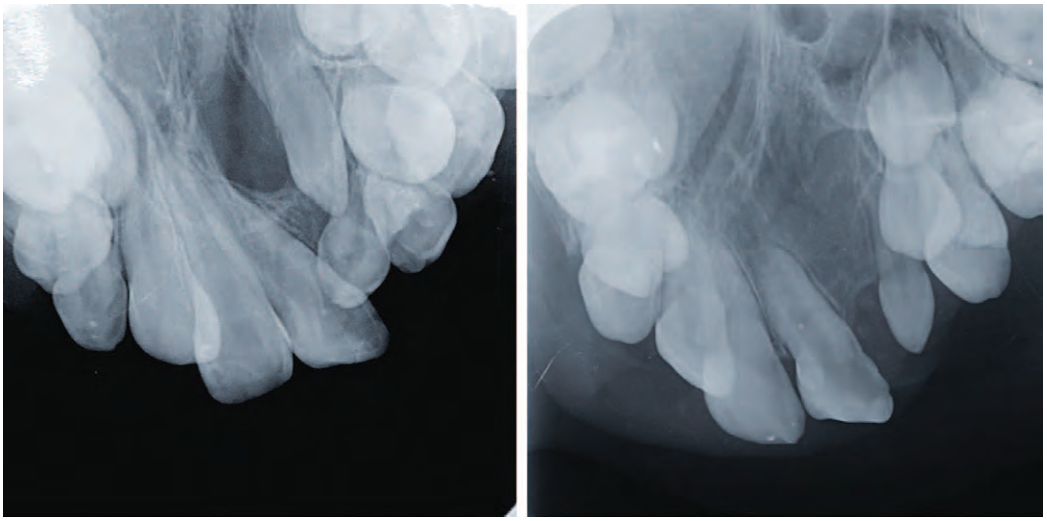

Fig. 4. Occlusal radiographs of a primary gingivoperiosteoplasty patient (*left*) and a secondary alveolar bone grafting patient (*right*). Note the apical cleft defect in the primary gingivoperiosteoplasty patient.

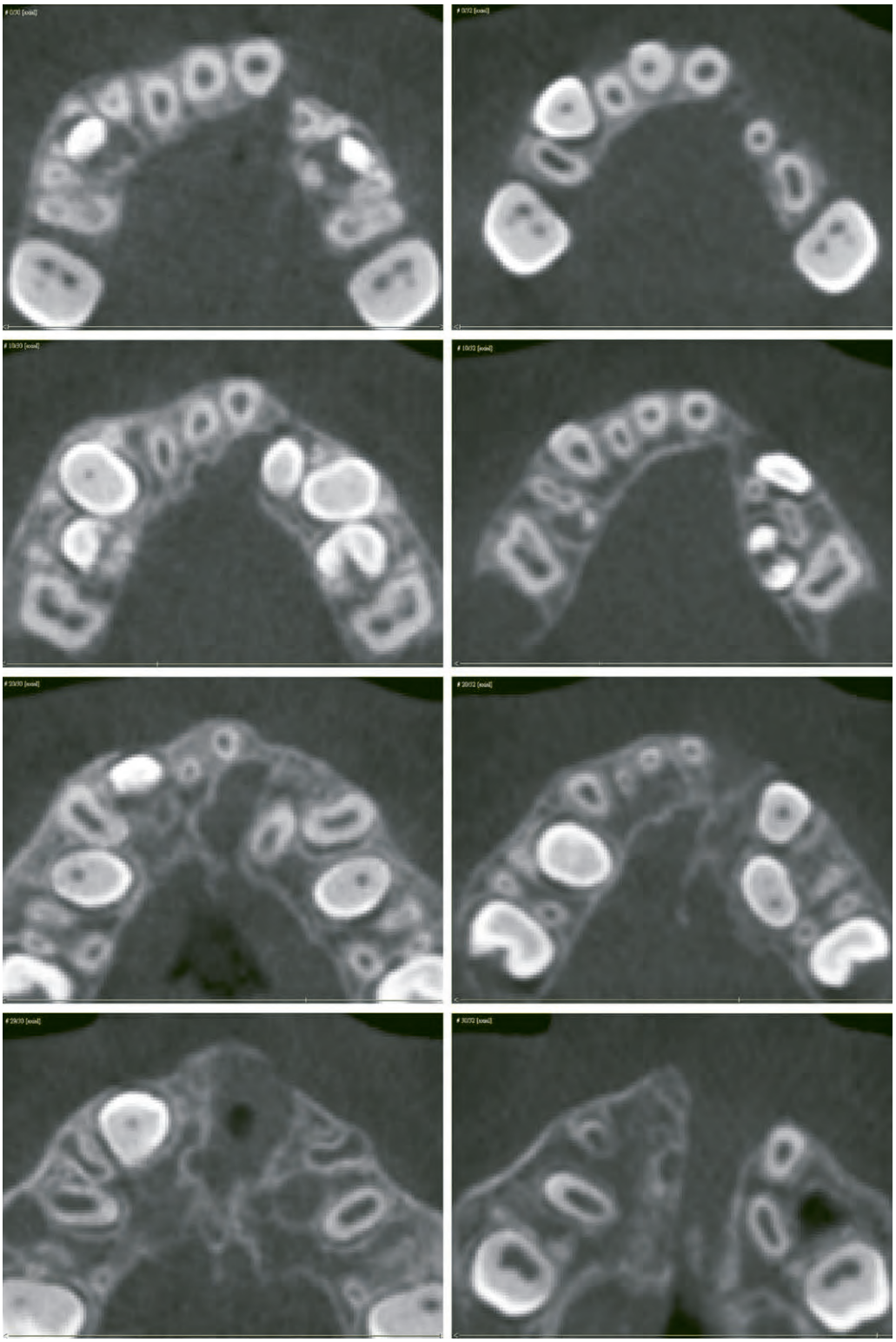

Fig. 5. Axial slices of cone-beam computed tomographic images of the same primary gingivoperiosteoplasty patient (*left*) and the same secondary alveolar bone grafting patient (*right*) in Figure 4. (*Above*) The level of the cement-enamel junction of incisor, (*second row*) 4 mm apical to the level of cement-enamel junction (of the incisor), (*third row*) 8 mm apical to the level of the cement-enamel junction (of the incisor), and (*below*) 12 mm apical to the level of the cement-enamel junction (of the incisor).

gingivoperiosteoplasty required later secondary alveolar bone grafting, our center has not practiced primary gingivoperiosteoplasty since 2003 because of its inhibitory effects on maxillary growth.[14–19] That is, primary gingivoperiosteoplasty eliminates the need for secondary alveolar bone grafting by 72 percent, but increases the need for Le Fort I procedures by at least 30 percent.[12] In contrast, primary gingivoperiosteoplasty has been advocated by another center because of reduced need for secondary alveolar bone grafting by 73 percent[6] and no significant impairment of maxillary growth.[20,21] Similarly, Meazzini et al.[22,23] claimed that gingivoperiosteoplasty is worth performing because more than 90 percent of patients had Bergland type I or II scores (i.e., clinical success) and only 17 percent more Le Fort I procedures are required. However, it should be noted that their timing for gingivoperiosteoplasty is relatively late (18 to 36 months) because gingivoperiosteoplasty is performed at the same time as repair of the hard palate (i.e., early secondary gingivoalveoloplasty).

This study had some limitations. First, although all the primary gingivoperiosteoplasty and secondary alveolar bone grafting procedures were performed by attending surgeons, five different surgeons were responsible for secondary alveolar bone grafting instead of only a single experienced surgeon involved in primary gingivoperiosteoplasty. Variation in the skill of the surgeon might influence the surgical outcome. However, if this had been a factor, the results would not have been affected because the secondary alveolar bone grafting led to more favorable outcomes than the primary gingivoperiosteoplasty. Second, before secondary alveolar bone grafting, 10 of 25 patients received orthodontic treatment to reposition severely tilted or rotated central incisors with simple bracket and archwire. Accordingly, the surgeon might have had better access for placing the graft and closing the soft tissue and thus greater surgical success.[24] However, this factor was likely insignificant because we found no difference in secondary alveolar bone grafting outcomes with and without pre–secondary alveolar bone grafting orthodontic treatment (209.6 ± 192.9 mm^3 versus 215.7 ± 129.0 mm^3 for residual cleft defect, $p = 0.61$; 3.4 ± 7.9 percent versus 0.5 ± 0.7 percent for unsupported root ratio; $p = 0.19$). Nevertheless, we still emphasize the need for randomized trials to confirm our findings. Finally, longer term follow-up is needed to evaluate surgical outcomes after full eruption of the canines adjacent to the cleft.

CONCLUSIONS

Our results suggest that in patients with unilateral cleft lip and palate, clinical success was higher with secondary alveolar bone grafting than with primary gingivoperiosteoplasty, and primary gingivoperiosteoplasty achieved 72 percent success. Primary gingivoperiosteoplasty resulted in more residual cleft defect in the previous alveolar cleft than secondary alveolar bone grafting, particularly on the palatal apical portion. Both primary gingivoperiosteoplasty and secondary alveolar bone grafting resulted in similarly good periodontal bone support of central incisors adjacent to the cleft.

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Discarded Wharton's Jelly of the Human Umbilical Cord: A Viable Source for Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) are multipotent cells that have the capability of differentiating into adipogenic, osteogenic, chondrogenic, and neural cells. With these multiple capabilities, MSCs have been highly regarded as effective transplantable cell source for regenerative medicine. A large bank of these cells can be found in several regions of the human umbilical cord (hUC) including the umbilical cord lining, the subendothelial layer, the perivascular zone, and most importantly in Wharton's jelly (WJ). These cells, all umbilical cord-derived MSCs, are very durable, have large loading capacities, and are considered ethical to harvest because the umbilical cord is often considered a waste. These logistical advantages make WJ as appealing source of stem cells for transplant therapy. In particular, WJ is a predominantly good source of cells because MSCs in WJ (WJ-MSC) are maintained in a very early embryological phase and therefore have retained some of the primitive stemness properties. WJ-MSCs can easily differentiate into a plethora of cell types leading to a variety of applications. In addition, WJ-MSCs are slightly easier to harvest compared to other MSCs (such as bone marrow-derived MSCs). The fascinating stemness properties and therapeutic potential of WJ-MSCs provide great promise in many aspects of regenerative medicine and should be considered for further investigations as safe and effective donor cells for transplantation therapy in many debilitating disorders, which are discussed here. We previously reviewed WJ-MSCs therapeutic potential [1] and now provide an update on their recent preclinical and clinical applications.

Keywords

MSCs; multipotent cells; proliferation; differentiation; transplantation

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Introduction

In recent years, medical research has focused on utilizing stem cell therapy to alleviate a number of debilitating disorders. In particular, recent efforts have turned to the human umbilical cord (hUC) for new sources of mesenchymal stem cells (MSCs). MSCs found in the hUC present several advantages over other stem cell tissue sources. First, hUC is seen as biological waste and typically discarded after birth. Its use therefore presents no ethical concerns [2]. Second, hUC cells exhibit reduced immunogenicity. Since these inactivated MSCs lack MHCII and other costimulatory molecules on their surface, they present no immune response in the host tissue. In laboratory studies, the allogenic transplantation of hUC cells into non-immune-suppressed animals did not produce rejection [3]. Third, hUC cells have an increased proliferative capacity, evidenced by a higher frequency of colony-forming-unit fibroblasts (CFU-F) and a shorter population doubling time than other cells [4].

MSCs can be isolated from the umbilical cord (UC) lining, subendothelial layer, perivascular zone, and the Wharton's Jelly (WJ) (the gelatinous matrix in the umbilical cord that provides insulation and protection of the vein and arteries of the umbilical cord) [5] (Figure 1). The MSCs found in these regions of the hUC are multipotent and can differentiate into adipogenic, osteogenic, chondrogenic, and neuronal cells [6].

However, limitations still remain for the isolation of UC-MSCs for clinical use. For cord lining MSCs, the isolation methods are incredibly time-consuming. In addition, the current procedure for isolation of WJ-MSCs involves fetal bone serum (FBS) as its nutrient enhancement. The problem that results from this supplement is that viral and prion diseases become a major concern. Thus, standard isolation still needs to be modified [7]. An additional problem with their ability for clinical uses is the UC-MSC's property to proliferate at high rates and, after tissue repair, differentiate into two daughter cells with asymmetric portions of the parent cell`s cytoplasm. Due to this, the MSCs present an enigmatic problem of not being capable to be tracked through MRIs after a short period of time elapses. Therefore determining whether the MSCs fully developed into the cell type it was primarily intended to and whether it is functioning correctly will be an arduous and inefficient process. Currently the stains of SPIO and Mn2+ are being used for the identification and tracking of MSCs. However these both present problems. The Mn stain has a high cytotoxicity and, although there is a solution to minimize the cytotoxicity, the impending risk is too high [8]. While SPIO stain does not possess this property it cannot track the MSC cells accurately and descends in clarity as more time elapses [9]. An experiment conducted with Prussian blue staining demonstrates this lack of consistency [10]. Therefore, future research efforts must focus on methods to more efficiently isolate MSCs from the umbilical cord [11] and an appropriate method of staining to enable MRI imaging of the MSCs.

Despite the setbacks in isolation, WJ-MSCs still present perhaps the best opportunity for cell therapy in the future. With a greater proliferative capacity and tri-lineage differentiation potential, these cells can be induced to form a diverse array of cell types than MSCs found in both the bone marrow and from different regions of the umbilical cord. This high

differentiation potential can be envisioned to target a variety of disorders (Table 1). For example, UC stem cells exhibit an interesting potency to heal cutaneous burn wounds [12]. Both UC-epithelial cells and UC-MSCs can be grown on scaffolds and grafted to treat partial thickness and full thickness burns [12].

Cell Therapy for Cancer

In comparison to other sources of MSCs derived from the bone marrow, WJ-MSCs exhibit reduced immunogenicity. WJ-MSCs lack costimulatory ligands which activate an immune response from both B and T cells [13]. At the same time, WJ-MSCs express HLA-G, a protein that induces the expansion of regulatory T cells and suppresses cytotoxic T cells and Natural Killer cells, at a high level [14].

Interestingly enough, WJ-MSCs (referred to as UCMSCs in [14]) also possess properties that make them potential tools for cancer therapy. Cancer cells secrete cytokines and growth factors, and WJ-MSCs have receptors for these molecules in the cell membrane [14]. This interaction between cytokines and growth factors and their receptors results in WJ-MSCs exhibiting a tropism towards the inflammatory cancer and tumor tissues [14].

Moreover, WJ-MSCs also present tumorcidal abilities. Although bone marrow-derived MSCs have been shown to stimulate tumor growth, WJ-MSCs have been demonstrated to attenuate the growth of tumors [14]. While most of these properties are not fully understood, there are two mechanisms for cancer suppression that are known. The first is that WJ-MSCs produce several secretory proteins which in turn promote the cell death of cancer cells and stop the cell cycle [14]. In addition, WJ-MSCs also caused more tumor suppressing genes to be expressed thus aiding in the cancer treatment. The second mechanism by which cancer can be suppressed is through the enhancement of the immune system reaction to the cancer cells [14]. Studies have shown that rats treated with rat WJ-MSC displayed great improvement to tumors that they have [15]. Those rats possessed a highly reduced tumor, and an abundant amount of lymphocytes in the area, which infiltrate the tissue of the tumor and assist in therapy. In addition to their anti-cancer benefits, WJ-MSCs are also considered safe therapeutic cells because they do not pose a risk of spreading tumor tissue into other parts of the body or other adverse effects [16]. If research efforts can develop tumor suppressor genes or anticancer drugs in the future, WJ-MSCs can potentially be utilized as vehicles for targeted cancer therapy because of their durability, large loading capacity, ability to be harvested in large numbers with no risk to the donor, and tumor tropism [14].

Cell Therapy for Liver Disease

WJ-MSCs have also been explored as a method to cure liver diseases. Because of WJ-MSCs excessive proliferation and ability to differentiate into different cells they are perfect candidates for this type of treatment. These specific stem cells have been known to differentiate into adipocytes, osteoblasts, and also neurons [17, 18]. Because of the need for donors of livers and the harmful side effects of the liver transplantations, stem cell therapies are becoming recognized to be a much better method of curing liver disease. Morphological analysis demonstrates that WJ-MSCs express markers that correlate with the phenotype of hepatoblasts (the precursor to hepatocytes, the cell of the primary liver tissue), suggesting

the ability for WJ-MSCs to differentiate into liver cells [19]. Hepatic commitment of these cells can potentially be induced in the presence of specific growth factors or the host liver cell environment. Moreover, in a model of fibrosis (the body's response to chronic liver damage), the introduction of WJ-MSCs into the injured livers of mice was able to relieve fibrosis and reduce hepatic inflammation [20], possibly by abrogating extracellular matrix accumulation (caused by fibrosis). However, these results are controversial because other studies found that MSCs produce no benefit to hepatic function in the long term [21].

Transplantation of WJ-MSCs has also been tested in liver fibrosis. Using carbon tetrachloride (CCl_4), rats were experimentally induced display liver fibrosis and 4 weeks later received WJ-MSCs injections [22]. After an additional 4 weeks, there was a remarkable decrease in the liver fibrosis in the rats treated with WJ-MSCs as compared to the rats that were not treated with the WJ-MSCs. Some WJ-MSCs exhibited phenotypes of the liver, and those WJ-MSCs that did not differentiate had the capability to secrete cytokines that have the potential to restore liver function [23]. These observations indicate a multi-pronged reparative mechanism of WJ-MSCs involving specific lineage differentiation and therapeutic molecules that are key pathways towards tissue repair.

Cell Therapy for Peripheral Nerve Damage

WJ-MSCs have also been proposed as a potential cure for peripheral nervous system (PNS) injuries. When a peripheral neuron is damaged, the Schwann cells lose contact to the next axon and therefore self-degrade their own myelin sheaths. The body responds with a proliferation of Schwann cells that support axonal regrowth and regeneration of myelin [24]. Therefore, research in cell therapy has explored the efficacy of transplanting Schwann cells to heal the injury. This is difficult, however, because isolation of Schwann cells can cause damage to other peripheral nerves, while the amount of Schwann cells able to be isolated is typically very low. MSCs offer a novel alternative stem cell source because they are easily accessible and highly proliferative. Because of the trans-differentiation potential of WJ-MSCs into ectoderm-derived cells, MSCs can potentially differentiate into functional Schwann cells and be applied to heal injured peripheral neurons [25]. To this end, the transplantation of WJ-MSCs into a rat with peripheral nerve damage revealed that the injected cells labeled with alentivirus green fluorescent protein to allow visualization of myelin of the regenerated axons, were able to differentiate and become functioning Schwann cells with an efficiency of 97%. These findings indicate that WJ-MSCs appear as effective donor cells for cell therapy in the future [25].

Cell Therapy for Cardiovascular and Connective Tissue Repair

Another potential use for WJ-MSCs is in cardiovascular tissue engineering. The cardiovascular system has low regenerative potential, which makes the use of WJ-MSCs an ideal alternative in cardiovascular tissue repair with their immunomodulatory properties and self-regenerating capacity [26]. Interestingly, biologically active heart valve leaflets could be engineered using only cells from the human umbilical cord [27]. These leaflets showed complex tissues that closely resembled native tissues. With such robust results, the use of WJ-MSCs became a large step in overcoming limiting factors in repairing congenital

malformations. These efficacy readouts have also been confirmed in large animal studies, where engineered heart tissues were successfully transplanted into sheep, even showing good functional performance after 20 weeks [28]. However, uncertainty still remains over whether these engineered tissues can survive successfully in the long-term [29]. Additional research studies have found that culturing UC-MSCs in the presence of certain growth factors and hormones more efficiently induces UC-MSCs to differentiate into cardiomyogenic lineages. In particular, the presence of the hormone oxytocin gave rise to the most efficient differentiation [30].

UC-MSCs may also be beneficial in the treatment of cartilage injuries. Cartilage injuries are the result of a metabolic imbalance of an organism's chondrocytes (the primary cell of cartilage tissue), and the natural growth and repair of cartilage tissue is slow [31]. Transplantation of UC-MSCs presents a potentially effective mechanism for cell therapy [32]. UC-MSCs, when cultured in a medium containing ascorbic acid, transferrin, dexamethasone, and other molecules, can differentiate into chondrocyte-like cells [33]. Therefore, stem cell transplantation stands as a strategy to substantially increase the number of chondrocytes, enabling a quicker recovery of cartilage diseases [34].

The same is true for tendon injuries. Human umbilical cord perivascular cells (HUCPVCs) have been shown to produce collagen that repairs tendon injuries in rats [35]. Additionally, the presence of HUCPVCs facilitated a change in structure and organization of the collagen fibers [35]. Instead of being disorganized, these collagen fibers were arranged in linear parallel bundles, thereby increasing the tendon's tensile strength in comparison to the control group [36].

Cell Therapy for Obesity and Diabetes

The prenatal differentiation of WJ-MSCs may play a factor in determining an individual's susceptibility to obesity and related disorders later in life. Obesity can be affected by increased adipogenesis, the early determination of MSCs to adipocytes before birth. This phenomenon is influenced heavily by the prenatal environment [37]. The changes in the prenatal environment have been largely ascribed to the mother's health condition. When healthy mothers were compared to diabetic mothers a major difference was observed in the prenatal environments were the protein levels [37]. The changes in the concentration of CD90 is related to the change in plasticity and the up-regulation of CD44, CD29, CD73, CD166, SSEA4 while TERT is related to the increase of the proliferative ability of the cells. Studies show that WJ-MSCs from mothers with hyperglycemia or gestational diabetes mellitus have a higher affinity towards adipocyte differentiation and increased adipocyte differentiation efficiency than those from lean mothers [37]. The findings suggest that the changes in the prenatal environment in obese mothers pre-dispose the child to becoming obese or having Type II Diabetes later in life [37]. Also additional research suggests that WJ-MSC may have the potential to benefit in the direct treatment of diabetes mellitus [38]. By using markers that indicate when certain genes are expressed, models have shown that WJ-MSCs have the capability to differentiate into all sorts of pancreatic cells including the insulin-producing β cells [39]. Using immunohistochemistry and ELISA assays, a significantly greater amount of insulin and C-peptide protein was released from the

differentiated cells than from the undifferentiated cells. These results suggest that the WJ-MSC did in fact turn insulin-producing cells. In just a week, WJ-MSCs can turn into the exact cells that are attacked by this aggressive disease. With more research and study, WJ-MSCs may provide a means to alleviate diabetes in the future [40].

Moreover, new cell isolation methods have emerged to more efficiently culture and expand the population of WJ-MSCs in a laboratory setting. A lower oxygen concentration (5%) than room air (21%), as well as a low plate density (10 cells/cm^2), has powerful effects on WJ cell expansion, shortening population doubling time and substantially increasing the colony forming efficiency of the cells [41]. These new methods are exciting and hold promise as researchers search for even more efficient methods to harvest stem cells for developing cell therapies [41].

As mentioned previously, WJ-MSCs are potentially viable resources as donor cells for clinical transplantation use, due primarily to their high proliferative capacity and ability to differentiate between three different tissue lineages (ectoderm, mesoderm, and endoderm). WJ-MSCs are likely more beneficial than some other sources of MSCs (Table 2). For example, for several years, bone marrow MSCs have been demonstrated as the future of hematopoietic stem cell transplantation, primarily due to their intrinsic micro-environmental support for hematopoietic stem cells and ability to differentiate into various mesodermal lineages, much like the WJ-MSCs, with limited difficulty in isolation. However these stem cells are inferior due to the invasive procedures which are required for its aspiration [9].

Another potent source of MSCs is the umbilical cord blood. Much like WJ-MSCs these are multi-faceted cells with the ability to differentiate into lineages whilst in either in vitro or in vivo condition and possess higher proliferative capacity than those MSCs of bone marrow [4]. However, studies suggest that MSCs derived from the umbilical cord blood are limited in their utility because technical challenges in extracting sufficient amounts of these cells.

Amniotic Fluid MSCs (AF-MSCs) are similar to those of Wharton’s Jelly in that they have a good differentiation capability and can be efficiently obtained from the placenta [11]. These multipotent stem cells may have a future in cell therapy and clinical use. However, since these cells have only recently emerged in the field, most researchers believe that further studies must be conducted to develop a proficient method to culture and isolate these cells [11].

Lastly, similarly to WJ-MSCs, Umbilical Cord Matrix MSCs (UCM-MSCs) are useful in tissue engineering and possibly cell therapy. They also have differentiation abilities, immunodulatory properties, and trophic activity [4]. However, the number of UCM-MSCs extracted is limited and unique ex-vivo expansion method is necessary to obtain sufficient numbers of UCM-MSCs [4].

Although all the sources of MSCs possess comparable stemness properties (differentiation, proliferation capacity), and present with potential for clinical cell-based therapeutic use, and all sources display nearly analogous post-transplantational effects, the ease in isolating and propagating ample supply of WJ-MSCs, combined with their high proliferative capacity and

ability to differentiate into the three germ lineages make WJ-MSCs appealing donor cells for transplantation therapy.

Conclusions

Recent evidence demonstrates that WJ-MSCs are potential transplantable cells for treatment of devastating diseases, such as cancer and diabetes. Their use in cell therapy will be an integral addition to the field of regeneration. WJ-MSCs have a multitude of benefits such as their high proliferation rate [42], lower doubling time, and ability to function with non-immune-suppressed animals [43]. However, there remains paucity in the translation of WJ-MSCs for clinical use, largely due to the cells' heterogeneity, which results from the current isolation methods and inefficient staining methods [44]. There are two primary explanations for heterogeneity. First, the hUC has multiple distinct anatomical zones, and previous attempts at isolating WJ-MSCs have inadvertently harvested cells from different anatomical structures of the hUC in addition to the Wharton's Jelly. Second, there is currently wide variation in procedures to harvest WJ-MSCs, and this variation can produce inconsistent results between studies [11]. Future research and refinement of isolation procedures can potentially overcome these obstacles [11]. Regardless of these drawbacks WJ-MCSs are still the ideal future for cell therapy; their properties of high proliferation capability and versatility to differentiate between three lineages allow them to lower immunogenicity and have the potential to treat an array of diseases and disorders [45].

In addition, WJ-MSCs stimulate immune responses from B and T cells [13] and suppress cytotoxic and natural killer cells [14]. WJ-MSCs possess cytokines and growth factor receptors, which allow them to be vital tools for cancer therapy. In such therapy, WJ-MCSs drastically weaken the cancerous tumors by secreting therapeutic proteins which promote the cancerous cells to undergo cell death and to stop the cell cycle [46]; moreover, WJ-MSCs enhance the immune response to cancer cells. With the minimum risk of spreading the cancer cells throughout the body or to the MSC donor, WJ-MSCs have the potential to serve as vehicles for delivery of tumor suppressive genes and anticancer drugs occur.

Apart from cancer treatment WJ-MCSs also can facilitate cell-based therapies for liver diseases and diabetes mellitus due to their high proliferation and differentiation ability [47], e.g., WJ-MSCs can express hepatoblastic phenotypes and can become liver cells or pancreatic cells [48].

As we recognized the many versatile capabilities of WJ-MSCs, their documented efficacy in animal models and limited clinical trials as therapeutic cells advances the field of regenerative medicine. With more research, WJ-MSCs may someday become recognized as routine donor cells for cell-based therapies [49, 50, 51].

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Abbreviations

hUC	Human Umbilical Cord
MSC	Mesenchymal Stem Cells
CFU-F	Colony-Forming-Unit Fibroblasts
FBS	Fetal Bone Serum
UC-MSC	Umbilical Cord Mesenchymal Stem Cells
WJ-MSC	Wharton’s Jelly Mesenchymal Stem Cells
WJ	Wharton's Jelly
HUCPVC	Human Umbilical Cord Perivascular Cells

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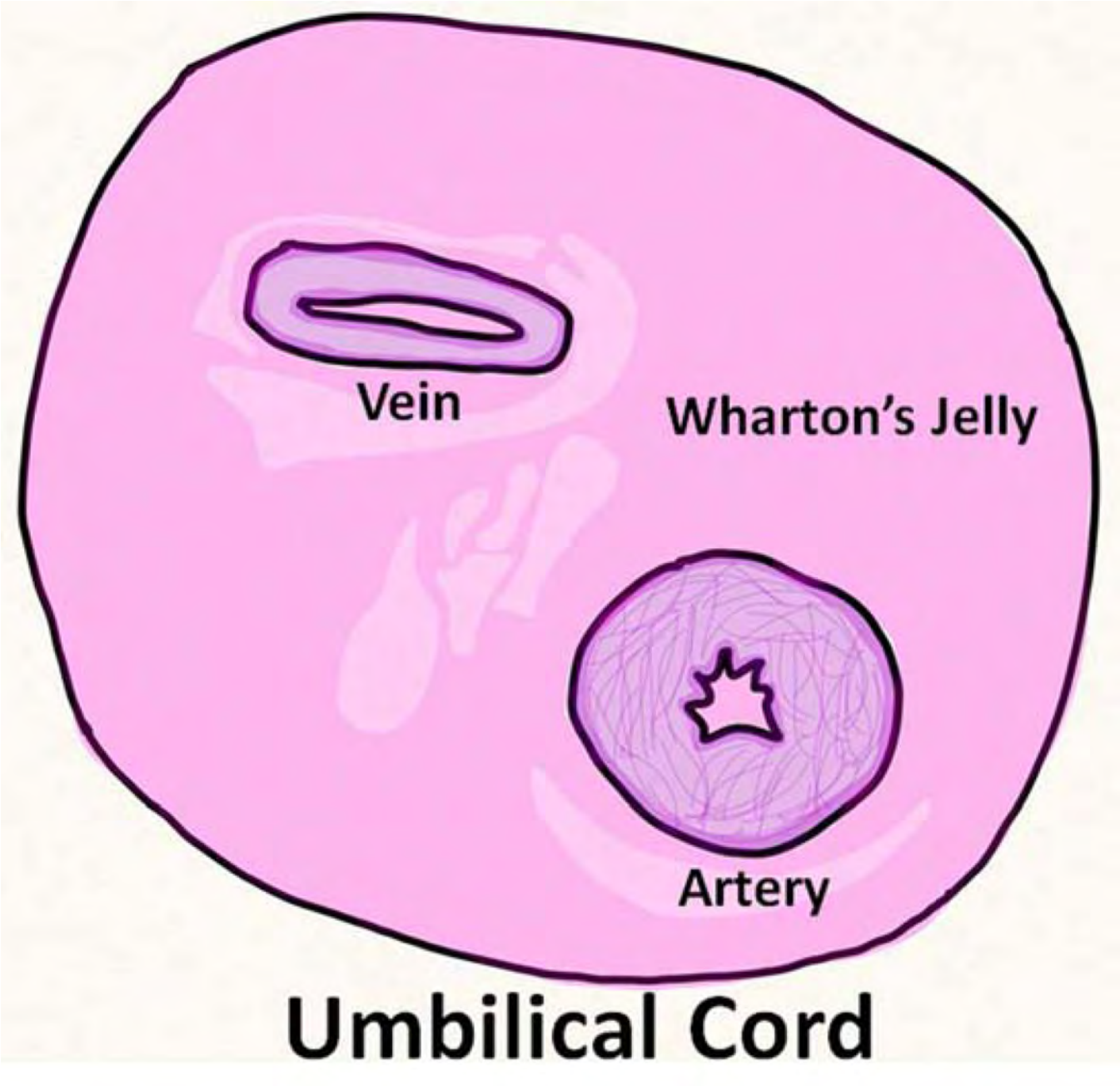

Figure 1.
Anatomy of the human umbilical cord showing Wharton's Jelly.

Table 1

Milestone Discoveries for WJ Transplantation

Disease Indication	WJ Cell Therapy Outcomes
Cancer	• Cells do not pose a risk for metastasis of tumor cells [15]. • Cells promote proteins that halt the cell cycle of cancer cells and promote tumor suppressing genes [14]. • Cells invoke the body's the immune system [14].
Liver Disease	• WJ-MSC's high proliferative capacity induces hepatocyte differentiation [19]. • The hepatocyte cells can reduce liver fibrosis [22, 23].
Peripheral Nerve Damage:	• The cell has the ability to differentiate into Schwann cells [25]. • These can be transferred to the body and applied to the naturally degrading Schwann cells [25].
Cardiovascular Repair:	• Biologically Cardio active leaflets can be manufactured using WJMSC's [27]. • The cells have been used in large animal studies with the sheep hearts functioning over 20 weeks into experimentation [28].
Connective Tissue Repair:	• Cartilage injuries are the result of a metabolic imbalance of chondrocytes and slow repair of the issue. WJ-MSC's can easily differentiate into the correct cell and be transplanted to repair the issue [32, 33]. • Tendon injuries are also caused by an imbalance and disorganization of the collagen fibers. Human Umbilical Cord Perivascular Cells can not only be transplanted to restore balance, but they help organize tendon collagen fibers [35, 36].
Obesity and Diabetes:	• In the prenatal environment, the growing child can have its cells be predisposed to grow into adipocyte cells based on the mother's conditions [37]. • Using WJ-MSC's differentiation ability, researchers can grow insulin producing cells in the lab that can be used to help treat obesity and type II diabetes [37, 39].

Table 2

Comparison of MSC Sources

MSC Source	Potential Benefits	Potential Drawbacks
WJ	• High proliferative capacity [4] • Tri-lineage differentiation ability [4, 6] • Provokes little immune response when transplanted [3, 4, 9]	• Currently no uniform isolation procedure [4, 7] • difficult to extract from other anatomical zones of the hUC [4]
Bone Marrow	• Useful for hematopoietic stem cell transplantation [9] • Ability to differentiate into multiple cell types in the mesodermal lineage [9]	• Difficult and invasive procedures needed for isolation [9] • Provokes a greater immune response than WJ-MSCs [9, 13]
UC Blood	• Exhibit properties similar to WJ-MSCs, especially in broad differentiation abilities [4]	• Difficult to extract and isolate cells in high enough amounts for transplantation [4]
Amniotic Fluid	• High differentiation capacity • Easy to obtain from the placenta [11]	• Very little research on functional effects conducted to date [11] • More research needed on their potential uses and potential methods for isolation [11]
UC Matrix	• High differentiation capacity [4] • Immunomodulatory properties similar to WJ-MSCs [3, 4, 9]	• Difficult to extract and isolate cells in high enough amounts for transplantation [4]