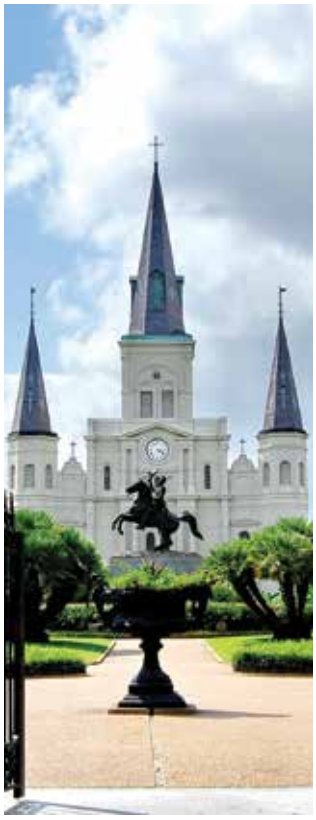
IFATS NEW ORLEANS 2015 CONFERENCE

13th Annual IFATS Meeting

IFATS

International Federation for
Adipose Therapeutics and Science

November 5-8, 2015
JW Marriott New Orleans
New Orleans, Louisiana
www.ifats.org

MARK YOUR CALENDAR

International Federation for
Adipose Therapeutics and Science

14th Annual Meeting

IFATS SAN DIEGO 2016

November 17-20, 2016
Westin Gaslamp
San Diego, California

Abstract Deadline:

Midnight EST, Wednesday, June 8, 2016

The Call for Abstracts will be sent this winter. All members of IFATS and all registered attendees of the 2015 IFATS Conference will be included in the mailing list. Any others who wish to be reminded to submit papers should contact the IFATS Executive Office.

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International Federation for
Adipose Therapeutics and Science

IFATS New Orleans 2015

November 5-8, 2015

JW Marriott New Orleans • New Orleans, Louisiana

Recording of any content presented at this educational program either by camera, video camera, cell phone, audio recorder, or any other device is strictly prohibited.

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IFATS
International Federation for Adipose Therapeutics and Science

It is my sincere pleasure to welcome you to the **13th International Federation for Adipose Therapeutics & Science (IFATS) Conference** at the JW Marriott in New Orleans, Louisiana. Building on our twelve previous successful conferences, we hope to facilitate meaningful interactions between clinicians and basic science investigators in the field of adipose therapeutics, while providing a platform for attendees to learn about advancing technologies in this rapidly evolving field.

A highlight of the meeting will be a special symposium on ***Adipose Stem Cells for Orthopedic Applications*** on Thursday, November 5th. We are fortunate to have Dr. Rocky Tuan from the University of Pittsburgh chairing this symposium. He heads a panel that includes: Dr. Nina Tandon of EpiBone, Dr. Elizabeth Loboa from North Carolina State University, Dr. Warren Grayson from Johns Hopkins University, and Dr. Ming Pei from the University of West Virginia. I would like to thank each of the speakers for their participation in this important symposium.

Another session will focus on regulatory issues and approval for clinical use of adipose tissue and adipose stem cells. Speakers in this session include regulatory experts and IFATS members from both academic and corporate environments. The session will feature a panel discussion to address specific questions from meeting attendees.

Additional highlights include state-of-the-art topics presented by our Keynote speakers. Rocky Tuan will discuss the use of adult stem cells for orthopedic applications. Steven Farmer from Boston University will focus on adipose tissue as a metabolic organ. Malcom Maden from The University of Florida will present mammalian organ regeneration.

The panel discussion sessions combined with free paper and poster presentation sessions, and other more informal opportunities, will provide ample time for conference attendees to exchange ideas with one another, and potentially provide a global perspective on currently available and future adipose stem cell applications.

I would also like to welcome and thank our exhibiting companies and sponsors for their support. This excellent educational program could not be provided without their invaluable contributions.

On behalf of the IFATS Board of Directors, we look forward to another successful meeting and to welcoming you to New Orleans. With world-class dining, music, and entertainment options all within walking distance of the meeting venue, the city of New Orleans extends its hospitality in a unique and fulfilling way.

As we say down here, *Laissez les bon temps roulez* (Let the good times roll!)

With best wishes,

Bruce Bunnell, PhD
IFATS President 2014-2015
Professor, Tulane University School of Medicine
Center for Stem Cell Research and Regenerative Medicine

SCIENTIFIC PROGRAM COMMITTEE

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Ricardo Rodriguez, MD
J. Peter Rubin, MD, FACS
Dmitry O. Traktuev, PhD
Stuart K. Williams, PhD
Kotaro Yoshimura, MD

INVITED SPEAKERS AND SESSION MODERATORS

Petra Bauer-Kreisel, PhD
Torsten Blunk, PhD
Annie C. Bowles, MS
Stephanie J. Bryant, PhD
Jeff W. Bulte, PhD
Bruce Bunnell, PhD
Mary Ann Chirba, JD, DSc, MPH
Sydney Coleman, MD
Alexandra Conde-Green, MD
Richard D'Amico, MD
Stephen R. Farmer, PhD
Anthony D. Foster, PhD
Julie Fradette, PhD
Jeffrey M. Gimble, MD, PhD
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Marco Helder, PhD
Kevin Hopkins, MD
Brian Johnstone, PhD
Adam J. Katz, MD, FACS
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Debasis Mondal, PhD
Walter Lee Murfee, PhD
Ming Pei, MD, PhD
Ivona Percec, MD, PhD
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Ricardo Rodriguez, MD
J. Peter Rubin, MD, FACS
Felix H. Savoie, MD
Nina Tandon, PhD
Susan Thibeault, PhD
Dmitry Traktuev, PhD
Rocky S. Tuan, PhD
Phyllis L. Warkentin, MD
Stuart K. Williams, PhD

DISCLAIMER

Papers are reprinted as they were submitted. IFATS takes no responsibility for typographical or other errors. All papers in this Program Book are listed in numerical order.

No one may present more than one paper at any IFATS Meeting, although an individual may be an author of more than one paper presented. The paper must be presented by one of the authors. If no alternate presenter is available, the paper will be replaced on the program.

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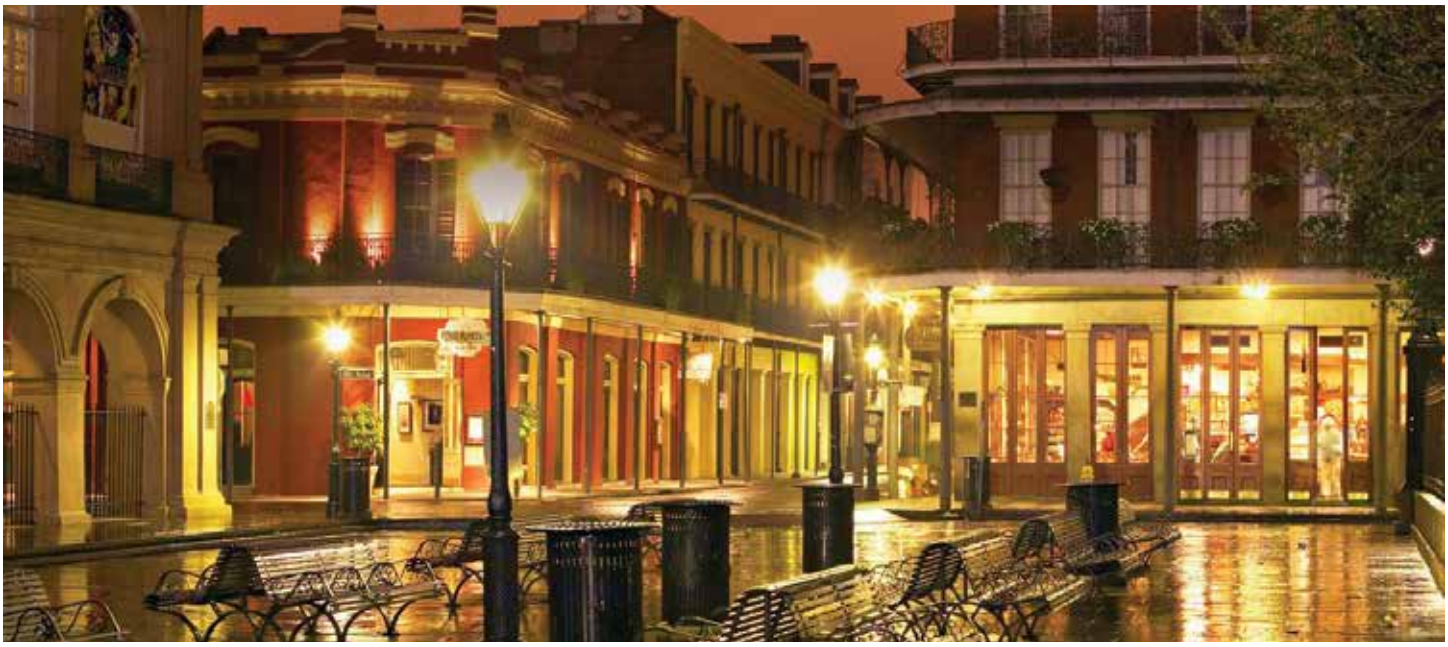

KEYNOTE SPEAKERS

Rocky S. Tuan, PhD - *University of Pittsburgh*
Dr. Rocky Tuan is the Director, Center for Cellular and Molecular Engineering, the Arthur J. Rooney, Sr. Professor and Executive Vice Chair, Department of Orthopedic Surgery, the Associate Director, McGowan Institute for Regenerative Medicine, the Director, Center for Military Medicine Research, and a Professor in the Departments of Bioengineering and Mechanical Engineering & Materials Science, University of Pittsburgh, Pittsburgh, PA

Lecture Title: Adult Stem Cells and Biomimetic Materials for Tissue Engineering and Modeling: Repair, Restore and Create
When: Thursday, November 5th - 3:15 to 4:15 pm

Stephen R. Farmer, PhD - *Boston University School of Medicine*
A professor of biochemistry at Boston University School of Medicine, Dr. Farmer earned his doctorate at the National Institute of Medical Research in London. His research focus is obesity and appropriate therapeutics. He is a founder of the Obesity Research Center at Boston University.

Lecture Title: Myocardin Related Transcription Factor A (MRTFA) Regulates Coversion of Progenitors to Adipocytes and Osteocytes
When: Friday, November 6th - 8:15 to 9:00 am

Malcolm Maden, PhD - *University of Florida*
Professor Maden studies the development and regeneration of various organ systems including the limb, the lung and the nervous system from the point of view of one important signaling molecule, retinoic acid. He is interested in the relationship between development and regeneration, whether regeneration can be induced in the adult organism by understanding the role of developmental signaling molecules and evolutionary aspects of retinoic acid signaling.

Lecture Title: Regeneration and Stem Cells in the Spiny Mouse: A Unique Mammalian Regeneration Model
When: Sunday, November 8th - 8:05 to 8:35 am

INVITED SPEAKERS

Felix H. "Buddy" Savoie, III, MD - *Tulane University School of Medicine*
Dr. Buddy Savoie is a graduate of the Louisiana State University School of Medicine and pursued an AO fellowship in Switzerland and Hand and Microvascular surgery and arthroscopy fellowships in the US. He is known as an accomplished author and teacher who comes to Tulane from Mississippi Sports Medicine and Orthopedic Center in Jackson where he has been a clinician, surgeon, and educator. He is the new Chief of the Section of Sports Medicine at Tulane with special interests in shoulder, elbow, and wrist surgery.

Lecture Title: Biologic Enhancement in Orthopaedic Shoulder and Elbow Surgery
When: Thursday, November 5th - 8:30 to 9:00 am

Nina Tandon, PhD - *EpiBone*
Nina Tandon is CEO and co-founder of EpiBone, the world's first company growing living human bones for skeletal reconstruction. She is the co-author of Super Cells: Building with Biology, a book that explores the new frontier of biotech. She is a TED Senior Fellow, Adjunct Professor of Electrical Engineering at the Cooper Union and a former Staff Associate Postdoctoral Researcher in the Laboratory for Stem Cells and Tissue Engineering, Columbia University. She has a Master's in Bioelectrical Engineering from MIT, a PhD in Biomedical Engineering, and an MBA from Columbia University. She was named one of the 100 Most Creative People in Business by Fast Company.

Lecture Title: Tissue Engineering of Bone from Autologous Adipose Derived Stem Cells for Maxillofacial Reconstruction
When: Thursday, November 5th - 9:00 to 9:30 am

INVITED SPEAKERS

Warren L. Grayson, PhD - *Johns Hopkins Biomedical Engineering*
Dr. Grayson received his B.Sc. in Chemical & Process Engineering at The University of the West Indies, his Ph.D. in Biomedical Engineering from Florida State University, and completed his postdoctoral training in the Department of Biomedical Engineering at Columbia University. He is currently the director of the Laboratory for Craniofacial and Orthopedic Tissue Engineering. His research on bioreactor design and engineering anatomically-shaped bone grafts has received widespread attention. His lab focuses on spatial and temporal regulation of stem cell differentiation in 3D constructs to generate clinically useful engineered grafts.

Lecture Title: Exploring the Translational Potential of Adipose-derived Stem Cells for Bone and Muscle Regeneration
When: Thursday, November 5th - 9:30 to 10:00 am

Elizabeth Loboa , PhD - *North Carolina State University*
Dr. Loboa is Professor and Associate Chair of the Joint Department of Biomedical Engineering at UNC-Chapel Hill and NCSU, and a Professor in the Department of Materials Science and Engineering at NCSU. She is the Founding Director of the Cell Mechanics Laboratory and holds adjunct faculty positions in the Departments of Fiber and Polymer Science (NCSU), Physiology (NCSU), Biotechnology (NCSU), and Orthopedics (UNC-CH). Research in her laboratory focuses on two areas: 1) empirical and computational approaches to quantitatively determine and deliver biomimetic mechanical, electrical, and material stimuli to human stem cells for functional tissue engineering of musculoskeletal tissues; and, 2) nanofibrous "smart bandages" as controlled release systems for wound healing and tissue engineering applications requiring antimicrobial, antibacterial, and/or anti-inflammatory treatment.

Lecture Title: Our Fat Future: Understanding and Translating ASC for Clinical Applications
When: Thursday, November 5th - 10:30 to 11:00 am

Ming Pei, MD, PhD - *West Virginia University*
Dr. Pei is an Associate Professor and a Director of Stem Cell and Tissue Engineering Laboratory in the Department of Orthopedics, West Virginia University. He also serves as an Adjunct Professor in Exercise Physiology and Mechanical & Aerospace Engineering at the West Virginia University. He spent three years as a Postdoctoral Associate in Harvard – M.I.T. Division of Health Sciences and Technology (HST) focusing on Bioreactor and Cartilage Tissue Engineering Projects, and three years as a Research Fellow in Department of Orthopedics, Brown University focusing on Stem Cell Projects. He received his PhD from Beijing University, China in 1999.

Lecture Title: Decellularized Cell Matrix and Cartilage Regeneration
When: Thursday, November 5th - 11:00 - 11:30 am

Phyllis L. Warkentin, MD - *University of Nebraska*
Dr. Warkentin is a founding member of the Foundation for the Accreditation of Cellular Therapy (FACT) and has been serving as a board member and chief medical officer since the organization's inception. In addition to her work with FACT, Dr. Warkentin has been the director of the unrelated bone marrow transplantation program at the University of Nebraska Medical Center since 1988 and a professor of pediatrics and pathology/microbiology at the University of Nebraska Medical Center since 1994.

Lecture Title: Adult Stem Cells - Regulatory Approval for Clinical Use
When: Saturday, November 7th - 9:30 to 10:00 am

Mary Ann Chirba, JD, DSc, MPH - *Boston College Law School*
Mary Ann Chirba holds a bachelor's degree in biology from Colgate University and a JD, magna cum laude, from Boston College of Law School. She hold a doctorate of Science in Health Policy and Management as well as a Master's in Public Health from the Harvard School of Public Health. She is the author, along with Alice Noble and Michael Maddigan, of *Health Care Reform: Law and Practice – A Comprehensive Guide to the Affordable Care Act and its Implementing Regulations.*

Lecture Title: FDA Update: Recent Guidance - Future Hindrance?
When: Saturday, November 7th - 10:00 to 10:30 am

PROGRAM IN BRIEF

The program is correct at the time of printing; however, the Program Chairman reserves the right to alter the schedule as necessary.

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Thursday, November 5, 2015

7:00 am - 6:00 pm	Registration
8:15 am - 12:00 pm	**SYMPOSIUM ON ADIPOSE STEM CELLS FOR ORTHOPEDIC APPLICATIONS** *Sponsored by the Musculoskeletal Transplant Foundation*
8:15 - 8:30 am	**Opening Remarks** Moderator: Rocky S. Tuan, PhD
8:30 - 9:00 am	**Biologic Enhancement in Orthopaedic Shoulder and Elbow Surgery** Speaker: Felix H. Savoie, MD *Chairman, Department of Orthopaedics, Tulane University School of Medicine*
9:00 - 9:30 am	**Tissue Engineering of Bone from Autologous Adipose Derived Stem Cells for Maxillofacial Reconstruction** Speaker: Nina Tandon, PhD, MBA *CEO and Co-founder of EpiBone*
9:30 - 10:00 am	**Exploring the Translational Potential of Adipose-derived Stem Cells for Bone and Muscle Regeneration** Speaker: Warren L. Grayson, PhD *Assistant Professor, Biomedical Engineering, John Hopkins University*
10:00 - 10:30 am	Coffee Break and Exhibits
10:30 - 11:00 am	**Our Fat Future: Understanding and Translating Adipose Stem Cells for Clinical Applications** Speaker: Elizabeth Loboa, PhD *Professor and Associate Chair, Joint Department of Biomedical Engineering, UNC-Chapel Hill and NCSU; Professor in the Department of Materials Science and Engineering, NCSU*
11:00 - 11:30 am	**Decellularized Cell Matrix and Cartilage Regeneration** Speaker: Ming Pei, MD, PhD *Associate Professor, Orthopaedics, West Virginia University*
11:30 am - 12:00 pm	**Panel Discussion - All Speakers**
12:00 - 1:00 pm	Lunch
1:00 - 2:45 pm	**Plenary Session 1: Basic Research: Soft Tissue & Other Organs** Moderators: Lauren Kokai, PhD & Ivona Percec, MD, PhD
2:45 - 3:15 pm	Coffee Break and Exhibits
3:15 - 4:15 pm	**Keynote Speaker** **Adult Stem Cells and Biomimetic Materials for Tissue Engineering and Modeling: Repair, Restore and Re-create** Rocky S. Tuan, PhD *Director, Center for Cellular and Molecular Engineering, University of Pittsburgh*
4:15 - 6:00 pm	**Poster Presentations** Moderators: Adam Katz, MD, FACS & Bruce Bunnell, PhD
6:00 pm	Adjourn

Friday, November 6, 2015

7:00 am - 5:00 pm	Registration
7:00 - 8:00 am	Continental Breakfast - Exhibit Hall
8:00 - 8:15 am	**Introductory Remarks** Bruce Bunnell, PhD
8:15 - 9:00 am	**Keynote Speaker** **Myocardin Related Transcription Factor A (MRTFA) Regulates Conversion of Progenitors to Adipocytes and Osteocytes** Stephen R. Farmer, PhD *Professor, Biochemistry, Boston University School of Medicine*

9:00 - 11:00 am	**Plenary Session 2: Highest Scoring Abstracts** *(mixed categories)*
	Moderators: Dmitry Traktuev, PhD & Kacey Marra, PhD
11:00 - 11:20 am	Coffee Break and Exhibits
11:20 am - 12:45 pm	**Plenary Session 3: Applied Research: Soft Tissue & Other Organs**
	Moderators: Brian Johnstone, PhD & Torsten Blunk, PhD
12:45 - 2:00 pm	Lunch
2:00 - 3:30 pm	**Concurrent Free Paper Session 1 - Clinical Research: Soft Tissue**
	Moderators: Ivona Percec, MD, PhD & Adam Katz, MD, FACS
2:00 - 3:30 pm	**Concurrent Free Paper Session 2 - Basic Research: Soft Tissue**
	Moderators: Dmitry Traktuev, PhD & Hebert Lamblet, MD
2:00 - 3:30 pm	**Concurrent Free Paper Session 3 - Applied Research: Soft Tissue**
	Moderators: Kevin Hopkins, MD, FACS & Bruce Bunnell, PhD
3:30 - 4:00 pm	Coffee Break and Exhibits
4:00 - 5:00 pm	**Small Panel Discussion 1: Clinical Aspects**
	Chair: J. Peter Rubin, MD, FACS Panelists: Sydney Coleman, MD, Kamran Khoobehi, MD, FACS, Ramon Llull, MD, PhD
4:00 - 5:00 pm	**Small Panel Discussion 2: Basic Research**
	Chair: Julie Fradette, PhD Panelists: Walter Lee Murfee, PhD, Susan Thibeault, PhD, Dan Hayes, PhD, Stephanie J. Bryant, PhD
4:00 - 5:00 pm	**Small Panel Discussion 3: Hot Topics**
	Chair: Jeffrey Gimble, MD, PhD Panelists: Jeff W. Bulte, PhD, Annie C. Bowles, MS, Anthony D. Foster, PhD, Debasis Mondal, PhD
5:00 - 6:30 pm	**Poster Session & Welcome Reception**

Saturday, November 7, 2015

7:00 - 8:00 am	Continental Breakfast - Exhibit Hall
8:00 - 9:00 am	**IFATS Members Meeting**
9:15 am - 1:30 pm	**REGULATORY AFFAIRS PANEL**
9:15 - 9:30 am	**Opening Remarks** Moderator: Stuart K. Williams, PhD
9:30 - 9:55 am	**Adult Stem Cells – Voluntary Standards to Regulatory Approval** Speaker: Phyllis I. Warkentin, MD - *University of Nebraska Medical Center/FACT*
9:55 - 10:20 am	**FDA Update: Recent Guidance - Future Hindrance? - FDA/US Perspective** Speaker: Mary Ann Chirba, JD, DSc, MPH - *Boston College Law School*
10:20 - 10:45 am	**Regulatory Affairs for Adult Stem Cells - European Perspective** Speaker: Marco Helder, PhD - *VU University Medical Center, Amsterdam*
10:45 - 11:10 am	**Regulatory Approval for Use of Adipose Stem Cells in Humans - Academic Perspective** Speaker: J. Peter Rubin, MD, FACS - *University of Pittsburgh*
11:10 am - 11:35 pm	**The Role of Pre-clinical Studies to Establish a Regulatory Strategy - Corporate Perspective** Speaker: Stuart K. Williams, PhD - *University of Louisville*
11:35 - 12:00 pm	**Regulatory Approval for Use of Adipose Stem Cells in Humans - Corporate Perspective** Speaker: Shah Rahimian, MD - *Antria*
12:00 - 12:25 pm	**FDA and Regulation of Adipose Tissue Procedures - ASPS Perspective** Speaker: Richard D'Amico, MD - *American Society of Plastic Surgeons*
12:25 - 1:00 pm	**Panel Discussion - All Speakers**
1:00 - 2:30 pm	Lunch

2:30 - 3:15 pm	**Concurrent Free Paper Session 4 - Regulatory Affairs/Innovation & Hot Topics/Applied Research**
	Moderators: Alexandra Conde-Green, MD & Marco Helder, PhD
2:30 - 3:15 pm	**Concurrent Free Paper Session 5 - Basic Research: Musculoskeletal**
	Moderators: Ricardo Rodriguez, MD & Stig-Frederik Trojahn Kolle, PhD NEW
2:30 - 3:15 pm	**Concurrent Free Paper Session 6 - Basic Research: Soft Tissue & Musculoskeletal**
	Moderators: Lauren Kokai, PhD & J. Peter Rubin, MD, FACS
3:15 - 3:45 pm	Coffee Break and Exhibits
3:45 - 4:30 pm	**Concurrent Free Paper Session 7 - Basic Research: Soft Tissue**
	Moderators: Petra Bauer-Kreisel, PhD & Adam Katz, MD, FACS
3:45 - 4:30 pm	**Concurrent Free Paper Session 8 - Basic Research: Soft Tissue**
	Moderators: Hebert Lamblet, MD & Torsten Blunk, PhD
3:45 - 4:30 pm	**Concurrent Free Paper Session 9 - Basic Research: Soft Tissue**
	Moderators: Julie Fradette, PhD & Ricardo Rodriguez, MD
6:00 - 9:00 pm	**A Taste of New Orleans -** *Sponsored by the Musculoskeletal Transplant Foundation*
	Muriel's - Jackson Square

Sunday, November 8, 2015

7:00 - 8:00 am	Continental Breakfast - Exhibit Hall
8:00 - 8:05 am	**Introductory Remarks** Bruce Bunnell, PhD
8:05 - 8:35 am	**Keynote Speaker**
	Regeneration and Stem Cells in the Spiny Mouse: A Unique Mammalian Regeneration Model Malcolm Maden, PhD *Professor, Department of Biology, University of Florida*
8:35 - 9:45 am	**Plenary Session 4: Clinical Research: Soft Tissue & Musculoskeletal/Innovation & Hot Topics**
	Moderators: Brian Johnstone, PhD & Jeffrey Gimble, MD, PhD
9:45 - 10:15 am	Coffee Break and Exhibits
10:15 - 11:25 am	**Plenary Session 5: Basic Research & Applied Research: Musculoskeletal**
	Moderators: Marco Helder, PhD & Ricardo Rodriguez, MD
11:25 am	**Closing Remarks** Bruce Bunnell, PhD

NOTES

NOTES

PROGRAM SCHEDULE

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Thursday, November 5, 2015

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9:30 - 10:00 am	**Exploring the Translational Potential of Adipose-derived Stem Cells for Bone and Muscle Regeneration** Speaker: Warren L. Grayson, PhD *Assistant Professor, Biomedical Engineering, John Hopkins University*
10:00 - 10:30 am	Coffee Break and Exhibits
10:30 - 11:00 am	**Our Fat Future: Understanding and Translating Adipose Stem Cells for Clinical Applications** Speaker: Elizabeth Loboa, PhD *Professor and Associate Chair, Joint Department of Biomedical Engineering, UNC-Chapel Hill and NCSU; Professor in the Department of Materials Science and Engineering, NCSU*
11:00 - 11:30 am	**Decellularized Cell Matrix and Cartilage Regeneration** Speaker: Ming Pei, MD, PhD *Associate Professor, Orthopaedics, West Virginia University*
11:30 am - 12:00 pm	**Panel Discussion - All Speakers**
12:00 - 1:00 pm	Lunch
1:00 - 2:45 pm	**Plenary Session 1: Basic Research: Soft Tissue & Other Organs** Moderators: Lauren Kokai, PhD & Ivona Percec, MD, PhD
1:00 pm	**1** **XENOTRANSPLANTATION OF INTEGRA® AND HUMAN SPHEROIDS FROM ADIPOSE-DERIVED STEM CELLS (S-ASCS) PROMOTES CALVARIAL BONE PRODUCTION IN A RABBIT MODEL** Presenter: Luigi Montesano Affiliation: Universita degli Studi di Palermo Authors: Montesano L, Di Stefano AB, Giammona A, Maenza A, Belmonte B, Moschella P, Cassata G, Florena AM, Todaro M, Stassi G, Cordova A, Moschella F, Leto Barone AA
1:10 pm	**2** **CELL SHEETS DERIVED FROM ADIPOSE-DERIVED STEM CELLS ACCELERATED WOUND HEALING AND REDUCED TISSUE FIBROSIS IN A MURINE MODEL** Presenter: Naichen Cheng, MD, PhD Affiliation: National Taiwan University Hospital Author: Cheng N
1:20 pm	**3** **A CELL EXPANSION PLATFORM GENERATED BY STROMAL CELLS PROMOTES MESENCHYMAL STEM CELL ISOLATION, EXPANSION, AND IL-10 EXPRESSION** Presenter: Rogelio Zamilpa, PhD Affiliation: StemBioSys Inc. Authors: Zamilpa R, Flores I, Navarro MM, Griffey S

1:30 pm

4
COMPARISON OF THE IMMUNOMODULATING EFFECTS OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS AND ADIPOSE DERIVED STEM CELLS IN VITRO ACROSS DIFFERENT HLA BARRIERS
Presenter: Matthias Waldner, MD
Affiliation: University of Pittsburgh Medical Center
Authors: Waldner M, Zhang W, Schweizer R, Almadori A, James IB, Plock JA, Solari MG, Marra KG, Gorantla VS, Rubin JP

1:40 pm

5
DEFINING THE ROLE OF ADIPOSE DERIVED STEM CELL TRANSPLANTATION IN A RADIATED TISSUE EXPANDER MODEL
Presenter: Abigail M. Cochran, MD
Affiliation: Southern Illinois University School of Medicine
Authors: Cochran AM, Yang MY, De La Garza MD, Cosenza NC, Richensperger JR, Harrison CH, Cox LC, Berry NB, Neumeister MN

1:50 pm

6
PARACRINE INTERACTIONS BETWEEN ADIPOSE-DERIVED STEM CELLS AND BREAST CANCER CELLS
Presenter: J. Peter Rubin, MD, FACS
Affiliation: University Hospital Zurich
Authors: Schweizer R, Tsuji W, Plock JA, Marra KG, Rubin JP

2:00 pm

7
3D CULTURED SVF SPHEROIDS SECRETE HIGH LEVELS OF GROWTH FACTORS INVOLVED IN ANGIOGENESIS
Presenter: Ning Yang, PhD
Affiliation: University of Florida
Authors: Yang N, Shang H, Katz A

2:10 pm

8
DEPOT-SPECIFIC DIFFERENCES OF OXIDATIVE STRESS IN HUMAN ADIPOSE-DERIVED STEM CELLS
Presenter: Shigeki Sugii, PhD
Affiliation: Singapore Bioimaging Consortium and Duke NUS Graduate Medical School
Authors: Sugii S, Toh SA, Sriram S

2:20 pm

9
POLOXAMER ENRICHMENT PRESERVES STROMAL VASCULAR FRACTION (SVF) CELL VIABILITY
Presenter: Isaac B. James, MD
Affiliation: University of Pittsburgh
Authors: James IB, Bourne DA, Sivak WN, Bliley JM, Wang S, Kokai LE, Schroth R, Mahoney C, Marra KG, Rubin JP

2:30 pm

10
ANTI-INFLAMMATORY MESENCHYMAL STEM CELLS (MSC2) DEMONSTRATED ENHANCED THERAPEUTIC EFFICACY IN THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL
Presenter: Xiujuan Zhang, PhD
Affiliation: Dalian University of Technology
Authors: Zhang X, Bowles AC, Betancourt AM, Bunnell BA

2:45 - 3:15 pm

Coffee Break and Exhibits

3:15 - 4:15 pm

Keynote Speaker

Adult Stem Cells and Biomimetic Materials for Tissue Engineering and Modeling: Repair, Restore and Re-create
Rocky S. Tuan, PhD
Director, Center for Cellular and Molecular Engineering, University of Pittsburgh

4:15 - 6:00 pm **Poster Presentations**
Moderators: Adam Katz, MD, FACS & Bruce Bunnell, PhD

~~4:15 pm~~

NOT PRESENTED

1P
NEURAL LINEAGE DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STROMAL CELLS PRELIMINARY RESULTS
Presenter: Luminita Labusca, ~~MD, PhD~~
Affiliation: University of Copenhagen
Authors: Labusca L, Hyttel P, Freude KK, Ostrup E

4:18 pm

2P
THE USE OF TISSUE SCAFFOLDS PLUS ENHANCED FRACTIONAL CELLS MAY BE AN AESTHETICALLY AND FUNCTIONAL ALTERNATIVE TO FREE TISSUE TRANSFERS IN BURN SCAR CONTRACTURE TREATMENT
Presenter: Mehmet Bozkurt, MD
Affiliation: Dr. Lutfi Kirdar Kartal Training and Research Hospital
Authors: Bozkurt M, Guvercin E, Taylan Filinte G, Ceran F, Basat SO

4:21 pm

3P
FEASIBILITY AND SAFETY OF ADIPOSE-DERIVED WOUND PASTE IN A MURINE MODEL OF FULL THICKNESS WOUNDING
Presenter: Ning Yang, PhD
Affiliation: University of Florida
Authors: Yang N, Shang H, Katz A

~~4:24 pm~~

NOT PRESENTED

4P
ALLOTRANSPLANTATION FOLLOWING RECELLULARIZATION OF DECELLULARIZED FLAP USING ADSCS
Presenter: Yungki Lee, MD
Affiliation: Seoul National University Bundang Hospital
Authors: Lee Y, Pak CS, Kim BK

4:27 pm

MOVED TO CONCURRENT SESSION 6

5P
NON-RESPONSIVE KNEE PAIN TREATED WITH AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE UNDER CONTINUOUS ULTRASOUND GUIDANCE
Presenter: Halland Chen, MD NEW
Affiliation: Optimum Joint Integrated Joint and Spine
Authors: Striano RD, Bilbool N, Chen H

~~4:30 pm~~

NOT PRESENTED

6P
THREE DIMENSIONAL CELL PROLIFERATION AND OSTEOGENESIS OF HUMAN ADIPOSE STEM CELLS ON POLY (L-LACTIC ACID) SCAFFOLDS PREPARED BY THERMALLY CONTROLLED METHODS
Presenter: Harish Chinnasami, PhD
Affiliation: Louisiana State University
Authors: Chinnasami H, Devireddy RV

4:33 pm

7P
INVISIBLE SCAR: INTRADERMAL INJECTION OF SVF COMBINED WITH SCAR REVISION CAN IMPROVE THE RESULT
Presenter: Wonyoung Yoon, MD, MS
Affiliation: Idea Plastic Surgery
Authors: Yoon W, Kook KS

4:36 pm

8P
DERMAL SCAFFOLD AND BONE MARROW ASPIRATE CONCENTRATE USAGE FOR SEVERE FACIAL BURNS MANAGEMENT AS A NEW PARADIGM FOR RESCUE PROCEDURE
Presenter: Mehmet Bozkurt, MD
Affiliation: Dr. Lutfi Kirdar Kartal Training and Research Hospital
Authors: Bozkurt M, Ceran F, Filinte GT, Guvercin E, Basat SO

4:39 pm

9P
A COMPARISON OF FAT GRAFTING METHODS ON OPERATING ROOM EFFICIENCY AND COST
Presenter: Mousam H. Parekh
Affiliation: Peace Health Medical Group
Authors: Gabriel A, Parekh MH, Champaneria MC, Macarios D, Griffin L, Maxwell GP

4:42 pm

10P
TREATMENT OF LYMPHEDEMA WITH CELL-ASSISTED LIPOTRANSFER WITH AUTOLOGOUS STROMAL VASCULAR FRACTION
Presenter: Navid M. Toyserkani, MD
Affiliation: Odense University Hospital
Authors: Toyserkani NM, Jensen CH, Sheikh SP, Sorensen JA

4:45 pm

11P
DERMAL WOUND PASTE FABRICATED WITH STROMAL VASCULAR FRACTIONS FOR THE POINT-OF-CARE WOUND THERAPY
Presenter: Ning Yang, PhD
Affiliation: University of Florida
Authors: Yang N, Patel N, Shang H, Katz A

4:48 pm

12P
GLUTEAL SCULPTING AUGMENTATION: A SYNERGY EFFECT OF THE COMBINATION BETWEEN LIPOSUCTION AND LIPOFILLING
Presenter: Marwan Abboud, MD
Affiliation: MA Clinic
Authors: Abboud M, Dibo S

4:51 pm

13P
THE EFFECTS OF THE CENTRIFUGATION SPEED ON THE SURVIVAL OF AUTOGENOUS FAT GRAFTS IN A RAT MODEL
Presenter: Mehmet Bozkurt, MD
Affiliation: Dr. Lutfi Kirdar Kartal Training and Research Hospital
Authors: Bozkurt M, Guvercin E, Kapi E, Sirinoglu H, Taylan Filinte G, Filinte D

4:54 pm

14P
THE APPLICATION OF AUTOLOGOUS FAT GRAFTING IN ACHIEVING SYMMETRY IN IMPLANT-BASED BREAST RECONSTRUCTION
Presenter: Biao Wang, MD
Affiliation: The First Affiliated Hospital of Fujian Medical University
Authors: Wang B, Shan X, Chen R

4:57 pm

15P
THE ROLE OF NOTCH IN REGULATING STEM CELL STATE
Presenter: Jamie J. Newman, PhD
Affiliation: Louisiana Tech University
Authors: Sandel D, Bunnell BA, Miele L, Newman JJ

5:00 pm

16P
FACTORS THAT MAY INFLUENCE YEILD AND VIABILITY OF EXTRACTED ADRCS PER GRAM OF TISSUE - FOLLOW UP STUDY
Presenter: Marcos Sforza, MD
Affiliation: BelPrime Clinic
Authors: Sforza M, Andjelkov K, Zaccheddu R

5:03 pm

NOT PRESENTED

17P
EVALUATION OF TWO PURIFICATION METHODS FOR ISOLATION OF HUMAN ADIPOSE-DERIVED STEM CELLS BASED ON RED BLOOD CELL LYSIS WITH AMMONIUM CHLORIDE AND HYPOTONIC SODIUM CHLORIDE SOLUTION
Presenter: Hongwei Liu, MD,PhD
Affiliation: The First Affiliated Hospital of Jinan University
Authors: Liu H, Li S, Liao X

5:06 pm

NOT PRESENTED

18P
~~**CLINICAL ANALYSIS OF USING WET AUTOLOGOUS FAT PARTICLES TRANSPLANTATION FOR TEMPORAL FILLING**~~
Presenter: Xunlei Huang, MD
Affiliation: The First Affiliated Hospital of Fujian Medical University
Authors: Huang X, Wang B, Zheng H

5:09 pm

19P
LIPOINJECTION VIA V SHAPED 3MM TULIP CANNULA FOR THE TREATMENT OF BURN SCARS
Presenter: Mehmet Bozkurt, MD
Affiliation: Dr. Lutfi Kirdar Kartal Training and Research Hospital
Authors: Bozkurt M, Ceran F, Filinte GT, Basat SO, Guvercin E

5:12 pm

NOT PRESENTED

20P
~~**FAT TRANSFER, GET RID OF YOUR WRINKLES**~~
Presenter: Aristides Arellano-Huacuja, Sr., MD
Affiliation: Clinica Dermatologica y Cirugia Estetica de Puebla
Author: Arellano-Huacuja A

5:15 pm

21P
INTERFERENCE OF THE FAT TISSUE IN CUTANEOUS REGENERATION AND ITS USE IN THE TREATMENT OF WOUNDS, BURNS AND SCARS
Presenter: Marco A. Pellon, MD
Affiliation: Clinica Sao Vicente
Author: Pellon MA

5:18 pm

22P
ISOLATION OF HUMAN ADIPOSE DERIVED STROMAL VASCULAR FRACTION: A CLINICAL APPLICATION

New Presenter

Presenter: Kwang Sik Kook, MD NEW
Affiliation: Idea Plastic Surgery
Authors: Jang S, Kook KS

5:21 pm

23P
DERMAL WHITE ADIPOSE TISSUE THE BEST CELLULAR SOURCE FOR SKIN REGENERATION PURPOSE
Presenter: Angelo Trivisonno, MD
Affiliation: Sapienza University
Author: Trivisonno A

5:24 pm

24P
HUMAN ADIPOSE-DERIVED MATRIX PROMOTES ANGIOGENESIS AND ADIPOGENESIS IN VIVO: A MULTI-WEEK ASSESSMENT OF VOLUME RETENTION AND HISTOPATHOLOGY
Presenter: Lauren E. Kokai, PhD
Affiliation: University of Pittsburgh
Authors: Kokai LE, Chnari E, Schilling BK, Minteer D, Mahoney C, Kelmendi-Doko A, Jacobs M, Marra KG, Rubin JP

5:27 pm

25P
THE IMPACT OF HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS ON MALIGNANT MELANOMA CELLS - IMPLICATIONS FOR CELL-BASED SKIN THERAPY
Presenter: Eva Koellensperger, MD
Affiliation: Heidelberg University Hospital
Authors: Koellensperger E, Preisner F, Sandmann S, Zoernig I, Germann G, Leimer U

5:30 pm

26P
THE THROMBIN EFFECT ON FAT GRAFT VIABILITY: IS THERE A CHANCE TO PROLONG FATS LIFE LENGTH?
Presenter: Mehmet Bozkurt, MD
Affiliation: Dr. Lutfi Kirdar Kartal Training and Research Hospital
Authors: Bozkurt M, Kacmaz C, Gideroglu K, Guvercin E, Taylan Filinte G, Filinte D

5:33 pm
NOT PRESENTED

27P
EFFECT OF 650 NM LOW-LEVEL LIGHT IRRADIATION ON THE THERAPEUTIC POTENTIAL OF ADIPOSE-DERIVED STEM CELLS IN REGENERATIVE MEDICINE
Presenter: Hongwei Liu, MD,PhD
Affiliation: The First Affiliated Hospital of Jinan University
Authors: Li S, Liu H

5:36 pm
NOT PRESENTED

28P
ALLOTRANSPLANTATION FOLLOWING RECELLULARIZATION OF DECELLULARIZED VESSEL USING ADSCS
Presenter: Daehee Kim, MD
Affiliation: Seoul National University Bundang Hospital
Authors: Kim D, Pak CS, Kim BK

5:39 pm
WINNER OF POSTER PRESENTATIONS

29P
BMP-2 INDUCED OSTEOGENIC AND ADIPOGENIC DIFFERENTIATION IN HUMAN ADIPOSE STEM CELLS
Presenter: Sari Vanhatupa, PhD
Affiliation: BioMediTech
Authors: Vanhatupa S, Ojansivu M, Autio R, Juntunen M, Miettinen S

5:42 pm
NOT PRESENTED

30P
LARGE VOLUME LIPOFILLING IN AESTHETIC PLASTIC SURGERY
Presenter: Aristides Arellano-Huacuja, Sr., MD
Affiliation: Clinica Dermatologica y Cirugia Estetica de Puebla
Author: Arellano-Huacuja A

5:45 pm

31P
SYNTHETIC MRNA TO DEDIFFERENTIATE EQUINE ADULT MULTIPOTENT STROMAL CELLS
Presenter: Mandi J. Lopez, DVM, MS, PhD
Affiliation: Louisiana State University
Authors: Jarazo J, Fargason C, Bondioli K, Lopez M

5:48 pm
NOT PRESENTED

32P
TREATMENT OF BRA STRAP SHOULDER GROOVE DEFORMITY BY AUTOLOGOUS FAT INJECTION SIMULTANEOUSLY WITH BREAST REDUCTION
Presenter: Yigit Ozer Tiftikcioglu, MD
Affiliation: Ege University Medical School
Authors: Tiftikcioglu YO, Erenoglu CM, Ozek C

6:00 pm — Adjourn

Friday, November 6, 2015

7:00 am - 5:00 pm — Registration

7:00 - 8:00 am — Continental Breakfast - Exhibit Hall

8:00 - 8:15 am — **Introductory Remarks**
Bruce Bunnell, PhD

8:15 - 9:00 am — **Keynote Speaker**

Myocardin Related Transcription Factor A (MRTFA) Regulates Conversion of Progenitors to Adipocytes and Osteocytes
Stephen R. Farmer, PhD
Professor, Biochemistry, Boston University School of Medicine

9:00 - 11:00 am — **Plenary Session 2: Highest Scoring Abstracts** *(mixed categories)*
Moderators: Dmitry Traktuev, PhD & Kacey Marra, PhD

9:00 am

11
ADIPOSE-TISSUE STROMAL VASCULAR FOR THE TREATMENT OF ACHILLES TENDINOPATHY: A RANDOMIZED PROSPECTIVE CLINICAL TRIAL
Presenter: Laura de Girolamo, PhD
Affiliation: Istituto Ortopedico Galeazzi
Authors: Usuelli FG, Grassi M, Alfieri Montrasio U, De Girolamo L

9:20 am	**12** **FLUORINE-19 LABELING OF STROMAL VASCULAR FRACTION CELLS FOR CLINICAL IMAGING APPLICATIONS** Presenter: Jeff W. Bulte, PhD Affiliation: Johns Hopkins University School of Medicine Authors: Kadayakkara DK, Wang G, Bar-Shir A, Helfer BM, Ohanlon C, Kraitchman DL, Rodriguez RL, Bulte JW
9:40 am	**13** **STROMAL VASCULAR FRACTION AS A NOVEL STEM CELL-BASED THERAPY FOR A MOUSE MODEL OF MULTIPLE SCLEROSIS** Presenter: Annie C. Bowles, MS Affiliation: Tulane University Authors: Bowles AC, Strong AL, Wise RM, Thomas RC, Gerstein BY, Dutreil MF, Lipschutz RS, Gimble JM, Bunnell BA
10:00 am	**14** **AUTOLOGOUS FAT GRAFTING FOR PEDAL FAT PAD ATROPHY: A PILOT STUDY** Presenter: Jeffrey A. Gusenoff, MD Affiliation: University of Pittsburgh Authors: Gusenoff JA, Mitchell R, Jeong K, Wukich D, Gusenoff BR
10:20 am	**15** **INCREASED GENOMIC STABILITY OF ADIPOSE DERIVED MESENCHYMAL STROMAL CELLS (MSCS) COMPARED TO BONE MARROW MSCS: INVOLVEMENT OF H19 LONG NON-CODING RNA AND P53 ACTIVITY** Presenter: Meirav Sela, MSc Affiliation: The Tel Aviv Sourasky Medical Center Authors: Sela M, Ravid O, Shoshani O, Weinstock A, Sadan TW, Gur E, Zipori D, Shani N
10:40 am	**16** **CIGARETTE SMOKING MARKEDLY REDUCES VASCULOGENIC ACTIVITY OF ADIPOSE STEM CELLS** Presenter: Daria Barwinska, BA Affiliation: Indiana University Authors: Barwinska D, Traktuev DO, Cook T, Merfeld-Clauss S, Petrache I, March KL
11:00 - 11:20 am	Coffee Break and Exhibits
11:20 am - 12:45 pm	**Plenary Session 3: Applied Research: Soft Tissue & Other Organs** Moderators: Brian Johnstone, PhD & Torsten Blunk, PhD
11:20 am	**17** **ALLOGENEIC ADIPOSE-DERIVED STROMAL CELLS PROMOTE IN VIVO FAT FORMATION IN DECELLULARIZED ADIPOSE TISSUE BIOSCAFFOLDS VIA INDIRECT MECHANISMS** Presenter: Lauren E. Flynn, PhD Affiliation: The University of Western Ontario Authors: Flynn LE, Han TT, Amsden BG
11:30 am	**18** **MECHANICALLY DISRUPTED HUMAN ADIPOSE TISSUE (LIPOGEMS®) EXHIBITS DISTINCT REGENERATIVE CELL CONTENT AND TROPHIC FACTOR SECRETION AS COMPARED WITH CONVENTIONALLY ENZYME-DISSOCIATED FAT** Presenter: Bruno Peault, PhD Affiliation: University of California Los Angeles Authors: Khan N, Lesme H, Krutchkoff B, Tremolada C, Peault B
11:40 am	**19** **ISOLATION OF A PURE POPULATION OF THERAPEUTICALLY POTENT CELLS FROM ADIPOSE TISSUE BASED ON CD140B ANTIGEN** Presenter: Dmitry O. Traktuev, PhD Affiliation: Indiana University Authors: Traktuev DO, Merfeld-Clauss S, Lupov IP, Cook T, March KL

11:50 am	**20** **A COMPARISON OF TOPICAL SPRAY AND LOCAL INJECTIONS OF ADIPOSE DERIVED REGENERATIVE CELLS (ADRCS) ON WOUND HEALING IN A PORCINE MODEL OF FULL THICKNESS BURN INJURY** Presenter: Philippe Foubert, PhD Affiliation: Cytori Therapeutics Authors: Foubert P, Gonzalez AD, Teodosescu S, Fraser JK
12:00 pm	**21** **EXTERNAL VOLUME EXPANSION AND FAT GRAFTING AS AN ALTERNATIVE FOR POST-RADIATION BREAST RECONSTRUCTION: EFFECTS ON A TRANSLATIONAL MODEL** Presenter: Jorge Lujan-Hernandez, MD Affiliation: University of Massachusetts Medical School Authors: Lujan-Hernandez J, Chin MS, Perry DJ, Chappell AG, Babchenko O, Bannon E, Lancerotto L, Lo YC, Fitzgerald TJ, Lalikos JF
12:10 pm	**22** **COORDINATE ISOLATION OF PERI-PANCREATIC FAT SVF AND PANCRETIC ISLETS FOR THE PREPARATION OF FUNCTIONAL PREVASCUALRIZED ISLET SPHEROIDS** Presenter: Stuart K. Williams, PhD Affiliation: University of Louisville Authors: Williams SK, Appakalai B, Hughes MG, Kleinert LB, Loganathan G, George NW, Tweed BT
12:20 pm	**23** **HASCS AND THEIR CONDITIONED MEDIUM ALLEVIATE THE PAINFUL SYMPTOMS IN TWO PRECLINICAL MODELS OF NEUROPATHY** Presenter: Anna T. Brini, PhD Affiliation: University of Milan/Galeazzi Orthopaedic Institute Authors: Brini AT, Niada S, Ferreira LM, Amodeo G, Milani A, Franchi S, Giannasi C, Sacerdote P
12:30 pm	**24** **HUMAN ADIPOSE STROMAL/STEM CELLS FROM OBESE DONORS SHOW REDUCED EFFICACY IN THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL OF MULTIPLE SCLEROSIS** Presenter: Amy L. Strong, PhD, MPH Affiliation: Tulane University School of Medicine Authors: Strong AL, Bowles AC, Wise RM, Morand JP, Dutreil MF, Gimble JM, Bunnell BA
12:45 - 2:00 pm	Lunch
2:00 - 3:30 pm	**Concurrent Free Paper Session 1 - Clinical Research: Soft Tissue** Moderators: Ivona Percec, MD, PhD & Adam Katz, MD, FACS
2:00 pm	**25** **CELL-ASSISTED LIPOTRANSFER: A CRITICAL APPRAISAL OF THE CLINICAL EVIDENCE FOR A NOVEL "STEM CELL THERAPY"** Presenter: Florian M. Lampert, MD Affiliation: University of Freiburg Medical Center Authors: Lampert FM, Grabin S, Antes G, Motschall E, Buroh S, Stark GB
2:08 pm	**26** **IS CENTRIFUGATION STILL PLAYING THE LEADING ROLE? A SYSTEMATIC REVIEW OF PROCESSING TECHNIQUES IN FAT GRAFTING** Presenter: Aartje J. Tuin, MD Affiliation: University Medical Center Groningen Authors: Tuin AJ, Domerchie PN, Schepers RH, Willemsen JC, Dijkstra PU, Spijkervet FK, Vissink A, Jansma J

2:16 pm

27
DERMAL SCAFFOLD AND ENHANCED FRACTIONAL CELLS USAGE FOR UPPER EXTREMITY BURN CONTRACTURES AS AN ALTERNATIVE PARADIGM TO FREE TISSUE TRANSPLANTS TO OBTAIN FUNCTIONAL AND AESTHETIC TISSUE HEALING
Presenter: Mehmet Bozkurt, MD
Affiliation: Dr. Lutfi Kirdar Kartal Training and Research Hospital
Authors: Bozkurt M, Ceran F, Filinte GT, Basat SO, Guvercin E

2:24 pm

NOT PRESENTED

28
A DURABLE TISSUE ENGINEERED INJECTABLE FILLER THAT PROMOTES ADIPOGENESIS AND ANGIOGENESIS IN DEEP WRINKLES
Presenter: Todd N. McAllister, PhD
Affiliation: Cytograft
Authors: McAllister TN, Pena G, Rodriguez R, Dusserre N, Lheureux N, Wiegering CE

2:32 pm

29
THE SURFACE AREA OF LIPOSUCTION CANNULA TIP PORT HAS AN EFFECT ON THE NUMBER OF SVF CELLS PER CC OF ADIOSE TISSUE HARVEST
Presenter: Ricardo L. Rodriguez, MD
Affiliation: Cosmeticsurgnet
Authors: Rodriguez RL, Dos Anjos S, Stambolstyan G, Llull R

2:40 pm

30
A SYSTEMATIC LITERATURE REVIEW ON THE USE OF FAT GRAFTING AND REGENERATIVE CELLS IN BURN WOUNDS AND SCARS
Presenter: Alexandra Conde-Green, MD
Affiliation: Rutgers New Jersey Medical School and Johns Hopkins University
Authors: Conde-Green A, Marano AA, Lee ES, Reisler T, Price LA, Milner SM, Granick MS

2:48 pm

WITHDRAWN

31
VOLUMETRIC ESTIMATION OF AUTOLOGOUS FAT FOR AUGMENTATION OF CONTOUR DEFECTS OF FACE
Presenter: Shilpi Bhadani, MCh
Affiliation: GNH Hospital
Authors: Bhadani S, Sarabahi S, Tiwari VK, Arora S

2:56 pm

32
THE USE OF MICRO FRACTURED FAT FOR GRAFTING OF THE PERIORBITAL REGION: 46 CONSECUTIVE CASES
Presenter: ChiaChi Kao, MD
Affiliation: Private Practice Santa Monica California
Author: Kao C

3:04 pm

33
EXPANDED MESENCHIMAL STEM CELLS FOR FAT GRAFTING - A NEW TECHNIQUE
Presenter: Maurizio Viel, MD
Affiliation: London Center for Aesthetic Surgery Gulf/Bioscience Clinic Dubai
Authors: Viel M, Viel R, Mucci G

3:12 pm

NOT PRESENTED

34
DEVELOPMENT AND EVALUATION OF THE AIRTIGHT, MINIMAL-INVASIVE AND FAST DEVICE HARVESTING ADIPOSE TISSUE FOR AUTOLOGOUS FAT GRAFTING
Presenter: Hongwei Liu, MD, PhD
Affiliation: The First Affiliated Hospital of Jinan University
Author: Liu H

2:00 - 3:30 pm

Concurrent Free Paper Session 2 - Basic Research: Soft Tissue
Moderators: Dmitry Traktuev, PhD & Hebert Lamblet, MD

2:00 pm

35
EVALUATION OF BIOMATERIAL, ADIPOCYTES AND ADSC INTERACTIONS IN PURE AUTOLOGOUS FAT GRAFTING MODEL THROUGH 7TMRI
Presenter: YoShen Chen, MD
Affiliation: National Taiwan University and Far Eastern Memorial Hospital
Authors: Chen YS, Hsueh YS, Chen YY, Tai HC, Lin FH

2:08 pm

NOT PRESENTED

36
MICROFAT AND NANOFAT GRAFTING MIXED WITH PRP: BASIC RESEARCH AND CLINICAL APPLICATIONS
Presenter: Mario Goisis, MD, MS
Affiliation: Doctors Equipe Milano
Authors: Di Petrillo A, Goisis M, Mele S, Guareschi M, Scarpa A

2:16 pm

37
CHARACTERIZATION AND COMPARISON OF FRESH STROMAL VASCULAR FRACTION FROM HUMAN SUBCUTANEOUS, OMENTAL AND PERIRENAL ADIPOSE TISSUE
Presenter: Jeremy Magalon, PharmD
Affiliation: Culture and Cell Therapy Unit/INSERM CBT1409
Authors: Magalon J, Arnaud L, Lyonnet L, Giraudo L, Chateau AL, Aboudou H, Bertrand B, Boissier R, Hardwigsen J, Casanova D, Karsenty G, Lechevallier E, Paul P, Veran J, Sabatier F

2:24 pm

38
ASSESSMENT OF THE IMPACT OF PRO-INFLAMMATORY CYTOKINES ON GENE EXPRESSION, SECRETION PROFILES AND ENDOTHELIAL NETWORKS WITHIN HUMAN TISSUE-ENGINEERED ADIPOSE SUBSTITUTES
Presenter: Julie Fradette, PhD
Affiliation: LOEX Centre de Recherche du CHU de Quebec Laval University
Authors: Safoine M, Aubin K, Mayrand D, Maux A, Audet-Casgrain MA, Fradette J

2:32 pm

39
COMPARISON OF COLLAGENASE NEUTRALIZATION METHODS FOR ADIPOSE DERIVED CELL ISOLATION
Presenter: Hulan Shang, MS
Affiliation: University of Florida
Authors: Shang H, Yang N, Katz A

2:40 pm

40
INVESTIGATING EARLY VASCULAR SPROUTING AND FAT GRAFT VOLUME RETENTION IN BOTH LIPOASPIRATED AND EXCISED EN BLOC ADIPOSE TISSUE
Presenter: Ava G. Chappell, BA
Affiliation: University of Massachusetts Medical School
Authors: Chappell AG, Lujan-Hernandez J, Rojas-Rodriguez R, Perry DJ, Min SY, Corvera S, Lalikos JF

2:48 pm

41
TISSUE FLAP PRECONDITIONING WITH ADIPOSE DERIVED STEM CELLS AND HYPERBARIC OXYGEN TREATMENT: A GUINEA PIG MODEL
Presenter: Nicole A. Gaid, MB BCh BAO, MMI
Affiliation: University of California San Diego
Authors: Gaid NA, Tenenhaus M, Grover I, Hayes S

2:56 pm

42
ADIPOSE TISSUE RETENTION VIA MICROSPHERE DRUG DELIVERY SYSTEM
Presenter: Arta Kelmendi-Doko, MD
Affiliation: University of Pittsburgh
Authors: Kelmendi-Doko A, Klett K, Wang SH, Marra KG, Rubin JP

3:04 pm

43
FEA ANALYSIS PREDICTS TAPERED STUMP ARCHITECTURE FOR BKA SHAPING WITH AUTOLOGOUS FAT TRANSFER
Presenter: Jessica Green, MD, PhD
Affiliation: Atlanta Medical Center
Authors: Green J, Reddy PR

3:12 pm

44
APPLICATION OF A NOVEL EX VIVO MODEL FOR EVALUATING STEM CELL FATE IN A MICROVASCULAR NETWORK
Presenter: Mohammad S. Azimi, MS
Affiliation: Tulane University
Authors: Azimi MS, Strong AL, Bunnell BA, Murfee WL

2:00 - 3:30 pm	**Concurrent Free Paper Session 3 - Applied Research: Soft Tissue** Moderators: Kevin Hopkins, MD, FACS & Bruce Bunnell, PhD
2:00 pm	**45** **NOVEL PRIMING METHOD FOR ADIPOSE-DERIVED STEM CELLS FROM THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MOUSE MODEL RESTORES ANTI-INFLAMMATORY PROFILE** Presenter: Rachel M. Wise, MS Affiliation: Tulane University Authors: Wise RM, Bowles AC, Bunnell BA
2:08 pm	**46** **ADIPOSE STROMAL CELLS FROM DONORS WITH METABOLIC SYNDROME CAN BE VALUABLE THERAPEUTIC MATERIAL FOR TREATMENT OF ACUTE RESPIRATORY DISTRESS SYNDROME** Presenter: Natalia V. Bogatcheva, PhD Affiliation: Indiana University Authors: Bogatcheva NV, Lu H, Merfeld-Clauss S, Traktuev DO, March KL
2:16 pm	**47** **LIPOFILLING, INTERNAL EXPANSION AND COMMON SENSE BASED ON TISSUE ENGINEERING FINDINGS IN AUTOLOGOUS BREAST RECONSTRUCTION** Presenter: Filip Stillaert, MD Affiliation: University Hospital Gent Author: Stillaert F
2:24 pm	**48** **LIPOASPIRATE AND ADIPOSE STEM CELLS AS POTENTIAL THERAPEUTICS FOR CHRONIC SCARS** Presenter: Dylan J. Perry, BA Affiliation: University of Massachusetts Medical School Authors: Perry DJ, Lujan Hernandez J, Chin MS, Min SY, Chappell AG, Appasani R, Rojas Rodriguez R, Corvera S, Lalikos JF
2:32 pm	**49** **IN VITRO EVALUATION OF PARTICULATE HUMAN ADIPOSE-DERIVED MATRIX SEEDED WITH HUMAN ADIPOSE-DERIVED STEM CELLS: DIFFERENTIATION INTO ADIPOCYTES AND FORMATION OF EXTRACELLULAR MATRIX** Presenter: Evangelia Chnari, PhD Affiliation: Musculoskeletal Transplant Foundation Authors: Huang YC, Lorenzo BC, Schilling BK, Kokai LE, Marra KG, Rubin JP, Chnari E
2:40 pm	**50** **IMPROVING FLAP SURVIVAL WITH FRESH AND CULTURE-EXPANDED ADIPOSE-DERIVED STROMAL CELLS IN A XENOTRANSPLANTATION RAT MODEL** Presenter: Navid M. Toyserkani, MD Affiliation: Odense University Hospital Authors: Toyserkani NM, Jensen CH, Sheikh SP, Sorensen JA
~~2:48 pm~~ **WITHDRAWN**	**51** **ACCESSING THE ONCOLOGICAL RISK OF FAT GRAFTING TO THE BREAST THROUGH IMMUNOHISTOCHEMISTRY AND GENE EXPRESSION VALUES OF CD68, KI67, ER+(1D5) AND PAI-1** Presenter: Francisco Claro Jr., MD Affiliation: State University of Campinas UNICAMP Authors: Claro F, Moreira LR, Morari J, Sarian LO, Pinto GA, Velloso LA, Pinto-Neto AM
2:56 pm	**52** **COMPARISON OF CURRENT PROTOCOLS FOR AUTOLOGOUS FAT GRAFTING** Presenter: Vincent Hivernaud, MS Affiliation: INSERM U791 LIOAD Authors: Hivernaud V, Lefourn B, Guicheux J, Girard AC, Weiss P, Roche R

3:04 pm	**53** **PAPER AS A SUITABLE MATRIX FOR ASC DELIVERY INTO WOUNDS** Presenter: Michael A. Zieger, PhD Affiliation: Indiana University School of Medicine Authors: Zieger MA, Rahimi R, Ochoa M, Campana G, Ziaie B, Sood R
3:12 pm	**54** **EVALUATION OF A POLOXAMER HYDROGEL FOR THE DELIVERY OF ADIPOSE-DERIVED STEM CELLS IN THE CONTEXT OF PERIPHERAL NERVE REGENERATION** Presenter: Kassandra L. O'Brien, BS Affiliation: University of Pittsburgh Authors: O'Brien KL, Bliley JM, Havis EM, James IB, Wang SS, Sivak WN, Rubin JP, Marra KG
3:30 - 4:00 pm	Coffee Break and Exhibits
4:00 - 5:00 pm	**Small Panel Discussion 1: Clinical Innovations in Fat Grafting and Stem Cells** Chair: J. Peter Rubin, MD, FACS Panelists: Sydney Coleman, MD *Fat Grafting for Aesthetic and Reconstructive Indications* Kamran Khoobehi, MD, FACS *Aesthetic Fat Grafting* Ramon Llull, MD, PhD *Stem Cells and Fat Grafting*
4:00 - 5:00 pm	**Small Panel Discussion 2: Challenges for Optimal Tissue Integration: From *In Vitro* to *In Vivo*** Chair: Julie Fradette, PhD Panelists: Walter Lee Murfee, PhD *Manipulating Microvascular Growth with MSCs: Do We Really Know What They Do?* Susan Thibeault, PhD *MSC's Interaction with Immune Cells and Mechanobiology* Daniel Hayes, PhD *Improving Graft Delivery: Semi-Synthetic Adipose Grafts* Stephanie J. Bryant, PhD *The Foreign Body Response to Cell-Laden Biomaterials*
4:00 - 5:00 pm	**Small Panel Discussion 3: Hot Topics & Innovations** Chair: Jeffrey Gimble, MD, PhD Panelists: Jeff W. Bulte, PhD *Tracking Stem Cells In Vivo* Annie C. Bowles, MS & Anthony D. Foster, PhD *Adipose-derived Cell Therapy in Inflammatory Diseases* Debasis Mondal, PhD *The Exosome and Micro RNA - New Insights Into the Adipose Secretome*
5:00 - 6:30 pm	**Poster Session & Welcome Reception**

Saturday, November 7, 2015

7:00 - 8:00 am	Continental Breakfast - Exhibit Hall
8:00 - 9:00 am	**IFATS Members Meeting**
9:15 am - 1:30 pm	**REGULATORY AFFAIRS PANEL**
9:15 - 9:30 am	**Opening Remarks** Moderator: Stuart K. Williams, PhD
9:30 - 9:55 am	**Adult Stem Cells – Voluntary Standards to Regulatory Approval** Speaker: Phyllis I. Warkentin, MD - *University of Nebraska Medical Center/FACT*
9:55 - 10:20 am	**FDA Update: Recent Guidance - Future Hindrance? - FDA/US Perspective** Speaker: Mary Ann Chirba, JD, DSc, MPH - *Boston College Law School*
10:20 - 10:45 am	**Regulatory Affairs for Adult Stem Cells - European Perspective** Speaker: Marco Helder, PhD - *VU University Medical Center, Amsterdam*
10:45 - 11:10 am	**Regulatory Approval for Use of Adipose Stem Cells in Humans - Academic Perspective** Speaker: J. Peter Rubin, MD, FACS - *University of Pittsburgh*
11:10 am - 11:35 pm	**The Role of Pre-clinical Studies to Establish a Regulatory Strategy - Corporate Perspective** Speaker: Stuart K. Williams, PhD - *University of Louisville*
11:35 - 12:00 pm	**Regulatory Approval for Use of Adipose Stem Cells in Humans - Corporate Perspective** Speaker: Shah Rahimian, MD - *Antria*
12:00 - 12:25 pm	**FDA and Regulation of Adipose Tissue Procedures - ASPS Perspective** Speaker: Richard D'Amico, MD - *American Society of Plastic Surgeons*
12:25 - 1:00 pm	**Panel Discussion - All Speakers**
1:00 - 2:30 pm	Lunch
2:30 - 3:15 pm	**Concurrent Free Paper Session 4 - Regulatory Affairs/Innovation & Hot Topics/Applied Research** Moderators: Alexandra Conde-Green, MD & Marco Helder, PhD
2:30 pm **WITHDRAWN**	**55** **~~RISK-BASED APPROACH: A "GENERIC" TEMPLATE FOR ADVANCED THERAPY MEDICINAL PRODUCTS~~** Presenter: AnneLine Chateau, PhD Affiliation: Assistance Publique des Hopitaux de Marseille Authors: Chateau AL, Veran J, Magalon J, Blanchet L, Giraudo L, Mendizabal H, Sabatier F
2:38 pm	**56** **PHENOTYPIC ANALYSIS OF STROMAL VASCULAR FRACTION AFTER MECHANICAL SHEAR: STRESS-INDUCED PROGENITOR TRANSDIFFERENTIATION?** Presenter: Derek A. Banyard, MD, MBA Affiliation: University of California Irvine Authors: Banyard DA, Widgerow AD, Wirth GA, Paydar KZ, Evans GR
2:46 pm	**57** **IMMEDIATE LARGE-VOLUME GRAFTING OF AUTOLOGOUS FAT TO THE BREAST FOLLOWING IMPLANT REMOVAL: FIVE YEARS EXPERIENCE** Presenter: Saad Dibo, MD Affiliation: MA Clinic Authors: Dibo S, Abboud M
2:54 pm	**58** **PHYSICAL, BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF FAT GRAFT PROCESSED VIA DIFFERENT METHODS** Presenter: Carrie H. Fang, PhD Affiliation: LifeCell Corporation An Acelity Company Authors: Fang CH, Collins S, Huang LT, Li H, Patel P, Wan H, Xu H

3:02 pm

59
ADIPOSE-DERIVED STEM CELLS: A COMPARISON OF ASC ISOLATION USING LABORATORY GRADE VERSUS MEDICAL GRADE COLLAGENASE
Presenter: Anna Wilson, MBChB, MRCS, MSc
Affiliation: UCL RAFT
Authors: Wilson A, Boyd A, Butler P, Seifalian A

2:30 - 3:15 pm

Concurrent Free Paper Session 5 - Basic Research: Musculoskeletal
Moderators: Ricardo Rodriguez, MD & & Stig-Frederik Trojahn Kolle, PhD NEW

2:30 pm

60
OSTEOINDUCTIVE EFFECTS OF GLYCEOLLINS ON ADIPOSE STROMAL CELLS
Presenter: Marjorie E. Bateman, BS
Affiliation: Tulane University School of Medicine
Authors: Bateman ME, Strong AL, Hunter RS, Burow ME, Boue SM, Hayes DJ, Bunnell BA

2:38 pm

61
DIFFERENT CULTURE CONDITIONS MODULATE IMMUNOLOGICAL PROPERTIES OF ADIPOSE STEM CELLS
Presenter: Mimmi Patrikoski, MSc
Affiliation: University of Tampere Finland
Authors: Patrikoski M, Sivula J, Mannerstrom B, Miettinen S

2:46 pm

62
PHYTOESTROGEN GLYCINOL ENHANCES OSTEOGENESIS AND ATTENUATES THE EFFECTS OF AGING ON BONE MESENCHYMAL STEM CELLS
Presenter: Robert B. Jones Jr., BA
Affiliation: Tulane University School of Medicine
Authors: Jones RB, Strong AL, Boue SE, Burow ME, Bunnell BA

2:54 pm

63
A NOVEL METHOD USING 3D PRINTING TO MAINTAIN IMPLANT SHAPE FOR EAR CARTILAGE RECONSTRUCTION
Presenter: Marco Helder, PhD
Affiliation: VU Medical Center
Authors: Visscher D, Bos EJ, Helder MN, Van Zuijlen PM

3:02 pm

64
THE ROLE OF MED31 IN REGULATING STEM CELL STATE
Presenter: Jamie J. Newman, PhD
Affiliation: Louisiana Tech University
Authors: Newman JJ, Beadle EP, Bunnell BA

2:30 - 3:15 pm

Concurrent Free Paper Session 6 - Basic Research: Soft Tissue & Musculoskeletal
Moderators: Lauren Kokai, PhD & J. Peter Rubin, MD, FACS

2:30 pm

65
OPTIMIZATION OF ADIPOSE PARTICLE SIZE FOR AUTOLOGOUS LIPOFILLING: THE IMPACT OF PARTICLE DIAMETER ON TISSUE METABOLISM, NECROSIS AND LONG TERM IN VIVO SURVIVAL
Presenter: Lauren E. Kokai, PhD
Affiliation: University of Pittsburgh
Authors: Kokai LE, Jones TL, Marra KG, Rubin JP

2:38 pm

WITHDRAWN

~~**66**~~
~~**THERAPEUTIC FAT GRAFTING IN A BREAST CANCER XENOGRAFT MODEL**~~
~~Presenter: Abigail M. Cochran, MD~~
~~Affiliation: Southern Illinois University School of Medicine~~
~~Authors: Cochran AM, Gupta AG, Yang MY, Cosenza NC, Richensperger JR, Harrison CH, Cox LC, De La Garza MD, Neumeister MN~~

2:46 pm

67
ENGINEERING VOLUME STABLE ADIPOSE TISSUE USING COMPOSITE CONSTRUCTS MADE FROM POROUS SCAFFOLDS AND FIBRIN HYDROGEL - A MATTER OF CELLS
Presenter: Petra Bauer-Kreisel, PhD
Affiliation: University of Wuerzburg
Authors: Bauer-Kreisel P, Fischer R, Wittmann K, Regn S, Wiese H, Maier G, Storck K, Blunk T

2:54 pm

68
INVESTIGATING THE MATRIX CHEMISTRY OF HUMAN ADIPOSE-DERIVED STEM CELLS: THE FIRST STEP TOWARD CREATING A SCAFFOLD FOR OPTIMAL ADIPOSE TISSUE ENGINEERING WITH A BIOMIMETIC APPROACH

WITHDRAWN

Presenter: Mary Frimpong, BSc (Hons), MBBS, MRCS
Affiliation: University of Aberdeen
Authors: Frimpong M, De Bari C, Curnier A

3:02 pm

69
CHANGES IN GENE EXPRESSION FOLLOWING CO-CULTURE OF ADIPOSE-DERIVED STROMAL/STEM CELLS WITH A FLOWABLE PLACENTAL CONNECTIVE TISSUE MATRIX
Presenter: Daniel R. Kuebler, PhD
Affiliation: Franciscan University of Steubenville
Authors: Kuebler DR, McLaughlin M, Damico M, Yeager R, Cunningham L

3:15 - 3:45 pm

Coffee Break and Exhibits

3:45 - 4:30 pm

Concurrent Free Paper Session 7 - Basic Research: Soft Tissue
Moderators: Petra Bauer-Kreisel, PhD & Adam Katz, MD, FACS

3:45 pm

70
ADIPOSE-DERIVED STROMAL/STEM CELLS ARE POSSIBLE RESERVOIRS FOR HUMAN CYTOMEGALOVIRUS
Presenter: Kevin Zwezdaryk, PhD
Affiliation: Tulane University
Authors: Zwezdaryk K, Ferris MB, Bunnell BA, Gimble JM, Sullivan DE

3:53 pm

71
NATURAL KILLER CELLS DIFFERENTIATE HUMAN ADIPOSE-DERIVED STEM CELLS AND MODULATE THEIR ADIPOGENIC POTENTIAL

WITHDRAWN

Presenter: Kameron Rezzadeh, BA
Affiliation: David Geffen School of Medicine at UCLA
Authors: Rezzadeh K, Hokugo A, Jewett A, Segovia LA, Kozlowska A, Zuk P, Zhou S, Jarrahy R

4:01 pm

72
EFFECT OF THERMAL PRECONDITIONING OF ADIPOSE TISSUE DERIVED STEM CELLS ON CRYOPRESERVATION

NOT PRESENTED

Presenter: Mullashahensha Shaik, MS
Affiliation: LSU
Authors: Shaik M, Gimble JM, Devireddy RV

4:09 pm

73
ANALYSIS OF FATTY ACIDS COMPOSITION IN HUMAN SUBCUTANEOUS ADIPOSE TISSUE

WITHDRAWN

Presenter: Natalie Khramtsova, PhD
Affiliation: Perm State University of Medicine
Authors: Khramtsova N, Plaksin SA

4:17 pm

74
THE IMPACT OF HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS ON BREAST CANCER CELLS - IMPLICATIONS FOR CELL-ASSISTED LIPOTRANSFERS IN BREAST RECONSTRUCTION
Presenter: Eva Koellensperger, MD
Affiliation: Heidelberg University Hospital
Authors: Koellensperger E, Bonnert LC, Marx J, Zoernig I, Sandmann S, Germann G, Leimer U

3:45 - 4:30 pm

Concurrent Free Paper Session 8 - Basic Research: Soft Tissue
Moderators: Hebert Lamblet, MD & Torsten Blunk, PhD

3:45 pm

75
CHARACTERIZATION OF ADIPOSE-DERIVED STROMAL VASCULAR FRACTION (SVF) CELLS ISOLATED WITH A NOVEL DISPOSABLE, CLOSED-SYSTEM DEVICE

NEW Presenter

Presenter: Christian Brown NEW
Affiliation: University of Florida
Authors: Shang H, Yang N, Yu A, Katz A

3:53 pm

76
EXTRACELLULAR MATRIX COMPOSITION AND DYNAMICS DURING ADIPOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STROMAL CELLS IN 3D CULTURE
Presenter: Torsten Blunk, PhD
Affiliation: University of Wuerzburg
Authors: Blunk T, Hoefner C, Wittmann K, Muhr C, Becker M, Bauer-Kreisel P

4:01 pm

WITHDRAWN

77
ANTI-ADIPOGENIC EFFECT OF OXY133 ON PRE-ADIPOCYTE COMMITMENT
Presenter: Akishige Hokugo, DDS, PHD
Affiliation: University of California Los Angeles
Authors: Hokugo A, Segovia LA, Rezzadeh K, Jarrahy R

4:09 pm

78
EVALUATION OF A SERUM-FREE MEDIUM FOR THE PRODUCTION OF TISSUE-ENGINEERED NATURALLY-DERIVED HUMAN CONNECTIVE SUBSTITUTES
Presenter: Meryem Safoine, BSc
Affiliation: LOEX Centre de recherche du CHU de Quebec Universite Laval
Authors: Safoine M, Aubin K, Trottier V, Fradette J

4:17 pm

79
THE ARCHITECTURE OF FAT GRAFTING: WHAT LIES BENEATH THE SURFACE
Presenter: Debra A. Bourne, MD
Affiliation: University of Pittsburgh Medical Center
Authors: Bourne DA, James IB, Wang SS, Marra KG, Rubin JP

3:45 - 4:30 pm

Concurrent Free Paper Session 9 - Basic Research: Soft Tissue
Moderators: Julie Fradette, PhD & Ricardo Rodriguez, MD

3:45 pm

80
NOX1-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION DICTATES THE PHENOTYPIC DIFFERENCE BETWEEN MESENCHYMAL STROMAL CELLS DERIVED FROM DIFFERENT FAT TISSUES
Presenter: Nir Shani, PhD
Affiliation: Tel Aviv Sourasky Medical Center
Authors: Shani N, Sela M, Tirza G, Ravid O, Volovitz I, Solodeev I, Friedman O, Krelin Y, Zipori D, Gur E

3:53 pm

81
ROLE OF NOTCH SIGNALING IN GLYCOLYSIS REGULATION OF ADIPOSE-DERIVED MESCENCHYMAL STEM CELLS UNDER HYPOXIC CONDITIONS
Presenter: Hiroyuki Moriyama, PhD
Affiliation: Pharmaceutical Research and Technology Institute
Authors: Moriyama H, Moriyama M, Ishihara S, Okura H, Ozawa T, Matsuyama A, Hayakawa T

4:01 pm

82
AN UPDATED EVIDENCE-BASED REVIEW OF ADIPOSE STEM CELL THERAPY IN CANCER RECONSTRUCTION
Presenter: Michael Alperovich, MD, MSc
Affiliation: NYU Langone Medical Center
Authors: Alperovich M, Lee ZH, Chiu ES

4:09 pm

NOT PRESENTED

83
EVALUATION OF THE EFFECTS OF ANTI-FIBROTIC AGENT ON ADIPOGENESIS AND FIBROSIS IN AN IN VIVO CHAMBER MODEL
Presenter: Wali S. Boodhun, MBBS
Affiliation: The OBrien Institute
Authors: Boodhun WS, Tan B, Han XL, Morrison W, Mitchell G, Abberton K

4:17 pm

WITHDRAWN

84
CHARACTERIZATION OF ADIPOSE-DERIVED STEM CELLS ISOLATED FROM SUPERFICIAL AND DEEP SUBCUTANEOUS FAT: EFFECTS OF CRYOPRESERVATION, BMI AND DONOR-AGE
Presenter: Wanda Lattanzi, MD PhD
Affiliation: Universita' Cattolica del Sacro Cuore School of Medicine
Authors: Quercia V, Pino V, Barba M, Di Pietro L, Visconti G, Di Taranto G, Salgarello M, Lattanzi W

6:00 - 9:00 pm	**A Taste of New Orleans -** *Sponsored by the Musculoskeletal Transplant Foundation* *Muriel's - Jackson Square*

Sunday, November 8, 2015

7:00 - 8:00 am	Continental Breakfast - Exhibit Hall
8:00 - 8:05 am	**Introductory Remarks** Bruce Bunnell, PhD
8:05 - 8:35 am	**Keynote Speaker**
	Regeneration and Stem Cells in the Spiny Mouse: A Unique Mammalian Regeneration Model Malcolm Maden, PhD *Professor, Department of Biology, University of Florida*
8:35 - 9:45 am	**Plenary Session 4: Clinical Research: Soft Tissue & Musculoskeletal/Innovation & Hot Topics** Moderators: Brian Johnstone, PhD & Jeffrey Gimble, MD, PhD
8:35 am	**85** **RECONSTRUCTION FOLLOWING CRANIOFACIAL TRAUMA WITH STROMAL VASCULAR FRACTION ENRICHED FAT GRAFTS: CORRELATING IN VITRO FINDINGS WITH CLINICAL OUTCOMES** Presenter: Jacqueline M. Bliley, BS, MS Affiliation: University of Pittsburgh Authors: Bliley JM, Bourne DA, James IB, Sivak WN, Wang S, Schroth R, Coleman S, Donnenberg A, Donnenberg V, Marra KG, Rubin JP
8:45 am	**86** **ADRCS AS AN ALTERNATIVE TO LATERAL INTERNAL SPHINCTEROTOMY IN PATIENTS WITH ANAL FISSURES** Presenter: Katarina Andjelkov, MD, PhD Affiliation: BelPrime Clinic Authors: Andjelkov K, Sforza M, Petrovic J, Zaccheddu R, Krivokapic Z
8:55 am	**87** **EXPANDING ADIPOSE-DERIVED STEM CELLS FOR HIGH-DOSE ENRICHMENT OF FAT GRAFTS TO THE HUMAN BREAST** Presenter: Peter V. Glovinski, MD Affiliation: Copenhagen University Hospital Rigshospitalet Authors: Glovinski PV, Herly M, Koelle SF, Svalgaard J, Elberg JJ, Drzewiecki KT, Fischer-Nielsen A
9:05 am	**88** **USE OF AUTOLOGOUS ASCS TO RECONSTRUCT CRANIO-MAXILLOFACIAL BONE DEFECTS OF 24 PATIENTS** Presenter: Susanna Miettinen, PhD Affiliation: REGEA Institute of Biomedical Technology University of Tampere Author: Miettinen S
9:15 am	**89** **ASC TREATMENT REDUCES THE SEVERITY OF MURINE GVHD THROUGH EARLY IMMUNOMODULATORY EFFECTS** Presenter: Anthony D. Foster, PhD Affiliation: Navy Medical Research Center Author: Foster AD, Clark N, Gimble JM, Davis TA
9:25 am **MOVED TO CONCURRENT SESSION 4 @ 2:30 PM**	**90** **SHORT-TERM CULTIVATED ASC-LIKE CELLS (SCALC) THE NATIVE STEM CELL FOR MEDICAL CURE** Presenter: Michael Krueger, MD.... NEW Presenter Affiliation: Klinik Sanssouci Author: Haschemi A, Born S, Meutsch J, Westphal J, Hartjen P, Smeets R, Krueger M
9:45 - 10:15 am	Coffee Break and Exhibits

10:15 - 11:25 am	**Plenary Session 5: Basic Research & Applied Research: Musculoskeletal** Moderators: Marco Helder, PhD & Ricardo Rodriguez, MD
10:15 am	**91** **ADIPOSE-DERIVED STEM CELL CONDITIONED MEDIUM (ASC-CM) PROMOTES REVASCULARIZATION OF ISCHEMIC TISSUES IN A RABBIT MODEL OF PERIPHERAL ARTERY DISEASE** Presenter: Brian Johnstone, PhD Affiliation: Indiana University School of Medicine Authors: Johnstone B, Jurcikova J, Lassak O, Vitkova K, Pavliska L, Porubova L, Buszman PP, Krauze A, Fernandez C, Jaluvka F, Spackova I, Merfeld-Clauss S, Traktuev DO, March KL, Prochazka V
10:25 am	**92** **TREATMENT OF DEGENERATIVE JOINT DISEASE (DJD) IN HORSES BY A MIXTURE OF PRP AND MICROFAT** Presenter: Guy Magalon, MD Affiliation: Amu Aix Marseille University Authors: Magalon G, Bembo F, Magalon J, Eraud J, Sabatier F
10:35 am **NOT PRESENTED**	**93** ~~**ENCAPSULATED HUMAN ADIPOSE DERIVED STEM CELLS IN AN INJECTABLE THIOL-ACRYLATE BASED SCAFFOLD FOR BONE TISSUE ENGINEERING**~~ Presenter: Anoosha Forghani, PhD Affiliation: Louisiana State University Authors: Forghani A, Garber L, Chen C, Devireddy RV, Pojman J, Hayes DJ
10:45 am	**94** **PRO-INFLAMMATORY AND OSTEO-INDUCTIVE CYTOKINES INDUCE BONE-RELATED EARLY RESPONSE GENES IN ADULT HUMAN ADIPOSE STROMAL CELLS** Presenter: Elizabeth C. Martin, PhD Affiliation: Tulane University Authors: Martin EC, Qureshi AT, King A, Krause P, Lee O, Dasa V, Freitas M, Forsberg JA, Elster E, Davis T, Gimble J
10:55 am	**95** **MICRORNA MEDIATED INHIBITION OF OSTEOBLAST MEDIATED BONE FORMATION** Presenter: Ammar T. Qureshi, PhD Affiliation: Naval Medical Research Center Authors: Qureshi AT, Martin EC, Gimble JM, Forsberg JA, Hayes DJ, Davis TA
11:05 am	**96** **IN VITRO CHARACTERIZATION AND BIOLOGICAL EVALUATION OF A THIOLATED ADIPOSE-DERIVED EXTRACELLULAR MATRIX HYBRID HYDROGEL FOR ADIPOSE-DERIVED STEM CELL DIFFERENTIATION** Presenter: John N. Poche, BS Affiliation: Louisiana State University Authors: Poche JN, Totaro NP, Hayes DJ
11:15 am	**97** **DEMINERALIZED HUMAN BONE MATRIX (DBM) WITH SYNGENEIC ADULT ADIPOSE-DERIVED MULTIPOTENT STROMAL CELLS (ASCS) ACCELERATES SPINAL FUSION** Presenter: Mandi J. Lopez, DVM, MS, PhD Affiliation: Louisiana State University Authors: Lopez MJ, Fargason C, Kelly L, Del Piero F, Dasa V, King A
11:25 am	**Closing Remarks** Bruce Bunnell, PhD

NOTES

AUTHOR INDEX

PAPER PRESENTATIONS

in numerical order

1

XENOTRANSPLANTATION OF INTEGRA® AND HUMAN SPHEROIDS FROM ADIPOSE-DERIVED STEM CELLS (S-ASCS) PROMOTES CALVARIAL BONE PRODUCTION IN A RABBIT MODEL

Presenter: Luigi Montesano
Authors: Montesano L, Di Stefano AB, Giammona A, Maenza A, Belmonte B, Moschella P, Cassata G, Florena AM, Todaro M, Stassi G, Cordova A, Moschella F, Leto Barone AA
Universita degli Studi di Palermo

Background: Calvarial bone reconstruction after craniotomies may benefit from tissue engineering techniques to restore native form and protective function. In search for a viable scaffold that is readily available in the clinical setting, our team has recently investigated that differentiation of spheroids from Adipose-derived Stem Cells (S-ASCs) toward the osteogenic lineage promoted osteoblasts growth within Integra® in vitro. Furthermore, bone regeneration of small laminectomy defects in mice occurred in vivo when S-ASCs were seeded in three-dimensional Integra® scaffolds. The aim of this study is to evaluate the osteoinductive properties of S-ASCs over a larger and standardized calvarial bone defects in a xenogeneic animal model.

Methods: New Zealand white rabbits (n=20) were anesthetized and a 1x1cm partial thickness calvarial defect was performed. The following groups were included in the study: group I (control): no treatment (n=8); group II: Integra® alone (n=8); group III: Integra® + undifferentiated S-ASCs (n=3); group IV: Integra® + S-ASCs-derived osteoblasts (n=2). Dermal matrices were implanted in the defect and subsequently explanted on post-operative day 45. Animals were immunosuppressed with dexamethasone 2 mg/kg q8hrs. Bone formation was evaluated using immunohistochemistry (Novocastra CD56).

Results: All rabbits in control groups I and II underwent limited spontaneous bone regeneration in the periphery (group I) or at the bone-Integra® interface (group II) without full bone regeneration. In contrast, preliminary data showed extensive colonization of Integra® with promotion of bone regeneration by either undifferentiated human S-ASCs (Group III, n=3), and S-ASCs pre-differentiated toward the osteogenic lineage (Group IV,n=2). Ex vivo CD56 positivity demonstrated that human S-ASCs-derived osteoblasts, and not host osteoblasts, were responsible for bone regeneration in groups III and IV.

Conclusions: S-ASCs represent a heterogenous spheroid-like population of stem-like cells with enhanced multilineage differentiation potential. Our preliminary data in vivo suggests that S-ASCs seeded in a dermal matrix such as Integra® were able to promote osteoblast production when seeded either undifferentiated or pre-differentiated in the osteoblastic lineage.

2

CELL SHEETS DERIVED FROM ADIPOSE-DERIVED STEM CELLS ACCELERATED WOUND HEALING AND REDUCED TISSUE FIBROSIS IN A MURINE MODEL

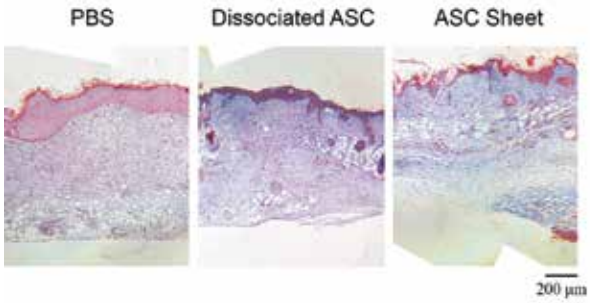
Presenter: Naichen Cheng, MD, PhD
Author: Cheng N
National Taiwan University Hospital

The abundance and easy accessibility of adipose-derived stem cell (ASC) have made it a promising candidate for stem cell therapies. However, transplantation of dissociated ASCs is frequently associated with early cell death with limited therapeutic effects. It has been proposed that the use of cell sheets is beneficial for cell transplantation, so we aimed to explore the regenerative capabilities of ASC sheets for cutaneous wound healing.

We stimulated extracellular matrix secretion of ASCs and fabricated cell sheets by treatment with a stable form of ascorbic acid, ascorbate 2-phosphate (A2-P). The ASC sheets was tested in a murine model of Mitomycin C-impaired cutaneous wound healing. The wound area was estimated by photography and wound tissue was harvested at predetermined time points for histology and immunohistochemistry. For in vitro study, we collected the conditioned medium of ASC sheet and apply in fibroblast and macrophage culture.

In the murine model of healing-impaired full-thickness cutaneous wound, faster wound healing was noted in the group that received ASC sheet treatment, and we observed significantly less tissue fibrosis with more engrafted ASCs. Immunohistochemical staining of the wound sections further revealed significantly lower TGF-beta1 and CD68 immunostaining in the ASC sheet-treated wound tissue. In vitro, fibroblasts treated by conditioned medium of ASC sheet exhibited lower expression of TGF-beta1 and alpha-smooth muscle actin. The inhibition of fibrosis was mediated by the higher secretion level of hepatocyte growth factor by ASC sheets. Moreover, the conditioned medium of ASC sheet significantly inhibited the transwell migration of macrophage.

In summary, ASC sheet can enhance wound healing and reduce wound tissue fibrosis in a murine model. Since decreasing tissue fibrosis may prevent the recurrence of ulcer, application of ASC sheet represents a promising approach treatment for difficult cutaneous wound.



3

A CELL EXPANSION PLATFORM GENERATED BY STROMAL CELLS PROMOTES MESENCHYMAL STEM CELL ISOLATION, EXPANSION, AND IL-10 EXPRESSION

Presenter: Rogelio Zamilpa
Authors: Zamilpa R, Flores I, Navarro MM, Griffey S
StemBioSys Inc.

Mesenchymal stem cell (MSC) applications for tissue repair and regeneration have shown great potential due to the ability of MSCs to immunomodulate the microenvironment and give to rise to multiple cell types. However, the critical barriers associated with such applications include obtaining high numbers and high quality, multipotent MSCs. We have developed a ready to use cell expansion platform generated by bone marrow stromal cells which we refer to as High Performance Micro-Environment (HPME). Scanning electron microscopy and mass spectrometry analyses indicate that the HPME is a three-dimensional, protein network composed primarily of extracellular matrix proteins. Culturing of adipose-, bone marrow-, and cord blood-derived MSCs on HPME improved the cell yield by a minimum of 2-fold when compared to tissue culture plastic. Specific for bone marrow MSCs, cells expanded on HPME had increased expression of stage specific embryonic antigen (SSEA)-4, a multipotent MSC marker (72±11% for ECM and 30±6% for tissue culture plastic). Interestingly, gene expression analysis demonstrated that bone marrow MSCs cultured on HPME had amplified expression of the anti-inflammatory cytokine interleukin-10 when compared to MSCs grown on plastic (n=3, 12±8 fold, $p<0.05$). These results indicate that the HPME is an ideal MSC expansion platform for therapeutic applications as it efficiently improves the yield and anti-inflammatory properties of MSCs.

4

COMPARISON OF THE IMMUNOMODULATING EFFECTS OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS AND ADIPOSE DERIVED STEM CELLS IN VITRO ACROSS DIFFERENT HLA BARRIERS

Presenter: Matthias Waldner, MD
Authors: Waldner M, Zhang W, Schweizer R, Almadori A, James I, Plock JA, Solari MG, Marra KG, Gorantla VS, Rubin JP
University of Pittsburgh Medical Center

Background: Mesenchymal stem cells deriving from bone marrow (BMSC) and adipose tissue (ASC) have immunomodulatory effects and low immunogenicity. Their ability to suppress innate and adaptive immune cells makes them ideal candidates for immunomodulative cytotherapy to reduce alloreactivity. BMSCs are already being used clinically for vascularized tissue allografts (VCA). ASCs represent an additional clinically useful and plentiful available source of cells for transplantation. The aims of this study are to assess and compare the immunomodulatory capacities of BMSCs and ASCs and their susceptibility to immunosuppressive drugs. This is the first study to compare paired ASC and BMSC specimens from the same human donors.

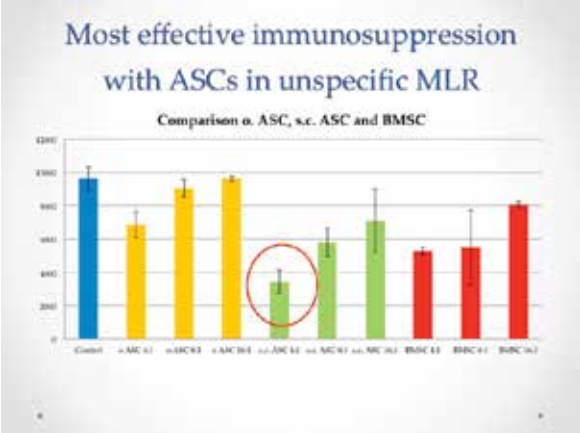
Methods: Tissue samples of omental fat, s.c. fat and bone marrow aspirate from 6 human organ donors where retrieved and MSCs isolated. Exclusion criteria for donors where age over 60 years or infectious serology. Cell characterization was performed using flow cytometry analysis. The effect of immunosuppressive drugs (Tacrolimus and Rapamycin) on survival and immunomodulative capacity of the MSCs were examined. In mixed lymphocyte reactions (MLR), the capacity of ASCs and BMSCs to suppress immune response was assessed and compared within individual donors. A combination of both cell types was tested in different MLR settings. Various HLA mismatched combinations of stimulating cells and responder cells were analyzed in co-culture with different concentrations of MSCs.

Results: The immunomodulating effects of all cell types where dose dependent and altered by the presence of immunosuppressants. Proliferation of responder cells was suppressed by both ASCs and BMSCs. Combination of both cell types resulted in a highly sufficient immunomodulation. Toxicity assays revealed a lower viability of s.c. ASCs after long time exposure to tacrolimus and a lower viability of s.c. ASCs and BMSCs after exposure to Rapamycin.

Conclusion: ASCs and BMSCs both showed effective immunomodulation across different HLA barriers. The combination of both cell types is a high efficient possibility to modulate the immune response. The large availability of adipose tissue and high cell yields make ASCs useful candidates for immunomodulative cell therapy in VCA.

4

COMPARISON OF THE IMMUNOMODULATING EFFECTS OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS AND ADIPOSE DERIVED STEM CELLS IN VITRO ACROSS DIFFERENT HLA BARRIERS



5

DEFINING THE ROLE OF ADIPOSE DERIVED STEM CELL TRANSPLANTATION IN A RADIATED TISSUE EXPANDER MODEL

Presenter: Abigail M. Cochran, MD
Authors: Cochran AM, Yang MY, De La Garza MD, Cosenza NC, Richensperger JR, Harrison CH, Cox LC, Berry NB, Neumeister MN
Southern Illinois University School of Medicine

Purpose: Fat grafting, commonly used to correct breast contour deformities, also seems to improve skin elasticity, scarring and sclerosis; the effects of which are attributed to Adipose Derived Stem Cells (ADSCs) within the transplant. ADSCs have also been shown to improve healing and treat radiation skin damage. However, the effects of ADSCs on expansion of irradiated skin remains unclear. We hypothesize that ADSC transplantation promotes tissue expansion of irradiated skin by differentiating into epidermal and dermal components.

Methods: A female Sprague Dawley (SD) tissue expansion model was used. Tissue expanders were placed subcutaneously on the dorsum of the rat, and weekly expansion ensued. Media: DMEM or ADSC from male SD rats was injected at 0, 14, 28 and 42 days. Three groups of animals (n=16) were examined: sham (no radiation, DMEM injection), control (radiation, DMEM injection) and treatment (radiation, ADSC injection). Four rats from each group were sacrificed at 7, 14 and 28 days. The final four rats were expanded to 60cc before undergoing flap surgery; 14 days later they were sacrificed. Skin was taken for histological analysis with H&E and trichrome staining. ADSC migration was observed via immunohistochemistry CM-Dii staining.

Results: Subcutaneous ADSC injection with tissue expanders resulted in thicker, more cellular epidermis and decreased fibrosis and inflammation compared to the control. At the time of expander removal, a larger surface area of expanded skin was observed in the ADSC group. ADSCs were present in epidermal and dermal and components including hair follicles, sweat glands and sebaceous glands by CMDii staining. Furthermore, ADSCs present at the time of flap surgery demonstrated continued proliferation at the time of sacrifice 14 days later.

Conclusion: ADSC survive radiation exposure in a tissue expander model. Subcutaneous ADSC injection promotes tissue regeneration by differentiating into epidermal and dermal components during tissue expansion post-radiation.

6

PARACRINE INTERACTIONS BETWEEN ADIPOSE-DERIVED STEM CELLS AND BREAST CANCER CELLS

Presenter: J. Peter Rubin, MD, FACS
Authors: Schweizer R, Tsuji W, Plock JA, Marra KG, Rubin JP
University Hospital Zurich

Aims: Autologous fat grafting is a promising method for breast reconstruction due to ease of procurement, natural appearance, and low immuno-response. Fat graft implementation with Adipose-derived Stem Cells (ASCs) has been proposed to enhance fat retention. However, the interplay between ASCs and breast cancer cells (BCCs) is still controversial and of utmost importance in the post-cancer setting. Epithelial-mesenchymal transition (EMT) has been investigated for a variety of tumors and thought to be involved in malignant transformation or progression. Herein we investigate the effects of human ASCs on human BCCs and whether they may induce EMT.

Methods: BT-474 and MDA-MB-231 BCC lines were co-cultured for 7 days using transwell basket systems to assess the paracrine interaction between ASCs and BCCs. Various ASC to BCC ratios were seeded and human fibroblasts used as a control. Change in morphology was assessed by microscopic evaluation. Any proliferative effect on BCCs was investigated by AlamarBlue. Co-culture supernatants were analyzed by ELISA to detect soluble factors involved in migration, inflammation and angiogenesis. RT-PCR was performed for EMT marker assessment.

Results: BT-474 changed from an epithelial- to a more mesenchymal-like morphology when co-cultured with ASCs and fibroblasts. Both BT-474 and MDA-MB-231 proliferation was tackled by ASCs and fibroblasts in indirect co-culture. TNF- was highly expressed in supernatants of BT-474 co-cultured with ASCs. Vimentin and Snail were up-regulated in BT-474 under ASC influence to a higher extent than fibroblasts.

Conclusions: In vitro, ASCs switch BT-474 phenotype to a more malignant, mesenchymal-like breast cancer type as depicted by morphological changes and increased proliferation, as well as cytokine regulation and EMT marker up-regulation. Fibroblasts had only partly similar effects. Additional in vitro and in vivo experiments are needed to clarify the role of ASCs in cancer progression.

7

3D CULTURED SVF SPHEROIDS SECRETE HIGH LEVELS OF GROWTH FACTORS INVOLVED IN ANGIOGENESIS

Presenter: Ning Yang, PhD
Authors: Yang N, Shang H, Katz A
University of Florida

Introduction: Cells cultured as 3D spheroids show many unique characteristics compared to monolayer culture. Although many 3D cell culture methods work on a variety of cell lines, forming spheroids from 'fresh' human stromal vascular fraction (SVF) cells is still difficult. We hypothesized that SVF spheroids could be reliably formed with the use of extracellular matrix, and that SVF-spheroids would secrete high levels of growth factors that were involved in angiogenesis.

Methods: Subcutaneous adipose tissue was obtained from patients under approved IRB protocol. SVF cells were isolated and spheroids were formed in medium with ASC culture medium (LM1) and endothelial cell culture medium at ratio 1:1 with 5% methocel. Each spheroid was formed with 100,000 viable SVF cells in non-attachment round-bottom 96-well plate and cultured in suspension for 14 days. Conditioned medium was collected at day 7 and day 14 of culture over 24 hours using culture medium without growth factor supplements.

Results: SVF cells formed spheroids in the first 3 days during culture period. Luminex assay indicated that SVF spheroids secreted high levels of EGF, PLGF, Angiopoietin-2 and other growth factors. H&E staining demonstrated the presence of SVFs embedded within a self-generated extracellular matrix. CD31 staining indicated that CD31 positive cells localized in the center area of the spheroid.

Conclusions: SVF spheroid culture maintained cell viability and secreted many growth factors that are involved in angiogenesis. These primary data indicate that SVF spheroids may be useful to stimulate/facilitate/enhance angiogenesis/vasculogenesis in vivo.

8

DEPOT-SPECIFIC DIFFERENCES OF OXIDATIVE STRESS IN HUMAN ADIPOSE-DERIVED STEM CELLS

Presenter: Shigeki Sugii, PhD
Authors: Sugii S, Toh SA, Sriram S
Singapore Bioimaging Consortium and Duke NUS Graduate Medical School

Subcutaneous (SC) and visceral (VS) depots of white adipose tissue differ in their pathophysiological contributions to metabolic homeostasis and aging process, but little is known about their molecular and metabolic mechanisms. We hypothesize that at least part of these differences originate from intrinsic properties of adipose-derived stem cells (ASCs), the precursor of adipocytes. We found that levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are higher in human ASCs from VS fat compared to ASCs from SC fat. SC-ASCs exhibit higher expression of genes involved in anti-oxidant and anti-inflammatory pathways, while VS-ASCs have increased expression of genes involved in pro-oxidant/ inflammatory ones. Treatment with anti-oxidant vitamins C and E drastically decreased ROS levels in ASCs, with the reduction being more prominent in VS-ASCs. These anti-oxidants also increased proliferation, migration and adipogenic capacity of VS-ASCs. Xanthine oxidase and NADPH oxidase were identified as major mediators of these processes. These findings establish depot-specific differences in ROS potential and oxidative capacity of adipose tissue at the level of stem cells. These results can also explain compromised adipogenic functions and inflammatory milieu of VS fat and offer a potential strategy in reversing aging-associated central obesity.

9

POLOXAMER ENRICHMENT PRESERVES STROMAL VASCULAR FRACTION (SVF) CELL VIABILITY

Presenter: Isaac B. James, MD
Authors: James IB, Bourne D, Sivak W, Bliley J, Wang S, Kokai LE, Schroth R, Mahoney C, Marra KG, Rubin JP
University of Pittsburgh

Background: Fat grafting is a powerful technique that allows autologous soft tissue augmentation with minimal donor site morbidity. The greatest limitation of fat grafting is unpredictable resorption. Fat undergoes variable periods of ischemia following harvest that may contribute to decreased viability. This may contribute to unpredictable engraftment and lead to significant volume loss. P188 is an FDA approved poloxamer that acts to stabilize cell membranes and has been shown to protect injured cells from apoptosis. Hypothermia, long useful in solid organ transplantation, can prolong cell viability during ischemic periods. We sought to investigate the capacity of P188 and cooling to protect fat graft from ischemic stress.

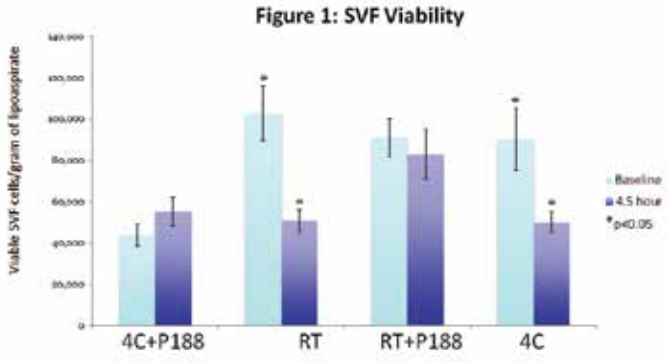
Methods: Fat was obtained under IRB approval from surgical patients via suction-assisted liposuction and processed through a Shippert filtration canister. Processed fat was incubated at either 4°C or room temperature (RT) with or without P188 (10mg/mL). The stromal vascular fraction (SVF) was then isolated and cell yield was assessed at baseline and 4.5 hours. Flow cytometry was conducted on SVF isolates at baseline and 24 hours.

Results: SVF viability decreased significantly from baseline to 4.5 hours in both the RT and 4C control groups (Figure 1). No decrease in cell viability was observed in the groups receiving P188 enrichment. The P188 group stored at 4C had significantly lower viability at baseline relative to other groups but did not decrease further thereafter. Viability of SVF and its cellular constituents (ASCs, pericytes, endothelial progenitors, and mature endothelial cells) were unchanged at 24 hrs in the RT+P188 group as demonstrated by flow cytometry. This preservation was not seen in the other groups.

Conclusions: P188 preserves fat viability during the ischemic period between harvest and engraftment. Cooling does not appear to improve and may decrease cell viability when combined with P188. Surgeons are not always able to inject fat immediately following harvesting in cases where they are performing multiple procedures, and cell death during this ischemic period may affect long term fat graft retention. These results suggest that adding P188 to fat following harvest may improve fat graft viability and subsequent retention.

9

POLOXAMER ENRICHMENT PRESERVES STROMAL VASCULAR FRACTION (SVF) CELL VIABILITY



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ANTI-INFLAMMATORY MESENCHYMAL STEM CELLS (MSC2) DEMONSTRATED ENHANCED THERAPEUTIC EFFICACY IN THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL

Presenter: Xiujuan Zhang, PhD
Authors: Zhang X, Bowles AC, Betancourt AM, Bunnell BA
Dalian University of Technology

Introduction: Multiple sclerosis (MS), characterized by inflammation, demyelination, and axonal damage, is a complicated disease of human central nervous system. Experimental autoimmune encephalomyelitis (EAE), exhibiting many clinical and histological features of MS, is the most used experimental animal model for MS. Mesenchymal stem cell (MSC) therapy for EAE has been extensively studied, however, traditional preparation of MSCs consist of mixed, poorly defined cell populations. We established a methodology that led to a consistent, uniform anti-inflammatory mesenchymal stem cells (MSC2). Because of the promising results of conventional MSC therapy for EAE, We hypothesized that a greater reduction of EAE symptoms would be seen with MSC2 treatment.

Methods: The production and analysis of anti-inflammatory MSC2 were described as in [1], and the induction of EAE was described as [2]. All the protocols were approved by the IACUC at Tulane University. Female C57Bl/6 mice, 6-8 weeks old, were used for the study. Along with the EAE induction, mice received an intraperitoneal injection of MSC2, conventionally prepared MSCs and vehicle (106 cells in 100ul HBSS). Mice were monitored daily for clinical signs by three independent blinded investigators. Thirty days post disease induction, animals were euthanized and perfused with sterile PBS. Tissues were removed and histological analyses were performed.

Results: TLR3 priming of conventional MSC into MSC2 did not change the stem cell properties. However, the MSC2 migration capacity and cytokine and chemokine secretion were significantly modulated. Although conventional MSC treatment (n=12) displayed therapeutic effects, MSC2 therapy (n=12) dramatically ameliorated clinical symptoms, delayed disease onset and progression. Moreover, immunohistochemistry analysis showed that MSC2 therapy demonstrated less demyelination and reduced the level of cell infiltrating.

Discussion: In this study, although conventional MSCs attenuated the clinical symptoms of EAE model and reduced demyelination and the level of cell infiltration, MSC2 treatment did so to a greater extent. Additionally, MSC2 yielded even greater improvement in disease onset and progression.

Reference: [1] PloS One 2010; 5:e10088?[2] PLoS ONE, 2014; 9:e85007.

11

ADIPOSE-TISSUE STROMAL VASCULAR FOR THE TREATMENT OF ACHILLES TENDINOPATHY: A RANDOMIZED PROSPECTIVE CLINICAL TRIAL

Presenter: Laura de Girolamo, PhD
Authors: Usuelli FG, Grassi M, Alfieri Montrasio U, De Girolamo L
Istituto Ortopedico Galeazzi

Introduction: Achilles tendinopathy commonly occurs in both active and inactive persons. It consists in the development of pain and inflammation in the early phases, with progression to the development of fibrotic tissue and degeneration of tendon matrix. Current conservative treatment approaches do not provide sustained satisfactory results, particularly in active patients, although platelet rich plasma (PRP) injection have shown to be effective in many cases. The therapeutic effect of adipose-derived mesenchymal stem cells (ASCs), either expanded or used directly within the stromal vascular fraction (SVF), have demonstrated to possess significant anti-inflammatory and immunomodulatory effects, mediated by the release of active factors, and thus potentially useful in the treatment of tendinopathy.

Method: To test this hypothesis, patients affected by non-insertional Achilles tendinopathy (range 18-55 y/o) were prospectively enrolled in this clinical study, and randomly assigned either to single PRP injection group (GPSIII kit, Biomet, USA) (n=28 tendons) or single adipose tissue SVF (FastKit, Corios, Italy) (n=28 tendons) injection group. All patients were assessed clinically pre-operatively and at 15, 30, 60, 120 and 180 days from treatment, using VAS Pain, VISA-A, AOFAS and SF-36 forms. Patients were also evaluated by ultrasound and magnetic resonance before treatment and after 4 and 6 months.

Results: Population background data and pre-operative scores were similar in the two groups ($p>0.05$). At final follow up both patients group showed significantly improvements in all the scores ($p<0.05$). In SVF patients these improvements were faster, with significantly results with respect to pre-injection level already starting 15 days after treatment. Indeed at this time point a significant difference between groups in term of VAS, AOFAS and VISA-A score was observed ($p<0.05$), with better results in the SVF group. After 6 months MR and ultrasounds showed an improvement of clinical signs in both groups, without relevant differences.

Conclusions: Both PRP and SVF are safe and effective treatments for Achilles tendinopathy. However, SVF allowed to obtain faster results, thus allowing to consider this treatment for patients requiring to come back to sport earlier.

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FLUORINE-19 LABELING OF STROMAL VASCULAR FRACTION CELLS FOR CLINICAL IMAGING APPLICATIONS

Presenter: Jeff W. Bulte, PhD
Authors: Kadayakkara DK, Wang G, Bar-Shir A, Helfer BM, Ohanlon C, Kraitchman DL, Rodriguez RL, Bulte JW
Johns Hopkins University School of Medicine

Stromal vacular fraction (SVF) cells are clinically used for various therapeutic targets. The location and presence of engrafted SVF cells is an important parameter for determining treatment failure vs. success. Using the GID SVF-1 platform, we describe here a clinical protocol to harvest and label SVF cells with the fluorinated (19F) agent CS-10000 as part of a first-in-man Phase I trial (NCT02035085) to track SVF cells with magnetic resonance imaging (MRI) during treatment of radiation-induced fibrosis in breast cancer patients. Flow cytometry revealed that SVF cells consisted of 25.0 +/- 15.8% CD45+, 24.6 +/- 12.5% CD34+, and 7.5 +/- 3.3% CD31+ cells, with 2.1 +/- 0.7 E5 cells per cc of adipose tissue obtained. CS-1000 labeled 87.0 +/- 13.5% of CD34+ progenitor cells, compared to 47.8 +/- 18.5% of hematopoietic CD45+ cells with an average of 2.8 +/12.0 E12 19F atoms per cell as determined from nuclear magnetic resonance (NMR) spectroscopy. The vast majority (92.7 +/- 5.0%) of CD31+ cells were also labeled, although the majority of these cells co-expressed CD34. Only 16.0 +/- 22.3% of CD45-/CD31-/CD34- cells were labeled with CS-1000. Following induction of cell death by either apoptosis or necrosis, >95% of 19F was released from the cells, indicating that fluorine retention may be used as a surrogate marker for cell survival. Labeled SVF cells engrafted in a silicone breast phantom could be visualized with a clinical 3T MRI scanner at a sensitivity of approximately 2E6 cells at a depth of 5 mm. The current protocol can be used to image transplanted SVF cells at clinically relevant cell concentrations in patients.

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STROMAL VASCULAR FRACTION AS A NOVEL STEM CELL-BASED THERAPY FOR A MOUSE MODEL OF MULTIPLE SCLEROSIS

Presenter: Annie C. Bowles, MS
Authors: Bowles AC, Strong AL, Wise RM, Thomas RC, Gerstein BY, Dutreil MF, Lipschutz RS, Gimble JM, Bunnell BA
Tulane University

Multiple sclerosis (MS) is the most common neurodegenerative disorder affecting young adults worldwide and remains an unmet clinical challenge. MS is caused by an autoimmune reaction against constituents of the central nervous system (CNS). Subsequent immune cell infiltration into CNS tissues leads to widespread inflammation, demyelination, and pathological lesions causing debilities such as tremors, fatigue, and progressive loss of motor functions. These pathological hallmarks are reproducible in the mouse experimental autoimmune encephalomyelitis (EAE) model of MS. Several clinical trials have demonstrated the safety and efficacy of adipose-derived stem cell (ASC) therapies in treating various disorders. ASC treatments are considered favorable due to their abundance in adult human adipose depots, less invasive harvest protocol, and potent immune-modulatory capabilities. However, due to the requisite for ex vivo culture isolation and expansion, alternative therapies are necessary. As such, the stromal vascular fraction (SVF) of adipose tissue is an ASC-based therapy directly administered to subjects. The SVF consists of ASC, adipocytes, and numerous leukocytes. The combination of cell types is believed to provide enhanced effectiveness by reducing inflammation and repairing CNS tissues. Currently, the SVF has advanced to clinical trials as a promising therapy for various medical indications including neurodegenerative diseases. Nevertheless, the efficacy of SVF therapy for MS patients or EAE mice has not been investigated. In this study, intraperitoneal injections of 1x106 SVF cells or ASC into EAE mice during the progression of disease, i.e. early, mid-stage, and chronic phases, were performed. Immune-modulatory activities of injected SVF and ASCs were determined using flow cytometry and qRT-PCR of lymphoid and CNS tissues and blood at the endpoint of the study. Both ASC-based treatments preserved myelin levels and reduced inflammatory mediators in CNS tissues. Results show greater efficacy for reducing disease severity, recovery of motor functions, and improvement in histological examinations from EAE mice injected with SVF than ASCs, regardless of the timing. Together, SVF cells are more comprehensive at treating the immunopathogenesis of EAE by producing

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AUTOLOGOUS FAT GRAFTING FOR PEDAL FAT PAD ATROPHY: A PILOT STUDY

Presenter: Jeffrey A. Gusenoff, MD
Authors: Gusenoff JA, Mitchell R, Jeong K, Wukich D, Gusenoff BR
University of Pittsburgh

Introduction: Pedal fat pad atrophy is associated with pain, decreased tissue thickness, and increased foot pressures. No objective studies investigating the use of fat grafting to the forefoot have been performed. We hypothesize that autologous fat injections can reduce pain, increase tissue thickness, and decrease pedal pressures in these patients.

Methods: A randomized cross-over study was performed to assess tissue thickness, pain and foot pressures. The intervention group (Group 1) underwent fat grafting immediately, followed by evaluation at 2 weeks, 1, 2, 6, 12, 18, and 24 months. The standard of care group (Group 2) underwent conservative management for 1 year, and then crossed over into the active group with 1 year of follow-up.

Results: 17 patients comprised Group 1 and 13 patients comprised Group 2. Mean age was 59±7.9 years in Group 1 and 63.2±14.1 years in Group 2 ($p=0.04$). 11 patients in Group 1 underwent bilateral injections with a mean volume of 4.7 cc per foot and 2 patients underwent bilateral injections in Group 2 with a mean volume of 4.6 cc per foot. At 6 months after fat injection, Group 1 demonstrated improved foot function ($p=0.01$), improved foot appearance ($p=0.019$), improved pain ($p=0.004$), and improved work/leisure activities ($p=0.006$). Group 2 demonstrated no significant changes in foot pain or disability over 6 months of standard of care. Mean tissue thickness over the metatarsals increased significantly in both feet immediately after fat injections ($p<0.004$), and at 2 months ($p<0.02$); however by 6 months, only the right foot maintained an increase in tissue thickness ($p=0.05$). There was no significant difference in tissue thickness in Group 2 at 6 months. At 6 months, Group 1 had significant decreases in standing foot pressure and force ($p<0.04$), while in Group 2, there were significant increases in foot pressure and force with both walking and standing ($p<0.02$).

Conclusion: Despite decreasing tissue thickness over time, pedal fat grafting significantly improves pain and disability outcomes, decreases foot pressures and forces, and prevents against worsening foot pressures and forces. Pedal fat grafting is a safe, minimally invasive approach to treat fat pad atrophy with minimal downtime.

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INCREASED GENOMIC STABILITY OF ADIPOSE DERIVED MESENCHYMAL STROMAL CELLS (MSCS) COMPARED TO BONE MARROW MSCS: INVOLVEMENT OF H19 LONG NON-CODING RNA AND P53 ACTIVITY

Presenter: Meirav Sela, MSc
Authors: Sela M, Ravid O, Shoshani O, Weinstock A, Sadan TW, Gur E, Zipori D, Shani N
The Tel Aviv Sourasky Medical Center

Introduction: Mesenchymal stem cells (MSCs) are multipotent and have been derived from various tissues. Although MSCs share many basic features, they often display tissue specific differences. Such differences may have important bearing on the efficacy of their utilization in the clinic. We previously demonstrated that bone marrow (BM) MSCs frequently become polyploid in culture. The transition to a polyploid state was mediated by H19 long non-coding RNA.

Methods: MSCs were derived from both adipose tissue and BM of mice and expanded under normoxic and hypoxic conditions. Cells were stained by propidium iodide and their ploidy was evaluated by flow cytometry. Gene expression profiles of independent MSC preparations was compared by quantitative real time PCR and protein expression levels by Western blot analysis. P53 silencing in MSCs was performed by a specific shRNA.

Results: We examined whether genomic instability is common to MSCs originating from different tissues. It is demonstrated that adipose derived MSCs (ASCs) tend to remain diploid during culture while a vast majority of BM MSCs become polyploid under both hypoxic (3% oxygen) and normoxic conditions. Increased RNA expression of H19 and VEGFa was observed under hypoxic conditions by both ASCs and BM MSCs. Importantly, ASCs gene expression is significantly less variable than BM MSCs under both oxygen conditions, indicating to their superior homogeneity. Gene expression analysis revealed that p53 target genes often induced by DNA damage, are up-regulated in ASCs under basal conditions compared to BM MSCs. The involvement of p53 in maintaining the stable diploid state of ASCs was further demonstrated as p53 shRNA induced ploidy changes in ASCs but not in BM MSCs.

Conclusions: The increased genomic stability of murine ASCs together with their lower H19 expression and relative homogeneity suggest a tissue specific higher stability of ASCs compared to BM MSCs, possibly due to higher activity of p53. The tissue specific differences between MSCs from different tissue source may have important consequences on their appropriate and efficient clinical use.

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CIGARETTE SMOKING MARKEDLY REDUCES VASCULOGENIC ACTIVITY OF ADIPOSE STEM CELLS

Presenter: Daria Barwinska, BA
Authors: Barwinska D, Traktuev DO, Cook T, Merfeld-Clauss S, Petrache I, March KL
Indiana University

Objective: It is known that cigarette smoking (CS) leads to dysfunction in angiogenic activity of endothelial progenitor cells and decrease in numbers of bone marrow hematopoietic stem cells. However, the effect of CS on vasculogenic activity of adipose stem cell (ASC) has not been studied yet. We hypothesized that human ASC derived from smoking donors will be inferior in their therapeutic vasculogenic properties when compared to ASC from non-smoking donors.

Methods: Human ASC were isolated from subcutaneous fat of smokers and non-smokers of both genders and expanded. Ability of expanded ASC to support vascular network formation (VNF) by human endothelial cells (EC) was evaluated. The potency of ASC conditioned media (CM) to support VNF in EC-fibroblast co-culture model was assessed. The levels of such vasculogenic factors as VEGF, HGF, SDF1, Ang-1, and Ang-2 in CM were analyzed by ELISA. The therapeutic effect of systemically delivered ASC on recovery of blood flow in mouse ischemic hindlimb was analyzed by Laser Doppler Imaging.

Results: We have shown that cultured ASC from smoking men and women have significantly diminished activity to support VNF compared to ASC harvested from non-smoking donors ($p<0.0001$). EC-fibroblast co-cultures incubated in the presence of CM generated by ASC from smoking women developed less dense vascular networks then ones incubated with CM from healthy donors ($p=0.001$). Moreover, all, except VEGF, angiogenic factors were decreased in CM generated by ASC from smoking women. In parallel, the in vivo studies revealed that ASC from non-smoking donors improved perfusion two-fold in the ischemic limb by day seven ($p<0.001$) compared to control media treatment, whereas ASC from smoking donors were unable to stimulate flow recovery.

Significance: Our data suggest that cigarette smoking had detrimental effect on human ASC therapeutic effects and specifically their vasculogenic activity. These findings should be taking in consideration when designing the treatment based on autologous ASC as a therapeutic agent. Further studies will be conducted to test whether substantial loss in therapeutic activity of ASC obtained from smoking donors can be compensated by increase in the number of treatments or by scaling up the dose.

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ALLOGENEIC ADIPOSE-DERIVED STROMAL CELLS PROMOTE IN VIVO FAT FORMATION IN DECELLULARIZED ADIPOSE TISSUE BIOSCAFFOLDS VIA INDIRECT MECHANISMS

Presenter: Lauren E. Flynn, PhD
Authors: Flynn LE, Han TT, Amsden BG
The University of Western Ontario

Introduction: There is a clinical need for new strategies to enable the stable and predictable regeneration of healthy adipose tissue for applications in plastic and reconstructive surgery. Over the past 5 years, our group has been developing decellularized adipose tissue (DAT) as a platform scaffolding technology for soft tissue regeneration. Supporting the rationale for a tissue-specific approach to scaffold design, DAT has been shown to provide a supportive microenvironment for the adipogenic differentiation of human adipose-derived stem/stromal cells (ASCs). Building from our previous work, the current study focused on exploring the mechanisms of ASC-mediated in vivo fat regeneration in DAT bioscaffolds.

Methods: A subcutaneous immunocompetent Wistar rat model was used to compare in vivo adipogenesis, angiogenesis and the macrophage response in ASC-seeded and unseeded DAT samples. DAT scaffolds were prepared from surgically-discarded human adipose tissue (REB# CHEM-002-07) using published protocols. The DAT was seeded with 1x10^6 allogeneic rat ASCs from male donors and implanted subcutaneously in female rats to enable cell tracking via the Y chromosome. ASC-seeded DAT and unseeded controls were explanted at 72 h, 1, 4, 8 and 12 weeks for blinded semi-quantitative histological and IHC analyses of the remodeling implant regions (N=4).

Results: Seeding the DAT with allogeneic ASCs enhanced angiogenesis and adipogenesis, with substantial remodeling of the ASC-seeded DAT into mature host-derived adipose tissue at 8 and 12 weeks (Fig. 1). Cell tracking studies indicated that the ASCs indirectly contributed to regeneration, promoting angiogenesis and modulating the inflammatory response to promote a more constructive macrophage phenotype within the ECM-derived bioscaffolds (Fig. 2). A subpopulation of macrophages was shown to express adipogenic markers and may be playing an active role in the observed fat formation.

Conclusions: Our findings demonstrate that the DAT scaffolds provide a conducive microenvironment for in vivo adipose regeneration and that allogeneic ASCs can help to orchestrate this response by promoting the recruitment of beneficial host cell populations that directly contribute to the developing adipose tissues.

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ALLOGENEIC ADIPOSE-DERIVED STROMAL CELLS PROMOTE IN VIVO FAT FORMATION IN DECELLULARIZED ADIPOSE TISSUE BIOSCAFFOLDS VIA INDIRECT MECHANISMS

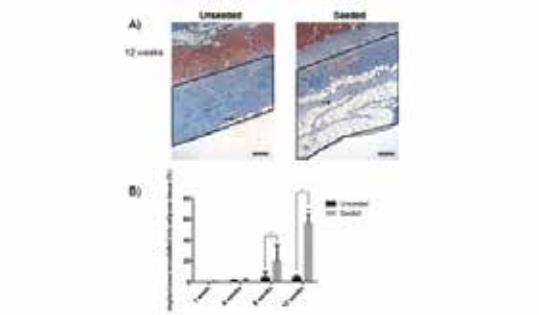

Figure 1. ASC seeding promotes adipose tissue formation in DAT scaffolds. A) Masson's trichrome staining with implant outlined in black, arrows indicate adipocytes. Scale bars represent 100 μm. **B)** Percentage of the DAT implant remodeled into adipose tissue. *, ** statistically significant (N=4, p<0.05).

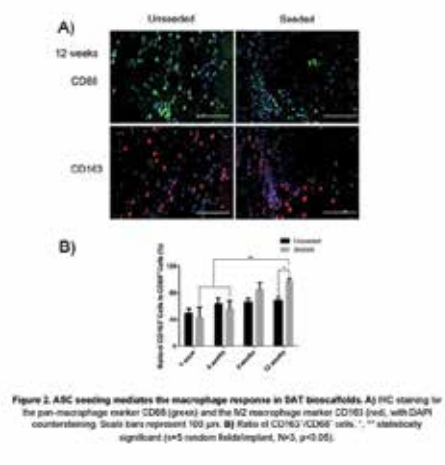

Figure 2. ASC seeding mediates the macrophage response in DAT bioscaffolds. A) IHC staining for the pan-macrophage marker CD68 (green) and the M2 macrophage marker CD163 (red), with DAPI counterstaining. Scale bars represent 100 μm. **B)** Ratio of CD163+/CD68+ cells. *, ** statistically significant (n=5 random fields/implant, N=3, p<0.05).

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MECHANICALLY DISRUPTED HUMAN ADIPOSE TISSUE (LIPOGEMS®) EXHIBITS DISTINCT REGENERATIVE CELL CONTENT AND TROPHIC FACTOR SECRETION AS COMPARED WITH CONVENTIONALLY ENZYME-DISSOCIATED FAT

Presenter: Bruno Peault, PhD
Authors: Khan N, Lesme H, Krutchkoff B, Tremolada C, Peault B
University of California Los Angeles

Introduction: Lipogems has developed a device for enzyme-free processing of human fat into an injectable therapeutic product. Lipogems®, already used in over 4000 patients and recently FDA-approved, has demonstrated great efficacy in plastic/reconstructive surgery, coloproctology, gynaecology, orthopaedics, otorhinolaryngology, urology and vulnology. We are characterizing Lipogems® in terms of native perivascular MSC content and growth factor/cytokine secretion.

Methods: Cosmetic lipoaspirates obtained with patient approval were processed either by collagenase II/dispase digestion into stromal vascular fraction (SVF), or Lipogems mechanical fragmentation, yielding discrete fat tissue clusters (diameter H 500 µm). Lipogems clusters were processed for immunohistochemistry. Both SVF and Lipogems clusters were analysed by FACS for presence of pericytes (CD146+ CD34- CD45-) and adventitial progenitors (CD146- CD34+ CD45-), and independently cultured. 8-day conditioned media were tested using the Human Angiogenesis and Human XL Cytokine Array Kits (R&D Systems).

Results: SVF contained 10.17 ± 6.49% adventitial progenitors and 0.83 ± 0.11% pericytes. Lipogems clusters were considerably enriched in the latter, including 12.48 ± 6.88% adventitial progenitors and 57.00 ± 29.13% pericytes (n=3). Strikingly, 36 growth factors/cytokines were secreted in higher amounts - or exclusively - by Lipogems clusters, as compared with SVF. These included factors produced by adipocytes, such as RBP4, leptin and adiponectin, but also other molecules involved in angiogenesis/tissue inflammation/regeneration, including CCL2, CXCL5, CXCL10, CXCL12, MMP9, GDF15, angiogenin, endoglin, MIF, angiopoietin 2, TIMP4, and PF4. Immunohistochemistry confirmed the presence in Lipogems clusters of microvascular structures ensheathed in pericytes, which partially or completely lost contact with microvessels over time.

Conclusions: As compared with SVF, fat mechanically dissociated into discrete tissue units containing intact microvessels is enriched in pericytes, known to play key roles in tissue development/regeneration. Such 3-D cell clusters secrete, qualitatively and quantitatively, more trophic factors involved in tissue healing, which may explain the therapeutic efficacy of Lipogems®.

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ISOLATION OF A PURE POPULATION OF THERAPEUTICALLY POTENT CELLS FROM ADIPOSE TISSUE BASED ON CD140B ANTIGEN

Presenter: Dmitry O. Traktuev, PhD
Authors: Traktuev DO, Merfeld-Clauss S, Lupov IP, Cook T, March KL
Indiana University

Multiple studies have shown that delivery of adipose tissue-derived stromal vascular fraction (SVF) to animals with induced pathologies improves the degree and rate of recovery. These findings supported the use of SVF for autologous cell therapies. However, SVF is composed of distinct cell sub-populations, the ratios of which vary significantly between isolations. To avoid inconsistency in SVF therapeutic effects and to facilitate the success of SVF adoptation in clinic, a method for isolation of adipose stromal cells (ASC), the therapeutically active fraction of SVF, needed to be established. Therefore we developed an algorithm to characterize SVF composition based on flow cytometry technique that will permit objective SVF separation into subpopulations, isolated pure population of ASC based on CD140b antigen and evaluated its therapeutic activity in mouse model of hindlimb ischemia. Labeling SVF with CD34-APC and [CD31/CD45]-PE easily separates endothelial and hematopoietic origin cells from ASC. CD34bright/CD31-/CD45- ASC were 100% positive for CD140b antigen. Analysis of CD140b+ cells revealed high homogeneity based on CD10, CD13, CD29, CD44, CD73, CD90. This finding justified the use of anti-CD140b antibodies for ASC isolation from SVF. Purification of SVF on MACS column based on CD140b eliminated tissue/cell debris as well as CD31+ and CD45+ cells; enriched SVF for CD140b+ cells from 37.4±7.9% to 87.4±3.3% and for CD34+ cells. Analysis of blood flow in the limbs revealed that mice treated with CD140b+ cells showed perfusion recovery to a greater degree than those treated with whole fresh or overnight pre-plated SVF. It is most likely that FDA will request to perform more stringent characterization of SVF preparation and establish a release criteria of the final product prior to approving SVF-based therapies for wide adoptation in clinic. Such requests may decrease the probability of inconsistency in SVF therapeutic effects due to the its composition. Here, we have shown that by enriching fresh preparations of SVF based on surface antigen CD140b, a highly homogenous cell population can be obtained in a single step, while preserving therapeutic activity of the selected cells.

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A COMPARISON OF TOPICAL SPRAY AND LOCAL INJECTIONS OF ADIPOSE DERIVED REGENERATIVE CELLS (ADRCS) ON WOUND HEALING IN A PORCINE MODEL OF FULL THICKNESS BURN INJURY

Presenter: Philippe Foubert, PhD
Authors: Foubert P, Gonzalez AD, Teodosescu S, Fraser JK
Cytori Therapeutics

Introduction: The use of non-cultured autologous Stromal Vascular Fraction (SVF) or clinical grade Adipose Derived Regenerative Cells (ADRCs) cells represents a promising strategy to promote wound healing and tissue repair. Nevertheless, issues regarding the optimal mode of administration remain unclear. The purpose of this study was to compare the effects of two modes of delivery of ADRCs (topical spray and local injection) in a porcine model of full thickness thermal burn injury.

Methods: Full thickness thermal burns were created on the backs of 10 Gottingen minipigs. Two days following injury wounds underwent fascial excision and were randomized to receive control vehicle or freshly isolated autologous ADRCs delivered by either multiple injections into and surrounding the wound bed or by spray onto the wound area. Healing was evaluated by planimetry, histopathology and immunohistochemistry at day 7, 12, 16, 21 and 28 post-treatment.

Results: In vitro analysis demonstrated that there was no substantial loss of cell number or viability attributable to the spraying application procedure. Using a matched pair analysis, planimetric wound healing assessment revealed that delivery of ADRCs by either local injection or topical spray increased wound re-epithelialization relative to control at day 14. No significant difference in wound epithelialization was observed between topical and local injection of ADRCs. In addition, on day 7 post-treatment, blood vessel density was on average 67% and 47% greater in wounds receiving local or topical spray ADRCs respectively than in the wounds treated with vehicle control. Histopathologic analysis of treated wounds suggests that ADRC treatment may modulate the inflammatory response by reducing neutrophil infiltration irrespective of the route of administration.

Conclusions: These data demonstrate that delivery of ADRCs by either local injection or topical spray modulates inflammation and improves wound angiogenesis and epithelialization. Importantly, both delivery routes exhibited similar effects on wound healing. Given the greater ease-of-use associated with topical spray delivery, these data support the use of a spray system for autologous ADRCs delivery.

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EXTERNAL VOLUME EXPANSION AND FAT GRAFTING AS AN ALTERNATIVE FOR POST-RADIATION BREAST RECONSTRUCTION: EFFECTS ON A TRANSLATIONAL MODEL

Presenter: Jorge Lujan-Hernandez, MD
Authors: Lujan-Hernandez J, Chin MS, Perry DJ, Chappell AG, Babchenko O, Bannon E, Lancerotto L, Lo YC, Fitzgerald TJ, Lalikos JF
University of Massachusetts Medical School

Introduction: Post-mastectomy radiation therapy (XRT) has improved breast cancer outcomes but has complicated the reconstruction process. Autologous fat grafting (FG) is an alternative or an adjunct to traditional reconstructive techniques, however results are currently unsatisfactory compared to non-irradiated patients. Pre-treatment with External Volume Expansion (EVE) has been proposed to improve graft take and cosmesis. EVE effects on radiation damaged tissue and its influence on fat graft take remain largely unexplored.

Methods: Forty two nude mice were divided into 4 groups. 50gy of localized dorsal XRT was applied and mice were monitored for 8 weeks until development of chronic fibrosis. Group 1 (n=10) received unilateral XRT with contralateral control. Group 2 (n=12) received bilateral XRT and EVE application for 5 days unilaterally with 25mmHg of negative pressure. Group 3 (n=10) was not irradiated and received 0.6 cc of human lipoaspirate as control. Group 4 (n=10) received bilateral XRT, then EVE protocol as group 2, followed by bilateral fat grafting. Skin perfusion and was measured using Hyperspectral Imaging. Fat graft volumes were quantified 8 weeks post-grafting using in-vivo microCT scans. Immunohistochemistry of harvested skin and grafts was performed to analyze vascularity (CD31) and cell proliferation (ki67).

Results: Group1: XRT skin was significantly more ischemic compared to control side ($p<0.05$).Group 2: EVE application induced a 37% increase in vascularity ($p<0.01$) in the overlying skin after 5 days of stimulation, a 45% increase in proliferating cells ($p<0.05$) with increased skin thickness in the XRT treated areas compared to XRT without EVE. Group 3: Fat graft retention after 8 weeks was of 74% in the control group. Group 4: Fat grafts on irradiated tissue had a retention of 66% whereas the XRT followed by EVE preconditioning had a 79% retention. ($p<0.05$).

Conclusion: Radiation injures microvasculature and reduces skin perfusion significantly reducing fat graft survival. EVE increased volume retention of fat grafts in our mouse XRT model. EVE likely works through cyclic non-lethal hypoxia and mechanotransductive preconditioning phenomena, inducing proliferation and angiogenesis of the recipient site.

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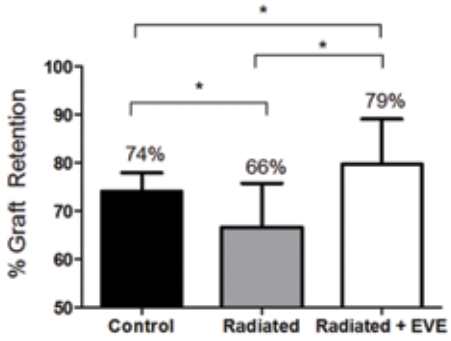
EXTERNAL VOLUME EXPANSION AND FAT GRAFTING AS AN ALTERNATIVE FOR POST-RADIATION BREAST RECONSTRUCTION: EFFECTS ON A TRANSLATIONAL MODEL



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COORDINATE ISOLATION OF PERI-PANCREATIC FAT SVF AND PANCRETIC ISLETS FOR THE PREPARATION OF FUNCTIONAL PREVASCUALRIZED ISLET SPHEROIDS

Presenter: Stuart K. Williams, PhD
Authors: Williams SK, Appakalai B, Hughes MG, Kleinert LB, Loganathan G, George NW, Tweed BT
University of Louisville

Introduction: Auto-islet transplantation is a procedure used to maintain insulin secretion in patients undergoing total pancreatectomy. The funciton of the transplanted islets is comprimised due to the slow revascularization of islets following transplantation. We have devloped a method to 3D bioprint pre-vascularized islets prior to implantation using adipose stromal vascular fraction (SVF) cells as a source of new microvasculature.

Methods: Human pancreata were grossly evaluated for the presence of peri-pancreatic fat, and fat deposits removed from the pancreas surface. A subsample of the fat was histologically evaluated (H&E staining) and the remainder of the fat subjected to enzymatic digestion to prepare a stromal vascular fraction cell population. Simultaneous with SVF prepration the islets were isolated using enzymatic digestion. The isolated islets and peri-pancreatic fat SVF were prepared for 3D bioprinting using a bioink that contained alginate and collagen. Spheroids containing islets and adipose SVF were bioprinted and placed in culture to evaluate cell viability and islet function.

Results: The preparation of human islets and human peri-pancreatic fat SVF cell populations can be performed simultaneously with the immediate preparation of prevascularized islet spheroids using 3D bioprinting technology. Morphologi differences were observed in the fat deposits on the surface of the human pancreas. The 3D bioprinted prevascularized islets remained viable in culture and, in the presence of a secretagogue, exhibit normal insulin synthesis and secretion. The 3D bioprinted islet-SVF spheroids were implanted subcutaneously in a rat tissue isolation device and exhibited maintenance of viability and function.

Conclusions: These studies illustrate the ability to simultaneously isolate human islets and human peri-pancreatic fat stromal vascular fraction cells, and a 3D bioprinter can be used to immediately produce prevascualrized islet spheroids. Functional studies establish the ability of 3D bioprinted islets to maintain viability and function.

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HASCS AND THEIR CONDITIONED MEDIUM ALLEVIATE THE PAINFUL SYMPTOMS IN TWO PRECLINICAL MODELS OF NEUROPATHY

Presenter: Anna T. Brini, PhD
Authors: Brini AT, Niada S, Ferreira LM, Amodeo G, Milani A, Franchi S, Giannasi C, Sacerdote P
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Introduction: We evaluated the effect of mesenchymal stromal cells isolated from human adipose tissue (hASC) and of their conditioned medium (CM-hASC) on the neuropathic symptomatology in two preclinical experimental models of neuropathic pain (NP): the sciatic nerve chronic constriction injury (CCI) and the diabetic neuropathy induced by Streptozotocin. When neuropathic pain was already established, the effect of hASCs was compared to the 40 times concentrated hASCs conditioned medium (CM).

Methods: Mice were i.v. injected with either 1x10^6 hASCs or CM-hASC (from 2x10^6 72-hours starved cells). The effect on mechanical allodynia (dynamic plantar aesthensiometer) and on thermal hyperalgesia (plantar test) was monitored over time.

Results: Regardless of the nature of NP, cells increased the hyperalgesic and allodynic thresholds with a fast onset (already 3 hours after treatment). In the CCI model, the antihyperalgesic and antiallodynic effect of a single shot of hASC lasted for two weeks, while in the STZ-induced neuropathic mice the antiallodynic effect lasted for 2 months and it was comparable to control. CM-hASC effect was evident for 1 month, and it was restored by additional treatment when the pain relief was decreased. Surprisingly, both hASCs and their CM were able to contrast the body weight loss in STZ-induced NP mice.

In order to elucidate their action in neuroinflammation, 7 days after the therapeutic treatment, six animals/group were sacrificed and the cytokines such as IL-1 and IL-10 were determined at the level of sciatic nerve, dorsal root ganglia and spinal cord: the main stations involved in pain transmission. In neuropathic mice the observed proinflammatory profile, (high IL-1 and low IL-10 levels), was counteracted by both hASC and CM–hASC which were able to restore a pro/antinflammatory cytokine balance.

Conclusion: hASC administration may have a future in neuropathic pain treatment, and since CM-hASC partially mimicked hASC effect in both NP experimental models, the involvement of a hASC-paracrine mechanism may eventually allow us to move toward a cell-free therapy.

References: Sacedote et al. Stem Cells Dev. 2013 15;22(8):1252-63Noh et al. Int J Biochem Cell Biol. 2013 45(8): 1538-1545

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HUMAN ADIPOSE STROMAL/STEM CELLS FROM OBESE DONORS SHOW REDUCED EFFICACY IN THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MODEL OF MULTIPLE SCLEROSIS

Presenter: Amy L. Strong, PhD, MPH
Authors: Strong AL, Bowles AC, Wise RM, Morand JP, Dutreil MF, Gimble JM, Bunnell BA
Tulane University School of Medicine

Multiple sclerosis is an autoimmune disease that affects the white matter of the central nervous system and involves inflammation and demyelination. The recent advances in our understanding of adipose-derived stromal/stem cells (ASCs) and the utilization of these cells in clinical settings to treat diseases have made it essential to identify the most effective ASCs for therapy. Studies have not yet investigated the impact of obesity on the therapeutic efficacy of ASCs. Obesity is characterized by adipocyte hyperplasia and hypertrophy and can extend to metabolic and endocrine dysfunction. Investigating the impact obesity has on ASC biology will determine whether these cells are suitable for use in regenerative medicine. The therapeutic efficacy of ASCs isolated from lean subjects (body mass index <25; lnASCs) and obese subjects (body mass index >30; obASCs) were determined in murine experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. Compared to the EAE disease-modifying effects of lnASCs, obASCs consistently failed to alleviate clinical symptoms or inhibit inflammation in the central nervous system. When activated, obASCs expressed higher mRNA levels of several pro-inflammatory cytokines compared to lnASCs. Additionally, conditioned media collected from the obASCs markedly enhanced the proliferation and differentiation of T cells; whereas, conditioned media from lnASC did not. These results indicate that obesity reduces, or eliminates, the anti-inflammatory effects of human ASCs such that they may not be a suitable cell source for the treatment of autoimmune diseases. The data suggests that donor demographics may be particularly important when identifying suitable stem cells for treatment.

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CELL-ASSISTED LIPOTRANSFER: A CRITICAL APPRAISAL OF THE CLINICAL EVIDENCE FOR A NOVEL "STEM CELL THERAPY"

Presenter: Florian M. Lampert, MD
Authors: Lampert FM, Grabin S, Antes G, Motschall E, Buroh S, Stark GB
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Background: Building on findings from in-vitro and in-vivo experiments, the so-called "Cell-assisted-Lipotransfer" (CAL) was developed to improve the clinical outcome of autologous fat transplantation by supplementation with adipose tissue derived mesenchymal Stem Cells (ADSCs). Its clinical use is flourishing worldwide regardless of the discrepancy between boastful marketing claims and poor clinical evidence regarding safety and efficacy.

Methods: We conducted a systematic review of the literature in collaboration with the German Cochrane Center. A thorough search strategy was conducted in eight major scientific databases. The obtained publications were screened by two independent reviewers and evaluated basing on objective criteria.

Results: 3161 publications were obtained, out of which 78 were judged relevant after screening of title and abstract and subsequent full text examination. Of the latter, only 13 were clinical studies, merely three of them reaching Evidence-Level II/III criteria. Considering all studies, a total of 286 CAL-procedures is described. No follow-up exceeded 42 months, neither could provide evidence on oncological safety.

Conclusions: No proof of a general superiority of CAL in comparison to the conventional fat transplantation could be brought to light; security issues are completely neglected or only inadequately referred to. Prior to the implementation of this unquestionably promising technique into the medical armamentarium, we have to gain possession of substantiated, high-grade evidence for its efficacy as well as on safety issues.

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IS CENTRIFUGATION STILL PLAYING THE LEADING ROLE? A SYSTEMATIC REVIEW OF PROCESSING TECHNIQUES IN FAT GRAFTING

Presenter: Aartje J. Tuin, MD
Authors: Tuin AJ, Domerchie PN, Schepers RH, Willemsen JC, Dijkstra PU, Spijkervet FK, Vissink A, Jansma J
University Medical Center Groningen

Background: Centrifugation is commonly used as processing method in fat grafting. The aim of this systematic review of literature is to search for the most optimal current method for fat graft processing.

Methods: A systemic review was performed in accordance with the PRISMA statement. PubMed, Embase, Cinahl, Cochrane, and Web of Science databases were searched until March 2015. Studies comparing different processing methods in fat grafting were included. Assessment of methodological quality was performed by MINORS. Eight processing categories were defined: "negative control", "decantation", "centrifugation", "mesh/gauze", "devices", "metal sieve", "wash", "wash and centrifugation". Outcomes were viability of adipocytes, adipose-derived stem cells and growth factors in vitro, volume and quality of the graft in animal studies, and satisfaction and volume retention in human.

Results: 382 unique studies were identified. Thirty-two records met the inclusion criteria and had sufficient methodological quality regarding MINORS. Thirty studies (94%) compared centrifugation with another processing method. The gauze/towel technique performed better than centrifugation regarding viability of adipocytes. The number of ADSC was the highest with the in gauze/towel method and in the pellet after centrifugation. The results of animal studies and the results of patients' satisfaction were not distinctive. In the only study assessing volume retention in humans, a wash-filter device performed significantly better than centrifugation.

Conclusion: Centrifugation was not the best technique for preserving viability of adipocytes. Processing methods using permeability principles seems to be auspicious. However, due to the great variety in processing methods and outcome variables reported no hard conclusion can't be drawn.

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DERMAL SCAFFOLD AND ENHANCED FRACTIONAL CELLS USAGE FOR UPPER EXTREMITY BURN CONTRACTURES AS AN ALTERNATIVE PARADIGM TO FREE TISSUE TRANSPLANTS TO OBTAIN FUNCTIONAL AND AESTHETIC TISSUE HEALING

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Ceran F, Filinte GT, Basat SO, Guvercin E
Dr Lutfi Kirdar Kartal Training and Research Hospital

Introduction: There is a significant increase in survival rates in acute burn treatment as a result of recent developments. Similar results are not observed in the long-term consequences of burn patients. In advanced stages of burn scars are likely to be encountered with extremity contractures, these contractures are especially common in the upper extremity and may lead to extensive functional impairment. Contractures tend to repeat likely, so reconstructive surgical initiatives should increase the function and reduce the recurrence. Therefore, dermal scaffold (DS), stem cell (SC) and fat graft applications, platelet rich plasma (PRP) injections are combined with conventional flap or graft surgeries to obtain better results. We have examined alternativeness of our treatment modalities that were created as a result of current strategies for the treatment of burn contractures to conventional treatments in this paper.

Methods: 15 Patients with complaints of the upper extremity burn contractures were admitted to our clinic. Patients were divided into 3 groups according to the presence of low (L), moderate (M) and severe (S) contractures. After contracture releasing, dermal scaffolds and split thickness skin grafts (STSG) were applied for L group. DS, PRP and STSG were applied for M group. DS, PRP, SC and STSG were applied for S group. Vacuum assisted closure was performed for all patients. Physical therapy was started on postoperative 1. week. Lipoinjection procedures were performed on postoperative 6 month for all groups to contour. Preoperative, early and late postoperative range of motion (ROM) was measured.

Results: Follow-up time was 18 months. Significant differences were found between preoperative and postoperative ROM. There was improvement in skin quality and flexibility, reduction of hypertrophic scarring.

Conclusions: Inadequate skin quality and flexibility, insufficient ROM and reccurence incidence are common problems in burn contracture treatment. Inability to use local or regional flaps is another trouble for extremities with wide hypertrophic scars. DS and STSG usage provides skin quality and flexibility, reduces hypertrophic scar formation and recurrence. PRP injection provides tissue regeneration and the integration of the graft-recipient site.

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A DURABLE TISSUE ENGINEERED INJECTABLE FILLER THAT PROMOTES ADIPOGENESIS AND ANGIOGENESIS IN DEEP WRINKLES

Presenter: Todd N. McAllister, PhD
Authors: McAllister TN, Pena G, Rodriguez R, Dusserre N, Lheureux N, Wiegering CE
Cytograft

NOT PRESENTED

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THE SURFACE AREA OF LIPOSUCTION CANNULA TIP PORT HAS AN EFFECT ON THE NUMBER OF SVF CELLS PER CC OF ADIOSE TISSUE HARVEST

Presenter: Ricardo L. Rodriguez, MD
Authors: Rodriguez RL, Dos Anjos S, Stambolstyan G, Llull R
Cosmeticsurgnet

To optimize fat graft results in a way that is reproducible, fat grafting must be understood as process engineering where every step is a variable that can contribute to the final result. The three major steps (aspiration, processing, reinjection) of fat grafting can each be broken down into multiple steps, each of which can have an effect on adipose tissue graft viability and characteristics. For the aspiration step little attention has been paid to the cannula port size and its effect on fragment size. Fragment size is of importance for fat grafting as Yoshimura has shown that smaller adipose fragments result in higher percent graft survival. Yet there is also question as to wheter fragment size affects stromal tissue harvest, which is of importance for SVF harvest.

Method: Liposuction harvest of specimens was carried out in 3 patients using a Sattler tip cannula with 1mm diameter port size openings and a Mercedes tip cannula with 4mm squared port openings. All other liposuction parameters were equal including aspiration pressure, harvest site, tumescent infiltration, etc. Specimens were obtained using the GID 700 system for harvesting fat graft. Specimens were from raw lipoaspirate, from inside the GID device after washing and processing, and from the waste cannister (material that had gone thru the GID mesh during processing). Cell counts, LDH and Triglyceride levels were obtained from all specimens.

Results: Total nucleated cell counts were higher for all specimens (raw aspirate, GID dvice, waste cannister) collected with the 4mm squared port size cannula ($P<0.01$). LDH and triglyceride levels were higher in the smaller diameter adipose tissue fragments. There was greater loss of tissue into the waste cannister with the Sattler tip cannula.

Discussion: Cannula port size determines fragment size and this should be taken into account when making a choice for harvesting fat graft and SVF harvest. Too small a port size (1mm diameter) may result in increased cell damage as measured by LDH and triglyceride levels. A larger 4mm squared port size is recommended for SVF harvest as the larger fragments have higher SVF content.

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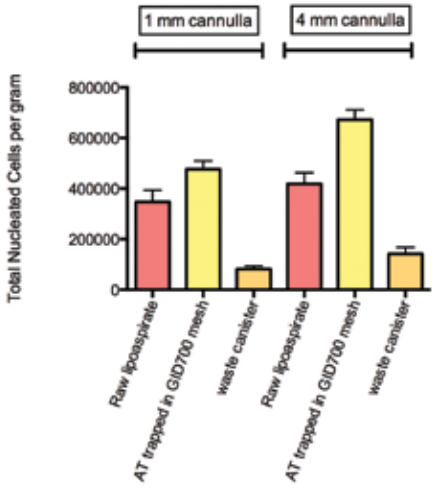
THE SURFACE AREA OF LIPOSUCTION CANNULA TIP PORT HAS AN EFFECT ON THE NUMBER OF SVF CELLS PER CC OF ADIOSE TISSUE HARVEST



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A SYSTEMATIC LITERATURE REVIEW ON THE USE OF FAT GRAFTING AND REGENERATIVE CELLS IN BURN WOUNDS AND SCARS

Presenter: Alexandra Conde-Green, MD
Authors: Conde-Green A, Marano AA, Lee ES, Reisler T, Price LA, Milner SM, Granick MS
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Background: There is an abundance of literature supporting the efficacy of fat grafting in aesthetic and reconstructive cases. There has been an emphasis on the regenerative capacity of adipose-derived stem cells (ASCs) and their utility in the improvement of wound healing and scarring provided by their cytokine and growth factor profiles. Despite the wealth of evidence supporting their efficacy, little attention has been paid to their utility in burn treatment. Our purpose is to provide an analysis of the literature regarding the use of fat grafting and ASCs in the treatment of burn wounds to guide surgeons and scientists on their clinical use, and on pursuing further investigation.

Methods: A systematic review of the literature was performed by a thorough search of 12 terms on PubMed, Medline and Cochrane databases. 241 articles were subject to evaluation by predetermined inclusion and exclusion criteria.

Results: Eighteen studies were selected, 6 murine and 12 human studies including case controls, case series and case reports. They describe histological and clinical effects of fat grafting and ASC therapy, including improvements in burn size and texture, enhance angiogenesis, decrease inflammation, allevaiton of pain and return of function.

Conclusions: There is a dearth of randomized controlled trials and quantitative analysis supporting the efficacy of fat grafting and ASCs in burns. However, the subjective improvements in scars are encouraging. It is our hope that this review will be a foundation for future studies and highlight the breath of knowledge yet to be explored by this therapy.

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VOLUMETRIC ESTIMATION OF AUTOLOGOUS FAT FOR AUGMENTATION OF CONTOUR DEFECTS OF FACE

Presenter: Shilpi Bhadani, MCh
Authors: Bhadani S, Sarabahi S, Tiwari VK, Arora S
GNH Hospital

WITHDRAWN

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THE USE OF MICRO FRACTURED FAT FOR GRAFTING OF THE PERIORBITAL REGION: 46 CONSECUTIVE CASES

Presenter: ChiaChi Kao, MD
Author: Kao CC
Private Practice Santa Monica California

Introduction: Within the last ten years, fat grafting to the face has become an integral adjunct to facelift surgery. Correction of the hollow upper and lower eyelid regions remain a challenge. Fat grafting inside the orbit is treacherous due to the potiential for globe injury, risk of blindness, graft take unpredictabilty, oil cyts, lumpiness, and uneveness of the fat graft. One strategy to minimize the risk of lumpiness is to reduce the parcel size of the harvested fat designated for the periorbita. Micronizing the fat can be accomplished by either reducing the size of the hole in the harvesting cannula to less than 3mm or by using a mechanical aggitation process via a transfer conduit where the fat is slushed back and forth between two syringes. Both techniques maybe too truamatic for the fat.

Method: Over the course of one year, we performed an IRB approved observational/safty study on 46 consecutive cases using Lipogems for rejuvenation of the upper/lower lids and periorbital region. This novel fat procesing device gently micro fractures the fat and yields vialble fat that are also rich in regenerative cells. All cases were performed in conjuction with endoscopic facelft surgery.

Results: None of the 46 patients experienced any acute adverse events, oil cyts, lumpiness, or uneveness of the graft. Follow up of these patients up to one year has demonstrated good retention of the graft. Patient satisfaction was extremely high.

Conclusion: The use of micro fractured fat allows greater ease and safety when grafting fat to the upper/lower lids and the periorbital region.

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EXPANDED MESENCHIMAL STEM CELLS FOR FAT GRAFTING - A NEW TECHNIQUE

Presenter: Maurizio Viel, MD
Authors: Viel M, Viel R, Mucci G
London Center for Aesthetic Surgery/Bioscience Clinic Dubai

Introduction: Combination of autologous in vitro-expanded ADSC with fat for soft tissue augmentation clinical applications overcomes some of the problems of fat graft alone, as variable resorption and survival rates. In-vitro expansion of ADSC increases the number of cells to the quantities required to achieve therapeutic results. Once injected, ADSC continue to proliferate, can differentiate or induce differentiation of endogenous stem cells and release angiogenetic and anti-apoptotic growth factors.

Method: ADSC were isolated from autologous fat and expanded according to GMP using a new expansion protocol from 5 patients. Before release, cells were tested for sterility and biological safety. In vitro and in vivo experiments were performed according to the European Pharmacopoeia and no signs of tumorigenesis were detected, suggesting the biological safety of expanded ADSC. Cells were administered subcutaneously in association with autologous fat to 5 patients for breast augmentation and 5 patients for facial rejuvenation.

Evaluation of the treated areas was performed prior and after treatment and 4 months after by means of MRI and bilateral mammography, in compliance with an established follow-up protocol.

Results: Follow-up reports highlight an increase in the volume of adipose tissue in treated areas, due to ADSC-mediated enhancement of fat graft viability and suggest that the ratio ADSC/fat is critical for graft retention.

Conclusion: Our study confirms that fat graft enriched with in vitro-expanded autologous ADSC, according to our procedure, is a safe and efficient approach for soft tissues augmentation.

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DEVELOPMENT AND EVALUATION OF THE AIRTIGHT, MINIMAL-INVASIVE AND FAST DEVICE HARVESTING ADIPOSE TISSUE FOR AUTOLOGOUS FAT GRAFTING

Presenter: Hongwei Liu, MD,PhD
Author: Liu H
The First Affiliated Hospital of Jinan University

NOT PRESENTED

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EVALUATION OF BIOMATERIAL, ADIPOCYTES AND ADSC INTERACTIONS IN PURE AUTOLOGOUS FAT GRAFTING MODEL THROUGH 7TMRI

Presenter: YoShen Chen, MD
Authors: Chen YS, Hsueh YS, Chen YY, Tai HC, Lin FH
National Taiwan University and Far Eastern Memorial Hospital

Introduction: The biomaterial added to the autologous fat grafting may act as both the supporting matrix for the grafted adipocytes and also as the cell carrier for introduction of additional cells such as the ADSC into the system. The purpose of this study is to carry out in vivo animal study and to evaluate the interactions between (1) mature adipocytes (2) biomaterial and (3) ADSC. The study is design in pure autologous fashion, each individual SD rat will only receive the fat graft or ADSC solely derived from its own body. All animals are immunocompetent so that the action of the immune reactions will be accounted for within the result, in order to simulate the possible human reaction to biomaterial and ADSC in current clinical practices.

Methods: In the study, three materials: autologous mature adipocytes (designate A for adipocyte), alginate beads (designate B for bead) and adipose-derived stem cells (designate C for cell) are transplanted into the back of the SD rat in either one of the five different combinations. Group A: adipocytes only; group B: alginate beads only; group AB: adipocytes and alginate beads; group BC: alginate beads laden with adipose-derived stem cells; group ABC: adipocytes and alginate beads laden with adipose-derived stem cells. Three animals are used for each group and they received 7T MRI at the 1th, 6th and 12th weeks after the transplantation. The images obtained through the MRI are used for the evaluation of graft condition in living host and for the calculation of grafts volume. At 12th weeks, the animals are sacrificed and the transplanted materials are retrieved for gross examination, weighting, and histological evaluation.

Results: In all aspect ABC group shows the closest resemblance to group A, and the increase of overall graft volume and near total resorption of biomaterial can be noted within that group. It seems without the additional induction from an external source, adipogenesis from the ADSC, encapsulated within the biomaterial, can only be evident when the mature adipocytes are also transplanted.

Conclusion: The interaction of cytokines and adipokines between the mature adipocytes and ADSC may be responsible for (1) faster disintegration of biomaterial (2) adipogensis from ADSC (3) better fat graft survival

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MICROFAT AND NANOFAT GRAFTING MIXED WITH PRP: BASIC RESEARCH AND CLINICAL APPLICATIONS

Presenter: Mario Goisis, MD, MS
Authors: Di Petrillo A, Goisis M, Mele S, Guareschi M, Scarpa A
Doctors Equipe Milano

NOT PRESENTED

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CHARACTERIZATION AND COMPARISON OF FRESH STROMAL VASCULAR FRACTION FROM HUMAN SUBCUTANEOUS, OMENTAL AND PERIRENAL ADIPOSE TISSUE

Presenter: Jeremy Magalon, PharmD
Authors: Magalon J, Arnaud L, Lyonnet L, Giraudo L, Chateau AL, Aboudou H, Bertrand B, Boissier R, Hardwigsen J, Casanova D, Karsenty G, Lechevallier E, Paul P, Veran J, Sabatier F
Culture and Cell Therapy Unit INSERM CBT1409

Introduction: Various depots of adipose tissue (AT) from different anatomic location, as visceral adipose tissue, or around solid organs are not well described. To address this issue, we compared phenotypes and clonogenic properties of freshly isolated SVF from SC, omentum (OM) and perirenal (PR) AT.

Method: A total of 11 samples were obtained: SC AT (n=4) were obtained by liposuction for plastic surgery, omental AT (n=4) from patients undergoing surgery necessitating OM removing, and perirenal AT (n=3) from living kidney donors. SVF was obtained using an already described manual AT digestion process. Viable nucleated cells were determined using the NC100 instrument. Combination of CD90 CD34 CD45 CD146 markers in flow cytometry was used to identify eight distinct cell subpopulations belonging to the following groups: leukocytes, vascular wall and subendothelial resident progenitors and mature cells. Frequency of adipose-derived mesenchymal-like stem cells was estimated using a CFU-F clonogenic assay.

Results: OM AT shows higher yield in total nucleated cells per g of AT (2,54 ± 1,53 x10ˆ6) compared to the other sources of SVF. Specific characteristics of CD45 negative subpopulations were observed by flow cytometry: CD34-/CD146+/CD90- was mainly present in SC AT (11,63 ± 8,13%) compared to OM AT (1 ± 1,18%) and PR AT (1,06 ± 1,04%); CD34-/CD146-/CD90- was mainly present in PR AT (30,58 ± 2,6%) compared to SC AT (2,62 ± 1,05%) and OM AT (5,27 ± 3,21%); CD34+/CD146-/CD90- was mainly present in OM AT (29,35 ± 9,62%) compared to SC AT (0 ± 0%) and PR AT (2,89 ± 5,01%). Clonogenicity assay shows a high proportion of CFU-F in SC AT (5,65 ± 2,1%) compared to OM (0,22 ±0,31%) and PR AT (1,04±0,45%).

Conclusions: SVF obtained from different fat depots contribute to different cell populations and may be of different interest from a therapeutic and/or diagnostic point of view. Potential and nature of each subpopulations deserve to be investigated. Low clonogenicity level of stromal cells in OM or PR AT currently regarded as a surgical waste, raise the perspective to be a relevant source of cultured endothelial cells. Such endothelial cells may be relevant to provide donor antigens and monitor humoral response in solid organ allograft.

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ASSESSMENT OF THE IMPACT OF PRO-INFLAMMATORY CYTOKINES ON GENE EXPRESSION, SECRETION PROFILES AND ENDOTHELIAL NETWORKS WITHIN HUMAN TISSUE-ENGINEERED ADIPOSE SUBSTITUTES

Presenter: Julie Fradette, PhD
Authors: Safoine M, Aubin K, Mayrand D, Maux A, Audet-Casgrain MA, Fradette J
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Introduction: Inflammation is a normal phase of the wound healing process likely to occur following tissue transplantation. Human reconstructed adipose tissues (hrAT) produced by tissue engineering represent a promising alternative to autologous fat grafts in reconstructive surgery. It is therefore important to determine the impact of inflammation on hrAT. We hypothesized that an in vitro inflammatory context will induce a modulation of the gene expression and secretion profile of hrAT and induce a degradation of their capillary network.

Methods: hrAT containing a capillary network or not were produced by tissue engineering using adipose-derived stem/stromal cells and microvascular endothelial cells. They were incubated with 10 or 100 ng/ml tumor necrosis factor α (TNFα) and 10 ng/ml interleukin-1β (IL-1β) for up to 6 days *in vitro*. Tissue samples and culture supernatants were collected and analyzed by quantitative real-time PCR, ELISA assays and confocal microscopy following an immunostaining for the endothelial cell marker CD31.

Results: In hrAT devoid of endothelial networks, quantitative PCR showed a decrease in expression of adipocyte's lipid metabolism related genes SLC2A4, FASN and LIPE. A robust increase in gene expression of 3 targets of the NF-κB pathway activation was observed after TNFα exposure (TNFAIP3, PTGS2, TRAF1). Culture supernatants from hrAT treated for 24h with 10 and 100 ng/ml TNFα revealed an increase in MCP-1 (3.3 and 5.0 fold, respectively), NGF (1.6 and 2.3 fold) and HGF (1.6 and 1.5 fold) while no differences were observed for VEGF or leptin secretion. For hrAT containing a preformed network of capillaries in vitro, a chronic 6-day incubation with IL-1β alone or in combination with TNFα revealed a less extended (53% and 64%, respectively) and less ramified capillary network (2.3 and 4.0 fold).

Conclusions: These results suggest that hrAT produce cell specific responses and an activation of the NF-κB pathway after exposure to an in vitro pro-inflammatory context created by TNFα and IL-1β supplementation. Using hrAT as a model will facilitate the study of the impact of inflammation on human adipose tissue such as after reconstructed tissue implantation or in the context of obesity. Supported by the CIHR.

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COMPARISON OF COLLAGENASE NEUTRALIZATION METHODS FOR ADIPOSE DERIVED CELL ISOLATION

Presenter: Hulan Shang, MS
Authors: Shang H, Yang N, Katz A
University of Florida

Introduction: Adipose-derived cell therapies are progressing toward the clinic for a variety of clinical indications. The isolation of good quality cells is critical for efficacy and reproducibility. Finding a simple and safe way to neutralize collagenase enzyme is a key step in cell isolation. Human serum albumin (HSA) is a suggested neutralizer but very expensive. EDTA is another way to neutralize enzyme activity but it may be associated with additional regulatory hurdles. We explored sample/enzyme dilution as a cheap, simple way to reduce enzyme activity.

Methods: Three neutralization methods were evaluated in parallel: HSA, EDTA and double volume dilution. Outcome measures included cell yield and cell viability and cell surface marker expression of CD45, CD31, CD34, CD146 and CD140b. Residual collagenase levels were quantified and compared for the three methods. Single factor ANOVA test was used for statistical analyses.

Results: There is no significant difference ($P>0.05$) between the three neutralization methods tested when evaluated for cell yield and viability. Percentage of white blood cells (CD45+ cells) and endothelial progenitor cells (CD45-CD31+CD34+) in total viable cells showed no significant difference ($p>0.05$) between the neutralization methods. Residual collagenase levels were less than 10CDU for all groups, with no significant difference between the three neutralization methods tested.

Conclusions: Dilution is as effective in neutralizing collagenase enzyme activity as more expensive serum albumin or EDTA methods. This finding may help facilitate the translation of adipose-derived therapies, with less cost burden.

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INVESTIGATING EARLY VASCULAR SPROUTING AND FAT GRAFT VOLUME RETENTION IN BOTH LIPOASPIRATED AND EXCISED EN BLOC ADIPOSE TISSUE

Presenter: Ava G. Chappell, BA
Authors: Chappell AG, Lujan-Hernandez J, Rojas-Rodriguez R, Perry DJ, Min SY, Corvera S, Lalikos JF
University of Massachusetts Medical School

Goals: Fat grafting is used in reconstructive surgery to restore soft tissue defects because of its regenerative potential and surgical accessibility. However, the survival of fat grafts is unpredictable. Liposuction is the standard surgical technique for harvesting the fat, yet, can be mechanically damaging to adipocyte architecture. The effect of the trauma caused by liposuction on the ADSC niche and the capacity to develop new vascular connections is not well known. Similarly, the impact of early revascularization in general on the long-term fat graft survival is not clearly understood. We hypothesize fat graft revascularization is impaired by liposuction trauma which could impair early vascular sprouting and ultimately impact long-term survival.

Methods: Tissue was obtained from discarded material from one panniculectomy procedure under approved IRB exemption. Liposuction and Excision techniques were compared. Fat grafts were obtained by liposuction using a 12-hole cannula and manual suction. Excised specimens were obtained by sharp-blade mincing of 0.5mm2 samples of dermal fat. Both samples were embedded in matrigel for 11 days and angiogenic potential was quantified as total mass of vascular sprouting using whole mount staining. In parallel, 11 immunodeficient mice received bilateral subcutaneous fat grafts (0.6cc) derived from each technique. Graft volume retention was calculated using in-vivo micro-CT scanning and 3D reconstruction at baseline and 8 weeks post-graft. Tissues were harvested after 8 weeks for histologic analysis using CD31 IHC.

Results: Both fat samples developed rapid vascular sprouting when placed in growth media. Excised adipose tissue showed a 69% higher vascular sprouting by day 7 and 16% by day 11 ($p<0.05$) compared to tissue obtained by liposuction. Moreover, excised fat grafts had higher volume retention than liposuction samples (88% vs 83%, $p<0.05$).

Conclusions: These results show excising adipose tissue en bloc has greater angiogenic potential in the initial phase after fat graft transplantation, which correlates with higher retention. This suggests traumatic harvesting may disturb the stem-cell niche, dampening early neovascularization and this could be one factor determining longterm graft survival.

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TISSUE FLAP PRECONDITIONING WITH ADIPOSE DERIVED STEM CELLS AND HYPERBARIC OXYGEN TREATMENT: A GUINEA PIG MODEL

Presenter: Nicole A. Gaid, MB BCh BAO, MMI
Authors: Gaid NA, Tenenhaus M, Grover I, Hayes S
University of California San Diego

Introduction: Complex wound, soft tissue reconstruction and transplacntation are inherently complicated by ischemia and reperfusion. Efforts to minimize these deleterious effects involve meticulous design and minimizing the ischemic period. Adjunctive measures have included free miccrovascular transfer, supercharging, caspase inhibitors and free oxygen radical scavengers. In this study, we hoped to characterize the potential complementary effects of Hyperbaric Oxygen (HBO) Therapy and Stem Cell (ADSC) delivery in tissue flap preconditioning.

Methods: In this animal trial, the study group received autologous ADSC injections subcutaneously whereas the control group received saline injections to the area of flap design. Subsequently, half of the animal subjects were exposed to four consecutive HBO treatments. Following preconditioning, a pedicled flap was elevated and clinically assessed via study-blinded observers, as well as by objective assessments of HSP 70 levels and quantification of TUNEL-positive cells.

Results: The presence of venous congestion was significantly greater in the flaps that did not receive HBO therapy (26.7% vs. 0%, $p=0.004$). Distal flap necrosis was more often noted in the animal subject who received ADSCs but were not exposed to HBO (46.7% vs. 6.7%, $p=0.013$). A statistically significant rise in HSP 70 levels in the 'No HBO and saline injection' group was detected ($p=0.042$), while the percentage of apoptotic cells was greater in the HBO and saline group ($p=0.003$) and compared to the HBO and ADSC injection group.

Conclusions: While further investigations with a larger sample size are underway, preliminary study findings support that the combination of ADSC injection and HBO preconditioning of flaps improves tissue viability and survival rates. The ongoing study also aims to elucidate the optimal preoperative time for the delivery of ADSC injection and HBO treatments.

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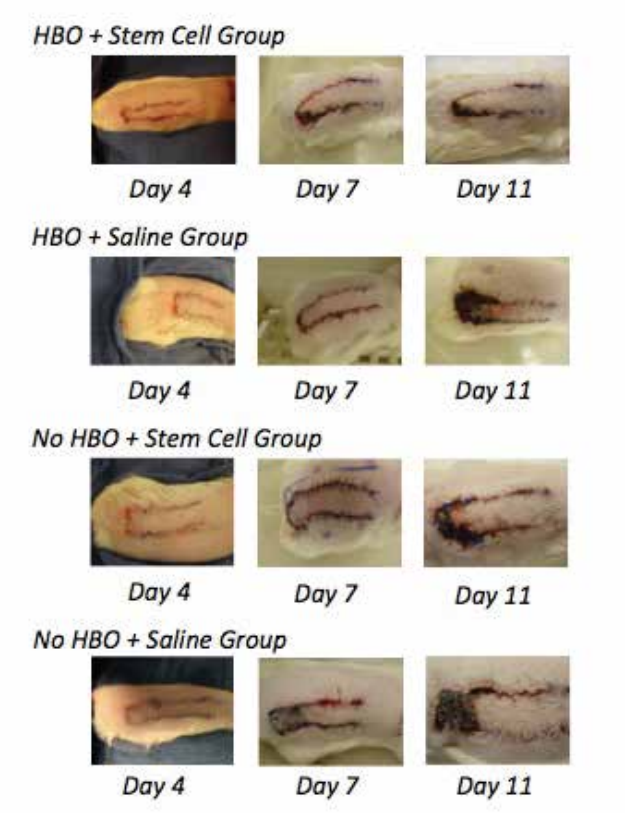
TISSUE FLAP PRECONDITIONING WITH ADIPOSE DERIVED STEM CELLS AND HYPERBARIC OXYGEN TREATMENT: A GUINEA PIG MODEL



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ADIPOSE TISSUE RETENTION VIA MICROSPHERE DRUG DELIVERY SYSTEM

Presenter: Arta Kelmendi-Doko, MD
Authors: Kelmendi-Doko A, Klett K, Wang SH, Marra KG, Rubin JP
University of Pittsburgh

Purpose: Adipose tissue is often a key structure to restore in reconstructive and augmentative surgeries. Current materials for soft tissue reconstruction suffer from shortcomings such as suboptimal volume retention, donor site morbidity, and poor biocompatibility. The aim of this study was to examine a controlled delivery system of adipogenic factors, namely dexamethasone (Dex), to generate stable adipose tissue when mixed with disaggregated human fat in a nude mouse model over 6 months. We hypothesized that the slow release of dexamethasone from polymeric microspheres would enhance both adipogenesis and angiogenesis, resulting in long term adipose volume retention.

Methods: In this study we tested two microsphere drug delivery systems. In the first one, dexamethasone was encapsulated in poly(lactic-co-glycolic acid) (PLGA) microspheres (Dex MS) where as in the second one dexamethasone was encapsulated in core of poly(lactic-co-glycolic acid) (PLGA), surrounded by the shell of poly-L-lactide (PLLA) to create the double wall polymer microsphere system for the purpose of slower and more sustained drug delivery. Dexamethasone-loaded PLGA (Dex MS) were prepared using single emulsion/solvent technique and dexamethasone-loaded PLGA – PLLA double wall microspheres (Dex DW MS) were prepared using a double emulsion/solvent extraction technique, and release kinetics was determined. Two different doses of both types of microspheres were examined, 50 and 27 mg of Dex DW MS mixed with 0.3 ml of human lipoaspirate along with 50 mg of empty MS and lipoaspirate-only controls. Samples were analyzed grossly and histologically after 6 months in vivo.

Results: Mass and volume were measured; dexamethasone microsphere-containing samples demonstrating greater adipose tissue retention compared to control group. Histological analysis including H&E and CD31 indicated increased vascularization ($p<0.05$) within the Dex MS-containing samples.

Conclusion: Controlled delivery of adipogenic factors such as dexamethasone via polymer microspheres can significantly effect retention of adipose tissue, maintaining healthy tissue formation and vascularization; therefor presenting a clinically relevant module of adipose retention.

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FEA ANALYSIS PREDICTS TAPERED STUMP ARCHITECTURE FOR BKA SHAPING WITH AUTOLOGOUS FAT TRANSFER

Presenter: Jessica Green, MD,PhD
Authors: Green J, Reddy PR
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Introduction: Autologous fat transfer is an outpatient procedure most commonly used to augment the contours of the breast or face but is considered a safe method of augmentation for a variety of medical conditions. We previously presented a novel application of autologous fat transfer to graft fat to a BKA stump to improve stump-prosthesis contact area. To determine the optimal shape for mechanical loading at the stump, we conducted a finite element analysis (FEA) model on a cylindrically-shaped stump and a tapered stump.

Methods: A cast model of two stump shapes, cylindrical and tapered, were made and topographical data was imported into Solidworks by 3D reconstruction of .jpg images. The shapes were assigned identical mechanical properties derived from literature review. Linear elastic isotropic deformation was assigned with a tensile strength of 3e7 N/m2. The models were subjected to an axial load of 338 N/m2 and Von Mises stresses were calculated.

Results: Results indicate that a tapered BKA stump is associated with reduced von Mises stress compared to the tapered stump. For tapering stumps, maximum von Mises stress was 1399.3 N/m2. For cylindrically shaped stumps, maximum von Mises stress was 1705.52 N/m2. Displacement mapping showed a maximum displacement of 1.63071e-5 mm for the tapered stump compared with 1.7268e-5 mm for the cylindrically shaped stump.

Conclusions: Autologous fat transfer for stump revision is an outpatient procedure with reduced recovery time and repeatability compared with the current standard of care. We have previously demonstrated a novel application of autologous fat transfer to tailor stump architecture to a prosthetic socket for improved prosthesis fitting and patient satisfaction. Using FEA analysis of BKA stump shape, we can determine the sites of increased mechanical loading and transfer fat to sites needed most to reduce risk of mechanical shear. Results of a simple linear elastic axial load model comparing cylindrical to tapered stump architecture demonstrated reduced von Mises stresses with tapered stumps.

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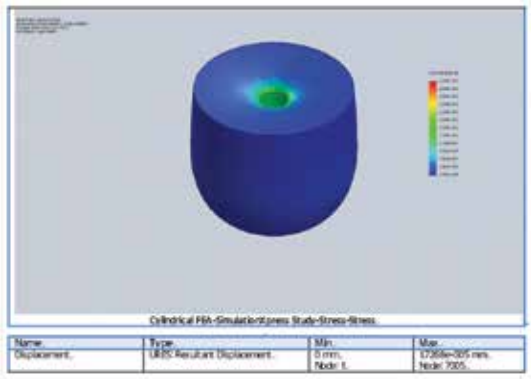
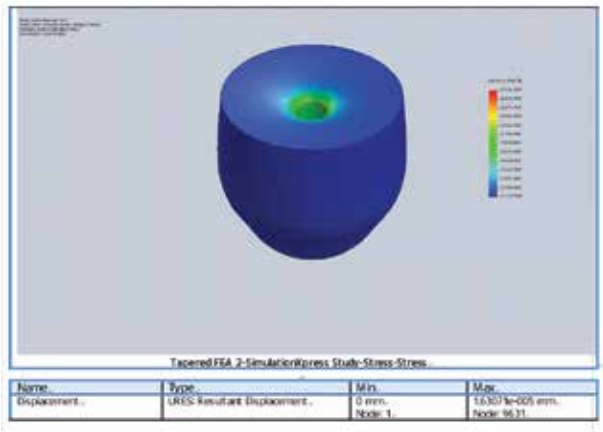
FEA ANALYSIS PREDICTS TAPERED STUMP ARCHITECTURE FOR BKA SHAPING WITH AUTOLOGOUS FAT TRANSFER





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APPLICATION OF A NOVEL EX VIVO MODEL FOR EVALUATING STEM CELL FATE IN A MICROVASCULAR NETWORK

Presenter: Mohammad S. Azimi, MS
Authors: Azimi MS, Strong AL, Bunnell BA, Murfee WL
Tulane University

Models that mimic angiogenesis are valuable for the investigation of underlying mechanisms and the pre-clinical development of therapies. Our laboratory recently developed a novel ex vivo tissue culture model that enables time-lapse investigation of mechanistic cell-cell interactions at specific locations across intact blood and lymphatic microvascular networks. The objective of this study was to demonstrate the usefulness of the rat mesentery culture model for evaluating mesenchymal stem cell differentiation into vascular pericytes and how aging affects differentiation dynamics. DiI-labeled cells were seeded onto adult Wistar rat mesenteric tissues and cultured in alpha-MEM + 1% serum for up to five days according to four experimental groups: 1) adult human adipose-derived stem cells (hASCs), 2) aged hASCs, 3) adult human bone marrow-derived stem cells (hBMSCs), and 4) aged hBMSCs. After one day, cells in all of the experimental groups displayed changes in cell morphology indicative of tissue integration. Angiogenesis per experimental group was supported by observation of increased vessel density and capillary sprouting. Also for each experimental group, a subset of cells was observed in typical pericyte location wrapped along blood capillaries. However, while no apparent differences existed between aged and adult hASC groups, the number of DiI-positive-covered vessels was increased in the aged hBMSC group versus the adult hBMSC group (Aged hBMSC=18.26±0.47 vessels/mm2 vs. Adult hBMSC=2.81±0.39 vessels/mm2; $p<0.05$). None of the cells in perivascular location were observed along initial lymphatic capillaries. Our results establish the rat mesentery culture model as a valuable tool for ex vivo screening of type-specific differences in stem cell fate across an intact microvascular network during angiogenesis.

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NOVEL PRIMING METHOD FOR ADIPOSE-DERIVED STEM CELLS FROM THE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS MOUSE MODEL RESTORES ANTI-INFLAMMATORY PROFILE

Presenter: Rachel M. Wise, MS
Authors: Wise RM, Bowles AC, Bunnell BA
Tulane University

Multiple sclerosis (MS) is a neurodegenerative disease characterized by inflammatory and demyelinating lesions in the central nervous system (CNS) causing symptoms including fatigue, muscle weakness, spasticity and progressive loss of motor function. Adipose-derived stem cells (ASCs) have been shown to reduce inflammation and myelin damage in the CNS, motor impairment, and disease severity in the experimental autoimmune encephalomyelitis (EAE) mouse, a model of MS. Recent studies characterizing ASCs from both wild-type and EAE mice (EAE-ASCs) harvested at 30 days post induction have revealed several cellular differences in the cells from EAE animals that make them unsuitable for autologous therapy. EAE-ASCs show decreased osteogenic differentiation capacity and elevated expression levels for pro-inflammatory cytokines in vitro, which diminishes their immuno-modulatory effects when administered in vivo. In this study, EAE-ASCs and wild-type ASCs were isolated at multiple time points during the progression of EAE and assessed in vitro for osteogenic and adipogenic differentiation capacity, colony forming units and proliferation assays, and qRT-PCR to contrast expression profiles for both pro and anti-inflammatory cytokines and chemokines. In parallel, the EAE-ASCs were primed with a toll-like receptor (TLR)-3 agonist and the same assessments were performed. It has been shown that upon activation of TLR-3 by this agonist, wild-type ASCs become "primed" toward an anti-inflammatory profile. This priming method used with stem cells promoted migration, enhanced expression of immuno-modulatory cytokines including CCL10, IL-1RA and IL-10, reduced expression levels of pro-inflammatory transforming growth factor beta (TGF√x) and Jagged 1, and reduced T-cell activation in a model of diabetic neuropathy. Thus, it is believed that priming of EAE-ASCs restores functionality to potentially recover their beneficial effects. Results show that primed EAE-ASCs were capable of producing a more anti-inflammatory profile, though not returning to wild-type levels. This study demonstrates the potential use of primed EAE-ASCs in future EAE treatment studies, and may elucidate a new method for autologous stem cell therapy in MS patients.

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ADIPOSE STROMAL CELLS FROM DONORS WITH METABOLIC SYNDROME CAN BE VALUABLE THERAPEUTIC MATERIAL FOR TREATMENT OF ACUTE RESPIRATORY DISTRESS SYNDROME

Presenter: Natalia V. Bogatcheva, PhD
Authors: Bogatcheva NV, Lu H, Merfeld-Clauss S, Traktuev DO, March KL
Indiana University

Introduction: Metabolic syndrome (MetS) is associated with higher risk of Acute Respiratory Distress Syndrome (ARDS) development in patients undergoing bariatric surgery. These patients may benefit from banking their adipose stromal cells (ASC) during surgery, with the purpose of using them for the therapy of ARDS if such complication develops. Assessment of therapeutic potency of ASC from donors with MetS is of ultimate importance for the development of autologous therapy.

Methods: Adipose tissue from MetS rats (ZDSD rats, PreClinOmics) was used to isolate rat MetS ASC. Lipoaspirate from patients with high BMI, hypertension, dyslipidemia and diabetes was used to isolate human MetS ASC. Stromal vascular fraction (SVF) yield, ASC proliferation rate and an ability of MetS ASC to secrete factors stabilizing vascular barrier were assessed and compared to healthy donor/control rat characteristics.

Results: Although subcutaneous fat yield from ZDSD rats was similar to that of control SD rats, cellular content of SVF per gram of fat was significantly (~40%) lower in ZDSD than in control rats. Moreover, proliferation rate assessed at passage 3 was markedly reduced in ZDSD ASC compared to ASC from control animals. Application of ASC conditioned media (ASC-CM) from control/healthy donor to the rat lung microvascular endothelium (RLEC) or human pulmonary artery endothelium (HPAEC) significantly reduced RLEC and HPAEC barrier dysfunction in response to H_2O_2 compared to the responses of un-treated endothelia. Importantly, application of ASC-CM from ZDSD rat or MetS donor to RLEC/HPAEC had similar barrier-protective effect.

Conclusions: Factors secreted by ASC derived from donors with MetS are capable of reducing H_2O_2-induced barrier dysfunction, linked to the development of lung edema in ARDS. Therefore, adipose tissue from donors with MetS can be a source of valuable therapeutic material for ARDS treatment. As patients with MetS are at higher risk of ARDS development, they will benefit from banking SVF/ASC during elective surgery, such as bariatric. This work was supported by Krannert Institute of Cardiology Endowment Fund, CTSI CECARE, Vascular and Cardiac Center for Adult Stem Cell Therapy (VC CAST), Cryptic Masons Medical Research Foundation, and PreClinOmics.

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LIPOFILLING, INTERNAL EXPANSION AND COMMON SENSE BASED ON TISSUE ENGINEERING FINDINGS IN AUTOLOGOUS BREAST RECONSTRUCTION

Presenter: Filip Stillaert, MD
Author: Stillaert F
University Hospital Gent

Introduction: Ideally, breast recontruction involves the use of autologous tissue to restore the anatomical landmarks and physical characteristics of the breast. Microsurgical tissue transfer is the standard procedure to achieve this goal. In a selected grooup of patients, the less invasive lipofilling technique offers new strategies in breast reconstruction. When fat grafting is chosen as an option in breast reconstruction the major concern is to create the ideal recipient site for fat grafts to survive.

Materials and methods: A specific group of patients (n=7) was treated according to a protocol distracted from tissue engineering research. This research showed that four basic requirements are necessary to support adipogenesis in vivo: 1/ a potent cell source, 2/ a supportive matrix, 3/ a well-defined space and 4/ vascularity. Internal expansion created a well-defined subcutaneous space and generated a highly vascularised recipient site to support the process of plasmatic imbibition and angiogenesis. Concomitant (plasma enriched) lipoaspirate injection and expander deflation (time interval of 3 months) reconstructed a natural looking breast. Endoscopic studies and MRI follow-up were included to study the process of fat grafting and the behaviour of injected fat.

Results: All patients were treated successfully with internal expansion and lipofilling to reconstruct an acceptable breast volume with natural contours. Endoscopic views revealed the importance of plasmatic imbibtion and the newly generated well-vascularised recipient site (capsula). MRI studies showed viable adipose tissue and volume measurments were performed to evaluate the graft survival rate.

Conclusion: The use of interal expansion and lipofilling is an attractive strategy to reconstruct a breast in a selected group of patients. Expansion generates a highly vasculairsed and well-defined recipient site to harbour the fat graft which leads to the generation of a homeostatic tissue construct mimicking the environment of the tissue engineering chamber.

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LIPOASPIRATE AND ADIPOSE STEM CELLS AS POTENTIAL THERAPEUTICS FOR CHRONIC SCARS

Presenter: Dylan J. Perry, BA
Authors: Perry DJ, Lujan Hernandez J, Chin M, Min SY, Chappell AG, Appasani R, Rojas Rodriguez R, Corvera S, Lalikos JF
University of Massachusetts Medical School

Introduction: Burn injuries can lead to hypertrophic or keloid scars which cause pain, pruritus, limited mobility across joints, and disfigurement. Current therapies are often unsatisfactory, costly, or excessively morbid. Prior studies suggest adipose derived stem cells (ADSCs) and lipoaspirate can improve healing and scar outcomes of acute thermal wounds. Clinical reports suggest lipoaspirate and ADSCs can improve chronic burn scar remodeling. However, this phenomenon has not been extensively studied in animal models. We sought to determine if adipose tissue can improve chronic scar remodeling and compare the effects of adipose derived stem cells and processed lipoaspirate.

Methods: 50 CD1 nu/nu athymic mice received a standardized deep partial thickness thermal burn and were monitored for 6 weeks as scars matured. Digital photographs and perfusion measurements by hyperspectral imaging (HSI) were taken over the entire study. Lipoaspirate and ADSCs (SVF and ex-vivo culture with flow cytometry confirmation) were obtained from a discarded human pannus specimen. After six weeks animals received a 0.6cc subcutaneous graft beneath the scar of either: human lipoaspirate processed with the Coleman technique, high dose (106) hADSCs in Matrigel, low dose (104) hADSCs in Matrigel, Matrigel only, or were not injected (n=10 per group). At 4 weeks post-injection animals were sacrificed and scar tissue samples were harvested for histological and molecular analysis.

Results: HSI oxygenated hemoblogin values in scars treated with lipoaspirate increased significantly more compared to 6-week pre-treatment baseline than all other groups ($p<0.05$). Scar planimetry analysis showed reduction in wound area in lipoaspirate treated mice compared to control groups ($p<0.01$). Blood vessel density quantification on Masson's trichrome stains suggest increased density in lipoaspirate treated scars compared to control (Fig 1, $p<0.01$).

Conclusion: HSI, blood vessel density, and scar area analysis suggest scar improvement in lipoaspirate treated scars compared to controls. No effect was seen with ADSCs suspended in Matrigel at either concentration at the analyzed timepoints. Additional molecular analyses are underway to investigate the effects on cellular mechanisms regulating scar remodeling

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LIPOASPIRATE AND ADIPOSE STEM CELLS AS POTENTIAL THERAPEUTICS FOR CHRONIC SCARS

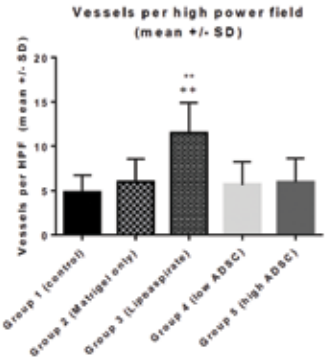

Figure 1. Blood vessel density per high power field quantified at 3 random 40x sections.

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IN VITRO EVALUATION OF PARTICULATE HUMAN ADIPOSE-DERIVED MATRIX SEEDED WITH HUMAN ADIPOSE-DERIVED STEM CELLS: DIFFERENTIATION INTO ADIPOCYTES AND FORMATION OF EXTRACELLULAR MATRIX

Presenter: Evangelia Chnari, PhD
Authors: Huang YC, Lorenzo BC, Schilling BK, Kokai LE, Marra KG, Rubin JP, Chnari E
Musculoskeletal Transplant Foundation

Introduction: There is a strong clinical need for improved therapies for fat retention after soft tissue loss. The use of autologous fat grafting results in variable and unpredictable outcomes. Adipose-derived matrix, retaining its adipogenic potential, may be a promising alternative to address this issue. This study examines the adipogenic capacity of a human adipose-derived matrix (Allograft Adipose Matrix - AAM) by seeding it with human adipose-derived stem cells (ASCs), and assesses the formation of extracellular matrix and the expression of adipogenic markers, signifying the differentiation of ASCs into adipocytes.

Methods: AAM was processed aseptically, milled into a particulate, reconstituted in basal medium and placed in insert wells. ASCs were seeded atop the matrix. Confocal microscopy, scanning electron microscopy (SEM) and histology were utilized to assess cell attachment and matrix production (collagen IV, laminin) at various time points. To assess adipogenesis, samples were characterized via immunohistochemical staining for adipogenic markers (adiponectin, leptin, FABP4, perilipin A).

Results: Confocal imaging confirmed cell attachment, proliferation, and infiltration into the matrix structure chronologically. Morphological changes suggested the differentiation of ASCs into adipocytes; SEM showcased cell attachment and proliferation within the matrix structure and by day 7, new matrix was visualized. Histology verified cell attachment and infiltration along with matrix deposition of collagen IV and laminin over time. H&E staining revealed adipocyte phenotype characterized by the nucleus located in the periphery within the cytoplasm. Expression of early-stage adipogenic markers (adiponectin), and late-stage adipogenic markers including leptin, FABP4, perilipin A was also confirmed.

Conclusions: This study demonstrates that aseptically processed human adipose-derived matrix retains critical adipogenic cues that facilitate ASC attachment, proliferation, matrix production, and the differentiation of ASCs into adipocytes in basal medium without exogenous adipogenic factors. This has significant implication for AAM to be used for adipose tissue regeneration. Animal studies are underway to correlate these in vitro results with in vivo efficacy.

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IMPROVING FLAP SURVIVAL WITH FRESH AND CULTURE-EXPANDED ADIPOSE-DERIVED STROMAL CELLS IN A XENOTRANSPLANTATION RAT MODEL

Presenter: Navid M. Toyserkani, MD
Authors: Toyserkani NM, Jensen CH, Sheikh SP, Sorensen JA
Odense University Hospital

Introduction: Flap necrosis due to limited vascular supply is an ongoing challenge in plastic surgery. In the last decade especially adipose-derived stromal cells have emerged as a possible solution to improve flap survival. The cells can be used freshly isolated as the stromal vascular fraction (SVF) or after culture-expansion of the adipose-derived stromal cells (ASCs). The aim of this study was to compare the efficacy of human SVF and ASCs for improving flap survival in a rat model.

Methods: Lipoaspirates from three human female donors were used for isolation of SVF. 36 male Sprague Dawley rats were randomized to three groups. A caudally based random flap measuring 2x7cm including a triangular area at the tip was elevated on the dorsum of each rat (flap 15cm^2). The flaps were injected with 5 x 10^6 SVF cells, 5 x 10^6 ASCs or phosphate buffered saline. Seven days later flap survival was analyzed with standardized photography. Flap skin was fixed for histologic analysis to examine vessel density with both alpha smooth muscle actin and CD31 staining and stem cell retention in the flap with human mitochondrial antigen.

Results: The mean survival rates ± SD were 55.0±7.2%, 50.4±9.1% and 45.7±9.5% for the SVF, ASC and control group respectively (SVF group significantly better than control group $p<0.05$). Vessel density assessed by alpha smooth muscle actin was ± SD 15.9±3.6, 19.6±4.7 and 13.7±3.2 vessels pr. high power field for SVF, ASC and control group respectively (ASC group significantly better than control group $p<0.01$). No human cells were stained in the flaps after 7 days.

Conclusions: Flap survival was significantly improved with SVF treatment. SVF treatment did not affect vessel density, which suggests that the mode of action was not stem cell induced angiogenesis. The clinical effect was modest and could represent the physiologic limits of angiogenesis in an acute setting. An alternative explanation for the benefit of SVF treatment could be the rodent immune response against human cells resulting in inflammation and vasodilation. Future research should elaborate this topic.

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ACCESSING THE ONCOLOGICAL RISK OF FAT GRAFTING TO THE BREAST THROUGH IMMUNOHISTOCHEMISTRY AND GENE EXPRESSION VALUES OF CD68, KI67, ER+(1D5) AND PAI-1

Presenter: Francisco Claro Jr., MD
Authors: Claro F, Moreira LR, Morari J, Sarian LO, Pinto GA, Velloso LA, Pinto-Neto AM
State University of Campinas UNICAMP

NOT PRESENTED

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COMPARISON OF CURRENT PROTOCOLS FOR AUTOLOGOUS FAT GRAFTING

Presenter: Vincent Hivernaud, MS
Authors: Hivernaud V, Lefourn B, Guicheux J, Girard AC, Weiss P, Roche R
INSERM U791 LIOAD

Background: Autologous Fat Grafting (AFG) is a widely used technique to naturally address soft-tissue deficit. However there is still no consensus on the procedure to use leading to unpredictable results due to resorption of the fat graft. In this study we compared 5 current protocols of AFG by analyzing the characteristics of the obtained adipose tissue and the outcome of the adipose graft in an in vivo model.

Methods: Lipoaspirate was harvested and processed according to 5 different protocols according to the manufacturer's instructions: (1) Bodyjet® combined with LipoCollector®, (2) Coleman's technique, (3) Macrofill® or (4) Microfill® kits and (5) PAL® combined with Puregraft®. This study was made with adipose tissue from 8 donors. After each protocols, quantity of adipose tissue, oil formation and cytokine secretion was assessed in vitro. Graft resorption rate and histological scoring (oil vacuole, fibrosis, infiltration and adipocytes homogeneity) were analyzed after subcutaneous injection in a murine model.

Results: In vitro, the adipose tissue phase was significantly greater after 2 days when using manual lipoaspiration and soft centrifugations (Microfill®/Macrofill®) compared to water-assisted lipoaspiration combined to decantation (BodyJet®). In vivo, one month after subcutaneous injection, the graft volume was greater with manual aspiration and centrifugation (Microfill®, Macrofill® and Coleman) compared to other aspirations, decantation and filtration (PAL®+PureGraft® and BodyJet®+LipoCollector®). The highest histological score was reached with the Macrofill® technique.

Conclusion: We confirmed that adipose tissue processing during lipotransfer technique can influence the AFG outcomes. There has been a great interest in protocols improvement in the past years. Among the new techniques, Macrofill® using gentle aspiration with multi-holes cannula and gentle centrifugation/ washing steps looks of great interest for AFG.

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PAPER AS A SUITABLE MATRIX FOR ASC DELIVERY INTO WOUNDS

Presenter: Michael A. Zieger, PhD
Authors: Zieger MA, Rahimi R, Ochoa M, Campana G, Ziaie B, Sood R
Indiana University School of Medicine

Introduction: Parchment paper is a low-cost substrate that has been used to fabricate flexible physical and chemical sensors for real-time monitoring of chronic wounds. An important characteristic of parchment paper is its silicone coating that gives it a natively hydrophobic property. Using a CO_2 laser the silicone coating can be selectively removed on desired locations, transforming hydrophobic areas to hydrophilic ones. We hypothesized that laser-modified parchment paper might be a suitable matrix for cell attachment and hence for delivery of adipose stem cells (ASCs) into the wound bed. ASCs secrete many types of growth factors and so have the potential to promote wound healing.

Methods: A pattern of hydrophilic dots was created as previously described (Chitnis et al., Lab Chip 11, 1161-1165 (2011)). The laser-treated paper (1.5-cm diameter discs) was placed in 24-well plates and sterilized overnight by exposure to a UV-C lamp. Sterile Teflon rubber rings (od=1.5cm; id=1.2cm) were used to anchor the paper to the well bottom. ASCs in tissue culture were trypsinized, resuspended and counted with trypan blue. 1 x 105 cells/ml were seeded per disc and cultured in EGM-2MV for up to 14 days. The medium was replaced every 24 h and the discs were collected over time and stained with eosin phloxine for histological assessment. On day 14, some samples were assessed for the cells' differentiation potential. The medium was changed to adipogenic or osteogenic induction medium and after additional culture time discs were processed using Oil Red O or von Kossa stain, respectively.

Results: Cells attached preferentially to hydrophilic regions and proliferated over time. The cells remained viable and were induced to differentiate into adipocytes or osteocytes. Some artifacts were present after prolonged culture such as the bridging of cells between regions and spheroids that appeared to sit on hydrophobic areas.

Conclusions: Laser-treated parchment paper is a suitable matrix for the attachment of ASCs and therefore may be an ideal material for developing a next-generation smart dressing that is low cost and incorporates autologous cells that promote wound healing.

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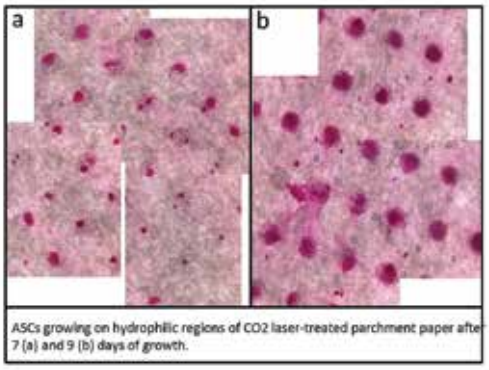
PAPER AS A SUITABLE MATRIX FOR ASC DELIVERY INTO WOUNDS



ASCs growing on hydrophilic regions of CO2 laser-treated parchment paper after 7 (a) and 9 (b) days of growth.

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EVALUATION OF A POLOXAMER HYDROGEL FOR THE DELIVERY OF ADIPOSE-DERIVED STEM CELLS IN THE CONTEXT OF PERIPHERAL NERVE REGENERATION

Presenter: Kassandra L. O'Brien, BS
Authors: O'Brien KL, Bliley JM, Havis EM, James IB, Wang SS, Sivak WN, Rubin JP, Marra KG
University of Pittsburgh

Introduction: Peripheral nerve damage is common in traumatic injuries and is associated with high long-term morbidity. The current gold standard of care for a severe transecting injury is transplantation of a nerve autograft. Unfortunately, this treatment does not typically result in full recovery. Several groups have shown therapeutic efficacy with the transplantation of adipose-derived stem cells (ASCs) in vivo. Despite this success, a clinically relevant method of cell delivery and maintenance has not been established. We investigated a thermoresponsive hydrogel based on the commercially available poloxamer 407, an FDA-approved inert pharmaceutical additive, for this purpose. Due to its thermoresponsive properties and the established safety profile of poloxamer 407, this material is attractive for cell delivery in a clinical setting.

Method: Survival of human ASCs in poloxamer hydrogel in vitro was characterized by proliferation analysis and confocal imaging. The potential use of this gel for ASC delivery in the context of peripheral nerve injury, as well as the efficacy of this intervention in promoting nerve regeneration, was evaluated in a rat model. A transecting injury was made to sciatic nerves of rats assigned to six treatment and control groups (n=6 per group), including ASCs delivered within poloxamer hydrogel and autograft. Gastrocnemius evoked muscle potentials were obtained at 6-wks post-op. Muscle and nerve tissue was explanted for gene expression and histological analysis.

Results: Human ASCs show up to 55% viability when grown within the gel over 72hrs. Imaging shows live ASCs with expected morphology. Explanted re-innervating gastrocnemius tissue shows activation of the muscle regeneration program in the group treated with ASCs delivered in poloxamer hydrogel vs. the group receiving gold stand of care (autograft), as assessed by expression levels of Pax7, MyoD, and Myogenin. Analysis of nerve and muscle histological samples and evoked muscle potentials data analysis are ongoing.

Conclusions: Poloxamer hydrogel is a promising potential method of therapeutic delivery of ASCs. Furthermore, these studies suggest that delivery of ASCs within poloxamer hydrogel at the site of peripheral nerve injury is an efficacious nerve regenerative treatment

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RISK-BASED APPROACH: A "GENERIC" TEMPLATE FOR ADVANCED THERAPY MEDICINAL PRODUCTS

Presenter: AnneLine Chateau, PhD
Authors: Chateau AL, Veran J, Magalon J, Blanchet L, Giraudo L, Mendizabal H, Sabatier F
Assistance Publique des Hopitaux de Marseille

WITHDRAWN

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PHENOTYPIC ANALYSIS OF STROMAL VASCULAR FRACTION AFTER MECHANICAL SHEAR: STRESS-INDUCED PROGENITOR TRANSDIFFERENTIATION?

Presenter: Derek A. Banyard, MD, MBA
Authors: Banyard DA, Widgerow AD, Wirth GA, Paydar KZ, Evans GR
University of California Irvine

Introduction: Optimization of fat grafting, especially with the introduction of cell-assisted lipotransfer, is a technique that continues to gain increasing attention in the field of regenerative medicine. One form introduced by Tonnard et al. in 2013, "nanofat grafting", implements mechanical emulsification and injection of standard lipoaspirate (LA) for the correction of superficial rhytides and pigmentation (1). Tonnard's group demonstrated good clinical outcomes, safety, and the ability to generate Adipose-derived stem cells (ADSCs) from this starting material; however, beyond the observation that nanofat is devoid of adipocytes, little work has been done to characterize the constituents of the stromal vascular fraction (SVF) resulting from this technique.

Methods: Standard vacuum-assisted liposuction was used to obtain 100 ml LA from six patients undergoing routine elective procedures. 50 ml of each LA was subjected to nanofat processing. The two fat samples from each patient were subjected to collagenase digestion, red blood cell lysis and the resulting SVF pellets were then stained with a multicolor fluorescence antibody panel (CD13-APC-Vio770, CD31-FITC, CD34-PerCP-Vio700, CD45-VioBlue, CD73-PE and CD146-APC). The SVF and subsequent cultured ADSCs were subjected to flow cytometry analysis at Time 0, Passage 0 (P0) and Passage 1 (P1).

Results: When compared to standard LA, the nanofat-processed SVF yielded a significantly higher proportion of CD34+ progenitor cells (5.09% vs 14.3%, p=0.003) as well as mesenchymal markers CD13 (2.58% vs 8.81%, p=0.002), CD73 (4.14% vs 10.0%, p=0.043) and CD146 (5.33% vs 10.5%, p=0.040). The CD34+ advantage persisted over multiple passages (P0 - 40.0% ± 36.6 vs 49.1% ± 15.0, P1 - 2.14% ± 1.24 vs 4.87% ± 2.85). Moreover, MSC subpopulation analysis of the SVF revealed a tendency towards adipose-derived stem (15.5% ± 10.2 vs 24.4% ± 8.2) and endothelial progenitor cell phenotypes (1.30% vs 6.42%, p=0.003) from the fat that was mechanically processed.

Conclusions: Mechanical processing of standard lipoaspirate appears to induce progenitor and mesenchymal stem transdifferentiation. Our data provides a possible explanation for the regenerative benefits realized through fat grafting with nanofat.

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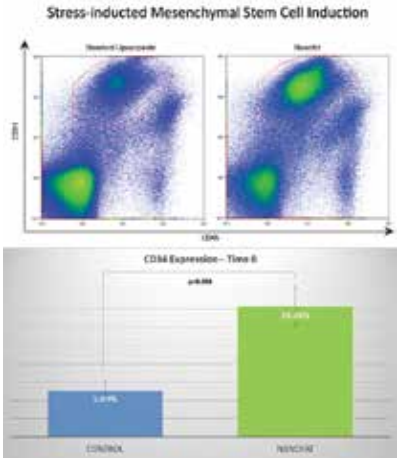
PHENOTYPIC ANALYSIS OF STROMAL VASCULAR FRACTION AFTER MECHANICAL SHEAR: STRESS-INDUCED PROGENITOR TRANSDIFFERENTIATION?



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IMMEDIATE LARGE-VOLUME GRAFTING OF AUTOLOGOUS FAT TO THE BREAST FOLLOWING IMPLANT REMOVAL: FIVE YEARS EXPERIENCE

Presenter: Saad Dibo, MD
Authors: Dibo S, Abboud M
MA Clinic

Background: To optimize autologous breast augmentation, a simple and reproducible surgical approach that maximizes the volume of fat transferred to the breast while minimizing the number of sessions and the operating time is needed.

Objectives: The authors share their experience with a novel approach for large-volume fat grafting to the expanded skin and subcutaneous tissue of the breast immediately after explantation, exchanging the volume provided by the implants with transplanted fat in a single session.

Methods: 105 patients (210 breasts) underwent explantation and autologous fat transfer between 2009 and 2014. Fat was harvested with power-assisted liposuction technology (Lipomatic Eva SP, Euromi SA, Verviers, Belgium) and was injected with simultaneous vibration and tunnelization of the recipient site with suction disabled. Changes in breast volume were measured in terms of bra cup size and MRI, and patients were monitored by mammography and ultrasonography.

Results: Injected fat volumes ranged from 300 to 650 mL per breast. Operating times ranged from 45 to 100 minutes. For all patients, one injection session was sufficient to replace the volume of the previous implant. Patients were monitored for an average of 2 years, and complications included cyst formation in 12 of 210 breasts (5.7%) and infection in 2 breasts (0.9%).

Conclusions: Power-assisted transfer of autologous fat to the breast improves the ability of the recipient site to receive the graft and allows for explantation and fat transplantation in a single session. This approach is suitable for patients who desire a natural-appearing breast that is similar in volume to their previous implant.

Reference: Immediate Large-Volume Grafting of Autologous Fat to the Breast Following Implant Removal. Aesthet Surg J.Accepted for publication March 2015. DOI:10.1093/asj/sjv073.

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PHYSICAL, BIOCHEMICAL AND BIOLOGICAL PROPERTIES OF FAT GRAFT PROCESSED VIA DIFFERENT METHODS

Presenter: Carrie H. Fang, PhD
Authors: Fang CH, Collins S, Huang LT, Li H, Patel P, Wan H, Xu H
LifeCell Corporation An Acelity Company

Background: Autologous fat transplantation has been used to address a variety of applications for soft tissue repair. However, fat graft viability and retention cannot be reliably predicted. Fat processing has been implicated for this variability. The purpose of this study is to characterize the in vitro physical and biological composition of lipoaspirate processed with different processing techniques.

Methods: Patients aged 30-50 were enrolled in this prospective study. Lipoaspirate from each consented patient was processed by four different methods: decantation, centrifugation, PureGraft™ and REVOLVE™ System. Biochemical characteristics and volume composition of free oil, adipose, and aqueous phases of the processed fat were determined. Each of the fat samples was further digested with collagenase for adipocyte and nucleated cell analysis. Specific stem cell populations were determined with cell markers CD45, CD105 and CD34. The functional stem cells were counted by CFU assay.

Results: Grafts obtained from all methods had good physiological properties with neutral pH. Each processing method yielded variable blood contaminants, different percentage of adipose tissue and free oil. Each processed graft was also demonstrated to contain different amounts of viable adipocytes and functional CD105+/CD34+/ CD45- stem cells. Among the four methods compared, Revolve™ System produced the most favorable preinjection graft: low blood contaminants, high content of adipose tissue, low free oil amount, more viable adipocytes and stem cells.

Conclusions: Different fat processing methods result in a fat graft with different physical and biological properties. The variability in fat processing therefore may contribute to fat graft viability and retention in vivo.

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ADIPOSE-DERIVED STEM CELLS: A COMPARISON OF ASC ISOLATION USING LABORATORY GRADE VERSUS MEDICAL GRADE COLLAGENASE

Presenter: Anna Wilson, MBChB, MRCS, MSc
Authors: Wilson A, Boyd A, Butler P, Seifalian A
UCL RAFT

Introduction: The use of adipose-derived stem cells (ASCs) as an autologous source of tissue provides much promise in reconstructive surgery. We have already demonstrated a time- and yield- efficient ASC isolation method. The aim of this research was the comparison of the use of laboratory grade and medical grade collagenase for ASC isolation, in order to make this procedure clinically applicable.

Methods: Three patients undergoing free fat transfer procedures donated surplus adipose tissue collected by the Coleman method from the abdomen. A previously tested one-hour digest ASC isolation protocol was used. Cells were counted using Trypan Blue at day 0 and day 7. Cells were characterised using flow cytometry at day 7 for markers CD19, CD34, CD45, CD73, CD90, CD105 and HLA-DR. ASC metabolism was analysed at day 14 using Alamar Blue assay. Cell differentiation was induced to adipogenic, chondrogenic and osteogenic lineages.

Results: ASCs were isolated by the use of both, lab and medical grade collagenase. Cell numbers from each group were comparable. All cells largely complied with the International Society for Cell Therapy guidelines of markers for mesenchymal stem cells. There was no statistically significant difference in cell metabolism between groups using lab and medical grade collagenase. ASCs from both groups differentiated into adipogenic, chondrogenic and osteogenic lineages.

Conclusion: An ASC isolation protocol suitable for use within the intraoperative timeframe has been tested using both, lab and medical grade collagenase. Both enzymes are comparable in terms of the yield, metabolism and differentiative capacity of ASCs obtained. These findings are promising for the future use of medical grade collagenase in the purification of ASCs within the intraoperative timeframe.

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OSTEOINDUCTIVE EFFECTS OF GLYCEOLLINS ON ADIPOSE STROMAL CELLS

Presenter: Marjorie E. Bateman, BS
Authors: Bateman ME, Strong AL, Hunter RS, Burow ME, Boue SM, Hayes DJ, Bunnell BA
Tulane University School of Medicine

Osteoporosis is characterized by destruction of bone architecture, resulting in decreased bone mass density (BMD) and increased fracture susceptibility. While current therapies focus on reducing bone resorption, the development of anabolic therapies to regenerate bone may also be beneficial. Promising anabolic therapeutic candidates include phytoestrogen compounds, such as daidzein and genistein, which have been shown to effectively induce osteogenic differentiation of adipose-derived stem cells (ASCs) and bone marrow-derived mesenchymal stem cells (BMSCs). Glyceollins are structural derivatives of daidzein, and herein, the osteoinductive effects of glyceollins I, II, and a mixture of I and II were assessed in ASCs and BMSCs and compared to estradiol. In adherent cultures, ASCs and BMSCs treated with estradiol, glyceollin I, glyceollin II, or glyceollin mix increased calcium deposition relative to vehicle-treated cells. ASCs and BMSCs were seeded onto PLGA scaffolds and differentiation in a three-dimensional environment was assessed by Alizarin Red staining. Osteoinductive effects of estradiol, glyceollin I, glyceollin II, and glyceollin mix were also observed relative to vehicle in the treated ASCs and BMSCs on scaffolds. It was shown that glyceollin II (1 πM) was most efficacious in inducing ASC and BMSC osteogenic differentiation in both adherent and scaffold culture. Dose-response analysis revealed that glyceollin II has highest potency at 10 nm in adherent culture and 1 πM in both ASCs and BMSCs in scaffold cultures. Inhibitor studies with fulvestrant were conducted, and at all doses tested, osteoinductive effects of glyceollin II were attenuated, indicating that glyceollin II acts through estrogen receptor-mediated pathways. Additionally, the effects of glyceollin II on lineage specific gene expression of key osteogenic genes were explored. These results indicate that glyceollins hold the potential for the development of pharmacological interventions and regenerative medicine approaches to improve clinical outcomes of patients diagnosed with osteoporosis.

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DIFFERENT CULTURE CONDITIONS MODULATE IMMUNOLOGICAL PROPERTIES OF ADIPOSE STEM CELLS

Presenter: Mimmi Patrikoski, MSc
Authors: Patrikoski M, Sivula J, Mannerstrom B, Miettinen S
University of Tampere Finland

Introduction: Adipose tissue is an abundant source of multipotent stem cells, known as adipose stem cells (ASCs). ASCs have excellent proliferation capacity, multilineage differentiation potential, low immunogenicity and ability for immunomodulation, which make them promising candidates for diverse clinical applications in regenerative medicine and immunomodulation therapies.

Methods: In the current study, immunogenicity and immunosuppression of ASCs were determined through mixed lymphocyte reactions. The immunogenic response was analyzed after cell isolation and expansion in fetal bovine serum (FBS), human serum (HS) supplemented medium, and in xeno-free and serum-free (XF/SF) conditions. Additionally, the immunophenotype as well as the cytokine secretion (CXCL8, CXCL9, CXCL10, CCL2, CCL5, IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α, and IFN-γ) of ASCs was analyzed under aforementioned conditions.

Results: ASCs were weakly immunogenic when expanded in any of the three conditions. The significantly strongest suppression was observed with cells expanded in FBS conditions, whereas higher ASC numbers were required to display suppression in HS or XF/SF conditions. Statistically significant differences in cytokine secretion were observed between different culture conditions, and between direct versus indirect co-cultures. The characteristic immunophenotype of ASCs was maintained in all conditions. However, in XF/SF conditions significantly lower expression of CD54 (ICAM-1) was observed at low passage number.

Discussion: Although culture conditions influence the immunogenicity, immunosuppression and cytokine secretion of ASCs, our findings demonstrated that ASCs have low immunogenicity and promising immunosuppressive potential whether cultured in FBS, HS or in XF/SF conditions. Consequently, ASCs are attractive candidates for allogenic clinical use and immunomodulation therapies.

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PHYTOESTROGEN GLYCINOL ENHANCES OSTEOGENESIS AND ATTENUATES THE EFFECTS OF AGING ON BONE MESENCHYMAL STEM CELLS

Presenter: Robert B. Jones Jr., BA
Authors: Jones RB, Strong AL, Boue SE, Burow ME, Bunnell BA
Tulane University School of Medicine

Osteoporosis is a progressive bone disease characterized by decreased bone mineral density and increased risk of fractures. Most prevalent in the elderly population, osteoporosis leads to significant morbidity and mortality. Recently, phytoestrogens have gained significant interest as an option for therapy due to their osteogenic potential. Glycinol is a phytoestrogen that accumulates in soybeans in response to stress or elicitor treatment. The interest in glycinol stems from its structural similarity to estradiol, which has been shown to increase osteogenic differentiation of bone marrow-derived mesenchymal stem cells (BMSCs). In this study, the effects of glycinol were investigated in human BMSCs derived from young subjects (<25 years old) and old subjects (>50 years old). BMSCs isolated from young (mean age: 22.3 ± 0.8 years) and old subjects (mean age: 56.0 ± 2.7 years) were characterized based on differentiation capacity, expression of key osteogenic and adipogenic genes, and expression of estrogen receptors and downstream estrogen effectors. BMSCs isolated from old subjects demonstrated reduced osteogenic and adipogenic differentiation potential in vitro, which correlated with a reduction in the mRNA levels for key osteogenic genes. BMSCs from both young and old subjects exhibited a dose-dependent response to β-estradiol and glycinol. The reduced osteogenic differentiation potential observed in the BMSCs derived from the old subjects was rescued by co-culture in the presence of glycinol. Additional studies demonstrated enhanced expression of key osteogenic transcription factors (e.g. osteonectin, osteopontin) following treatment with glycinol, which provides mechanistic support for the increased osteogenesis observed in BMSCs following treatment. Furthermore, glycinol induced the expression of estrogen receptor ERα, and estrogen receptor antagonist studies further support that glycinol promotes osteogenesis through estrogen receptor signaling pathways. Together, these studies demonstrate the osteoinductive effects of glycinol on BMSCs and support the further investigation of glycinol as a potential treatment for osteoporosis. In particular, these data suggest that glycinol may represent a therapeutic modality for targeting age-diminished osteogenesis.

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A NOVEL METHOD USING 3D PRINTING TO MAINTAIN IMPLANT SHAPE FOR EAR CARTILAGE RECONSTRUCTION

Presenter: Marco Helder, PhD
Authors: Visscher D, Bos EJ, Helder MN, Van Zuijlen PM
VU Medical Center

Cartilage reconstruction following ear defects caused by congenital deficiency or traumatic injury is a major challenge in the field of plastic and reconstructive surgery. In recent years, tissue engineering has shown promising results for reconstruction of cartilage. However, two key challenges in the field of auricular cartilage tissue engineering are preservation of adequate auricular shape and production of biologically and mechanically adequate cartilage. In this study we developed and tested a combined construct consisting of a porous 3D-printed polycaprolactone (PCL) mold encapsulating a collagen I/III backbone scaffold and hyaluronan hydrogel for form-retention and cartilage tissue engineering.

Goat ear chondrocytes (C), perichondrocytes (P), and adipose-tissue-derived mesenchymal stem cells (ASC) were culture-expanded, encapsulated in hydrogel, either isolated or in combination of C-ASC (20:80 ratio), P-ASC (20:80 ratio) or C-P (20:80 ratio) and cultured in PCL-encaged collagen I/III scaffolds at 37 degrees for 28 days. The constructs were analyzed at day 14 and 28 with Alcian Blue, multi-photon laser scanning microscopy (MLSM) and macroscopic imaging.

Results showed that encaging the scaffolds with 3D-printed porous PCL molds completely prevented scaffold contraction after 28 days of in vitro culturing. Histological and MLSM analysis confirmed glycosaminoglycan and collagen production in most, but not all scaffolds. Less extracellular matrix formation was seen in scaffolds seeded with ASC alone and C-ASC scaffolds.

This study showed that a combined construct consisting of a 3D-printed PCL mold, collagen scaffold and hydrogel can support combinations of different cell types to produce auricular cartilage with adequate biological properties.

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A NOVEL METHOD USING 3D PRINTING TO MAINTAIN IMPLANT SHAPE FOR EAR CARTILAGE RECONSTRUCTION

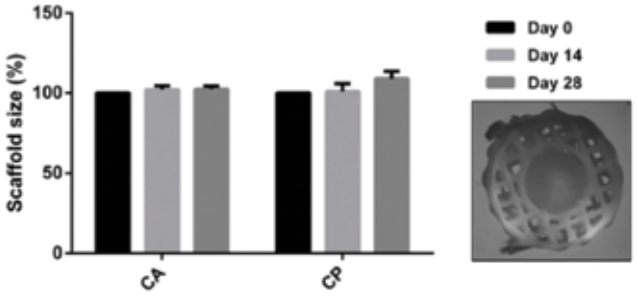
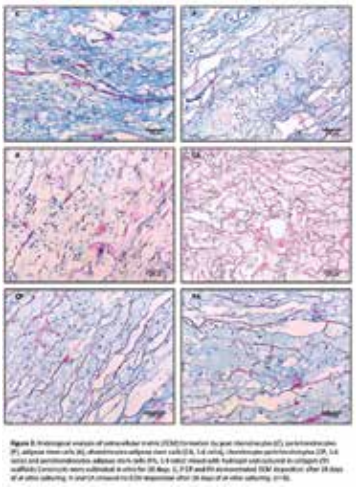
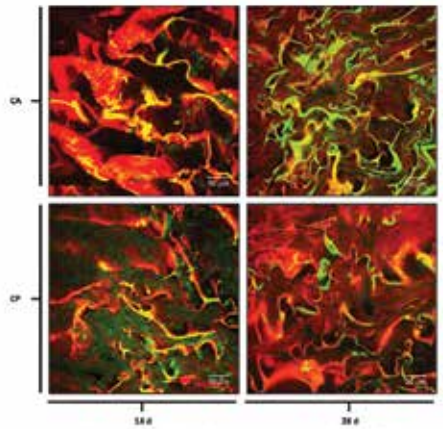


Figure 1. *In vitro* scaffold contraction of collagen I/III scaffolds in combination with hyaluronan hydrogel encapsulated in 3D printed PCL scaffolds. After 14 days and 28 days of *in vitro* culturing none of the hydrogel-collagen I/III scaffold groups showed any change in scaffold size. Picture shows macroscopic morphology of PCL mold with scaffold centrally located. CA: chrondrocytes-adipose stem cells (1:4), CP: chrondrocytes-perichondrocytes (1:4). n=3 for both groups.





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THE ROLE OF MED31 IN REGULATING STEM CELL STATE

Presenter: Jamie J. Newman, PhD
Authors: Newman JJ, Beadle EP, Bunnell BA
Louisiana Tech University

Introduction: Mediator is a highly conserved, multi-subunit, protein complex which functions as a transcriptional co-factor in eukaryotes to bridge long range interactions between enhancers and promoters in a cell-type specific manner. Alterations or deletions in any one of Mediator's ~30 subunits may affect gene expression patterns and result in developmental defects or disease. Mediator has been shown to play a critical role in embryonic stem cell maintenance and if disrupted, may affect the cell's pluripotent state and differentiation potential. We are interested in extending this knowledge to adult stem cells and understanding Mediator's role in the maintenance and differentiation of human bone marrow-derived mesenchymal (BMSCs) and adipose stem cells (ASCs).

Method: We are studying how Mediator regulates cell state by investigating a well characterized subunit, MED31. We will study the effects of siRNA-mediated knockdown of Med31 on stem cell self-renewal and differentiation towards adipocyte and osteocyte lineages. We will further investigate the role of MED31 by performing RNA-Seq following knockdown to identify which genes are likely directly regulated by MED31 and account for the observed phenotype. Finally, we will monitor the levels of Med31 gene and protein expression in different cell lines and during differentiation to determine if MED31 may be a marker for determining potency of adult stem cells.

Results: Preliminary results indicate MED31 may have a role in cell proliferation and osteocyte differentiation. Specifically, MSCs transfected with MED31 siRNA showed less growth under standard culture conditions at 72 hours compared to the control (Figure 1) and at 21 days of osteocyte differentiation, there was less Alizarin Red staining observed in knockdown samples compared to controls (Figure 2).

Conclusion: As mesenchymal lineage stem cells (BMSCs and ASCs) move into the clinic it is necessary to understand the mechanisms that regulate their biology and factors that identify a clinically viable cell line. This project will contribute to a more detailed understanding of how Mediator acts to promote healthy gene expression and influence differentiation pathways improving their potential for use in the clinic and the laboratory.

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THE ROLE OF MED31 IN REGULATING STEM CELL STATE

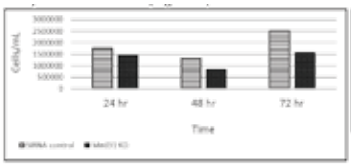

Figure 1. Cell viability following MED31 knockdown

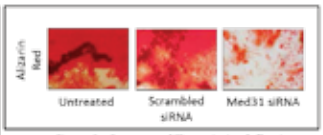

Figure 2. Osteocyte differentiation following knockdown. Staining performed at 21 days.

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OPTIMIZATION OF ADIPOSE PARTICLE SIZE FOR AUTOLOGOUS LIPOFILLING: THE IMPACT OF PARTICLE DIAMETER ON TISSUE METABOLISM, NECROSIS AND LONG TERM IN VIVO SURVIVAL

Presenter: Lauren E. Kokai, PhD
Authors: Kokai LE, Jones TL, Marra KG, Rubin JP
University of Pittsburgh

Introduction: In autologous lipofilling, excess adipose tissue is extracted, processed to remove tumescent fluid, oil and cellular debri, and then injected into a volume void. A major limitation with this procedure is rapid volume loss of grafts over the first 3-6 months post injection. Literature suggests that adipose particle size may have a direct impact on the long term survival of fat grafts. When fat particles are very large, oxygen and nutrient diffusion to the interior particle core is insufficient to maintain tissue viability, resulting in inferior long term fat grafting outcomes. We hypothesize that small particles (3-5 mm diameter) will produce superior fat grafting outcomes when compared to large (10+ mm) fat particles.

Methods: An ex vivo study was conducted with fat particles of variable diameter to evaluate the impact of particle size on tissue viability. Adipose tissue obtained from panniculectomy was cut into particles of three diameter ranges, 1-3mm, 5-8mm, 10-13mm (small, medium and large, respectively). Fat particles were placed in a dynamic culture for 28 days, with media samples measured every 2 days for VEGF secretion. At four time points, 3, 7, 14 and 28 days, tissue samples were removed and markers of inflammation (IL1), hypoxia (SOD1 and GPX1) and adipogenesis (FABP4) were quantified with qPCR. In a subsequent in vivo study, fat particles of known size were implanted subcutaneously in immunocompetent, Balb/c mice. Study outcomes included graft retention at 1, 4, 8 and 12 weeks, tissue histology, and markers of inflammation, apoptosis, and tissue regeneration (qPCR).

Results and Conclusions: In vitro, fat particle size impacts the secretion of VEGF, a growth factors essential for tissue revascularization. Further, small fat particles have reduced inflammatory signaling and increased tissue adipogenesis compared with medium or large particles. In vivo, large fat particles had increased fibrosis and visible oil cysts, indications of inflammation and fat tissue necrosis. No statistical differences were observed between group volume retention at any time point, however, qPCR data suggested smaller fat particles had superior tissue survival and increased recovery from hypoxic stress. We are currently repeating this study out to 24 weeks.

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THERAPEUTIC FAT GRAFTING IN A BREAST CANCER XENOGRAFT MODEL

Presenter: Abigail M. Cochran, MD
Authors: Cochran AM, Gupta AG, Yang MY, Cosenza NC, Richensperger JR, Harrison CH, Cox LC, De La Garza MD, Neumeister MN
Southern Illinois University School of Medicine

WITHDRAWN

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ENGINEERING VOLUME STABLE ADIPOSE TISSUE USING COMPOSITE CONSTRUCTS MADE FROM POROUS SCAFFOLDS AND FIBRIN HYDROGEL - A MATTER OF CELLS

Presenter: Petra Bauer-Kreisel, PhD
Authors: Bauer-Kreisel P, Fischer R, Wittmann K, Regn S, Wiese H, Maier G, Storck K, Blunk T
University of Wuerzburg

Introduction: Adipose tissue engineering aims to develop functional adipose tissue constructs for the treatment of soft tissue defects and contour deformities. In order to achieve volume stability of the engineered tissue we developed composite constructs consisting of biodegradable porous polyurethane (PU) scaffolds as support structure and fibrin hydrogel as cell carrier, which were evaluated in vitro and in vivo with a particular focus on the impact of the implanted cells for adipose tissue development.

Methods: Human adipose-derived stromal cells (hASC) obtained from abdominal lipoaspirates were seeded in fibrin/PU composite constructs. After adipogenic cultivation in vitro, adipogenesis was analyzed by histology, gene expression (qRT-PCR) and leptin secretion (ELISA). For in vivo studies seeded and acellular fibrin/PU constructs were subcutaneously implanted in nude mice around the femoral vessels (i.e. integrated arteriovenous bundle). Constructs were cultivated in vitro with or without adipogenic stimulus for 7 days. After 12 weeks in vivo explanted constructs were evaluated by histological analysis. The origin of the developed tissue was assessed by human-specific vimentin staining.

Results: Characterization of ASC-seeded composite constructs in vitro demonstrated volume maintenance and high cell viability. Adipogenic differentiation was confirmed histologically and by expression of adipogenic marker genes and leptin secretion. After 12 weeks in vivo all constructs displayed excellent volume stability. Apparently, the fibrin gel was replaced by coherent tissue perfused by a network of capillaries. Developed adipose tissue was shown to be exclusively of host origin, however delivered ASC promoted tissue development significantly as shown by histomorphometric quantification of adipocytes. Adipogenesis in vivo was further enhanced by adipogenic precultivation of the constructs in vitro.

Conclusions: Using porous PU scaffolds and a stable fibrin gel, volume-stable adipose tissue constructs were sucessfully developed in a nude mouse model. Implanted cells were shown not to build up the newly formed tissue, but to have a significant impact on tissue development likely by secreting cytokines stimulating the invasion and differentiation of host cells.

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INVESTIGATING THE MATRIX CHEMISTRY OF HUMAN ADIPOSE-DERIVED STEM CELLS: THE FIRST STEP TOWARD CREATING A SCAFFOLD FOR OPTIMAL ADIPOSE TISSUE ENGINEERING WITH A BIOMIMETIC APPROACH

Presenter: Mary Frimpong, BSc (Hons), MBBS, MRCS
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WITHDRAWN

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CHANGES IN GENE EXPRESSION FOLLOWING CO-CULTURE OF ADIPOSE-DERIVED STROMAL/STEM CELLS WITH A FLOWABLE PLACENTAL CONNECTIVE TISSUE MATRIX

Presenter: Daniel R. Kuebler, PhD
Authors: Kuebler DR, McLaughlin M, Damico M, Yeager R, Cunningham L
Franciscan University of Steubenville

Background: The use of ASCs to treat orthopedic injuries is likely to be augmented by the use of a scaffolding matrix. The matrix not only can serve as a site for ASC adhesion, but also as a substrate for tissue remodeling and repair. While a variety of possible scafolds and scaffolding substrates exist, properly processed placental membranes are an attractive option. In addition to being composed of a collagen-based matrix, these tissues contain a number of growth factors and cytokines that are known to play a role in the normal healing process.

Methods: To test the effect placental tissue had on the behavior of ASCs, ASCs were cultured in the presence of a novel flowable placental connective tissue matrix, PX50 (Human Regenerative Technologies).

Results: Upon addition of PX50 to cultured ASCs, over 90% of the ASCs bound to the placental tissue matrix components suspended in the flowable product. The ACS did not display any changes in morphology but did display alterations in the expression of two genes involved in bone and cartilage repair, insulin-like growth factor-1 (IGF-1) and bone morphogenetic protein-2 (BMP-2). BMP-2 expression was elevated more than four fold over ASCs cultured in fetal bovine serum, while IGF-1 expression was elevated more than seven fold. The expression of four other genes, fibroblast growth factor-2, platelet-derived growth factor-B, transforming growth factor beta-1, and bone morphogenetic protein-4, was not altered upon exposure to PX50.

Conclusions: ASCs in culture adhere to the placental tissue matrix components of PX50 and display upregulation of genes known to be involved in bone and cartilage healing. The co-injection of PX50 and ASCs may be a promising minimally invasive option for treating orthopedic defects.

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ADIPOSE-DERIVED STROMAL/STEM CELLS ARE POSSIBLE RESERVOIRS FOR HUMAN CYTOMEGALOVIRUS

Presenter: Kevin Zwezdaryk, PhD
Authors: Zwezdaryk K, Ferris MB, Bunnell BA, Gimble JM, Sullivan DE
Tulane University

Introduction: Human adipose-derived stromal/stem cells (ASCs) show enormous potential for use in cellular and regenerative therapies. Specifically, ASCs may be used to reduce inflammation and immune response in numerous pathologies. Human cytomegalovirus (HCMV) is known to reactivate during immunosuppression. Reactivation of HCMV occurs in CD34+ progenitor cells and/or monocytes. Therefore, we investigated if early passage ASCs that are CD34+ could be potential latency-associated reservoirs for HCMV.

Methods: ASCs were infected with a GFP-labeled strain of HCMV. Infected cells were sorted before characterization by flow cytometry. Lytic viral infection of ASCs was confirmed by detection of viral gene products using western blotting and immunofluorescence. Expression of HCMV latency-associated genes was measured by qRT-PCR.

Results: We report that HCMV can infect ASCs at very low titers. Additionally, preliminary studies indicate that HCMV infection of ASCs impairs proliferation and differentiation but does not result in immediate apoptosis or necrosis. Viral associated genes at different stages of viral replication were detected suggesting that it is not an abortive infection.

Conclusions: Our results indicate that prior to using ASCs for therapeutic purposes, they should be screened for the presence of HCMV.

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NATURAL KILLER CELLS DIFFERENTIATE HUMAN ADIPOSE-DERIVED STEM CELLS AND MODULATE THEIR ADIPOGENIC POTENTIAL

Presenter: Kameron Rezzadeh, BA
Authors: Rezzadeh K, Hokugo A, Jewett A, Segovia LA, Kozlowska A, Zuk P, Zhou S, Jarrahy R
David Geffen School of Medicine at UCLA

WITHDRAWN

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EFFECT OF THERMAL PRECONDITIONING OF ADIPOSE TISSUE DERIVED STEM CELLS ON CRYOPRESERVATION

Presenter: Mullashahensha Shaik, MS
Authors: Shaik M, Gimble JM, Devireddy RV
LSU

Human adipose-derived stromal/stem cells (huASCs) are multipotent cells that can differentiate into adipogenic, chondrogenic, and osteogenic lineages. Latest advancements in the fields of tissue engineering and regenerative medicine have increased the potential of stem-cell-based therapies as a standard of care for a wide variety of diseases. Therefore, appropriate cryopreservation protocols will be necessary to increase patient access, reduce cost, and enhance the safety and effectiveness of huASCs. Recent studies from our laboratories have described the in vitro cryopreservation of huASCs frozen in a media consisting of minimal amounts of dimethyl sulfoxide (DMSO) and serum (animal or human). However, the transplantation of cells cryopreserved in DMSO has been associated in nearly all patients with nausea, chills, hypotension, dyspnea and cardiac arrythmia. Therefore, the practical goals of this study are the development and optimization of a serum-free and DMSO-free cryoprotective medium. Multiple reports in literature suggest that the up regulation of Heat Shock Proteins (HSPs) can provide cytoprotection in various diseases. In this study, the huASCs are thermally preconditioned by exposing them to 43°C for 1 hour to up regulate HSPs prior to cryopreservation. The huASCs are cryopreserved in different groups with 10% DMSO, 10% PVP, 1% DMSO, and no cryoprotectants (CPAs) at a cooling rate of -1°C /min to -35°C followed by plunging in liquid nitrogen. The cell viability of huASCs post thawing is determined using apoptosis assay kit with flow cytometry and compared with the groups that are not thermally preconditioned. The post thaw viability of thermally preconditioned huASCs with 10% DMSO, 10% PVP and 1% DMSO resulted in 93.6±1.33, 79.33±3.65 and 72.9±4.63 percent whereas the huASCs cryopreserved with no thermal preconditioning showed the cell viability of 91.6±1.67, 74.92±3.86 and 70.9±9.68. However, the cells cryopreserved without CPAs showed poor cell viability even with the heat shock. Western blot and qPCR are carried out on thermally preconditioned cells to compare the expression of heat shock proteins especially HSP90, HSP70, and HSP27. Further studies have to be done to understand the role of HSPs in improving the cell viability of cryopreserved cells so that a cryopreservation protocol for huASCs with reduced amounts of cryoprotectants and serum can be developed.

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ANALYSIS OF FATTY ACIDS COMPOSITION IN HUMAN SUBCUTANEOUS ADIPOSE TISSUE

Presenter: Natalie Khramtsova, PhD
Authors: Khramtsova N, Plaksin SA
Perm State University of Medicine

WITHDRAWN

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THE IMPACT OF HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS ON BREAST CANCER CELLS - IMPLICATIONS FOR CELL-ASSISTED LIPOTRANSFERS IN BREAST RECONSTRUCTION

Presenter: Eva Koellensperger, MD
Authors: Koellensperger E, Bonnert LC, Marx J, Zoernig I, Sandmann S, Germann G, Leimer U
Heidelberg University Hospital

Introduction: In this study we evaluated the interactions of human adipose tissue derived stem cells (ADSCs) and different human breast cancer cell lines (BRCAs), with regard to the safety of cell-assisted lipotransfers for breast reconstruction and a thereby unintended co-localization of ADSCs and BRCAs.

Methods: ADSCs were co-cultured with five different human BRCAs (MCF-7, MDA-MB-231, SK-BR-3, ZR-75-30, EVSA-T) and primary BRCAs from one patient in a transwell system. Cell-cell-interactions were analyzed by assessing proliferation, migration and invasion, angiogenesis, quantitative real time-PCR of > 300 tumor-associated genes, and multiplex protein assays of 20 chemokines and growth factors and eight matrix metalloproteinases (MMPs). Results of co-culture were compared to those of the respective mono-culture.

Results: Quantitative real time-PCR revealed remarkable changes in the expression of multiple tumor-associated genes in co-culture of both, ADSCs and BRCAs. Concomitantly, the concentration of several tumor-associated proteins, such as cytokines and MMPs, were strongly increased in co-culture. Furthermore, exclusively in co-culture with ADSCs, the different BRCAs were exposed to several important tumor-modulating proteins, such as CCl2, HGF or interleukins. Co-culture did not significantly affect cellular proliferation of neither ADSCs nor BRCAs ($p>0.05$). The migration of MCF-7- and MDA-MB-231-BRCAs was significantly increased in co-culture with ADSCs by a mean of 11% and 23% respectively ($p=0.04$ / 0.012), as well as that of ADSCs in co culture with MDA-MB-231, ZR-75-30, and EVSA-T (+11-15%, $p = 0.035$-0.045). Co-culture with MDA-MB-231-, SK-BR-3-, and EVSA-T-BRCAs significantly increased the invasive behavior of ADSCs by a mean of 24 - 41% ($p=0.014$-0.039). There were no significant differences in the in vitro invasive properties of BRCAs in co-culture compared to mono-culture. An in vitro angiogenesis assay revealed an increased tube formation of conditioned media from co-culture.

Conclusion: This study elucidates the possible interactions of primary human ADSCs with human BRCAs, pointing towards a potential increased oncological risk, which should not be neglected considering a clinical use of cell-assisted lipoaspirates in breast reconstruction.

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CHARACTERIZATION OF ADIPOSE-DERIVED STROMAL VASCULAR FRACTION (SVF) CELLS ISOLATED WITH A NOVEL DISPOSABLE, CLOSED-SYSTEM DEVICE

Presenter: Adam Katz, MD
Authors: Shang H, Yang N, Yu A, Katz A
University of Florida

Introduction: Adipose-derived cells lend themselves to a variety of translational strategies - especially autologous "point-of-care" (PoC) strategies using uncultured cells. The purpose of this study was to characterize the identity, purity and reproducibility of adipose-derived stromal vascular fraction (SVF) cells isolated using a novel, scalable closed system 'point-of-care' disposable device.

Methods: Liposuctioned adipose tissue was obtained under IRB approval. The tissue was subjected to enzymatic digestion to isolate and concentrate SVF cells. Cell identity was evaluated by measuring cell yield, % viability, and cell surface phenotype using flow cytometry. Purity was evaluated by assays of endotoxin, gram stain and residual collagenase.

Results: Tissue was harvested from 23 patients, with an average age of 44, an average BMI of 28. The average initial yield of viable nucleated cells was 9.55 x 10^5 cells/gram of dry tissue processed, with an average viability of 81.5%. WBCs constituted approximately 14% of total viable nucleated cells and nearly 10% of cells stain with markers that are suggestive of endothelial cells/progenitor cells. An average of 16% of cells stain positive for CD34, marking the putative adipose stem/progenitor cell. All isolates but one was negative for endotoxin and final residual collagenase levels were minimal in the final cell suspension.

Conclusions: We show that the isolation of SVF cells using a novel disposable 'point-of-care' device and standardized methods is safe, yielding a viable but mixed population of cells that are free of microbial contaminants.

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EXTRACELLULAR MATRIX COMPOSITION AND DYNAMICS DURING ADIPOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STROMAL CELLS IN 3D CULTURE

Presenter: Torsten Blunk, PhD
Authors: Blunk T, Hoefner C, Wittmann K, Muhr C, Becker M, Bauer-Kreisel P
University of Wuerzburg

Introduction: In adipose tissue engineering for reconstructive surgery, a detailed understanding of adipose tissue development is of key importance. The extracellular matrix (ECM) is an important modulating factor in the process of adipogenesis and the function of mature adipocytes. However, most of the preexisting studies regarding ECM development during adipogenesis were done in 2D culture. Therefore, to get an insight in ECM and tissue development in a more in-vivo like context, the dynamics of major ECM components during adipogenic differentiation of adipose-derived stromal cells (ASC) in a 3D spheroid culture system were investigated in comparison to conventional 2D monolayer culture.

Methods: hASC obtained from human abdominal lipoaspirates were either cultured as 3D spheroids or in 2D monolayer culture. Adipogenic induction was performed using a common hormonal cocktail. Adipogenesis was assessed by lipid staining and quantification of total trigyceride content. At various time points during differentiation, major ECM components were analyzed by immunohistochemistry and gene expression analysis (qRT-PCR).

Results: During adipogenesis of hASC dynamic changes in expression and structure of major ECM components (laminin, collagen type I and IV) were revealed by immunohistochemical analysis and gene expression analysis in 2D and 3D cultures. Whereas the development of Col IV was highly linked to adipogenesis regardless of culture dimensionality, distinct differences emerged in the expression of Col I and laminin during adipogenesis between 2D and 3D culture. This differential expression coincided with a distinct differentiation behavior of the cells depending on the culture system.

Conclusion: Based on the differences in ECM development (notably Col I and laminin) in coincidence with a different adipogenic behavior of the cells in 2D and 3D culture, a specific role of these ECM components in the process of adipogenesis is suggested. Especially laminin is in the focus of current investigations in order to elucidate its possible role in the adipogenic differentiation of hASC. The results of this work may aid in the understanding of adipose tissue development and the rational engineering of adipose tissue implants in the long term.

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ANTI-ADIPOGENIC EFFECT OF OXY133 ON PRE-ADIPOCYTE COMMITMENT

Presenter: Akishige Hokugo, DDS, PHD
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WITHDRAWN

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EVALUATION OF A SERUM-FREE MEDIUM FOR THE PRODUCTION OF TISSUE-ENGINEERED NATURALLY-DERIVED HUMAN CONNECTIVE SUBSTITUTES

Presenter: Meryem Safoine, BSc
Authors: Safoine M, Aubin K, Trottier V, Fradette J
LOEX Centre de recherche du CHU de Quebec Universite Laval

Introduction: Restoring soft tissue defects caused by tumor resections, extensive burns, and congenital or acquired lipodystrophies is a major challenge in reconstructive surgery. Tissue engineering is a promising option considering the high variability of fat transplantation success. The actual production method for soft tissue reconstruction uses fetal bovine serum (FBS). However, use of animal derivatives can result in infectious agents transmission or in immunological reactions triggered by xenogenous proteins, and is therefore not recommended in a clinical application context. We hypothesized that the important parameters of tissue reconstruction that are cell expansion, extracellular matrix formation and tissue functionality will be maintained during tissue production in serum-free conditions using the self-assembly method of tissue engineering.

Methods: Adipose-derived stromal/stem cells (ASC) stimulated with ascorbic acid to secrete and organize their own extracellular matrix were used to produce connective cell sheets, which were superposed in group of 3 to form human reconstructed connective tissues (hrCT). A commercially available serum-free medium (SFM) was used for the entire tissue production and compared to the standard medium containing 10% FBS.

Results: ASC proliferation was evaluated at the end of passages 1, 2 and 3 and was 2 to 4 fold higher with SFM as compared to standard medium. Extracellular matrix formation was evaluated by measuring the tissue thickness. hrCT were 4.4 fold thicker and 1.6 fold less contractile when produced in SFM. Moreover, cell sheets cultured in SFM could be manipulated earlier, therefore diminishing hrCT production time by 50%. Furthermore, the enhanced secretion of the angiogenic factors Ang-1 (1.5 fold, $p<0.0001$), VEGF (6.4 fold, $p<0.0001$), and PAI-1 (3.0 fold, $p<0.001$) by hrCT in SFM reflects their therapeutic potential for angiogenesis stimulation.

Conclusion: FBS substitution considerably reduces production time while allowing the adaptation of our tissue production method to conditions compatible with the clinical context. In addition to their use as graft substrates, those tissues can also provide stromal support for skin or vesical reconstruction. Supported by CIHR.

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THE ARCHITECTURE OF FAT GRAFTING: WHAT LIES BENEATH THE SURFACE

Presenter: Debra A. Bourne, MD
Authors: Bourne DA, James IB, Wang SS, Marra KG, Rubin JP
University of Pittsburgh Medical Center

Background: Fat grafting in a powerful and versatile procedure, however it is limited by unpredictability of resorption. The loss of volume after fat grafting is related to necrosis of a portion of the transplanted fat. Grafted tissue survives via plasmatic imbibition until neovascularization creates a capillary network to provide nourishment. Fat that is deposited in bulky clumps undergoes central necrosis before neovascularization can occur. This study aims to better understand the architecture of fat deposits within the recipient bed following fat grafting, specifically the size of individual grafts and how this is related to the total volume of tissue grafted.

Methods: Fat was harvested by liposuction and stained with methylene blue. Stained fat was grafted into 4x4x2 cm sections of pannus at six volume ratios relative to the recipient bed 1:1, 1:2, 1:4, 1:6, 1:8 and 1:10. The diameters of fat deposits were measured and the means compared by ANOVA. The number of fat deposits with a radius greater than 2 mm was calculated and compared using Fisher Exact comparisons. A p-value 0.05 was significant.

Results: As the volume ratio of fat grafted decreased, the mean fat deposit diameter decreased from 0.57 ± 0.02 cm (1:1), to 0.40 ± 0.01 cm (1:2), to 0.31 ± 0.01 cm (1:4), to 0.26 ± 0.01 cm (1:6), to 0.25 ± 0.01 cm (1:8), to 0.20 ± 0.01 cm (1:10) (figure 1). All comparisons reached statistical significance except 1:8 vs 1:6 (p=0.96). Similarly, as the volume ratio decreased, the percentage of deposits with a radius greater than 2mm decreased from 68.3% (1:1), to 42.0% (1:2), to 20.9% (1:4), to 9.5% (1:6), to 5.3% (1:8), to 3.0% (1:10). All percentage comparisons were significant except 1:10 vs 1:8 (p=0.098).

Conclusions: Loss of volume after fat grafting is related to central necrosis of bulky fat deposits. This study demonstrates that as the total volume of fat transferred reaches or exceeds a ratio of 1:4 the average diameter of individual fat deposits increases and the percentage of deposits with a radius greater than 2 mm increases signficantly. Central areas of these larger depostis are expected to undergo necrosis leading to fibrotic scar tissue formation and volume loss.

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THE ARCHITECTURE OF FAT GRAFTING: WHAT LIES BENEATH THE SURFACE

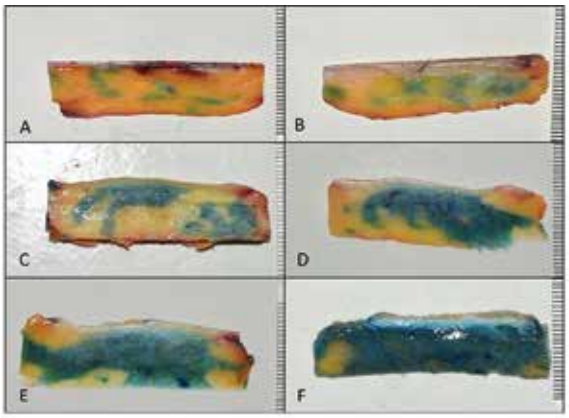

Figure 1: Representative sections of pannus. A) fat grafted at a volume ratio of 1:10. B) fat grafted at a volume ratio of 1:8. C) fat grafted at a volume ratio of 1:6. D) fat grafted at a volume ratio of 1:4. E) fat grafted at a volume ratio of 1:2. F) fat grafted at a volume ratio of 1:1.

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NOX1-INDUCED REACTIVE OXYGEN SPECIES PRODUCTION DICTATES THE PHENOTYPIC DIFFERENCE BETWEEN MESENCHYMAL STROMAL CELLS DERIVED FROM DIFFERENT FAT TISSUES

Presenter: Nir Shani, PhD
Authors: Shani N, Sela M, Tirza G, Ravid O, Volovitz I, Solodeev I, Friedman O, Krelin Y, Zipori D, Gur E
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Introduction: Mesenchymal stromal cells (MSCs) are multipotent progenitors, which can be isolated from a wide range of adult tissues. MSCs derived from different tissues often differ both in their in situ and in vitro phenotypes. However, little is known about the molecular basis of tissue specificity. Reactive oxygen species (ROS) are produced mostly during mitochondrial oxidative phosphorylation or as cellular signaling molecules mainly by the family of NOX NADPH oxidases. NOX-induced ROS accumulation in fat tissue during obesity was shown to influence the development of the metabolic syndrome.

Methods: Rat adipose derived MSCs (ASCs) were produced from abdominal or subcutaneous fat. RNA and protein were extracted from ASCs at different passages and analyzed. ROS cellular levels were analyzed by fluorescence microscopy or flow cytometry. Apoptosis was examined by cell-cycle, Annexin/PI or caspase activation analyses. Fat differentiation was induced and differentiation levels were evaluated by oil red O staining.

Results: Isolation of human ASCs is currently performed from multiple subcutaneous fat depots employing various fat extraction modalities. Our data demonstrate different characteristics of ASCs derived from abdominal or subcutaneous fat. Rat abdominal ASCs (aASCs) demonstrate poor culture expansion and a weak fat differentiation and migration potentials, compared to ASCs produced from subcutaneous fat. The poor propagation, differentiation and migration potentials of aASCs resulted from their tendency to overproduce reactive oxygen species (ROS) by NOX1 during culture. NOX1 involvement in the phenotype of aASCs was confirmed when cells that were expanded in the presence of a specific NOX1 inhibitor, demonstrated reduced ROS accumulation, reduced apoptosis, long-term expansion and improved fat differentiation and migration potentials.

Conclusions: NOX-induced ROS over-accumulation can dictate the tissue specific phenotype of ASCs. Thus, ASCs rom different adipose tissues possess specific signatures that control their phenotypes. Gaining deeper understanding of the tissue specific traits of MSCs may therefore assist in designing improved propagation and treatment protocols and may shed light on the physiological properties of MSC progenitors in situ.

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ROLE OF NOTCH SIGNALING IN GLYCOLYSIS REGULATION OF ADIPOSE-DERIVED MESCENCHYMAL STEM CELLS UNDER HYPOXIC CONDITIONS

Presenter: Hiroyuki Moriyama, PhD
Authors: Moriyama H, Moriyama M, Ishihara S, Okura H, Ozawa T, Matsuyama A, Hayakawa T
Pharmaceutical Research and Technology Institute

Human adipose-derived multilineage progenitor cells (hADMPCs) are an attractive material for cell therapy and tissue engineering because of their multipotency and ease of availability without serial ethical issues. However, their limited lifespan in in vitro culture system hinders the therapeutic applications of hADMPCs. Some somatic stem cells including hADMPCs are are known to be localized in hypoxia regions. These stem cells exhibit a high level of glycolytic metabolism despite the presence of high oxygen, and further increase their glycolysis rate under hypoxia. However, physiological role of glycolytic activation and its regulatory mechanisms is still incompletely understood.

Here we show that Notch signaling is required for glycolysis regulation under hypoxic condition. Our results demonstrate that hypoxia dramatically increased the glycolysis rate, improved the proliferation efficiency, prevented the senescence, and maintained the multipotency of hADMPCs. These effects were mediated by Notch signaling pathway. Hypoxia significantly increased the level of activated Notch1 and expression of its downstream gene, HES1. Furthermore, hypoxia markedly increased glucose consumption and lactate production of hADMPCs, which decreased back to normoxic levels upon treatment with a γ-secretase inhibitor. We also found that HES1 was involved in induction of GLUT3, TPI, and PGK1 in addition to reduction of TIGAR and SCO2 expression. In addition, we also found that HES1 was involved in the induction of GLUT3 expression through NF-κB signaling. Finally, 2-deoxy-glucose, an inhibitor of glycolysis, attenuates the proliferation rate of hADMPCs, whereas the aerobic respiration block by NaN_3 did not decrease the proliferation; rather, it increased proliferation at a low concentration, which may support our data indicating that the metabolic switch from mitochondrial respiration to glycolysis provides a growth advantage to hADMPCs. These results clearly suggest that Notch signaling regulates glycolysis under hypoxic conditions and thus likely affects the cell lifespan via glycolysis.

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AN UPDATED EVIDENCE-BASED REVIEW OF ADIPOSE STEM CELL THERAPY IN CANCER RECONSTRUCTION

Presenter: Michael Alperovich, MD, MSc
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Introduction: The use of adipose stem cells (ASCs) in autologous fat transfers is a favorable technology for reconstruction. However, much of the growing enthusiasm has been tempered by studies demonstrating a possible increased risk of growth and metastasis in cancer. We present a systematic review of the literature evaluating the safety of ASC therapy in cancer patients.

Methods: A systematic literature review was performed independently by two authors. PubMed/MEDLINE, Ovid and other relevant plastic surgery databases were searched using the following terms: adipose AND "stem cells" OR ("stem" AND "cells") OR "stem cells" AND ("tumour" OR "neoplasms" OR "tumor") AND ("disease progression"). We identified all relevant in vivo studies and the bibliographies of these articles were cross-referenced.

Results: Eleven in vivo studies examined the role of ASCs on tumor growth and proliferation. The studies utilized variable ASC preparations, sources, doses, and delivery methods in different types of tumor models. Eight of these studies demonstrated pro-tumor effects of ASCs, two studies showed anti-tumor effects and one study demonstrated no change. The main proposed mechanisms for enhanced tumorigenesis included increased tumor stroma formation and neovascularization. Various in vivo tumor models were used including breast (5), prostate (2), lung, pancreas, brain, and leukemia cells. In all studies except one, ASCs were isolated from human tissue (abdominoplasty, mammoplasty, etc). There was no significant relationship between the tumor model, source or type of ASC preparation and effect on tumor formation. Notably, the two studies demonstrating anti-tumor effects introduced the ASCs several weeks after tumor engraftment while all of the pro-tumor studies co-injected the ASCs with the tumor cells suggesting that timing of delivery may be critical in determining the anti- or pro-tumor fate of these cells.

Conclusions: While ASCs have enormous potential in regenerative medicine, there is no clear consensus on the effect of ASCs on tumor growth. Until sufficient evidence can be obtained, the greatest caution must be exercised before the widespread use of ASC technology in an oncologic patient setting.

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EVALUATION OF THE EFFECTS OF ANTI-FIBROTIC AGENT ON ADIPOGENESIS AND FIBROSIS IN AN IN VIVO CHAMBER MODEL

Presenter: Wali S. Boodhun, MBBS
Authors: Boodhun WS, Tan B, Han XL, Morrison W, Mitchell G, Abberton K
The OBrien Institute

WITHDRAWN

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CHARACTERIZATION OF ADIPOSE-DERIVED STEM CELLS ISOLATED FROM SUPERFICIAL AND DEEP SUBCUTANEOUS FAT: EFFECTS OF CRYOPRESERVATION, BMI AND DONOR-AGE

Presenter: Wanda Lattanzi, MD PhD
Authors: Quercia V, Pino V, Barba M, Di Pietro L, Visconti G, Di Taranto G, Salgarello M, Lattanzi W
Universita' Cattolica del Sacro Cuore

WITHDRAWN

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RECONSTRUCTION FOLLOWING CRANIOFACIAL TRAUMA WITH STROMAL VASCULAR FRACTION ENRICHED FAT GRAFTS: CORRELATING IN VITRO FINDINGS WITH CLINICAL OUTCOMES

Presenter: Jacqueline Bliley, BS, MS
Authors: Bliley J, Bourne D, James I, Sivak WN, Wang S, Schroth R, Coleman S, Donnenberg A, Donnenberg V, Marra KG, Rubin JP
University of Pittsburgh

Background: Autologous fat grafting is a minimally invasive technique that can be used to repair craniofacial soft tissue defects. However, graft retention is variable with 20-70% of the graft being resorbed over time. The use of fat grafts concentrated with the stromal vascular fraction (SVF) is hypothesized to help maintain tissue volume over time. The purpose of this study was to evaluate the efficacy of SVF-enriched fat grafts compared to standard fat grafting, as well as to correlate in vitro findings of the isolated SVF and adipose-derived stem cells (ASCs) with clinical outcomes such as graft retention.

Methods: Subjects (n=5) were enrolled into the clinical trial if they possessed bilateral malar soft tissue defects. Paired defects were randomized to either SVF enriched fat grafting or standard fat grafting. Volume retention was followed over time using computerized topography. Flow cytometry was used to assess subpopulations within the SVF. For each subject, cultured ASCs were also subjected to functional tests, including proliferation, adipocyte differentiation, and VEGF secretion under hypoxic conditions.

Results: The proliferation of isolated ASCs showed an approximate 2-fold increase in cell growth over a 7-day period. The differentiation potential of ASCs showed a 30-40 fold increase in lipid accumulation when stimulated with differentiation medium, compared to the baseline ASC medium control. No differences were observed between ASC proliferation and differentiation among patients ($p<0.05$). Based on flow cytometry, the proportion of SVF (45-) and ASCs (34+31-) within the SVF-enriched graft strongly correlated with graft retention at 3 months postoperatively; however, in the non-enriched graft there was not a correlation between SVF subpopulation percentage and eventual graft retention.

Conclusions: Future work will assess graft retention between cell-enriched and standard fat grafting groups in a larger subset of patients. In vitro assays will also be correlated with volume retention in order to develop predictive tools to help determine clinical outcomes.

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ADRCS AS AN ALTERNATIVE TO LATERAL INTERNAL SPHINCTEROTOMY IN PATIENTS WITH ANAL FISSURES

Presenter: Katarina Andjelkov, MD, PhD
Authors: Andjelkov K, Sforza M, Petrovic J, Zaccheddu R, Krivokapic Z
BelPrime Clinic

Introduction: An anal fissure is a tear in the lining of the lower rectum (anal canal). It is one of the most common anorectal diseases and occurs most frequently in young adults. The anal fissure impairs the quality of life, mostly due to often severe pain, itching and sometimes bleeding during or after bowel movements and may need a medical treatment. The results of similar studies showed efficacy in treatment of anal fistulas and significant improvement in sphincter incontinence using ADRCs. The treatment of anal fissures with ADRCs is unprecedented.

Method: We treated 6 patients (4 female and 2 male) age between 25-50 years old, with chronic fissure (duration more than 8 months) that didn't respond to conventional conservative therapy. All patients were candidates for lateral internal sphincterotomy. Exclusion criteria were: autoimmune diseases, presence of malignant or chronic infectious disease, or immunosuppressive therapy. Before the procedure all routine preoperative tests were done. Patients were asked to quantify their pain using VAS scale of pain before and on each follow up consultation. Approximately 150mL of lipoaspirate from the abdominal area was harvested under local anesthesia and sedation. ADRCs were extracted using Celution 800/CRS device and were immediately reinjected directly in the fissure and as enriched fat graft in subcutaneous layer underneath the fissure. The average number of cells reinjected was 3,20x107. The patients were followed up after 1 week, 1, 2, 6 and 12 months after the procedure.

Results: Complete cicatrization was achieved in all cases, with the average time of 7 weeks. Detailed analysis of each case is given.

Conclusion: Application of ADRCs is the method of great potential when considering chronic wounds including anal fissures. Further studies need to be done to obtain more accurate data, but we strongly believe that the stem cells will fulfill a clear unmet medical need and will help improve the healing and hence the quality of life of patients with anal fistula, fissure and sphincter dysfunction.

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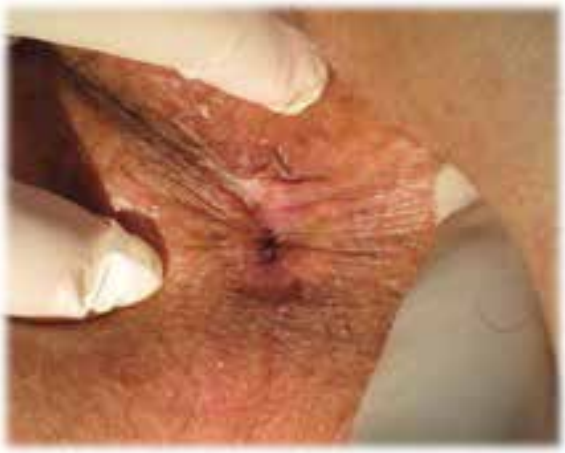
ADRCS AS AN ALTERNATIVE TO LATERAL INTERNAL SPHINCTEROTOMY IN PATIENTS WITH ANAL FISSURES



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EXPANDING ADIPOSE-DERIVED STEM CELLS FOR HIGH-DOSE ENRICHMENT OF FAT GRAFTS TO THE HUMAN BREAST

Presenter: Peter V. Glovinski, MD
Authors: Glovinski PV, Herly M, Koelle SF, Svalgaard J, Elberg JJ, Drzewiecki KT, Fischer-Nielsen A
Copenhagen University Hospital Rigshospitalet

Introduction: We previously demonstrated that high-dose enrichment of human fat grafts with ex-vivo expanded autologous adipose-derived stem cells (ASCs) remarkably improves graft survival. We plan to use this method for breast augmentation in a clinical trial. This requires significantly larger fat grafts and proportionally higher amounts of ASCs. For high-dose ASC-enriched fat grafting, we estimate that 3 x 10^9 ASCs are needed per breast. This amount of ASCs corresponds to SVF isolated from 50-200 L of lipoaspirate. To meet this number of ASCs we explored the proliferative capacity of new cell culture vessels for the necessary scale up of ex-vivo ASC expansion.

Method: The stromal vascular fraction (SVF) was isolated from the lipoaspirate of nine healthy women (age 25-52 years). On average 1.6 x 10^6 SVF-cells per mL of lipoaspirate (range (1.0 - 2.6) x 10^6 SVF/mL) was isolated from each donor. Cells were seeded at a density of 4-8.000 SVF cells/cm^2 and cultured for 14-17 days in standard culture media supplemented with 10% pooled human platelet lysate. Seeding was performed in four different types of cell culture vessels including gas permeable HYPERStacks® and CellSTACKS® with traditional airflow (Corning Life Science). The vessels were tested in different sizes and surface materials and harvested in the primary passage (P0).

Results: The CellSTACK® 10 (TCT surface) had the highest proliferative capacity for ASC expansion and produced 697,5 x 10^6 live ASCs (range (612,70 - 783,11)x 10^6 ASCs) with a viability above 90%. ASC cultured in the HYPERStack®-12 layer and -36 layer and the CellSTACK 10 (CellBIND surfaces) showed a lower and more variable yield. The control flasks (Millicell® HY Flasks) produced an average of 87,2 x 10^6 ASCs (range (51,2-120,9) x 10^6 ASCs). This range was comparable to the CellSTACK-10 (TCT) in yield per cm^2.

Conclusion: With the use of SVF isolated from 120 mL lipoaspirate and subsequent ASC expansion in five CellSTACK® 10 (TCT), we were able to produce more than 3 x 10^9 ASCs in P0. This result suggests how to produce the necessary amount of ASCs required for a clinical trial of high-dose ASC-enriched fat grafting for breast augmentation.

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USE OF AUTOLOGOUS ASCS TO RECONSTRUCT CRANIO-MAXILLOFACIAL BONE DEFECTS OF 24 PATIENTS

Presenter: Susanna Miettinen, PhD
Author: Miettinen S
REGEA Institute of Biomedical Technology University of Tampere

Introduction: Cranio-maxillofacial skeletal defects can arise as a consequence of congenital malformations, trauma, neoplastic growth, or following severe infection. These skeletal defects create functional and esthetic problems for the patient, for which few effective treatments are currently available. This study aimed to review our experience with 24 consecutive cases of cranio-maxillofacial hard-tissue defects at four anatomically different sites, namely frontal sinus (3 cases), cranial bone (5 cases), mandible (10 cases), maxilla (4 cases) and nasal septum (2 cases).

Methods: Autologous adipose tissue was harvested from the abdominal wall, and adipose-derived stem cells were cultured, expanded, and then seeded onto porous resorbable scaffold materials for subsequent reimplantation into defects. The defects were reconstructed with either bioactive glass or -tricalcium phosphate scaffolds seeded with adipose-derived stem cells (ASCs), and in some cases with the addition of recombinant human bone morphogenetic protein-2. Production and use of ASCs were done according to good manufacturing practice guidelines. The ASCs were expanded for 3-5 weeks. Multipotency (adipogenic, osteogenic and chondrogenic differentiation) and mesenchymal stem cell surface marker profile (CD14, CD19, CD34, CD45, CD73, CD90, CD105, HLA-ABC-PE, and HLA-DR) of ASCs was analyzed. Moreover, chromosomal integrity, sterility, endotoxins and mycoplasma were determined before cell transplantation. Follow-up time ranged from 12 to 52 months.

Results: Successful ossification and integration of the construct to the surrounding skeleton was noted in 18 of the 24 cases. In unsuccessful cases 1 patient developed infection, 1 nasal septum transplant was lost because of nose picking habit of the recipient and 4 patients did not produce sufficient volume of bone.

Conclusion: These cases show that the use of autologous adipose-derived stem cells combined with osteostimulative biomaterial results in generation of new viable bone.

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ASC TREATMENT REDUCES THE SEVERITY OF MURINE GVHD THROUGH EARLY IMMUNOMODULATORY EFFECTS

Presenter: Anthony D. Foster, PhD
Authors: Foster AD, Clark N, Gimble JM, Davis TA
Navy Medical Research Center

Introduction: Acute bone marrow toxicity, due to clinical treatment or accidental exposure, requires a hematopoietic stem cell transfer (HSCT) as a life saving measure. A major complication of allogeneic (non-self) HSCT is graft vs. host disease (GvHD), a significant cause of morbidity and mortality despite aggressive immunosuppression therapies. As such, options that reduce the risk or severity of GvHD are currently of great interest. Adipose derived stem cells (ASCs) have an established anti-inflammatory and immune regulatory phenotype that may be beneficial in the prevention or mitigation of acute GvHD. However, little is known about the efficacy and early immunological changes induced by ASC treatment in GvHD.

Methods: An established murine model of GvHD was used, wherein recipient C57BL/6 mice receive lethal irradiation followed by a fully mismatched transfer of mature T cells and bone marrow cells from BALB/c donors. Transfer groups included syngeneic (C57BL/6 to C57BL/6), allo only (BALB/c to C57BL/6), and allo + 2.5 x 10^6 human ASCs. At indicated time points mice were euthanized, and tissues were collected for mRNA, protein expression, and flow cytometry analysis. The severity of disease was determined by histological and clinical analysis.

Results: ASC treatment significantly improved disease features both histologically and clinically. A time course analysis of T cell kinetics as well as gene and protein expression revealed that ASC treatment significantly reduced donor CD8 T cell expansion and several inflammatory cytokines (including IFNg) vs. the allo only injection group by day 7 post transfer.

Conclusions: These findings demonstrate the efficacy of ASCs as a treatment option for reducing the severity of acute GvHD with only a single treatment. It is well recognized that donor T cells initiate and drive GvHD. A > 2-fold reduction in the number of donor CD8 T cells at day 7 due to treatment strongly supports a role for ASCs that includes donor T cell suppression. A concomitant reduction in inflammatory cytokines supports this conclusion as well. These data suggest a mechanism of action that includes differential donor T cell expansion during the early phase of GvHD induction due to ASC treatment, though more research is necessary to define this.

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SHORT-TERM CULTIVATED ASC-LIKE CELLS (SCALC) - THE NATIVE STEM CELL FOR MEDICAL CURE

Presenter: Michael Krueger, MD
Authors: Haschemi A, Born S, Meutsch J, Westphal J, Hartjen P, Smeets R, Krueger M
Klinik Sanssouci

Introduction: MSCs and ASCs are encouraging tools for regenerative medicine because of their manifold immunomodulatory and regenerative properties. Easy access and high cell yields make adipose tissue an ideal source for MSCs. Usually either the Stromal vascular fraction (SVF), cells sorted for specific surface markers or cultivated ASCs are used. However, there are speculations about cultivation leading to changes in the phenotype. Therefore we tried a new approach, creating a homogeneous native cell fraction without the necessity to use antibodies or cultivation.

Method: SVFs were isolated from lipoaspirate as described in literature. SVF cells were seeded in plastic flasks, adherent cells were harvested within less than 20h. We call this fraction Short-term cultivated ASC-like cells (SCALC). The SCALC were seeded for further analysis in different densities and split at 90% confluence. Analysis was performed before seeding and at splitting. Differentiation experiments were done according to Zhu et al. 2013.

Results: Identification of the surface markers revealed that SCALCs are less heterogenic than SVF. They were positive for CD13, CD34, CD44, CD73, CD90, and negative for CD31, CD45, CD105 and CD235a. The CD34+ population overlapped with the CD90+ population but was strictly CD45- (no hematopoietic lineage). The capability for self-renewal was shown in long-term cultivation for several weeks. The cells were able to proliferate in-vitro with a doubling time of approximately 1.6 days. They exhibited a fibroblastic morphology after 7 days, their size increasing 5 to 10-fold in the process. Surface markers changed during cultivation, adopting the phenotype for ASCs suggested by IFATS & ISCT. Capability for multilineage differentiation was also verified.

Conclusions: We found that SVF cells cultivated for less than 20h show similar properties to ASCs. SCALC are much more homogenous than the SVF, at the same time exhibiting a more "native" phenotype (CD34+, CD105-) compared to cultivated ASCs. They are also substantially smaller than ASCs. The shorter cultivation time reduces the risk for contamination, and the smaller size could enhance the cell distribution after i.v. administration. This has to be verified in future studies.

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ADIPOSE-DERIVED STEM CELL CONDITIONED MEDIUM (ASC-CM) PROMOTES REVASCULARIZATION OF ISCHEMIC TISSUES IN A RABBIT MODEL OF PERIPHERAL ARTERY DISEASE

Presenter: Brian Johnstone, PhD
Authors: Johnstone B, Jurcikova J, Lassak O, Vitkova K, Pavliska L, Porubova L, Buszman PP, Krauze A, Fernandez C, Jaluvka F, Spackova I, Merfeld-Clauss S, Traktuev D, March KL, Prochazka V
Indiana University School of Medicine

Introduction: Transplantation of adipose-derived stem cells (ASC) is an emerging therapeutic option for addressing intractable diseases such as critical limb ischemia (CLI) resulting from peripheral arterial disease. Evidence suggests that therapeutic effects of ASC are primarily mediated through paracrine-mechanisms. These paracrine factors can be captured in conditioned medium (CM) comprised of a cocktail of beneficial growth factors and cytokines that individually and in combination demonstrate disease-modifying effects in animal models. The ability of ASC-CM to promote reperfusion in a rabbit model of CLI was evaluated in this study.

Methods: CM was collected from cultured ASC (passage 6), filtered and concentrated 50-fold. A total of 27 adult female rabbits underwent surgery to induce ischemia in the left limb. An additional 5 rabbits served as sham controls. One week after surgery, the ischemic limbs were injected i.m (4 sites in gastrocnemius, 1 site in tibialis anterior) with either: (1) placebo control, (2) 0.4 ml ASC-CM/ site (low dose) or (3) 0.8 ml ASC-CM/ site (high dose) (9 animals/group). Medial gastrocnemius muscle perfusion was serially assessed over 1 month with a Doppler probe. Blood samples were analyzed for growth factors and cytokines. Post-mortem tissues were histochemically assessed for CD31+ capillary density.

Results: At 1 month after treatment, tissue perfusion in ischemic limbs of rabbits treated with high dose ASC-CM was almost double ($P<0.05$) that of the placebo group (58.8 + 23 versus 30.7 ± 13.6; mean perfusion units ± sd). The level of perfusion in the limbs treated with high dose ASC-CM was no different than in sham control limbs (54.3 ± 15.4). This effect correlated with a greater microvascular density ($P<0.01$) as well as transient increases in levels of such angiogenic and pro-survival factors as VEGF and HGF in the blood.

Conclusions: A single bolus administration of ASC-CM restored tissue reperfusion in in the ischemic limbs in a rabbit model of CLI. We hypothesize that neo-angiogenesis was promoted by growth factors and cytokines present in ASC-CM as well as by stimulation of endogenous host responses. These data suggest that ASC-CM may be a potent therapy for patients with CLI who are at risk for amputation.

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TREATMENT OF DEGENERATIVE JOINT DISEASE (DJD) IN HORSES BY A MIXTURE OF PRP AND MICROFAT

Presenter: Guy Magalon, MD
Authors: Magalon G, Bembo F, Magalon J, Eraud J, Sabatier F
AMU AIX Marseille University

This technique allows harvesting and injections in less than an hour of autologous cell therapy product with immediate use on standing horse for severe Degenerative Joint Disease. The Injection is mixing PRP (platelets rich plasma) and microfat harvested in sterile closed method from tail area filtered in a double layer pocket. A blood sample (64 ml), collected from the jugular vein, permits to obtain 3,5-4,5 billion platelets in 5 ml of plasma after elimination of the PPP (Platelets Poor Plasma). Fat is harvested on local anesthesia with a single use, 14 G cannula, connected to a 10 ml syringe Luer-Lock, to enable the collection of the fat, under gentle aspiration and directly injected into a sterile single used closed circuit with a double layer pocket filtration. The quantity of harvested fat is about 30 ml permitting to obtain final volume of pure fat around 10 ml. PRP obtained at first step is mixed with purified fat in equal parts 5 ml PRP/5 ml purified fat. Sterile injection is prepared with a 21 G, 0.8 mm, 50 mm needle. The synovial fluid is retrieved and 10 ml of preparation injected into the joint. In the laboratory, we characterize fat horses and we count the number of platelets. Ten horses thoroughbred, French trotters, endurance Arab and jumping horses with severe DJD. Inclusion criterias were severe DJD confirmed by radiography and ultrasound and a clinical lameness 1/5 to 3/5. 1 carpus and 9 fetlocks were treated during the period 2014-2015. No flare and adverse reactions were noticed. After 2 months, all horses were improved with a shown better comfort, walking. 4 horses are back in competition and the others still in the rehabilitations protocol period.

This study shows that autologus adipose tissue micro-injection, mixed with PRP, is safe and can improve degenerative joint disease in athlete horses. The trophic properties of adipose tissue are mainly due to SVF (AD-SVF). PRP improves tissue regeneration, due to the presence essential cytokines and grows factors.

This study showed that this innovative treatment was not only safe and well tolerated but also provided encouraging preliminary evidence of efficacy. We want to translate this preclinical model on a clinical model for treatment of osteoarthritis of the knee.

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ENCAPSULATED HUMAN ADIPOSE DERIVED STEM CELLS IN AN INJECTABLE THIOL-ACRYLATE BASED SCAFFOLD FOR BONE TISSUE ENGINEERING

Presenter: Anoosha Forghani, PhD
Authors: Forghani A, Garber L, Chen C, Devireddy R, Pojman J, Hayes D
Louisiana State University

NOT PRESENTED

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PRO-INFLAMMATORY AND OSTEO-INDUCTIVE CYTOKINES INDUCE BONE-RELATED EARLY RESPONSE GENES IN ADULT HUMAN ADIPOSE STROMAL CELLS

Presenter: Elizabeth Martin, PhD
Authors: Martin E, Qureshi A, King A, Krause P, Lee O, Dasa V, Freitas M, Forsberg J, Elster E, Davis T, Gimble J
Tulane University

Background: Traumatic heterotopic ossification (HO) is the formation of ectopic bone in non-osseous structures. The precise mechanism for the development of traumatic HO is unknown however, musculoskeletal trauma is critical to the development of most forms of HO. Data collected from combat patients with blast injury suggests that there may be an environment conducive to bone development with osteogenic signaling factors such as BMP2 in wound effluent and IL6 in serum. This suggests that HO development may be systemic in origin.

Methods: For all experiments human adipose stromal cells (ASCs) were grown in 5% dextran stripped FBS media 48 hours prior to stimulation. Gene expression profiles were obtained following stimulation with BMP2 (200ng/ml), IL6 (20ng/ml) or 5% human serum for 4 and 24 hours. Osteo induction was induced for 4, 24, 48 hours and 2 weeks. Human serum was derived from civilian patients who suffered orthopaedic trauma without an incidence of HO (n=3) and those that developed HO (n=4). Comparison was to pooled serum from individuals without trauma. Gene expression analysis was determined through qPCR.

Results: Evaluation of the effects of BMP2 and IL6 signaling on non-injured ASCs was determined through qPCR analysis. There was an observed difference in expression of early response AP1 complex genes. ASCs grown in osteogenic induction media demonstrated a similar increase in gene expression compared to control media. Serum from a cohort of civilian patient samples was collected 24-48 hours post trauma to determine if an early induction gene signature existed in orthopaedic trauma induced HO. ASCs were stimulated with human serum and a PCR gene panel was evaluated. Serum from individuals who developed HO had alterations in AP1, integrin, and uPA signaling. PCR validation demonstrated a significant reduction in the AP1 family and enhanced expression of mitogen-activated protein kinase 8 and plasminogen in individuals with trauma induced HO.

Conclusion: MAPK signaling is altered in ASCs following osteogenic stimulation and serum exposure from individuals with trauma induced HO. These results provide an initial gene signature for the development of HO and support ongoing work linking the systemic response to injury with wound specific outcomes.

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MICRORNA MEDIATED INHIBITION OF OSTEOBLAST MEDIATED BONE FORMATION

Presenter: Ammar T. Qureshi, PhD
Authors: Qureshi AT, Martin EC, Gimble JM, Forsberg JA, Hayes DJ, Davis TA
Naval Medical Research Center

Background: Heterotopic Ossification (HO), mineralization within extra-skeletal soft tissue leading to the formation of mature ectopic bone in muscle, tendon and ligaments is a critical problem for the combat-injured service members. MicroRNAs, post-transcriptional gene regulators, have shown to control multiple transcriptional factors, signaling pathways and regulatory networks involved in normal bone formation. To date, a few multifunctional and antagonistic miRNAs (miR-211, miR-204, miR-21) have been identified that suppress osteogenesis via pathways that do not disrupt or inhibit adipogenesis and can be used to reduce the osteogenic differentiation of cells. Our aim is to evaluate the modulation of osteoblastic gene regulation with miRNAs with the goal to redirect MSC proliferation in an osteogenic environment toward adipogenic differentiation.

Methods: Human adipose stromal cells (ASCs) and rat muscle-derived mesenchymal cells (rMSCs) were grown in normal stromal media (SM) and stimulated with miR-21, miR-204, miR-211, miR-34a (positive control) and miR-148b (negative control) for 48 hrs. The miRNA stimulation either occurred in SM or osteogenic media (OM) and the cultures were maintained for 21 days. The osteogenic and adipogenic differentiation were measured by Alizarin red staining and oil red staining respectively and expression levels of gene markers were quantified using qRT-PCR.

Results: The transfection of rMSC with miR-204 and miR2-11 in OM resulted in decreased osteogenic gene expression (OP, ALPL and JAG1) and increased expression of adipogenic marker CFD. These findings were confirmed by reduced Alizarin red and increased oil red staining. Similar trends were noted using ASCs wherein expression of adipogenic markers PPAR^3 and CFD were increased and osteogenic markers (ALPL, ON, OP, SMAD1 and Col11) were decreased.

Conclusion: We demonstrated that miR-204/211 have the in vitro potential to redirect rMSC and hASC from osteogenic to adipogenic lineages in an osteogenic microenvironment. Moreover, these results validate that miR-204/211 are important regulatory factors in controlling osteoblastic differentiation networks, a potential clinical application for anti-HO therapy targeting osteogenic progenitor cell development in traumatized tissue

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IN VITRO CHARACTERIZATION AND BIOLOGICAL EVALUATION OF A THIOLATED ADIPOSE-DERIVED EXTRACELLULAR MATRIX HYBRID HYDROGEL FOR ADIPOSE-DERIVED STEM CELL DIFFERENTIATION

Presenter: John N. Poche, BS
Authors: Poche JN, Totaro NP, Hayes DJ
Louisiana State University

For more accurate biomimetic materials, 3D scaffolds are being used in tissue engineering to provide structural support and signaling molecules to adult-derived stem cells for targeted tissue differentiation. With increased bioactivity observed in biologically-derived gels, and structual integrity and mechanical tunabilty gained through synthetic gels, a thiolated adipose-derived extracellular matrix (taECM) and a multifunctional acrylate were used to synthesize a hybrid hydrogel for use as a multi-tissue directing scaffold. Raw adipose tissue from two donors was decellularized and processed using a modified version of the Matrigel procedure. The resultant proteinaceous pellet was thiolated, then cross-linked with poly (ethylene glycol) diacrylate (PEGDA) via radical-induced photopolymerization. Five hydrogels were synthesized comprised of 0:4:4, 1:3:4, 2:2:4, 3:1:4, and 4:0:4 taECM: dithiothreitol (DTT): PEGDA by molar functionality. Fourier Transform Infared Spectroscopy, compression testing, and swelling and mass loss studies were used to define the chemical, mechanical, and degradation properties of the samples. In addition, an in vitro characterization of the chemical, mechanical, and cytotoxic properties of the hydrogel was conducted with adipose-derived mesenchymal stem cells (hASCs). Furthermore, an in vitro evaluation with hASCs was performed to identify the differentiation potential of the scaffolds. This study displayed the potential of utilizing an adipose-derived hybrid hydrogel for tunable tissue regeneration. As many studies are necessary to represent the importance of both mechanical and biological signals to hASCs, future efforts will employ signaling molecules and controllable mechanical stability to target a specific differentiation pathway, i.e. chondrogenesis versus osteogenesis. Also, by generating a hydrogel with varying mechanical strength, multi-tissue types may be formed, i.e. cartilage to bone, over the gradient generated.

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DEMINERALIZED HUMAN BONE MATRIX (DBM) WITH SYNGENEIC ADULT ADIPOSE-DERIVED MULTIPOTENT STROMAL CELLS (ASCS) ACCELERATES SPINAL FUSION

Presenter: Mandi J. Lopez, DVM, MS, PhD
Authors: Lopez MJ, Fargason C, Kelly L, Del Piero F, Dasa V, King A
Louisiana State University

Osteogenesis by adult adipose-derived multipotent stromal cells (ASCs) supports their ability to augment bone formation. Demineralized bone matrix (DBM) carriers for clinical application combine the osteogenic capabilities of bone and ASCs. This investigation tested the hypothesis that human DBM+syngeneic ASCs augment spinal fusion in an athymic rat model over either component alone 4, 8 and 12 weeks after implantation.

Lumbar spinal fusion was performed on adult male rats in four cohorts, saline, ASCs, DBM or DBM + ASCs. Radiographic spinal fusion was scored and total bone volume and surface area were measured on 3-D models of micro computed tomography images. One fusion callus of each spine was stained with Masson's trichrome and Picrosirius red for light microscopy. Expression of target genes alkaline phosphatase (ALP), osteopontin (OP), collagen I alpha I (COL1A1) and osteoprotegerin (OPG) were determined in the other fusion callus.

DBM and DBM + ASCs significantly increased fusion and callus volume and surface area compared to saline or ASCs at 4, 8 and 12 weeks. Based on micro-CT and light microscopy, fusion callus was most mature and organized in the DBM + ASCs cohort at all times. Target genes were significantly higher in the DBM + ASCs and DBM cohorts at all times, with OPG expression highest in the DBM+ASCs cohort at 4 weeks.

Combining syngeneic ASCs and DBM appears to significantly augment spinal fusion in an athymic rat model over either treatment alone, with the most marked effect within 4 weeks after implantation.

NOTES

POSTER PRESENTATIONS

in numerical order

1P

NEURAL LINEAGE DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STROMAL CELLS - PRELIMINARY RESULTS

Presenter: Luminita Labusca, Md PhD
Authors: Labusca L, Hyttel P, Freude KK, Ostrup E
University of Copenhagen

NOT PRESENTED

2P

THE USE OF TISSUE SCAFFOLDS PLUS ENHANCED FRACTIONAL CELLS MAY BE AN AESTHETICALLY AND FUNCTIONAL ALTERNATIVE TO FREE TISSUE TRANSFERS IN BURN SCAR CONTRACTURE TREATMENT

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Guvercin E, Taylan Filinte G, Ceran F, Basat SO
Dr. Lutfi Kirdar Kartal Training and Research Hospital

Introduction: Despite current developments in acute burn injury treatment resulted in significant increase in survival rates, similar gains can not be achived in long-term treatment results of burn patients. It is possible to encounter with limb contractures in the advanced stage of burn treatment; these contractures are especially prevelant in upper extremities and can lead to extensive functional impairment. Because contractures in these patients are likely to recur; reconstructive surgical interventions should give results in terms of increasing the function, while reducing recurrence. Current studies are aimed to improve the long-term results by blending traditional methods with new technologies. We have created our approach to the treatment modalities and examine whether it is an alternative to conventional treatments for burn contractures.

Material and Method: Patients divided into 3 groups for severity of contracture. After scar excision and contracture release; we used dermal scaffold with sptli thickness skin graft (STSG) in 1st group. In 2nd group PRP and thrombine is added to 1st groups treatment protocole. Mesenchymal or bone marrow derived stem cells are added in the 3rd group (Table 1). Fat grafts are injected under grafts in postopertive 6th month. Static splints were used to stabilize joints. NPWT used as dressing. Range of motion before and after the operation are measured and compared.

Result: 15 patients were included in the study. Patients age were between 2 and 41 (avarage 13.8). Follow up period was 18-30 months. Range of motions before and after the operation were measured and difference was calculated and difference found statistically significant (Table 2). It showed improvement in skin quality and elasticity. A decrease was found in hypertrophic scarring.

Conclusion: Conventional treatments often results in recurrences and failures in burn recontruction. Development of cellular therapies, use of dermal scaffolds and alternative treatments in wound management has enabled us to give more options to patients which came to an end point with conventional treatment modalities. In particular, hypertrophic scars are potential in children due to fast growth so alternative treatment options should be kept in mind and added to treatment.

2P
THE USE OF TISSUE SCAFFOLDS PLUS ENHANCED FRACTIONAL CELLS MAY BE AN AESTHETICALLY AND FUNCTIONAL ALTERNATIVE TO FREE TISSUE TRANSFERS IN BURN SCAR CONTRACTURE TREATMENT

3P
FEASIBILITY AND SAFETY OF ADIPOSE-DERIVED WOUND PASTE IN A MURINE MODEL OF FULL THICKNESS WOUNDING

Presenter: Ning Yang, PhD
Authors: Yang N, Shang H, Katz A
University of Florida

Introduction: Our goal is to leverage adipose tissue into novel point-of-care therapies for full thickness wounds. Based on FDA guidance, we performed an initial safety study in mice using adipose-derived, 'point-of-care wound paste' in full thickness wounds. Primary outcome objectives were safety and cell migration. Secondary objectives were wound healing rate, wound contraction, angiogenesis, and scar quality.

Methods: Mice with full thickness wounds were randomized to 4 treatment groups: 1) secondary healing; 2) 'adipose-derived wound paste'; 3) wound paste with keratinocytes; and 4) acellular wound paste (matrix only). Wound paste was made using GFP labeled cells. Healing rates and wound contraction were measured using digital imaging at 1, 2, 3, 4 and 6 weeks. Scar quality was measured at week 6. Cell migration was evaluated with PCR and histology.

Results: Wound paste did not have a detrimental effect on either animal survival. Adipose-derived cells survived real-time formulation into wound paste and implantation into open wounds, and proliferated over time. There were no significant differences between treatment groups for wound healing rate, wound contraction, or scar quality.

Conclusion: This initial in vivo feasibility and safety study confirmed that adipose-derived cells can be formulated in real-time into wound paste, applied to an open wound, and survive and proliferate in the wound over time. Although cells seem to remain localized to the wound and contribute to neo-vessels, they did not seem to alter healing kinetics or quality in this study. Future studies will evaluate both human-derived cells, as well as the effect of increasing cell dose.

4P

ALLOTRANSPLANTATION FOLLOWING RECELLULARIZATION OF DECELLULARIZED FLAP USING ADSCS

Presenter: Yungki Lee, MD
Authors: Lee Y, Pak CS, Kim BK
Seoul National University Bundang Hospital

NOT PRESENTED

5P

NON-RESPONSIVE KNEE PAIN TREATED WITH AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE UNDER CONTINUOUS ULTRASOUND GUIDANCE

Presenter: Richard D. Striano, DC, RMSK
Authors: Striano RD, Bilbool N, Chen H
OptimumJoint Integrated Joint Spine

Purpose: The use of autologous adipose has gained interest as a source for ortho-biologic therapies. Fat is readily accessible and simple to harvest. This adipose can be used to provide cushion and filling in of structural defects. Adipose has been shown to have an abundance of regenerative perivascular cells. In this knee pain case report we initiate our evaluation of the potential benefits of using micro-fragmented non-digested adipose tissue to obtain minimally manipulated, micronized fat tissue with an intact stromal vascular niche harboring regenerative cellular elements.

Background: This case is the first in a 100 subject IRB approved by IRCM. The patient is a 59 year old male with severe knee pain failing prior treatments including arthroscopic surgery following a prior MRI 5 months before treatment showing evidence of osteoarthritis, meniscal tear and Chondromalacia patella.

Method: The micro-fragmented fat is derived using minimal manipulation in a closed kit system, (Lipogems®) without the addition of enzymes. The final product consists of fat droplets with preserved vascular lumina at 300-500 microns. In this treatment protocol, the Lipogems were injected with a 23-gauge needle under continuous ultrasound guidance into the joint and filling the meniscal defects. No other agents were combined with the fat. Outcomes were measured immediately following the treatment, at 24 hours, 1 week, 5 weeks, 3 months and 6 months. At 6 months, repeat MRI is performed.

Results: In the first patient at 6 months whose positive outcome may have been questionable, improvement in all measured scores. VAS pain score on a 1-10 scale with 10 being worse improved from 8/10 to 2/10, knee injury and osteoarthritis outcome score (KOOS) improved from 44.6/100 to 96.3/100. MRI 6 months post treatment revealed the hyaline cartilage over the femoral condyle showed improved signal and thickness with a widened joint space. The meniscus appearance is consistent with prior surgery.

Discussion: Due to arthroscopic surgery following the MRI prior to the treatment, the change in the meniscus 6 months after adipose is not comparable. While further study is underway, these results in a case with multiple abnormalities treated with autologous micro-fragmented fat appear promising.

5P
NON-RESPONSIVE KNEE PAIN TREATED WITH AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE UNDER CONTINUOUS ULTRASOUND GUIDANCE

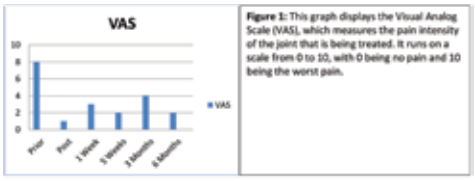


Figure 1: This graph displays the Visual Analog Scale (VAS), which measures the pain intensity of the joint that is being treated. It runs on a scale from 0 to 10, with 0 being no pain and 10 being the worst pain.

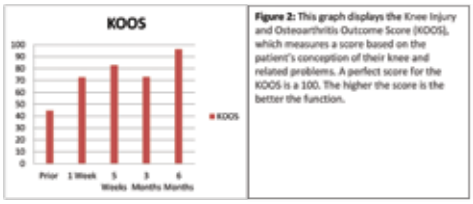


Figure 2: This graph displays the Knee Injury and Osteoarthritis Outcome Score (KOOS), which measures a score based on the patient's conception of their knee and related problems. A perfect score for the KOOS is a 100. The higher the score is the better the function.

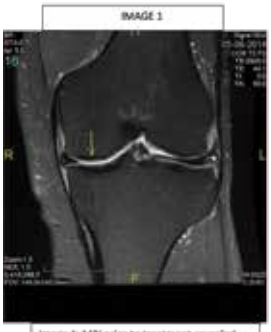


Image 1: MRI prior to treatment revealed medial compartment degeneration (yellow arrow).

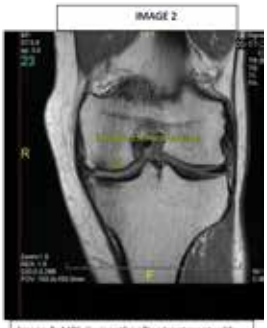


Image 2: MRI six months after treatment with adipose revealed widening of the joint space and increased Hyaline cartilage on the femoral condyle (yellow arrow).

6P
THREE DIMENSIONAL CELL PROLIFERATION AND OSTEOGENESIS OF HUMAN ADIPOSE STEM CELLS ON POLY (L-LACTIC ACID) SCAFFOLDS PREPARED BY THERMALLY CONTROLLED METHODS

Presenter: Harish Chinnasami, PhD
Authors: Chinnasami H, Devireddy RV
Louisiana State University

NOT PRESENTED

7P

INVISIBLE SCAR: INTRADERMAL INJECTION OF SVF COMBINED WITH SCAR REVISION CAN IMPROVE THE RESULT

Presenter: Wonyoung Yoon, MD, MS
Authors: Yoon W, Kook KS
Idea Plastic Surgery

Introduction: Scars on the skin cause patients considerable distress, resulting in unpleasant aesthetics, restriction of movement cross joints and adverse psychological effects. The cutaneous wound healing process causes scar formation, during which exaggerated inflammation has been demonstrated to have an important role. Mesenchymal stem cells (MSCs) have recently been shown to mitigate inflammatory phase during the wound healing process. To counter this, we locally injected adipose stem cells (ASCs) to treat cutaneous scars together with scar revision sugery. We sought to demonstrate the effectiveness of ASCs in improving the repair of scars.

Methods: A total of 16 scars from 11 patients with post-traumatic or post-surgical scars was treated with scar revision. On the day of surgery, fat was harvested from the patients and stromal vascular fraction (SVF) was extracted. SVF injection was done with the scar revision. SVF injection alone was given twice a week for 3 consecutive weeks after scar revision. Thereafter, patients visited the clinic once a month for 3 months to receive SVF injection. About 3 million cells/cm was injected in average. Photographs were taken on each visit. Scars were also assessed with the patient and observer scar assessment scale (POSAS) before the surgery, and upon completion of the treatment.

Result: Upon photographic analysis, scars were improved aesthetically in all cases. The decrease of POSAS scores were statistically significant. No major complications were observed.

Conclusion: Scar revision combined with serial SVF injection showed better results than surgical revision alone. Intradermal SVF injection can be safely added with scar revision surgery. Regulating the inflammatory phase of wound healing and promoting the proliferative phase appears to be a key effect of SVF in reducing scars. Further studies should be followed to find more efficient ways for the delivery of SVF.

8P

DERMAL SCAFFOLD AND BONE MARROW ASPIRATE CONCENTRATE USAGE FOR SEVERE FACIAL BURNS MANAGEMENT AS A NEW PARADIGM FOR RESCUE PROCEDURE

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Ceran F, Filinte GT, Guvercin E, Basat SO
Dr. Lutfi Kirdar Kartal Training and Research Hospital

Introduction: Facial burns often get an inextricable situation in terms of healing and long-term morbidity. Many different procedures were used to overcome these challenging topics. Although results were more promising in terms of plastic surgeons, is inadequate for patients. We present the effectiveness of the use of dermal scaffold and bone marrow aspirate concentrate for severe facial burns management.

Methods: Written informed consents were obtained from all patients for the surgical procedures. Three in acute, three in subacute phase, a total of six patients with severe facial burns were treated by using dermal scaffold (DS) (Matriderm® collagen-elastin matrix), bone marrow aspirate concentrate (BMAC) and immediate split thickness skin grafting after serial debridements. 120 cc of bone marrow was harvested from the anterior iliac crest in adults and 30 cc from anteromedial face of the tibia in children younger than 2 years of age and processed using the SmartPReP BMAC® system (Harvest Technologies Corp.) to obtain BMAC. Lipoinjection procedure was performed in subacute facial burns to contour in addition. Postoperative results were archived by using photographic documentation.

Results: Average follow-up period was 16 months. Average operation number per patient was 4. A considerable improvement in skin softness, thickness, elasticity, color was obtained in all patients.

Conclusions: DS creates a perfect ground for full-thickness facial burns to obtain elasticity and acceptable thickness. Grafted body parts undergo pigmentation and contractures as is known. DS usage reduces pigmentation, contracture development and pressure garment time as a result. These features facilitate a return to social life and promote a better quality of life for people living with burns. BMAC may develop graft survival and integration. The results of the study demonstrate that combining the dermal scaffold with bone marrow aspirate concentrate improves the outcomes of severe facial burns management.

9P

A COMPARISON OF FAT GRAFTING METHODS ON OPERATING ROOM EFFICIENCY AND COST

Presenter: Mousam h. Parekh
Authors: Gabriel A, Parekh, MH, Champaneria MC, Macarios D, Griffin L, Maxwell GP
Peace Health Medical Group

Objectives: Centrifugation (Cf) is the standard method of fat processing but is cumbersome and time consuming, especially when large volumes are processed. A new autologous fat processing system (Rv) that incorporates fat harvesting and processing in a single unit offers a simple, more efficient system. This study compared the efficiency and economics of using Rv with CF in terms of fat grafting and operating room (OR) costs.

Methods: Data were collected over 2 years from consecutive breast surgery patients undergoing autologous fat grafting: January to December 2012 with the Cf method and January to December 2013 with Rv method. The volume of fat harvested, volume of fat injected after processing, and time taken to complete fat grafting (from harvest to injection) were determined. Standard OR costs ($15-$20 per minute for basic surgical procedure, excluding physician costs) were used to estimate potential savings.

Results: There were a total of 239 cases: Cf=118 and Rv=121 patients. There were no significant differences between Cf and Rv patients in mean age, BMI, smoking status, diabetes, and prior radiation. Mean volume of fat harvested (499.5 vs 122.9 ml) and injected (178.9 vs 78.0 mL) were significantly higher ($p<0.0001$) in the Rv group compared to the Cf group, respectively. Mean time to complete fat grafting was significantly shorter in the Rv group compared to the Cf group (35.3 vs 89.8 minutes, respectively; $p<0.0001$). Mean OR costs with Rv were estimated to be $529-$706 vs $1347-$1796 with Cf. After taking into consideration the cost of Rv ($470), the potential net savings in mean OR costs were $347-$620 per case.

Conclusions: The Rv fat processing system decreased operative time, which translated into a potential cost savings, and allowed for a larger volume of fat to be processed for injection compared to standard centrifugation.

10P

TREATMENT OF LYMPHEDEMA WITH CELL-ASSISTED LIPOTRANSFER WITH AUTOLOGOUS STROMAL VASCULAR FRACTION

Presenter: Navid M. Toyserkani, MD
Authors: Toyserkani NM, Jensen CH, Sheikh SP, Sorensen JA
Odense University Hospital

Introduction: Lymphedema is one of the most frequent side effects of breast cancer treatment. At present standard of care is compression therapy and manual lymph drainage, none of which are curative. The present sparse evidence suggests that stem cells could be a possible solution to alleviate lymphedema. We hypothesized that the scar tissue in the axillary region could block the regeneration of lymph vessels. Our aim was therefore to loosen this scar tissue with a cell-assisted lipotransfer using autologous stromal vascular fraction (SVF)

Methods: The patient was a 48 year old female with previous unilateral breast cancer treated with lumpectomy and complete lymph node dissection on the right side. She was treated with radiotherapy and had been recurrence free for 5 years. Preoperatively arm circumferences were measured and a DXA scan was performed. A cell-assisted lipotransfer to the axillary region was planned as a single-stage procedure. Autologous SVF was isolated using a closed automated system. Additional SVF was kept for laboratory characterization including flow cytometry and colony forming units assay.

Results: For SVF isolation 320ml lipoaspirate was obtained from the abdomen. The scar tissue in the axilla was loosened with lipotransfer, where 10ml of fat was injected using the Coleman method. In total 4.07 x 10^7 SVF was injected subcutaneously at 8 pre-defined spots in around the axillary scar. One fifth of the obtained cells were saved for laboratory characterization. The remaining cells were injected immediately after isolation. There were no complications or any adverse reactions related to the procedure. At one month follow up the patient felt less pressure in her lymphedema affected arm. Further results will be shown at the conference including a 3 months postoperative DXA scan for an objective measure of arm volume.

Conclusions: Adipose-derived stromal cells have so far shown great potential in Regenerative Medicine. If cell-assisted lipotransfer proves to be efficacious at alleviating lymphedema, it could revolutionize the way lymphedema is treated. A proper clinical phase 1 study is being planned.

11P

DERMAL WOUND PASTE FABRICATED WITH STROMAL VASCULAR FRACTIONS FOR THE POINT-OF-CARE WOUND THERAPY

Presenter: Ning Yang, PhD
Authors: Yang N, Patel N, Shang H, Katz A
University of Florida

Introduction: Adipose tissue is uniquely amenable to autologous point-of-care treatment strategies due to ease of harvest and abundance. Fresh isolated human stromal vascular fraction (SVF) cells have variety of cell lineages, which secreted tremendous of growth factor and cytokines which are essential for wound healing. Seeding the SVFs in real time into wound dermal paste and maintaining cell activities provide a platform for point-of-care treatment.

Methods: Fresh isolated SVF cells were mixed with acellularized dermal scaffold and hyaluronic acid (HA) gel and formed the paste-like paste (Dermal Wound Paste, DWP). DWP were cultured for 14 days in DMEM/F12 with 1% human serum and antibiotic. Cell number was evaluated on day 1, 7 and 14 using PrestoBlue assay. Conditioned medium (CM) was collected for cell migration, proliferation, endothelial angiogenesis and Luminex assays. On day 14, DWP were fixed and processed for histological analysis.

Results: The average initial cell number (n=5) within DWP formulations was 1.76 million per disc. At day 14, the cell number had increase 193% to an average of 3.40 million cells per disc ($p<0.005$). SVF cells were distributed evenly within DWP. CD31 staining demonstrated the presence of endothelial cells and/or monocytes. Luminex results demonstrated that DWP secreted a variety of growth factors that are highly implicated in tissue repair and wound healing. Fibroblast and microvascular endothelial cells (HMVECs) migration assays demonstrated that DWP secreted growth factors that stimulated fibroblast and endothelial cell migration as compared to acellular control constructs (147% of fibroblast and 135% of HMVECs at day 3, n=3). Cell proliferation assay demonstrated that DWP CM facilitated fibroblast and endothelial cells proliferation by 158% (fibroblast) and 174% (HUVEC) at day 3 compare to ADM CM. The matrigel angiogenesis assay showed HUVECs formed more complex and intact network with longer branches/tubes in DWP CM.

Conclusions: Our results demonstrate that freshly isolated human SVF cells can be reproducibly formulated into combinatorial constructs in real time at the point-of care; and, that these constructs possess enhanced biological activity as a result of these cells.

12P

GLUTEAL SCULPTING AUGMENTATION: A SYNERGY EFFECT OF THE COMBINATION BETWEEN LIPOSUCTION AND LIPOFILLING

Presenter: Marwan Abboud, MD
Authors: Abboud M, Dibo S
MA Clinic

Introduction: The purpose is to share the authors' five years experience with gluteal augmentation by simultaneous liposuction and lipofilling.

Material and Methods: The Power Assisted Liposuction machine (Lipomatic) is used for liposuction of the zones surrounding the buttock, harvesting and injection of fat as well as preparation of the recipient site. Fat is harvested using a 3 mm 9 holes cannula by liposuction along a triangle joining the mid axilla superiorly down to the superior intergluteal fold posteriorly and the inguinal crease anteriorly enabling sculpting of these zones at the same time. The lipoaspirate is strained and is then filled into 60 ml syringes. Following that, preparation of the recipient site is achieved through multidirectional and multilayered tunneling through multiple access points, in a way to fashion a matrix for fat grafting. Lipofilling is carried out in multiple planes with a custom-made V-shaped 3 mm multi-hole-cannula, enabling simultaneous vibration and tunnelization of the buttock during fat injection.

Results: The power assisted buttock augmentation technique was applied for 110 bilateral buttock augmentations. Liposuction volumes ranged between 1400 and 5000 ml and the injected volumes per side and per session ranged from 300 to 900 ml. The operative time ranged between 60 to 120 min. An average of 1.2 sessions were required to achieve the final desired outcome. The follow up period ranged between 12 and 48 months.

Conclusion: Ideal gluteal sculpting should combine large volume fat grafting to the buttock with liposuction of the surrounding zones to achieve a cosmetically pleasing outcome. Combining tunnelization and vibration with fat grafting improves the dispersion of fat in the recipient site and increases the recipient site grafting capacity while maintaining a reduced operative time.

13P

THE EFFECTS OF THE CENTRIFUGATION SPEED ON THE SURVIVAL OF AUTOGENOUS FAT GRAFTS IN A RAT MODEL

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Guvercin E, Kapi E, Sirinoglu H, Taylan Filinte G, Filinte D
Dr. Lutfi Kirdar Kartal Training and Research Hospital

Introduction: Transplantation of fatty tissues is one of the most commonly performed methods in plastic surgery used both for reconstructive and cosmetic purposes. The most important problem is the durability rates of the transplanted graft in late term and this rate is closely associated with the applied technique and centrifugation is an essential part of the preparation process. This study includes the comparison of different centrifugation speeds on the survival of autogenous fat grafts in rats.

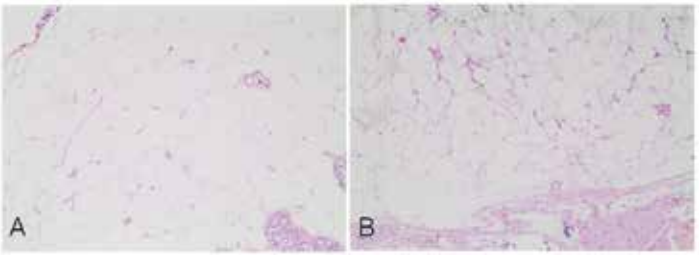
Method: 49 Sprague-Dawley rats between 250 to 500 gr were divided in to 7 groups. All rats were operated under general anesthesia and left inguinal fat pad was extracted. In all groups, the extracted fatty tissues were reimplanted to the subcutaneous area of the rats scalp after performing appropriate preparation process. In the first group the fatty tissue was reimplanted in en-bloc type and in the second group after trimming. 1000 rpm of centrifugation speed was performed to the fat grafts in the third group, 2000 rpm in the fourth group, 3000 rpm in the fifth group, 4000 rpm in the sixth group and 5000 rpm in the seventh group for 4 minutes. In these groups, the solid composite part at the top of the tube was reimplanted to the scalp area. The fat grafts were taken after three months under general anesthesia and histopathological and statistical evaluations were performed.

Results: The histopathological evaluation revealed that the rate of viable fat grafts was found to be significantly increased in the 4th and the 5th groups comparing to the other groups. The evaluation of fat graft volumes and weights revealed that total weight and volume amounts of the 4th, 5th and 6th groups were significantly high in comparison with the other groups.

Conclusion: Maximal long-term durability and fat cell viability results were obtained in the groups with 2000 rpm/4 minutes and 3000 rpm/4 minutes centrifugation speed indicating that 4 minutes centrifugation with an average speed of 2500 rpm provides the best results for the survival of autogenous fat autografts in rats.

13P

THE EFFECTS OF THE CENTRIFUGATION SPEED ON THE SURVIVAL OF AUTOGENOUS FAT GRAFTS IN A RAT MODEL



14P

THE APPLICATION OF AUTOLOGOUS FAT GRAFTING IN ACHIEVING SYMMETRY IN IMPLANT-BASED BREAST RECONSTRUCTION

Presenter: Biao Wang, MD
Authors: Wang B, Shan X, Chen R
The First Affiliated Hospital of Fujian Medical University

Objective: To discuss the clinical application of autologous fat grafting in achieving symmetry in implant-based breast reconstruction.

Methods: To acquire symmetry of the breasts, in terms of volume and shape, we performed fat grafting on the reconstructed and/or the contralateral breast in 15 cases of implant-based breast reconstruction. 12 cases underwent simultaneous fat grafting with the post-expansion prosthesis implantation while 3 cases had the fat transplanted in a separate 2nd stage of operation. In simultaneous fat transplantation, fat granule is injected to the subcutaneous layer as well as the post- pectoralis major space, before the tissue expander is removed. By comparison, postponed autologous fat grafting usually involves mammoplasty on the contralateral breast and the fat granule is injected in the subcutaneous layer only. The therapeutic effects are evaluated through the comparison of pre- and post-operative photography, self-evaluation of the patients, and the post-operative adverse reaction of fat granule absorb, cystic fibrosis.

Results: Between 2012 and 2014, we have performed simultaneous fat grafting on 12 cases with implant-based breast reconstruction, while on 3 cases fat grafting is postponed to a 2nd stage of surgery. After a follow-up ranging from 1 to 12 months, all the 15 reconstructed breasts have achieved satisfactory appearances as well as symmetry concerning volume and location.

Conclusion: We recommend autologous fat grafting as an ideal solution to adjusting the appearance of the reconstructed breast, so that symmetry of the breasts, concerning shape, volume, location as well as flexibility, can be acquired. The breast reconstruction procedure involving simultaneous fat grafting is particularly appealing to the patients. Moreover, the surgical technique is simple and there is hardly any scar formed in the donor site.

15P

THE ROLE OF NOTCH IN REGULATING STEM CELL STATE

Presenter: Jamie J. Newman, PhD
Authors: Sandel D, Bunnell BA, Miele L, Newman JJ
Louisiana Tech University

Introduction: Notch is a contact-dependent, developmental signaling pathway responsible for many cell fate decisions including maintenance of stemness in neural stem cells and differentiation of muscle stem cells. Recent studies have shown that Notch signaling is involved in the osteogenic and immunomodulatory characteristics of umbilical cord-derived MSCs. Notch pathway dysregulation has been implicated in a number of human diseases where altered cell fate decisions result in pathological phenotypes. Modulating Notch signaling may be a useful strategy to prevent or induce differentiation in adult stem cells for regenerative medicine applications. Ex-vivo Notch1 activation with immobilized Notch ligand Delta-like 1, for example, improves the engraftment of hematopoietic progenitors in patients. It is therefore necessary to characterize the stem cell niche in relation to how the Notch signaling pathway contributes to stem cell survival and differentiation and to better understand how signaling pathways can be used to optimize the therapeutic potentials of adult stem cells.

Method: We will explore Notch signaling in adult stem cells by first determining the levels of expression for each of the four Notch receptors. We will then use a gamma secretase inhibitor to determine the effects of blocking all Notch signaling on self-renewal and differentiation and follow up these experiments with lentiviral shRNA-mediated knockdown of each of the individual receptors to determine which receptors are most involved in each of the cellular properties. Finally, RNA-Seq will be performed for each knockdown to identify a likely set of Notch target genes responsible for the observed phenotypes.

Results: Preliminary results indicate that all four Notch receptors are expressed in human bone marrow derived mesenchymal stem cells. Additional studies are underway to validate expression in adipose stem cells and induce knockdowns for each of the receptors.

Conclusion: Signaling pathways modulate cell behavior in vivo and can be used to modulate phenotype in culture. A better understanding of a critical developmental pathway, its role in adult stem cell biology, and how it can be used to influence cell fate in the lab can greatly improve the success of regenerative medicine.

16P

FACTORS THAT MAY INFLUENCE YIELD AND VIABILITY OF EXTRACTED ADRCS PER GRAM OF TISSUE - FOLLOW UP STUDY

Presenter: Marcos Sforza, MD
Authors: Sforza M, Andjelkov K, Zaccheddu R
BelPrime Clinic

Introduction: This study aims at providing some more contribution in patient's depending factors that may influence ADRCs cell yield and their viability (age, sex, Body Mass index (BMI), smoking and physical activity) within the same amount of processed fat. Furthermore, we observed how cell yield and their viability changes in time (after 1, 12 and 24 hours after processing). After the preliminary results presented on IFATS in 2014, the authors intend to present the expanded follow up results after 18 months.

Method: We analyzed 80 patients in total, both male and female. Age, BMI, sport activity and smoking habits data were collected. They were divided in two groups depending on BMI values (normal or overweight). There were no underweight or obese patients. All 80 patients had the ADRCS extracted for therapeutically use, but with different indications. All of them had liposuction done by the author and in the same manner (tumescent technique, same cannula) to obtain 150ml of fat. The cells were digested to obtain ADRCS using (Cellution 800, Cytori Therapeutics). The cell counting was performed using a Nucleocounter (NC100, Chemotec). The cells were maintained for 24h on a Rocker at 250C with 25 rocking cycles per minute.

Results: Cell yield, cells' viability, ADRCS for active dose and total volume for cell therapy were statistically analyzed in correlation with patients' BMI, sport activity, sex, age and smoking habits and results are presented. Cell yield and viability changes in time were recorded (1, 12 and 24h after processing).

Conclusion: There doesn't seem to be a final conclusion of what is the minimal dosage of ADSCs necessary to achieve good therapeutically results. Moreover, it is expected that different pathologies would require different doses, what is yet to be established. This study is a good starting point when analyzing the exact amount of fat need to be harvested in order to obtain the certain number of ADRCS depending the patients' biotype and their habits.

17P

EVALUATION OF TWO PURIFICATION METHODS FOR ISOLATION OF HUMAN ADIPOSE-DERIVED STEM CELLS BASED ON RED BLOOD CELL LYSIS WITH AMMONIUM CHLORIDE AND HYPOTONIC SODIUM CHLORIDE SOLUTION

Presenter: Hongwei Liu, MD,PhD
Authors: Liu H, Li S, Liao X
The First Affiliated Hospital of Jinan University

NOT PRESENTED

18P

CLINICAL ANALYSIS OF USING WET AUTOLOGOUS FAT PARTICLES TRANSPLANTATION FOR TEMPORAL FILLING

Presenter: Xunlei Huang, MD
Authors: Huang X, Wang B, Zheng H
The First Affiliated Hospital of Fujian Medical University

NOT PRESENTED

19P

LIPOINJECTION VIA V SHAPED 3MM TULIP CANNULA FOR THE TREATMENT OF BURN SCARS

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Ceran F, Filinte GT, Basat SO, Guvercin E
Dr. Lutfi Kirdar Kartal Training and Research Hospital

Introduction: Burn is one of the few most excruciating traumatic life events that a person can go through regardless of their demographic differences such as age, economic status, social status, job. And apart from other traumas such an injury has major chronic sequeale socially and pschologically due to loss of aesthetics and function of the skin and underlying tissues. Today burn scar and contracture are still a major obstacle. This problem grows when the affected organ is one that is seen by our social counterparts everday such as face or a functional unit such as hands. This study was planned to investigate a recent therapy option for burn patient, subcutaneous tunnel formation and lipofilling, to verify previous results of lipofilling and to investigate if tunnel formation augments treatments benefit.

Methods: 9 adult patients with varying areas of burn were selected randomly. Tunnelization and lipofilling was performed on the facial and hand burns to see the aesthetic and functional outcomes. The target tissue was tunnelized at the dermal-hypodermal scarred layer with 3mm V shaped Tulip® dissector liposuction cannula from multiple entry points towards diffusely divided angles destructing some of the subdermal fibrotic tissue and creating subdermal pocket for the adipose tissue to be injected. Harvested and processed adipose tissue was injected diffusely to these tunnels.

Results: The subjective aesthetic feeling and clinical appearence were improved in all patients. Patient observer scar assesment scores were higher correlated with the literature. The skin thickness and rough texture was thinner and softer, the skin was more elastic in terms of patients feeling, eritematous color was decreased to natural color.

Conclusions: The subdermal tunnelization with 3mm canula destroys the subdermal fibrotic bands and inorganized collagen nodules and creates a pocket for the harvested fat graft. It is easy to inject adipose tissue diffusely in these pockets.

20P
FAT TRANSFER. GET RID OF YOUR WRINKLES
Presenter: Aristides Arellano-Huacuja Sr., MD
Author: Arellano-Huacuja A
Clinica Dermatologica y Cirugia Estetica de Puebla

NOT PRESENTED

21P
INTERFERENCE OF THE FAT TISSUE IN CUTANEOUS REGENERATION AND ITS USE IN THE TREATMENT OF WOUNDS, BURNS AND SCARS
Presenter: Marco A. Pellon, MD
Author: Pellon MA
Clinica Sao Vicente

Introduction: The author discusses the physical and metabolic properties of adipose tissue, its interference in skin regeneration and shows how to take advantage of these properties in order to accelerate and improve the quality of healing of deep wounds, burns and scars.

Ischemia, acidosis and changes in cell membrane produced by the trauma of suction and manipulation of the fat tissue alter the basal physiological behavior of adipocytes, adipose-derived stem cells and other cells present in the liposuctioned adipose tissue, resulting in activation of these cells, increasing the expression of angiogenic, trophic and immunological factors and triggers the tissue regeneration process in the recipient area.

Method: The author conducted a clinical trial in patients with deep wounds, burns and scars who were subjected to application of fat grafts sessions above and below their injuries, chronologically registering the vascular and morphological changes which resulted in a faster and good quality healing as well as improving the appearance of hypertrophic scars. It is also described a simple and very efficient technique of applying the fat graft upon the wound.

Results: The author observed a significant improvement in the speed and quality of the healing of varicose ulcers, acute wounds, burns, skin grafts and hypertrophic scars with the application of these fat grafts series.

Conclusion: When a damage of the skin's structures occurs, that information gets to the dermal and subcutaneous adipocytes via paracrine signaling, extracellular components, and cell-cell interactions. The presence of these factors on and below the injury will attract the factors released by the cells of the grafted fat tissue, resulting in a powerful stimulus to regeneration of these tissues. The immunomodulation managed by the grafted fat cells provide healing with less inflammatory reaction which will result in better scar quality.

22P

ISOLATION OF HUMAN ADIPOSE DERIVED STROMAL VASCULAR FRACTION: A CLINICAL APLLICAION

Presenter: Kwang Sik Kook
Authors: Jang S, Kook KS
Idea Plastic Surgery

Introduction: Adipose derived stromal vascular fractions are widely used for cell therapy. So we found optimal method of isolation and compared cell number from different donor site and repeat aspiration number using 3 years clinical data.

Method: In this study, we using collagenase (liberase™), isolated SVF from Human Adipsoe Tissue, by changing different concentration, centrifuging time and speed washing versus non washing adipose tissue. And we compared with patients' age, donor site. We can also observed that SVF cell number by increasing repeated number of aspiration from same donor site.

Results: This data indicated that increase of collagenase (liberase™) concentration, cell number was increased until 0.2%. Over 0.2% concentration, SVF cell number was not increased. And there was almost no difference cell number until 40s. But after 50s, cell cumber was decreased. We can also observed that SVF cell number was donor site depedent. Thigh and side site's average SVF cell number was more than 2.0*10^6 and abdomen's average SVF cell number was 0.6*10^6. Interestingly, we can also investigated that there was a little reduced SVF cell number by increasing repeated number of aspiration.

Conclusion: Based on this data, SVF cell number was affected donor site and little affect of age. This was indicated that SVF cell number was nearly affected an old age for cell therapy treatment. And indicated that little decresed SVF cell number by increasing repeated number of aspiration, we can use SVF without regared to number of aspiration.

23P

DERMAL WHITE ADIPOSE TISSUE THE BEST CELLULAR SOURCE FOR SKIN REGENERATION PURPOSE

Presenter: Angelo Trivisonno, MD
Author: Trivisonno A
Sapienza University

The superficial layer of adipose tissue in contact with deep dermis is more vascularized, and with more important staminality than deeper fat (1). Driskell et al. consider the layer of adipose tissue underlying the reticular dermis and that encases mature hair follicles, as a Dermal White Adipose Tissue, independent from deeper subcutaneous white adipose tissue. Called intradermal adipocytes that have a common precursor with dermal fibroblasts, and that coordinate keratinocyte and fibroblast proliferation and migration (2). These superficial adipose cells have distinct morphologies and physiological characteristics, than deeper ones; and have role in homeostasis and wound healing of the skin, and hair follicle growth (3). In the rodents are separated from underlying subcutaneous by a muscular layer, the panniculus carnosus. Correspondent to human superficial lamellar layer of the adipose tissue. Genetic and pharmacological inhibition of mature adipocytes formation abrogates fibroblast presence and ECM deposition in the regenerating dermis.

To for the availability of this superficial layer we used a microcannula with few and small holes arranged in a only single raw, to harvest in contact with deep dermisin 102 patients (4). And we have used this specific tissue in smaller fragments both volume replacement in difficult areas such as tear trough, lid/cheek junction, temple, etc. but also for lip contour or wrinkles as a filler, with needles from 21 to 27 gauge. But also for skin regeneration purpose, by intradermal injection. We report our results, evaluated by profilometry with follow up to 8 months.

We observed good results in volume augmentation in difficult areas as tear trough, and in improvement of skin quality.

The use of microcannula compared traditional cannula destroys more the adipocytes, but not influence the viability of SVF cells (1).

24P

HUMAN ADIPOSE-DERIVED MATRIX PROMOTES ANGIOGENESIS AND ADIPOGENESIS IN VIVO: A MULTI-WEEK ASSESSMENT OF VOLUME RETENTION AND HISTOPATHOLOGY

Presenter: Lauren E. Kokai, PhD
Authors: Kokai LE, Chnari E, Schilling B, Minteer D, Mahoney C, Kelmendi-Doko A, Jacobs M, Marra KG, Rubin JP
University of Pittsburgh

Introduction: Autologous fat has been used for aesthetic and reconstructive procedures with success for many years. To avoid a secondary procedure, risks of donor-site morbidity, and provide an off-the-shelf alternative, a method has been developed to produce a human adipose-derived matrix which maintains structural components and an endogenous milieu important for cellular ingrowth and adipogenesis. The goal of this in vivo study was to evaluate the biocompatibility and soft tissue-volumizing performance of multiple formulations of human adipose-derived matrix (Allograft Adipose Matrix - AAM).

Methods: AAM was reconstituted in sterile saline at variable protein concentrations and implanted subcutaneously bilaterally on the dorsal flank of 5-7 week female Nu/Nu or Balb/C mice. Volume retention was measured at 3, 6 and 12 weeks with gas pycnometry. A histological review with H&E, Masson's trichrome and perilipin immunohistochemistry was performed at each study time point.

Results: No adverse events in mice were observed over the course of this study and inflammation and histiocytes typical of a normal wound healing response were observed at all time points. Macroscopic observation during material extraction showed a distinct boundary for most implants, however instances of complete integration of AAM with native fat pads were also observed. Perilipin-positive adipocytes within AAM were observed at 3 weeks and increased in number at each consecutive time point. All formulations of AAM remained present at 12 weeks, with the experimental group containing the highest percentage of reconstituted protein maintaining 70% of the original injection volume.

Conclusions: We have shown that AAM can successfully generate adipocyte-laden tissue in situ in a small animal model at 12 weeks. Additional experimental time points of 18 and 24 weeks are underway.

25P

THE IMPACT OF HUMAN ADIPOSE TISSUE-DERIVED STEM CELLS ON MALIGNANT MELANOMA CELLS - IMPLICATIONS FOR CELL-BASED SKIN THERAPY

Presenter: Eva Koellensperger, MD
Authors: Koellensperger E, Preisner F, Sandmann S, Zoernig I, Germann G, Leimer U
Heidelberg University Hospital

Introduction: This study evaluates the interactions of human adipose tissue derived stem cells (ADSCs) and human malignant melanoma cells (MMCs), with regard to a prospective cell-based skin regenerative therapy and a thereby unintended co-localization of ADSCs and MMCs.

Methods: ADSCs were co-cultured with multiple MMC lines and primary MMCs (pMMCs) in a transwell system, and cell-cell-interactions were analyzed by assessing proliferation, migration and invasion, angiogenesis, quantitative real time PCR of > 230 tumor associated genes, and multiplex protein assays of 20 chemokines and growth factors and eight MMPS. Results of co-culture were compared to those of the respective mono-culture.

Results: Co-culture significantly increased pMMCs proliferation (p=0.015 / p=0.003) about 23-30% and significantly reduced the proliferation of ADSCs about 19-29% (p=0.005-0.02), as well as that of G-361-MMCs about 30% (p=0.002). In co-culture the migration of all MMC-lines (32-68%, p<0.001-0.005), and pMMCs (23-68%, p=0,031-0.008) was significantly increased. The migration of ADSCs was significantly increased in co-cultures with three MMC-lines [11-61%, p<0.001 - p=0.021)] as well as with the two pMMCs (23-33%, p=0.003-0.013), too. The invasive behavior of three MMC-lines was significantly increased in co-culture (117-23%, p=0.002-0.019). ADSCs invasive features were significantly increased by co-culture with three MMC-lines (45-12%, p=0.001-0.013), and the two pMMCs (41-23%, p<0.001-0.002). Furthermore, an in vitro angiogenesis assay revealed an increased tube formation by conditioned media from co-culture. Quantitative real time-PCR showed remarkable changes in the expression of multiple tumor-associated genes in co-culture compared to mono-cultures of both, ADSCs and MMCs. The concentration of several tumor-associated proteins, such as cytokines and MMPs, were strongly increased in co-culture. Furthermore, exclusively in co-culture the MMCs were exposed to several important tumor-modulating proteins.

Conclusion: This study evaluates the possible interactions of primary human ADSCs with human MMCs, pointing towards an increased oncological risk, which should not be neglected considering a clinical use of isolated human ADSCs in skin regenerative therapies.

26P

THE THROMBIN EFFECT ON FAT GRAFT VIABILITY; IS THERE A CHANCE TO PROLONG FATS LIFE LENGTH?

Presenter: Mehmet Bozkurt, MD
Authors: Bozkurt M, Kacmaz C, Gideroglu K, Guvercin E, Taylan Filinte G, Filinte D
Dr. Lutfi Kirdar Kartal Training and Research Hospital

Introduction: Autologous fat tissue transfer has been used frequently both in reconstructive and aesthetic surgical procedures. There are many advantages of autologous fat tissue transfer; a wide donor area, practical extraction, and the availability from the person's own tissue. The most important disadvantage of fat graft, which is also a late term complication, is graft resorption. The purpose of this study is to evaluate the effect of thrombin, which is reported to increase the tissue regeneration and angiogenesis in many areas, to viability of fat graft.

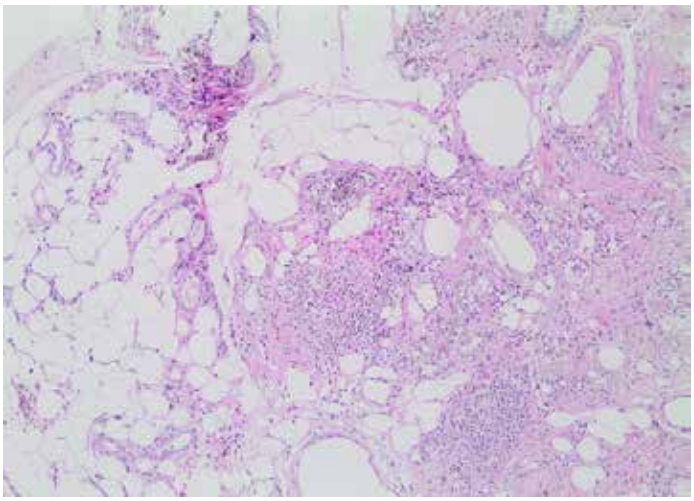
Methods: Twenty Wistar-Albino type adult male rats were used in the study. They were divided into 4 groups, one control and 3 experimental groups. Inguinal fat pads were excised from all animals. Fat pads were reduced to 500 milligrams (+/- 1 milligrams). In order to obtain thrombin, 3 animals were sacrificed to use their whole body blood. %1 amount of 0.25 mg lidocaine hydrochloride was injected tothe scapular regions of the animals. Afterwards subcutaneous cavities were formed there to place the fat tissue inside. After placing the fat graft, varying proportions of thrombin were injected to the animals in the experimental groups. No thrombin was not injected to the control group. No complications were observed postoperatively. After 90 days, the experimental animals were sacrificed and the fat grafts were removed. They were washed with %0.9 saline, then they were dried and weighed, the values found were recorded separately for each experimental animal. Then, all fat grafts in each groups were fixed with %10 formalin solution for histological evaluation.

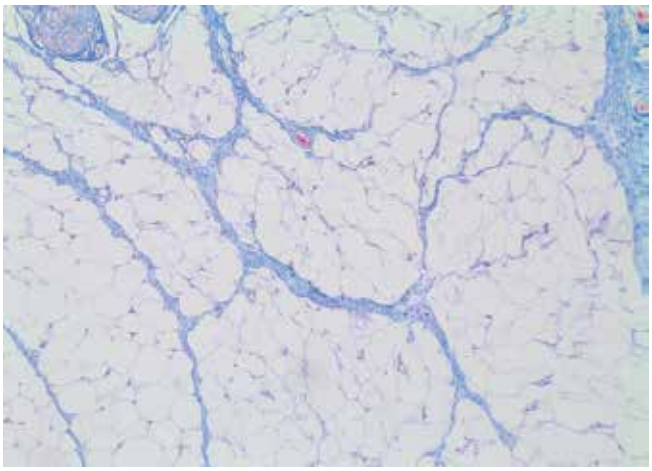
Results: Macroscopic, histological and statistical evaluations showed that thrombin has reduced the the weight and volume loss on fat graft, increased viable fat cell amount and reduced inflammation on receptive area.

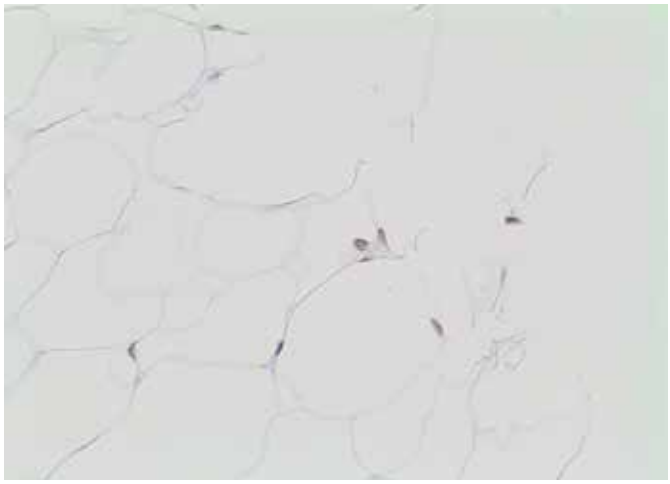
Discussion: One of the most important problems encountered in fat graft procedures is graft loss. It is known that the thrombin is already reported to have positive effects of on angiogenesis and tissue regeneration. However; there wasn't any work studying the effect of thrombin on fat graft viability. The positive effects of thrombin on viability of fat graft has given us courage to use it in further studies. Longer follow-ups are necessary.

26P

THE THROMBIN EFFECT ON FAT GRAFT VIABILITY; IS THERE A CHANCE TO PROLONG FATS LIFE LENGTH?







27P

EFFECT OF 650 NM LOW-LEVEL LIGHT IRRADIATION ON THE THERAPEUTIC POTENTIAL OF ADIPOSE-DERIVED STEM CELLS IN REGENERATIVE MEDICINE

Presenter: Hongwei Liu, MD,PhD
Authors: Li S, Liu H
The First Affiliated Hospital of Jinan University

NOT PRESENTED

28P

ALLOTRANSPLANTATION FOLLOWING RECELLULARIZATION OF DECELLULARIZED VESSEL USING ADSCS

Presenter: Daehee Kim, MD
Authors: Pak CS, Kim BK
Seoul National University Bundang Hospital

NOT PRESENTED

29P

BMP-2 INDUCED OSTEOGENIC AND ADIPOGENIC DIFFERENTIATION IN HUMAN ADIPOSE STEM CELLS

Presenter: Sari Vanhatupa, PhD
Authors: Vanhatupa S, Ojansivu M, Autio R, Juntunen M, Miettinen S
BioMediTech

Introduction: Bone morphogenetic protein (BMP)-2 is a growth factor used to stimulate bone regeneration in clinical applications. However, there are contradicting reports on the functionality of BMP-2 in human adipose stem cells (hASCs).

Methods: In this study we analyzed the effects of BMP-2 on Smad1/5 signaling, proliferation and differentiation in hASCs, in vitro. BMP-2 induced Smad1/5 activation was analyzed in different serum conditions (fetal bovine serum; FBS and human serum; HS) with hASC lines derived from several donors by Western Blotting of phosphorylated Smad1/5 protein (p-Smad1/5) and by immunofluorescence microscopy of the p-Smad1/5 nuclear translocation. The BMP-2 induced differentiation of hASCs was analyzed in basal growth medium (BM) and osteogenic medium (OM) by quantitative alkaline phosphatase assay (qALP), Alizarin Red mineralization assays, Oil Red O lipid formation assays and expression of osteogenic and adipogenic marker genes.

Results: Our results indicate that the functionality of BMP-2 is strongly dependent on serum and culture conditions. BMP-2 induced Smad1/5 activation and nuclear translocation in all cell lines studied, but Smad activation was prominent only in HS conditions compared to FBS conditions. Interestingly, BMP-2 exhibits a dual role in differentiation process of hASCs, and the effect on differentiation fate is donor cell line dependent. BMP-2 stimulated osteogenic effect in some cell lines by inducing ALP activity and mineralization, whereas in other donor lines, BMP-2 induction clearly promoted also adipogenic differentiation. Our results clearly indicate that hASCs responds to mammalian produced BMP-2 more efficiently when compared to growth factor produced in bacterial system.

Conclusions: Based on our results BMP-2 is performing a dual action inducing osteogenic and adipogenic differentiation of hASCs. The molecular functionality of the BMP-2 in our experiments indicated tight dependency on culture conditions and production origin of the growth factor. These results partially explain the existing contradictory in results of the previous BMP-2 studies and indicate variability in functional mechanisms of BMP-2 signaling in hASCs.

30P

LARGE VOLUME LIPOFILLING IN AESTHETIC PLASTIC SURGERY

Presenter: Aristides Arellano-Huacuja Sr., MD
Author: Arellano-Huacuja A
Clinica Dermatologica y Cirugia Estetica de Puebla

NOT PRESENTED

31P

SYNTHETIC MRNA TO DEDIFFERENTIATE EQUINE ADULT MULTIPOTENT STROMAL CELLS

Presenter: Mandi J. Lopez, DVM, MS, PhD
Authors: Jarazo J, Fargason C, Bondioli K, Lopez MJ
Louisiana State University

Introduction and Hypothesis/Objectives: Equine adult multipotent stromal cell (MSC) therapies are limited by cell yield and loss of cell potency with culture expansion. Dedifferentiation of adult MSCs may improve retention of cell potency during in vitro expansion. This study was designed to test the expansion capacity and potency of equine adult MSCs from adipose tissue (ASCs), bone marrow (BMSCs) and skin (FSCs) following chemical transfection with synthetic mRNA of human sequences for embryonic genes, OCT 4, KLF4 and SOX2.

Materials and Methods: Target gene mRNA and cell protein expression was compared among passage (P) 3 cells from the different tissue sources before and after 7 and 14 days of transfection. Additionally, multilineage capacity and cell proliferation were compared in P10 cells.

Results: Target protein expression localized to the nucleus was higher in transfected cells after 7 and 14 days of transfection compared to untreated cells based on immunocytochemistry. Based on qRT-PCR, OCT4 and SOX expression was significantly higher in transfected cells after 14 days of treatment compared to untreated cells. Multilineage differentiation was detected in transfected but not untreated cells at P10. Expansion rates of P10 transfected and untreated cells were not significantly different with the exception of higher proliferation of treated BMSCs after 21 days of culture.

Discussion/Conclusions: Based on these results, dedifferentiation of equine MSCs with synthetic mRNA increases in vitro potency and expansion capacity.

32P

TREATMENT OF BRA STRAP SHOULDER GROOVE DEFORMITY BY AUTOLOGOUS FAT INJECTION SIMULTANEOUSLY WITH BREAST REDUCTION

Presenter: Yigit Ozer Tiftikcioglu, MD
Authors: Tiftikcioglu YO, Erenoglu CM, Ozek C
Ege University Medical School

NOT PRESENTED

NOTES

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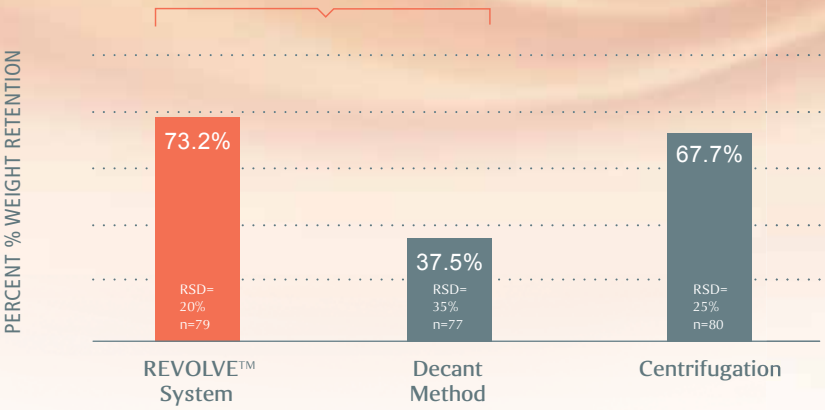

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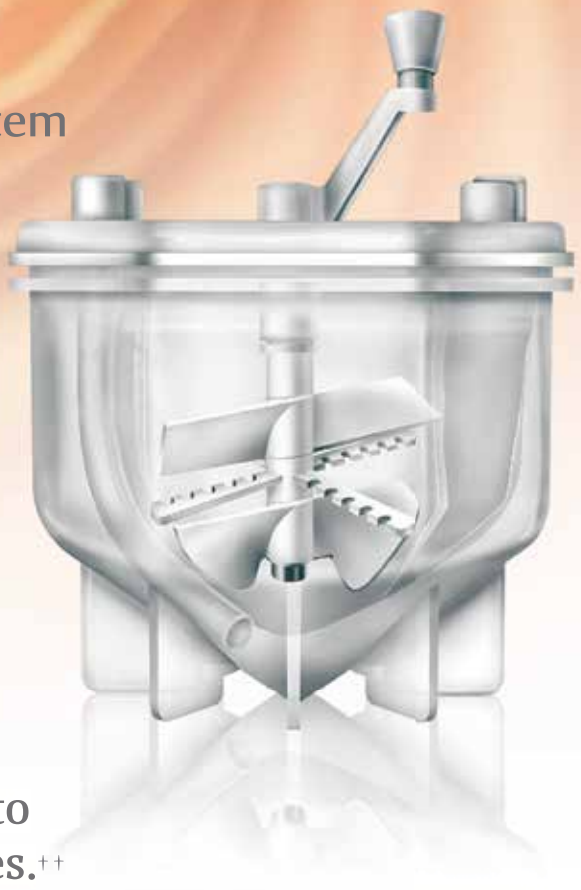

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†Ansorge H, Garza JR, McMormack MC, et al. Autologous fat processing via the revolve system: quality and quantity of fat retention evaluated in an animal model. *Aesthet Surg J.* 2014 Mar 1;34(3):438-47.

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