IFATS NEW YORK 2013 CONFERENCE

11TH ANNUAL MEETING

November 21-24, 2013

Conrad Hotel New York • New York City, New York
MARK YOUR CALENDAR

International Federation for Adipose Therapeutics and Science

12th Annual Meeting
IFATS AMSTERDAM 2014

November 13-16, 2014
NH Grand Hotel Krasnapolsky
Amsterdam, The Netherlands

ABSTRACT DEADLINE:
Midnight EST, Wednesday, June 4, 2014

The Call for Abstracts will be sent this winter. All members of IFATS and all registered attendees of the 2013 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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Founders Board

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New Orleans, Louisiana, USA

Catherine Foss
Executive Director
Hanover, New Hampshire, USA
Welcome from the 2013 IFATS Co-Presidents

We have the honor of Co-Chairing the 11th Annual Meeting of the International Federation for Adipose Therapeutics and Science (IFATS) to be held in New York City.

Highlights of this year’s conference include a strong focus on both clinical translation and basic science, as evidenced by our Invited Speaker, Damian Garcia-Olmo, MD, PhD, and our Keynote Speaker, David Kaplan, PhD. The program includes a special short course on the fundamentals of adipose-derived stem cells, offered as a focused tutorial for clinicians not versed in stem cell terminology. Additionally, we have scheduled over a dozen sessions on hot topics such as stem cell enriched fat grafting, aging, bone regeneration and wound healing.

We are also providing a pre-conference symposium entitled The Business of Adipose-derived Stem Cells: Perspectives from Industry and Clinicians. The symposium will cover several key, timely topics, including an introduction to companies in the adipose stem cell field, perspectives from both established and new entry companies, and what changes in cell therapies are needed for treating the wounded warrior.

The meeting will take place the weekend prior to Thanksgiving. Participants will have the opportunity to remain in New York City to attend the famous and highly entertaining Macy’s Thanksgiving Day Parade – one of the most exciting events on this great city’s annual calendar.

We look forward to seeing you in New York,

Sydney Coleman, MD
Assistant Professor
New York University Medical Center
University of Pittsburgh Medical Center
TriBeCa Plastic Surgery

Kacey G. Marra, PhD
Associate Professor
Department of Plastic Surgery
Adipose Stem Cell Center
University of Pittsburgh
SCIENTIFIC PROGRAM COMMITTEE

Spencer Brown, PhD
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Sydney Coleman, MD
Paul J. DiMuzio, MD, FACS
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Kacey G. Marra, PhD

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J. Peter Rubin, MD, FACS
Andre Tchernof, PhD
Dmitry O. Traktuev, PhD
Christopher West, MBChB, MRCS
Stuart K. Williams, PhD

INVITED SPEAKERS AND SESSION MODERATORS

Jacqueline Bliley, MS
Anne Bouloumie, PhD
Spencer Brown, PhD
Lee Buckler, LLB
Bruce Bunnell, PhD
Louis Casteilla, PhD
Mary Ann Chirba, JD, DSc, MPH
Sydney Coleman, MD
Daniel Del Vecchio, MD
Kentaro Doi, MD
Julie Fradette, PhD
Trivia Frazier-Wiltz, PhD
Damian Garcia-Olmo, MD, PhD
David Genecov, MD

Marco Helder, PhD
Jae-Ho Jeong, MD, PhD
David Kaplan, PhD
Adam J. Katz, MD, FACS
Lauren Kokai, PhD
Pamela Layton
Han Tsung Liao, MD, PhD
Ramon Llull, MD, PhD
Guy Magalon, MD
Keith March, MD, PhD
Kacey Marra, PhD
Stephen Minger, PhD
Mary Pat Moyer, PhD
Benno Naaijkens, MSc

Marie-Eve Ouellette, MSc
Ivona Percec, MD, PhD
Sudheer Ravuri, PhD
Jonathan Schwartz
Mark Spilker, PhD
Joseph M. Rosen, MD
J. Peter Rubin, MD, FACS
Dmitry Traktuev, PhD
Stig-Frederik Trojahn Kolle, MD
Wakako Tsuji, MD, PhD
Jolene Valentin, PhD
Christopher West, MBChB, MRCS
Kotaro Yoshimura, MD

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.

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.
KEYNOTE SPEAKER

David Kaplan, PhD holds an Endowed Chair, the Stern Family Professor of Engineering, at Tufts University. He is Professor and Chair of the Department of Biomedical Engineering and also holds faculty appointments in the Department of Chemical and Biological Engineering, Department of Chemistry, the Tufts University School of Medicine and the Tufts University School of Dental Medicine. His research focus is on biopolymer engineering to understand structure-function relationships, with emphasis on studies related to self-assembly, biomaterials engineering and functional tissue engineering. He has published more than 400 papers and edited eight books. He directs the NIH P41 Tissue Engineering Resource Center (TERC) that involves Tufts University and Columbia University, and the Bioengineering and Biotechnology Program at Tufts University. He serves on the editorial boards of numerous journals and is Associate Editor for the journal Biomacromolecules. He has received a number of awards for teaching, was Elected Fellow, American Institute of Medical and Biological Engineering (2003) and received the Society for Biomaterials Clemson Award for contributions to the literature in 2007.

Lecture Title: Adipose Derived Stem Cells and Regenerative Medicine
When: Friday, November 22nd - 8:30 to 9:30 am

INVITED SPEAKERS

Dr. Trojahn Kølle obtained his bachelor degree and MD from the University of Copenhagen and graduated in January 2006. He started his training in Plastic and Reconstructive surgery in 2008 at the Department of Plastic Surgery, Breast Surgery & Burns at Copenhagen University Hospital, Rigshospitalet and is currently working as a resident doctor. He is also employed at Aleris-Hamlet Private Hospital training in cosmetic plastic surgery. During his residency, he spent three years as a research fellow conducting the clinical trial “Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial”. During his fellowship he initiated the laboratory setup for producing adipose tissue-derived stem cells for clinical use in plastic surgery. His results were recently published in the The Lancet and in Cytotherapy. His PhD thesis was submitted at the University of Copenhagen in October 2013. Dr. Trojahn Kølle is currently supervisor of two initiated clinical trials concerning clinical application of adipose derived mesenchymal stem cells (ASCs) and is the primary investigator of a new trial applying ASC-enriched lipofilling for breast reconstruction. He is a member of the Danish Society of Plastic and Reconstructive Surgery (DSPR) and the European Society of Plastic, Reconstructive and Aesthetic Surgery (ESPRAS).

Lecture Title: Culture Expansion of Adipose Tissue-derived Stem Cells for Clinical Use and their Potential in Fat Grafting
When: Friday, November 22nd - 9:30 to 10:30 am

Since 1984, Professor Mary Ann Chirba has taught at Boston College Law School and in 1999 was appointed Assistant Professor of Legal Reasoning, Research & Writing and continues to teach a variety of health law courses. Professor Chirba currently lectures at Harvard Medical School, Children’s Hospital of Boston, and Tufts Medical School. A former litigator, she has also been certified by the American Health Law Association as a mediator and arbitrator. Her current research interests include legal protections for disabled children, the use of law and regulation to promote medical product safety, emerging federal and international guidelines for stem cell research, and federal and state health care reform. Professor Chirba holds a bachelor’s degree in biology from Colgate University and a J.D., magna cum laude, from Boston College Law School, a Doctorate of Science in Health Policy and Management and a Master’s in Public Health from the Harvard School of Public Health.

Lecture Title: Evolving Global Regulations for Stem Cells
When: Saturday, November 23rd - 4:00 to 5:00 pm

Professor Damian Garcia-Olmo, MD, PhD, is presently Chief of Colorectal Surgery and Director of the Cell Therapy Unit at La Paz University Hospital (Madrid). Dr. Garcia-Olmo obtained his PhD degree (1982) from the University of Murcia and has spent periods of complementary formation at the John Radcliffe Hospital in Oxford (UK) and Saint Marks (London-UK) as well as in the Massachusetts General Hospital in Boston (USA). In addition Dr. Garcia-Olmo, is a General Surgeon with more than 25 years of experience both in the clinical setting as well as in research. Currently he has performed more than 6,000 surgical interventions in general surgery. His main clinical and scientific interests rely in the development of advanced treatments for colorectal pathologies, including innovative surgical approaches and frontier developments in regenerative medicine using stem cells.

Lecture Title: Clinical Trials Using Adipose Derived Mesenchymal Stem Cells for Fistulae Treatment
When: Sunday, November 24th - 8:00 to 9:30 am
## Thursday, November 21, 2013

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<tr>
<td>10:00 am - 12:00 pm</td>
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**PRE-CONFERENCE SYMPOSIUM**

**The Business of Adipose-derived Stem Cells: Perspectives from Industry and Clinicians**  
Moderators: Sydney Coleman, MD, Kacey Marra, PhD & J. Peter Rubin, MD, FACS

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<tr>
<td>7:00 - 9:00 pm</td>
<td>Welcome Reception - Conrad Hotel</td>
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<td>6:30 am - 6:00 pm</td>
<td>Registration</td>
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<td>7:00 - 8:00 am</td>
<td>Continental Breakfast - Exhibit Hall</td>
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<td>8:15 - 8:30 am</td>
<td>Welcome and Introduction</td>
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<td><em>Sydney Coleman, MD &amp; Kacey Marra, PhD - Co-Presidents</em></td>
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<td>8:30 - 9:30 am</td>
<td><strong>KEYNOTE SPEAKER</strong></td>
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<td>Adipose Derived Stem Cells and Regenerative Medicine</td>
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<td><em>David Kaplan, PhD - Tufts University</em></td>
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<td><em>Stig-Frederik Trojahn Kølle, MD - Copenhagen University Hospital</em></td>
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<td>10:30 - 11:00 am</td>
<td>Coffee Break and Exhibits</td>
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<td>11:00 am - 12:30 pm</td>
<td><strong>CONCURRENT SESSION C1B</strong></td>
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<td>ASCs and Tissue Engineering</td>
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<td><em>Moderators: Keith March, MD, PhD &amp; Jolene Valentin, PhD</em></td>
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<td>11:00 am - 12:30 pm</td>
<td><strong>CONCURRENT SESSION C1C</strong></td>
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<td>ASC Molecular Biology, Cancer and Gene Therapy</td>
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<td><em>Moderators: Bruce Bunnell, PhD &amp; Trivia Frazier-Wiltz, PhD</em></td>
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<td>12:30 - 1:30 pm</td>
<td>Lunch and Exhibits</td>
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<td><strong>CONCURRENT SESSION C2A</strong></td>
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<td>Clinical Fat Grafting for Breast and Gluteal Reconstruction</td>
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<td><em>Moderators: Kotaro Yoshimura, MD &amp; Kentaro Doi, MD</em></td>
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<td>1:30 - 3:30 pm</td>
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<td>Adipose Tissue as a Scaffold</td>
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<td><em>Moderators: Jullie Fradette, Ph.D &amp; Marie-Eve Ouellette, MSc</em></td>
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<td>1:30 - 3:30 pm</td>
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<td>ASCs and Vascularization</td>
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<td><em>Moderators: Dimitry Traktuev, Ph.D &amp; Sudheer Ravuri, Ph.D</em></td>
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<td>3:30 - 3:45 pm</td>
<td>Coffee Break and Exhibits</td>
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<tr>
<td>3:45 - 5:15 pm</td>
<td><strong>Clinical Panel: Maximizing Fat Graft Survival</strong></td>
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<td><em>Moderators: Sydney Coleman, MD &amp; J. Peter Rubin, MD, FACS</em></td>
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<td>5:15 - 6:15 pm</td>
<td><strong>Panel: Fundamental Tools, Methodologies and Challenges in Adipose Stem Cell Research and Translation for the Clinician</strong></td>
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<td><em>Moderator: J. Peter Rubin, MD, FACS</em></td>
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<tr>
<td>7:00 - 10:00 pm</td>
<td><strong>Dinner - Conrad Hotel</strong></td>
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### Saturday, November 23, 2013

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<td>Continental Breakfast - Exhibit Hall</td>
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| 8:00 - 9:45 am  | **PLENARY SESSION 2 - Part I** Best Papers Submitted Focusing on Clinical Translation  
|                | Moderators: Sydney Coleman, MD & Adam J. Katz, MD, FACS              |
| 9:45 - 10:10 am | Coffee Break and Exhibits                                             |
| 10:10 am - 12:00 pm | **PLENARY SESSION 2 - Part II** Best Papers Submitted Focusing on Basic Science Translation  
|                | Moderators: Anne Bouloumie, PhD & Kacey Marra, PhD                    |
| 12:00 - 1:30 pm | Lunch and Exhibits                                                    |
| 1:30 - 3:30 pm  | **CONCURRENT SESSION C3A** Clinical Fat Grafting to the Face          
|                | Moderators: Ramon Llull, MD, PhD & J. Peter Rubin, MD, FACS           |
| 1:30 - 3:30 pm  | **CONCURRENT SESSION C3B** ASCs and Wound Healing: Skin and Other Tissues  
|                | Moderators: Jae Ho Jeong, MD, PhD & Wakako Tsuji, MD, PhD            |
| 1:30 - 3:30 pm  | **CONCURRENT SESSION C3C** Hot Topics: ASCs, PRP and Nerve Repair      
|                | Moderators: Louis Casteilla, PhD & Han Tsung Liao, MD, PhD           |
| 3:30 - 4:00 pm  | Coffee Break and Exhibits                                             |
| 4:00 - 5:00 pm  | **INVITED SPEAKER** Evolving Global Regulations for Stem Cells        
<p>|                | Mary Ann Chirba, JD - Boston College                                  |
| 5:00 pm        | Free Evening to Enjoy New York                                         |</p>
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<td><strong>INVITED SPEAKER</strong>&lt;br&gt;Clinical Trials Using Adipose Derived Mesenchymal Stem Cells for Fistulae Treatment&lt;br&gt;<em>Damian Garcia-Olmo, MD, PhD - Madrid, Spain</em></td>
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<td>Coffee Break and Exhibits</td>
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<td>10:00 - 11:30 am</td>
<td><strong>CONCURRENT SESSION C4A</strong>&lt;br&gt;ASCs Fat Graft Harvesting and Storage Methods&lt;br&gt;Moderators: <em>Lauren Kokai, PhD &amp; Christopher West, MBChB, MRCS</em></td>
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<td>10:00 - 11:30 am</td>
<td><strong>CONCURRENT SESSION C4B</strong>&lt;br&gt;Musculoskeletal Regeneration&lt;br&gt;Moderators: <em>Marco Helder, PhD &amp; Benno Naaijkens, MSc</em></td>
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<td>10:00 - 11:50 am</td>
<td><strong>CONCURRENT SESSION C4C</strong>&lt;br&gt;ASCs: Aging and Passage Effects&lt;br&gt;Moderators: <em>Kacey Marra, PhD &amp; Jacqueline Bliley, MS</em></td>
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<td>12:00 pm</td>
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### PRE-CONFERENCE SYMPOSIUM

**The Business of Adipose-derived Stem Cells: Perspectives from Industry and Clinicians**

**Moderators:** Sydney Coleman, MD, Kacey Marra, PhD & J. Peter Rubin, MD, FACS

The Pre-Conference Symposium will cover several key, timely topics:
- Introduction to companies in the adipose stem cell field
- Demonstration of company products explaining how they fit in the adipose stem cell community
- Perspectives from both established and new entry companies
- Definition of how venture capitalists envision the future of adipose stem cell therapies
- What changes in cell therapies are needed for the wounded warrior

*The program begins with a buffet lunch at noon. The panel format will allow both presentations and roundtable discussions.*

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<tr>
<td>12:20 - 12:30 pm</td>
<td>Welcome and Introduction</td>
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<tr>
<td>12:30 - 2:00 pm</td>
<td>Perspectives of Adipose Cell Therapies from Industry</td>
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<td><strong>Panelists:</strong> William Cimino, PhD - CEO, The GID Group</td>
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<td>Pamela Layton - Founder and CEO, Parcell Laboratories</td>
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<td>Stephen Minger, PhD - GE Healthcare Life Sciences, UK (Global Head of Research and Development for Cell Technologies)</td>
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<td>Mary Pat Moyer, PhD - Founder and CEO, INCELL Corporation</td>
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<td>Mark Spilker, PhD - Vice President of Research &amp; Development, Musculoskeletal Transplant Foundation (MTF)</td>
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<td>Colin White, PhD - Vice President, Research and Development at Parcell Laboratories</td>
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<tr>
<td>2:00 - 3:15 pm</td>
<td>Perspectives on Adipose Cell Therapies from Venture Capitalists and Investors</td>
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<td><strong>Panelists:</strong> Lee Buckler, LLB - Founder and Managing Director, Cell Therapy Group</td>
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<td>Jao Ho Jeong, MD - Director, Oblige Plastic Surgery Clinic &amp; StemTec Korea</td>
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<td>Efrem J. Kamen - Founder &amp; Managing Member, Pura Vida Investments</td>
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<td>Jonathan Schwartz - International Business Consultant for Development of Regenerative Medicine</td>
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<td>3:15 - 3:30 pm</td>
<td>Coffee Break</td>
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<tr>
<td>3:30 - 5:00 pm</td>
<td>Developing New Therapies for our Wounded Warriors: An Opportunity for Commercial Partners Interested in Stem Cell Technologies</td>
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<td><strong>Nerve Regeneration Strategies</strong></td>
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<td>Speaker: Kacey G. Marra, PhD - Associate Professor, University of Pittsburgh, Department of Plastic Surgery &amp; Bioengineering; Co-Director, Adipose Stem Cell Center; Faculty, McGowan Institute for Regenerative Medicine</td>
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<td><strong>Wound Healing and Burns</strong></td>
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<td>Speaker: Adam J. Katz, MD, FACS - Associate Professor, Plastic and Reconstructive Surgery, University of Florida and Co-Founder, The GID Group and Co-Founder, StemSource, Inc.</td>
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<td><strong>Craniofacial Reconstruction</strong></td>
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<td>Speaker: J. Peter Rubin, MD, FACS - Chair, Department of Plastic Surgery, Director of the Center for Innovation in Restorative Medicine, University of Pittsburgh</td>
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<td>5:00 pm</td>
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**11:00 am**

1.
**LONG-TERM ADIPOSE TISSUE RETENTION IN A MOUSE MODEL ENHANCED BY CONTROLLED DRUG DELIVERY**

*Presenter: Arta Kelmendi-Doko, MD*

*Affiliation: University of Pittsburgh*

*Authors: Kelmendi-Doko A, Marra KG, Rubin JP*

**11:10 am**

2.
**ASSESSMENT OF ANGIOGENIC FACTORS IN DE-NOVO TISSUE ENGINEERED VASCULARIZED ADIPOSE TISSUE**

*Withdrawn*

*Presenter: Juergen H. Dolderer, MD*

*Affiliation: Center of Plastic Hand and Reconstructive Surgery*

*Authors: Dolderer JH, Klein S, Schiller SM, Schroeder UH, Siegel-Axel D, Prantl L*

**11:20 am**

3.
**IDENTIFICATION OF THE MINIMUM CELL DOSE NECESSARY TO INCREASE FAT GRAFT RETENTION**

*Presenter: Jacqueline M. Bliley, BS, MS*

*Affiliation: University of Pittsburgh*

*Authors: Bliley JM, Grahovac TL, McLaughlin MM, Kling RE, Philips BJ, Day JR, Marra KG, Rubin JP*

**11:30 am**

4.
**LIDOCAINE IMPACT IN FAT GRAFTING: AN IN VIVO STUDY**

*Withdrawn*

*Presenter: AnneClaire Girard, PhD*

*Affiliation: Stemcis*

*Authors: Girard AC, Atlan M, Mirbeau S, Delarue P, Hulard O, Festy P, Roche R*
11:40 am
5 DEVELOPMENT AND OPTIMIZATION OF A NOVEL BIODEGRADABLE POROUS SILK CATHETER FOR THE INFUSION OF ADIPOGENIC AGENTS INTO ADIPOSE TISSUE GRAFTS
Presenter: Jolene Valentin, PhD
Affiliation: University of Pittsburgh
Authors: Valentin J, Philips BJ, McLaughlin MM, Gil ES, Kaplan DL, Marra KG, Rubin JP

11:50 am
6 DELIVERY OF ADIPOSE DERIVED STEM CELLS VIA HYALURONIC ACID HYDROGEL MICROCARRIERS
Presenter: Thomas Zarembinski, PhD
Affiliation: BioTime, Inc
Authors: Hsiung MC, Zarembinski T, Tew WP, Erickson IE

12:00 pm
7 THE EFFECTS OF SYNTHETIC POLYMER BASED BIOMATERIALS ON THE BEHAVIOR OF ADIPOSE DERIVED PERI-VASCULAR STEM CELLS (PSC) IN VITRO
Presenter: Christopher C. West, MBChB, BMedSci, MRCS(Eng)
Affiliation: The University of Edinburgh
Authors: West CC, Murray IR, Jiang Z, Zhang R, Stewart KJ, Hay DC, Bradley M, Peault B

12:10 pm
8 AUTOMATED ENCAPSULATION OF ADIPOSE STROMAL VASCULAR FRACTION CELLS IN ALGINATE HYDROGEL SPHEROIDS USING A DIRECT-WRITE 3D PRINTING SYSTEM
NEW Presenter: Stuart Williams, MD
Affiliation: University of Louisville
Authors: Touroo JS, Hoying JB, Williams SK

12:20 pm
9 COLORECTAL TISSUE ENGINEERING: COMPARATIVE IN VIVO AND IN VITRO STUDY FOR OPTIMAL SCAFFOLD SELECTION
Presenter: Quentin Denost, MD
Affiliation: INSERM Bioingenierie Tissulaire U1026
Authors: Denost Q, Adam JP, Montembault A, Bareille R, Siadous R, Delmont S, Rullier E, David L, Bordenave L

11:00 am - 12:30 pm
CONCURRENT SESSION C1C
ASC Molecular Biology, Cancer and Gene Therapy
Moderators: Bruce Bunnell, PhD & Trivia Frazier-Wiltz, PhD

11:00 am
10 THE EFFECTS OF THE CHEMOTHERAPEUTIC AGENT TAMOXIFEN ON ADIPOSE-DERIVED STEM CELLS
Presenter: Steven Pike, MD
Affiliation: Cooper University Hospital and Cooper Medical School at Rowan University

11:10 am
11 LIDOCAINE EFFECTS ON HUMAN ADIPOSE-DERIVED STEM CELLS: THE PHANTOM MENACE?
Presenter: AnneClaire Girard, PhD
Affiliation: Stemcis
Authors: Girard AC, Gunasekaran M, Roche R, Hoareau L, Festy F
11:20 am 12
NEUROGENIC STEM CELL (NSC) AND MESENCHYMAL STEM CELL (MSC)-MEDIATED TUMOR-TARGETED GENE THERAPY BASED ON LARGE-SCALE TRANSFECTION OF PLASMID DNA INTO PRIMARY ADIPOCYTES
Presenter: Patricia Aubanel, MD
Affiliation: Invitrx Therapeutics Inc
Authors: Soriano RA, Aubanel P, Haghighat N, Torfi H

11:30 am 13
ADMINISTRATION OF THE STROMAL VASCULAR FRACTION (SVF) AMELIORATES CHRONIC AUTOIMMUNE ENCEPHALOMYELITIS
Presenter: Bruce A. Bunnell, PhD
Affiliation: Tulane University School of Medicine
Authors: Bunnell BA, Semon J, Strong AL, Zhang X, Gimble JM

11:40 am 14
HYPOXIA INFLUENCES ADIPOKINE PRODUCTION; IMPLICATIONS FOR BREAST CANCER PREVENTION
Presenter: Irene Pien, BS, BA
Affiliation: Duke University School of Medicine
Authors: Pien I, Fisher M, Bond J, Ibarra-Drendall C, Klitzman B, Seewaldt V, Hollenbeck ST

11:50 am 15
HUMAN METABOLICALLY ACTIVE BROWN ADIPOSE TISSUE DERIVED STEM CELLS
Presenter: Francisco Silva, BS
Affiliation: BioRestorative Therapies
Authors: Silva F, Vargas V, Grainger D, Bull D, Patel A

12:00 pm 16
DEVELOPMENT OF AN AUTOLOGOUS THERAPEUTIC COCKTAIL FROM SVF-DERIVED CONDITIONED MEDIUM
Presenter: Brian Johnstone, PhD
Affiliation: Indiana University School of Medicine
Authors: Johnstone BH, Van Natta B, March KL

12:10 pm 17
EFFECT OF DOXORUBICIN ON ADIPOSE-DERIVED STEM CELLS AND BREAST CANCER CELL LINES: CAN WE INCORPORATE CHEMOTHERAPY INTO OUR RECONSTRUCTIVE STRATEGIES?
Presenter: Wakako Tsuji, MD, PhD
Affiliation: University of Pittsburgh
Authors: Tsuji W, Chung CW, McLaughlin MM, Valentin JE, Marra KG, Rubin JP

12:20 pm 18
ROLE OF PATIENT DEMOGRAPHICS AND DEPOT SITE ON ADIPOSE STEM CELL FUNCTIONALITY AND TUMORIGENESIS
Presenter: Amy F. Lin, MPH
Affiliation: Tulane University School of Medicine
Authors: Lin AF, Strong TA, Rhodes LV, Semon JA, Zhang X, Shi Z, Zhang S, Gimble JM, Burow ME, Bunnell BA

12:30 - 1:30 pm Lunch and Exhibits
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Presenter</th>
<th>Affiliation</th>
<th>Authors</th>
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</thead>
<tbody>
<tr>
<td>1:30 pm</td>
<td>C2A</td>
<td>Clinical Fat Grafting for Breast and Gluteal Reconstruction</td>
<td>Moderators: Kotaro Yoshimura, MD &amp; Kentaro Doi, MD</td>
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<tr>
<td>1:30 pm</td>
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<td>FAT GRAFTING FOR TREATMENT OF POSTOPERATIVE SOFT TISSUE CONTOUR DEFORMITIES AGGRAVATED BY LATE SIDE EFFECTS OF RADIATION THERAPY</td>
<td>Viacheslav S. Vasilyev, PhD</td>
<td>South Ural State Medical University</td>
<td>Vasilyev VS, Vasilyev SA, Vasilyev YS, Vasilyev IS, Karpov IA, Kazachkov EL, Orlova SS, Migranova NV</td>
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<tr>
<td>1:40 pm</td>
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<td>IS THERE AN IDEAL DONOR SITE OF FAT FOR SECONDARY BREAST RECONSTRUCTION?</td>
<td>Kevin H. Small, MD</td>
<td>New York Presbyterian Hospital</td>
<td>Small KH, Petruolo O, Choi M, Karp N</td>
</tr>
<tr>
<td>1:50 pm</td>
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<td>IMMEDIATE LIPOFILLING OF LATISSIMUS DORSI (LD) FLAPS AND PRE-EMPTIVE LIPOFILLING OF MASTECTOMY SKIN FLAPS IN BREAST RECONSTRUCTION</td>
<td>Stephen J. Goldie, MBChB, MRCS, PhD</td>
<td>St. Johns Hospital</td>
<td>Goldie SJ, Raine C, Dixon JM</td>
</tr>
<tr>
<td>2:00 pm</td>
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<td>IMMEDIATE MEGA VOLUME FAT GRAFTING TO THE BREAST FOLLOWING REMOVAL OF BREAST IMPLANTS</td>
<td>Marwan Abboud, MD</td>
<td>MA Clinic</td>
<td>Abboud M, Dibo SA</td>
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<tr>
<td>2:10 pm</td>
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<td>10 YEAR EXPERIENCE WITH MASSIVE FAT GRAFTING (&gt;1000CC/PATIENT) TO BUTTOCKS: RESULTS, COMPLICATIONS, AND LESSONS LEARNED</td>
<td>Ricardo L. Rodriguez, MD</td>
<td>Cosmeticsurgnet</td>
<td>Rodriguez RL, Conde Green A</td>
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<td>2:20 pm</td>
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<td>AESTHETIC GLUTEAL LIPOAUGMENTATION OR AESTHETIC GLUTEAL REMODELING BY FAT GRAFTING: THE “FRENCH TOUCH”</td>
<td>Christophe Ho Quoc, MD</td>
<td>Leon Berard Center</td>
<td>Ho Quoc C, Dlimi C, Delay E</td>
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<tr>
<td>2:30 pm</td>
<td></td>
<td>NANOFAT GRAFTING: BASIC RESEARCH AND CLINICAL APPLICATIONS</td>
<td>Patrick Tonnard, MD</td>
<td>Coupure Centrum Plastic Surgery Gent</td>
<td>Tonnard P, Verpaele AV, Peeters GP</td>
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<td>2:40 pm</td>
<td></td>
<td>IN VIVO IMAGING OF APOPTOSIS AFTER LIPOTRANSFER WITH/WITHOUT STEM CELLS</td>
<td>Keisuke Takanari, MD, PhD</td>
<td>Nagoya University Graduate School of Medicine</td>
<td>Takanari K, Toriyama K, Yagi S, Sato H, Yamamoto T, Funahashi Y, Gotoh M, Kamei Y</td>
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</tbody>
</table>
2:50 pm  27  
A PLEA FOR STANDARDIZATION IN FAT GRAFT REPORTING; A SIMPLE METHOD OF CREATING A STANDARD FAT VOLUME UNIT  
Presenter: Jeffrey M. Hartog, MD  
Affiliation: The Adrecyte Regenerative Medicine and Surgery Center  
Author: Hartog JM  

3:00 pm  28  
A NOVEL THERAPY FOR CORRECTION OF POST-LIPOSUCTION CONTOUR DEFECTS: CASE SERIES AND REVIEW OF THE LITERATURE  
Presenter: Som Kohanzadeh, MD  
Affiliation: University of Alabama Birmingham  
Authors: Kohanzadeh S, Martin MS, Collawn S  

3:10 pm  29  
HYBRID AUGMENTATION MAMMOPLASTY  
Presenter: Sungsoo Park, MD  
Affiliation: Bong Bong Plastic Surgery Clinic  
Author: Park S  

3:20 pm  30  
THE IMPACT OF COLD STORAGE ON HUMAN LIPOASPIRATES  
Presenter: Wei Z. Wang, MD  
Affiliation: University of Nevada School of Medicine  
Authors: Wang WZ, Fang XH, Williams SJ, Stephenson LL, Baynosa RC, Jaeger N, Khiabani KT, Zamboni WA  

1:30 - 3:10 pm  CONCURRENT SESSION C2B  
Adipose Tissue as a Scaffold  
Moderators: Julie Fradette, PhD & Marie-Eve Ouellette, MSc  

1:30 pm  31  
SIGNALING PATHWAYS TO ACTIVATE AND DIFFERENTIATE ADIPOSE-DERIVED STEM/STROMAL CELLS (ASCs) AFTER ADIPOSE TISSUE INJURY  
Presenter: Shinichiro Kuno, MD  
Affiliation: University of Tokyo School of Medicine  
Authors: Kuno S, Doi K, Mineda K, Kinoshita K, Kato H, Yoshimura K  

1:40 pm  32  
DECELLULARIZED ADIPOSE TISSUE AS A PLATFORM TECHNOLOGY FOR SOFT TISSUE RECONSTRUCTION AND AUGMENTATION  
Presenter: Lauren E. Flynn, PhD  
Affiliation: Queens University  
Authors: Flynn LE, Fuetterer L, Brown C, Yu C, Han T, Bianco J, Watkins JF  

1:50 pm  33  
UNMODIFIED ADIPOSE TISSUE-DERIVED ECM INTRINSICALLY FACILITATES REMODELING OF ADIPOSE TISSUE CONSTRUCTS BUT NOT BONE  
Presenter: Courtney Kim, PhD  
Affiliation: Tissue Genesis, Inc  
Authors: Kim C, Lee JQ, Shimoda C, Paek HJ
2:00 pm

34
COMPARATIVE STUDY OF TISSUE-ENGINEERED ADIPOSE SUBSTITUTES AND HUMAN NATIVE FAT THROUGH TRANSCRIPTOMICS AND LIPID PROFILING
Presenter: Marie-Eve Ouellette, MSc
Affiliation: Centre LOEX de l'Université Laval CHU de Québec
Authors: Ouellette ME, Berube JC, Kirouac F, Aubin K, Vallee M, Berthiaume L, Julien P, Bosse Y, Fradette J

2:10 pm

35
LIVING SCAFFOLDS: SCAFFOLD SEEDING WITH HUMAN ADIPOSE-DERIVED STEM CELLS (ASCs) FOR SURGICAL REPAIR APPLICATIONS
Presenter: Aaron L. Klinger, MD
Affiliation: Cooper University Hospital and Cooper Medical School at Rowan University

2:20 pm

36
NEW FAT-DERIVED PRODUCTS FOR TREATING INDUCED-SKIN LESIONS OF SCLERODERMA IN NUDE MICE
Presenter: Nicolas Serratrice, MS
Affiliation: Hôpital de la Conception
Authors: Serratrice N, Bruzzese L, Magalon J, Veran J, Daumas A, Andrac-Meyer L, Magalon G

2:30 pm

37
LATTICE ACELLULAR DERMAL MATRIX FOR STRUCTURAL SUPPORT IN A FAT GRAFT
Presenter: Joonkyu Park, MD
Affiliation: Seoul National University Hospital
Authors: Park J, Kwon ST

2:40 pm

38
ANALYSES OF HUMAN ADIPOSE TISSUE-DERIVED BIOSCAFFOLDS
Presenter: Caasy Thomas-Porch, BS
Affiliation: Tulane University
Authors: Thomas-Porch C, Shah F, Frazier T, Hayes D, Scherp P, Flynn LE, Bunnell BA, Gimble JM

2:50 pm

39
IN VITRO GENERATION OF MESENCHYMAL NEOTISSUES BY ADIPOSE AND BONE MARROW DERIVED ADULT EQUINE MULTIPOTENT STROMAL CELLS ON COLLAGEN SCAFFOLDS
Presenter: Mandi J. Lopez, DVM, MS, PhD
Affiliation: Louisiana State University

3:00 pm

40
EVALUATION OF THE EFFECTS OF ADIPOGEL (ADIPOSE-DERIVED MATRIX) ON DIFFERENT CELL POPULATIONS, WITH OR WITHOUT THE USE OF ADDITIVES
Presenter: Beryl H. Tan, MD
Affiliation: O’Brien Institute
Authors: Tan BH, Han XL, Morrison WA, Abberton KM
CONCURRENT SESSION C2C
ASCs and Vascularization
Moderators: Dimitry Traktuev, PhD & Sudheer Ravuri, PhD

1:30 pm

41
TRANSPLANTATION OF MOUSE ADIPOSE DERIVED STEM CELLS IMPROVED CARDIAC FUNCTION IN RATS WITH ACUTE MYOCARDIAL INFARCTION AND MODULATED EXPRESSION OF INFLAMMATORY CYTOKINES AND IMMUNE CELLS
Presenter: JongHo Kim, MS
Affiliation: Korea University
Authors: Kim JH, Park CY, Park JH, Choi SC, Choi JH, Hong SJ, Lim DS

1:40 pm

42
DIRECTING STEM CELLS TO THE INFARCTED HEART USING TARGETED MICROBUBBLES: DEVELOPMENT OF A NEW MOLECULAR THERAPEUTIC TECHNIQUE
Presenter: Benno A. Naaijkens, MSc
Affiliation: VU Medical Center Amsterdam
Authors: Naaijkens BA, Bogaards SJ, Krijnen PA, Kamp O, Musters RJ, Kokhuis TJ, de Jong N, Niessen HW, van Dijk A, Juffermans LJ

1:50 pm

43
PROLIFERATION OF ADIPOCYTE PROGENITORS FROM HUMAN ADIPOSE TISSUE CAPILLARY NETWORKS INDICATES A VASCULAR NICHE FOR HUMAN ADIPOCYTE STEM CELLS
Presenter: Silvia Corvera, MD
Affiliation: University of Massachusetts Medical School
Authors: Corvera S, Min SY, Gealekman O, Lalikos J, Fudem G, Chouinard M

2:00 pm

44
DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS (ASCs) TO ENDOTHELIUM BY SPHINGOSINE-1-PHOSPHATE FOR SEEDING ONTO A BIOLOGICAL SCAFFOLD
Presenter: Ping Zhang, PhD
Affiliation: Cooper Medical School at Rowan University

2:10 pm

45
ACTIVIN A IS KEY FACTOR RESPONSIBLE FOR ADIPOSE STROMAL CELLS ACQUISITION OF SMOOTH MUSCLE CELL PHENOTYPE AND FOR MODULATION OF THEIR ANGIOGENIC ACTIVITY AS A RESULT OF CONTACT WITH ENDOTHELIAL CELLS
Presenter: Dmitry O. Traktuev, PhD
Affiliation: Indiana University
Authors: Traktuev DO, Merfeld-Clauss S, Lupov IP, Lu H, Compton-Craig P, March KL

2:20 pm

46
EXAMINATION OF VASCULAR REMODELING EVENTS IN A MURINE FLAP DELAY MODEL
Presenter: Scott A. Seaman, BS
Affiliation: University of Virginia
Authors: Seaman SA, Peirce SM
2:30 pm

47 CREATION OF AN ARTIFICIAL ARTERY USING ADIPOSE-DERIVED STEM CELLS (ASCs) AND SMALL INTESTINE SUBMUCOSA (SIS)
Presenter: Kathleen M. Lamb, MD
Affiliation: Thomas Jefferson University Hospital
Authors: Lamb KM, Policha A, Chang L, Zhang P, Jimbo M, Abai B, Salvatore D, Tulenko T, DiMuzio P

2:40 pm

48 ANTI-INFLAMMATORY PROTEIN TSG-6 SECRETED BY ADIPOSE STROMAL CELLS INHIBITS NEUTROPHIL TRANSMIGRATION ACROSS ENDOTHELIAL MONOLAYER
Presenter: Jie Xie, MD
Affiliation: Indiana University School of Medicine
Authors: Xie J, Yi R, Feng D, Clauss MA, March KL

2:50 pm

49 THE FUTURE OF ENDOTHELIAL CELLS INDUCED FROM ADIPOSE DERIVED STEM CELLS: A POTENTIAL TREATMENT FOR HEMOPHILIA A
WITHDRAWN
Presenter: Brittany Busse, MD
Affiliation: UC Davis Health System
Authors: Busse B, Miguelino M, Powell J, Sahar DE

3:00 pm

50 USE OF ADIPOSE EXPLANTS TO TEST WOUND HEALING POTENTIAL OF LYMPHATIC OPTIMIZED ADIPOSE DERIVED SVF
Presenter: Catherine J. Baty, DVM, PhD
Affiliation: University of Pittsburgh
Authors: Baty CJ, Karlsson JM, Finegold DN, Acarturk TO, Futrell WJ

3:10 pm

51 COMPARISON OF BONE MARROW AND ADIPOSE TISSUE DERIVED STEM CELLS
Presenter: Ziya Saylan, MD
Affiliation: Office Dr. Saylan
Author: Saylan Z

3:20 pm

52 LIPOSHIFTING, TREATMENT OF POSTLIPOSUCTION IRREGULARITIES
WITHDRAWN
Presenter: Ziya Saylan, MD
Affiliation: Office Dr. Saylan
Author: Saylan Z

3:30 – 3:45 pm Coffee Break and Exhibits

3:45 – 5:15 pm Clinical Panel: Maximizing Fat Graft Survival
Moderators: Sydney Coleman, MD & J. Peter Rubin, MD, FACS
Panelists: Daniel Del Vecchio, MD
Ramon Llull, MD, PhD
Guy Magalon, MD
Ivona Percec, MD, PhD
Kotaro Yoshimura, MD

5:15 – 6:15 pm Panel: Fundamental Tools, Methodologies and Challenges in Adipose Stem Cell Research and Translation for the Clinician
Moderator: J. Peter Rubin, MD, FACS
Panelists: Spencer Brown, PhD
Adam J. Katz, MD, FACS
Kacey Marra, PhD

7:00 – 10:00 pm Dinner - Conrad Hotel
Saturday, November 23, 2013

6:30 am - 5:00 pm  Registration

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

8:00 - 9:45 am  **PLENARY SESSION 2 - Part I**  

Best Papers Submitted Focusing on Clinical Translation  
Moderators: Adam J. Katz, MD, FACS & Sydney Coleman, MD

8:00 am  

53  
A PHASE I CLINICAL TRIAL FOR MAXILLARY BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE SCAFFOLDS; AN INTRA-OPERATIVE CONCEPT  
Presenter: Marco N. Helder, PhD  
Affiliation: VU University Medical Center  
Authors: Helder MN, Prins HJ, Ten Bruggenkate CM, Overman JR, Klein-Nulend J, Schulten EA

8:12 am  

54  
EXPANDED ALLOGENEIC ADIPOSE-DERIVED STEM CELLS (EASCS) FOR THE TREATMENT OF COMPLEX PERIANAL FISTULA IN CROHN'S DISEASE: RESULTS FROM A MULTICENTER PHASE I/IIA CLINICAL TRIAL  
Presenter: Xavier X. Gonzalez-Argente, MD  
Affiliation: University Multicenter Study; Hospital Son Espases; Hospital Virgen del Rocio; Hospital San Juan de Dios Hospital la Paz; Hospital Virgen de la Macarena Hospital de Valme  
Authors: Gonzalez-Argente XX, De La Portilla F, Alba F, Garcia-Olmo D, Herrerias JM, Galindo A

8:24 am  

55  
AURAL TISSUE ENGINEERING USING ADSCS AND 3D SCAFFOLD  
NOT Presented  
Presenter: Beatriz Nicaretta, MD  
Affiliation: IASO General Hospital  
Authors: Sterodimas A, Nicaretta B

8:36 am  

56  
FIVE YEARS EXPERIENCE OF HIGH SEPTAL FAT INJECTIONS VERSUS STEM CELL ENRICHED FAT INJECTIONS FOR FACIAL REJUVENATION: WHAT WORKS AND WHAT DOESN'T WORK!  
Presenter: Tunc Tiryaki, MD  
Affiliation: Istanbul Academy of Plastic Surgery  
Authors: Tiryaki T, Aksungur E, Oymak O, Tiryaki D

8:48 am  

57  
DIABETES MELLITUS IMPAIR SURVIVAL OF AN AUTOLOGOUS FAT GRAFT IN ANIMAL MODEL: A PILOT STUDY  
WITHDRAWN  
Presenter: HoSeong Shin, MD, PhD  
Affiliation: Soonchunhyang University Bucheon Hospital  
Authors: Shin HS, Choi YD, Han BR, Hong SJ, Kim JY

9:00 am  

58  
ADVERSE EFFECTS OF SVF ON INTERVERTEBRAL DISC REGENERATION IN A LARGE ANIMAL MODEL  
Presenter: Suzanne E. Detiger, MD  
Affiliation: VU University Medical Center  
Authors: Detiger SE, Hoogendoorn RJ, Mevorat Kaplan K, van Royen BJ, Smit TH, Yayon A, Helder MN
<table>
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<tr>
<th>Time</th>
<th>Session Title</th>
<th>Presenter(s)</th>
<th>Affiliation</th>
<th>Authors</th>
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<tr>
<td>9:12 am</td>
<td>AUTOLOGOUS ADIPOSE STEM CELLS IN TREATMENT OF FEMALE STRESS URINARY INCONTINENCE; RESULTS OF A PILOT STUDY</td>
<td>Susanna Miettinen, PhD</td>
<td>University of Tampere BioMediTech</td>
<td>Miettinen S, Kuismanen K, Sartoneva R, Mannerstrom B, Haimi S, Nieminen K</td>
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<tr>
<td>9:36 am</td>
<td>IMPROVEMENT OF SURVIVAL RATE OF FAT TRANSPLANTATION BY OXYGEN-RELEASING MICROSPHERES AND ADIPOSE-DERIVED STEM CELLS</td>
<td>DongWoo Jung, MD, PhD</td>
<td>College of Medicine Yeungnam University Daegu Korea</td>
<td>Jung DW, Kim YH, Lim JO, Kim TG, Lee JH</td>
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<td>9:48 - 10:10 am</td>
<td>Coffee Break and Exhibits</td>
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<td>10:10 am</td>
<td>PLENARY SESSION 2 - Part II Best Papers Submitted Focusing on Basic Science Translation</td>
<td>Julie Fradette, PhD &amp; Kacey Marra, PhD</td>
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<td>10:10 am</td>
<td>ADIPOSE-DERIVED MESENCHYMAL STEM CELLS PROMOTE BREAST CANCER GROWTH AND METASTATIC SPREAD</td>
<td>Ricardo Schweizer, MD</td>
<td>University of Zurich</td>
<td>Kamat P, Schweizer R, Kaenel P, Salemi S, Eberli D, Andres AC, Giovanoli P, Plock JA</td>
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<tr>
<td>10:22 am</td>
<td>UPSTREAM/PROMOTER ANALYSIS OF WNT SIGNALING ANTAGONIST, SFRP1 IN ADIPOSE DERIVED STEM CELL BIOLOGY: IMPLICATIONS FOR SOFT TISSUE RECONSTRUCTION</td>
<td>Sudheer K. Ravuri, PhD</td>
<td>University of Pittsburgh</td>
<td>Ravuri SK, Philips BJ, Li X, Marra KG, Donnenberg VS, Donnenberg AD, Rubin JP</td>
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<tr>
<td>10:34 am</td>
<td>PREDICTABLE &amp; DURABLE FAT GRAFTING BY TARGETED PROTECTION OF THE DONOR MICROVASCULATURE USING PHOSPHODIESTERASE 5 INHIBITORS</td>
<td>Marc A. Soares, MD</td>
<td>New York University School of Medicine</td>
<td>Soares MA, Ezeamuzie O, Ojo C, Ham M, Saadeh PB, Ceradini DJ</td>
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<tr>
<td>10:46 am</td>
<td>THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE STROMAL CELLS IN RETINOPATHY OF PREMATURITY</td>
<td>Rajashekhar Gangaraju, PhD</td>
<td>Indiana University School of Medicine</td>
<td>Gangaraju R, Callaghan B, Rogers P, Samuels B, March K</td>
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</table>
10:58 am 66
THE NOVEL APPROACH OF GENERATING SKIN FROM FAT
NOT Presented
Presenter: Claudia Chavez-Munoz, MD, PhD
Affiliation: Northwestern University
Authors: Chavez-Munoz C, Nguyen K, Xu W, Hong SJ, Mustoe TA, Galiano RD

11:10 am 67
RNA-SEQ ANALYSIS OF ALDEFLUOR-BRIGHT AND -DIM ADIPOSE STEM CELLS AND FUNCTIONAL ANALYSIS OF DIFFERENTIATION POTENTIAL AND PROLIFERATION IN CLONES DERIVED FROM THESE SUBPOPULATIONS
Presenter: Winters R. Hardy, PhD
Affiliation: IUPUI and UCLA
Authors: Hardy WR, Tran B, Traktuev D, Peault B, March KL

11:22 am 68
ENGINEERING VASCULARIZED ADIPOSE TISSUE IN A FLOW-THROUGH BIOREACTOR SYSTEM USING DECELLULARIZED JEJUNAL SEGMENTS AS CELL CARRIER
Presenter: Torsten Blunk, PhD
Affiliation: University of Wuerzburg
Authors: Blunk T, Werner K, Reboredo J, Ruecker C, Bauer-Kreisel P, Walles H

11:34 am 69
CELLULAR STRESS INCREASES TNF-ALPHA RESPONSE IN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS
NOT Presented
Presenter: Tomas Robredo, BSc
Affiliation: La Paz University Hospital Research Institute
Author: de Miguel F, Robredo T

12:00 - 1:30 pm Lunch and Exhibits

1:30 - 3:30 pm CONCURRENT SESSION C3A
Clinical Fat Grafting to the Face
Moderators: Ramon Llull, MD, PhD & J. Peter Rubin, MD, FACS

1:30 pm 70
FAT GRAFTING: SKIN AND SCAR REGENERATIVE PROPERTIES
Presenter: Mohammad Nassimizadeh, MBChB, Bsc
Affiliation: University Hospital Birmingham
Authors: Nassimizadeh M, Nassimizadeh A, Dancey A

1:40 pm 71
FACIAL FAT GRAFT: A VARIETY OF SITE AND PURPOSE
Presenter: Marco Klinger, MD
Affiliation: Universita Degli Studi di Milano Scuola di Specialit di Chirurgia Plastica Ricostruttiva ed Estetica
Author: Klinger M

1:50 pm 72
FAT GRAFTING AS THE LAST RESOURCE FOR FACIAL REJUVENATION IN PATIENTS WITH SEVERE CO-MORBIDITIES
Presenter: Marcos Sforza, MD
Affiliation: Dola Park Hospital
Authors: Sforza M, Andjelkov K, Zaccheddu R
EVALUATION OF THE HISTOLOGICAL CHANGES OF THE FAT GRAFTED FACIAL SKIN: CLINICAL TRIAL
Presenter: Patricio Covarrubias, MD
Affiliation: DIPRECA Hospital
Authors: Covarrubias P, Cardenas-Camarena I, Guerrerosantos J, Valenzuela I, Espejo I, Robles JA, Gioia S

EVALUATION OF CLINICAL OUTCOME OF AUTOLOGOUS FAT TRANSPLANTATION WITH SMALL-NEEDLE-KNIFE IN RECONSTRUCTION OF BODY SURFACE CONCAVE DEFORMITY
Presenter: Jianhong Long, MD
Affiliation: Xiangya Hospital of Central South University
Authors: Long J, Sun Y

A PHASE ONE, OPEN LABEL, SINGLE ARM STUDY TO DEMONSTRATE THE SAFETY OF THE ANTRIA CELL PREPARATION PROCESS DURING FACIAL FAT GRAFTING ASSISTED WITH AUTOLOGOUS, ADIPOSE-DERIVED STROMAL VASCULAR FRACTION (SVF)
Presenter: Shah Rahimian, MD, PhD
Affiliation: Antria, Inc
Authors: Rahimian S, Maliver L, Johns F, Bizousky D, Gore R, Johnson T, McNitt D, Quist L

AESTHETIC AND FUNCTIONAL RECOVERY OF CONGENITAL MUSCULAR TORTICOLLIS TREATED WITH INTRAMUSCULAR FAT GRAFTING
Presenter: Juan Monreal, MD
Affiliation: Hospital San Rafael
Author: Monreal J

LIPOSUCTION AND LIPOFILLING FOR THE TREATMENT OF SYMPTOMATIC SILICONE TOXICOSIS OF THE GLUTEAL REGION
Presenter: Christopher J. Salgado, MD
Affiliation: University of Miami
Authors: Salgado CJ, Sinha V, Sanchez P, Desai U

COMPOSITE BODY CONTOURING ASSISTED BY STROMAL ENRICHED LIPOGRAFT
Presenter: Aris Sterodimas, MD, MSc, PhD
Affiliation: IASO General Hospital
Authors: Sterodimas A, Illouz YG

CLINICAL OBSERVATION OF DIFFICULTIES & COMPLICATIONS OF FAT GRAFTING PROCEDURES
Presenter: Eva A. Siolo, MD, MBChB, FCS Plast (SA)
Affiliation: Inkosi Albert Luthuli Central Hospital UKZN
Author: Siolo EA

FAT GRAFTING AS ADJUVANT TO REDUCE SCARS IN ARM LIFTS
Presenter: Katarina Andjelkov, PhD
Affiliation: Private Clinic
Authors: Andjelkov K, Sforza M, Kuka G, Zaccheddu R
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Presenter</th>
<th>Affiliation</th>
<th>Authors</th>
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<tr>
<td>3:20 pm</td>
<td></td>
<td><strong>COMPARISON OF BOTULINUM TOXIN VERSUS STEM CELL GRAFTING IN WIDE FACIAL SCARS</strong></td>
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<td>Presenter: Adel M. Wilson, MD, FRCS</td>
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<td></td>
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<td>Affiliation: Cairo University</td>
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<td>Author: Wilson AM</td>
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<td>1:30 - 3:30 pm</td>
<td><strong>CONCURRENT SESSION C3B</strong></td>
<td><strong>ASCs and Wound Healing: Skin and Other Tissues</strong></td>
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<td>Moderators: Jae Ho Jeong, MD, PhD &amp; Wakako Tsuji, MD, PhD</td>
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<td>1:30 pm</td>
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<td><strong>ADIPOSE DERIVED STEM CELL THERAPY AMELIORATES DELAYED WOUND HEALING AFTER RADIATION BY MODULATING TGF-BETA EXPRESSION</strong></td>
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<td>Presenter: Alex K. Wong, MD</td>
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<td></td>
<td></td>
<td>Affiliation: Keck School of Medicine of USC</td>
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<td>Authors: Ragina N, Hoang D, Hill C, Lee YS, Kim G, Han B, Hong Y, Chuong CM, Senagore A, Urata MM, Wong AK</td>
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<td>1:40 pm</td>
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<td><strong>A NOVEL KERATIN MICROSPHERE DELIVERY OF ADIPOSE-DERIVED STEM CELLS FOR ACCELERATED HEALING OF DIABETIC FOOT ULCERS</strong></td>
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<td>Presenter: Hilal Arnouk, MD, PhD</td>
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<td></td>
<td>Affiliation: Cell Constructs, Inc.</td>
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<td>Authors: Arnouk H, Bharawaj S, Nagy T, Barrows TH</td>
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<td>1:50 pm</td>
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<td><strong>IMPROVEMENT OF THE FIBROTIC DERMAL TISSUE BY A COMPOSITE GRAFT MADE OF AUTOLOGOUS ADIPOSE MESENCHYMAL STEM CELLS (AMSCS) SEEDED ON HUMAN ACELLULAR COLLAGEN MATRIX (HACM): APPLICATION IN LARGE CRITICAL SIZE OF CHRONIC WOUNDS</strong></td>
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<td>Presenter: Aurore Lafosse, MD</td>
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<td>Affiliation: University Clinical Hospital Saint Luc UCL</td>
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<td>Authors: Lafosse A, Aouassar N, Hanet MS, Andre W, Lengele B, Vanwijck R, Dufrane D</td>
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<td>2:00 pm</td>
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<td><strong>OUTCOMES OF FAT GRAFTING IN NON-HEALING WOUNDS</strong></td>
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<td>Presenter: Kristen Aliano, MD</td>
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<td></td>
<td>Affiliation: Long Island Plastic Surgical Group</td>
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<td>Authors: Aliano K, Bassiri-Tehrani B, Stavrides S, Mathews B, Davenport T</td>
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<td>2:10 pm</td>
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<td><strong>DIABETIC ULCER TREATMENT BY INJECTABLE COMMINUTED ADIPOSE CONNECTIVE TISSUE (CAC): A THERAPEUTIC TOOL OF ADIPOSE-DERIVED STEM CELLS (ASCS) WITHOUT NEED OF CELL ISOLATION</strong></td>
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<td>Presenter: Kentaro Doi, MD</td>
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<td>Affiliation: University of Tokyo</td>
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<td>Authors: Doi K, Yoshimura K, Kato H, Kuno S, Mineda K, Kinoshita K</td>
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<td>2:20 pm</td>
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<td><strong>POINT-OF-CARE DERMAL REPLACEMENT CONSTRUCTS CONTAINING ADIPOSE-DERIVED SVF CELLS</strong></td>
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<td>Presenter: Ning Yang, PhD</td>
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<td>Affiliation: University of Florida</td>
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<td>Authors: Yang N, Yu A, Shang H, Katz AJ</td>
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2:30 pm  
88  
FAT GRAFTING A CURE FOR ALOPECIA?  
Presenter: Abdul Karim Nassimizadeh  
Affiliation: University Hospital Birmingham  
Authors: Nassimizadeh M, Nassimizadeh A, Dancey A  

2:40 pm  
89  
EFFICACY OF ENDOSCOPIC SUBMUCOSAL INJECTION OF HUMAN ASC FOR THE TREATMENT OF EXPERIMENTAL COLITIS IN RATS  
Presenter: Fernando de Miguel, PhD  
Affiliation: La Paz University Hospital Research Institute  
Authors: de Miguel F, Martin Arranz E, Robredo T, Mancheno P, Menta R, Diez J, Lombardo E  

2:50 pm  
90  
ADIPOSE STEM CELL IN LIMITATION OF ACUTE LUNG INJURY  
Presenter: Natalia V. Bogatcheva, PhD  
Affiliation: Indiana University  
Authors: Bogatcheva NV, Lu H, Poirier C, Traktuev DO, Cook T, Merfeld-Clauss S, Petrache I, March KL  

3:00 pm  
91  
INTRALYMPHATIC ADMINISTRATION OF ADIPOSE MESENCHYMAL STEM CELLS SHOWS THERAPEUTIC EFFECTS IN EXPERIMENTAL COLITIS AND ARTHRITIS  
Presenter: Eleuterio Lombardo, PhD  
Affiliation: TiGenix  
Authors: Escolano A, Garin M, Lopez-Santalla M, Menta R, DelaRosa O, Redondo JM, Dalemans W, Lombardo E  

3:10 pm  
92  
COMPARISON OF THE THERAPEUTIC EFFECTS OF HUMAN AND MOUSE ADIPOSE STEM CELLS IN A MURINE MODEL OF ACUTE LUNG INJURY  
Presenter: Trivia Frazier, MD  
Affiliation: Tulane University School of Medicine  
Authors: Frazier T, Bunnell BA, Zhang S, Danchuk S, Gimble J, Betancourt A, Sullivan D  

3:20 pm  
93  
EARLY PASSAGE ADIPOSE-DERIVED STROMAL CELLS ACCELERATE HEALING IN WOUNDED 3-D CULTURES  
Presenter: Sherry S. Collawn, MD, PhD  
Affiliation: UAB  
Authors: Collawn SS, Chow LT, Banerjee NS  

1:30 - 3:30 pm  
CONCURRENT SESSION C3C  
Hot Topics: ASCs, PRP and Nerve Repair  
Moderators: Louis Casteilla, PhD & Han Tsung Liao, MD, PhD  

1:30 pm  
94  
THE COMBINED USE OF ENHANCED STROMAL VASCULAR FRACTION AND PLATELET-RICH PLASMA IMPROVES FAT GRAFTING MAINTENANCE IN BREAST RECONSTRUCTION: CLINICAL AND INSTRUMENTAL EVALUATION  
Presenter: Pietro Gentile, MD, PhD  
Affiliation: University of Rome Tor Vergata  
Authors: Gentile P, Cervelli V
1:40 pm  95  
EFFECT OF PLATELET-RICH PLASMA ON ADIPOGENIC AND ANGIGENIC GENE EXPRESSION IN ADIPOSE-DERIVED STEM CELLS  
Presenter: Han Tsung Liao, PhD  
Affiliation: University of Pittsburgh  
Authors: Liao HT, Ravuri SK, Kokai LE, Marra KG, Rubin JP

1:50 pm  96  
AUTOLOGOUS PLATELET RICH PLASMA (PRP) IMPROVES ADIPOSE-DERIVED MESENCHYMAL STEM CELLS PROLIFERATION  
Presenter: Ali Modarressi, MD  
Affiliation: University Hospitals of Geneva  
Authors: Modarressi A, Atashi F, Pittet B

2:00 pm  97  
PLASMA ENRICHED LIPOFILLING (PEL): TRANSLATIONAL RESEARCH OR JUST ANOTHER IDEA?  
Presenter: Filip B. Stillaert, MD  
Affiliation: University Hospital Gent  
Authors: Stillaert FB, Roche N, Van Landuyt K, Blondeel P, Monstrey S, Doornaert M, De Pypere B

2:10 pm  98  
THE EFFECT OF PLATELET-RICH PLASMA FOR BONE REGENERATION WITH ADIPOSE-DERIVED STEM CELLS  
Presenter: Morikuni Tobita, DDS, PhD  
Affiliation: Juntendo University School of Medicine  
Authors: Tobita M, Tajima S, Mizuno H

2:20 pm  99  
IS THE COMBINATION OF FAT GRAFTS AND PLATELET RICH PLASMA EFFECTIVE AND SAFE? AN EXPERIMENTAL STUDY IN RATS  
WITHDRAWN  
Presenter: Alexandre Blumenschein, MS  
Affiliation: Universidade Federal de Goias  
Authors: Blumenschein A, Freitas R, Moreira MA, Cysneiros MA, Tufanin AT

2:30 pm  100  
EFFECT OF THE ADIPOSE TISSUE STROMAL VASCULAR FRACTION COMBINED WITH PLATELET-RICH PLASMA ON IRRADIATION-INDUCED CAPSULAR CONTRACTURE AROUND SILICONE IMPLANTS: AN EXPERIMENTAL STUDY  
Presenter: Ozlem Gundeslioglu, MD  
Affiliation: NE University Meram Medical Faculty  
Authors: Gundeslioglu O, Inan I, Tezcan Y, Toy H, Emlik D, Aktan M, Duman S

2:40 pm  101  
EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS TREATMENT IN A MOUSE MODEL OF NEUROPATHIC PAIN  
Presenter: Anna T. Brini, PhD  
Affiliation: Department of Biomedical Surgical and Dental Sciences University of Milan  
Authors: Brini AT, Niada S, Rossi A, Arrigoni E, Franchi S, Panerai AE, Sacerdote P

2:50 pm  102  
PREVENTION OF FAT GRAFT ABSORPTION BY EPINEURAL SHEATH TUBE - A PRELIMINARY REPORT  
Presenter: Maria Siemionow, MD  
Affiliation: Cleveland Clinic  
Authors: Siemionow M, Uygur S, Kwiecien G, Bobkiewicz A, Madajka M
PERIPHERAL NERVE REPAIR: MULTIMODAL COMPARISON OF THE REGENERATIVE POTENTIAL OF ADIPOSE TISSUE DERIVED CELLS IN A BIODEGRADABLE CONDUIT
Presenter: Elizabeth Kappos, MD
Affiliation: University Hospital Basel
Authors: Kappos E, Engels PE, Meyer zu Schwabedissen M, Tremp M, Fischmann A, Schaefer DJ, Kalbermatten DF

MRI IS A VALUABLE TOOL TO MONITOR ENHANCED EARLY PERIPHERAL NERVE REGENERATION
Presenter: Mathias Tremp, MD
Affiliation: University Hospital Basel
Authors: Tremp M, Meyer zu Schwabedissen M, Kappos EA, Engels PE, Fischmann A, Scherberich A, Schaefer DJ, Kalbermatten DF

TREATMENT OF SYSTEMIC SCLEROSIS PATIENTS WITH MICROFAT GRAFTING AND SVF
Presenter: Guy Magalon, MD
Affiliation: APHM
Authors: Magalon G, Nguyen P, Daumas A

Coffee Break and Exhibits

INVITED SPEAKER
Evolving Global Regulations for Stem Cells
Mary Ann Chirba, JD - Boston College

Free evening to enjoy New York
**Sunday, November 24, 2013**

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<tr>
<td>6:30 am - 12:00 pm</td>
<td>Registration</td>
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<td>7:00 - 8:00 am</td>
<td>Continental Breakfast - Exhibit Hall</td>
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| 8:00 - 9:30 am  | **INVITED SPEAKER**  
Clinical Trials Using Adipose Derived Mesenchymal Stem Cells for Fistulae Treatment  
*Damian Garcia-Olmo, MD, PhD - Madrid, Spain* |
| 9:30 - 10:00 am | Coffee Break and Exhibits                                           |
| 10:00 - 11:30 am| **CONCURRENT SESSION C4A**  
ASCs Fat Graft Harvesting and Storage Methods  
Moderators: Lauren Kokai, PhD & Christopher West, MBChB, MRCS |

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<th>Affiliation</th>
<th>Authors</th>
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<tr>
<td>10:00 am</td>
<td>106 POINT OF CARE DEVICE FOR CONCENTRATING AND HARVESTING MESENCHYMAL STROMAL CELLS FROM LIPOASPIRATE</td>
<td>John Chapman, PhD</td>
<td>California State University Sacramento</td>
<td>Chapman J, Showalter M, Horton K</td>
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<tr>
<td>10:10 am</td>
<td>107 PROCESSING TECHNIQUE INFLUENCES ADIPOSE DERIVED STEM CELL CONCENTRATION AND CELL VIABILITY IN LIPOASPIRATE</td>
<td>Elizabeth Zellner</td>
<td>Yale University School of Medicine</td>
<td>Wu W, Zellner, E, Steinbacher D</td>
</tr>
<tr>
<td>10:20 am</td>
<td>108 IN VITRO TISSUE QUALITY ASSESSMENT OF AUTOLOGOUS FAT GRAFT PREPARED USING TRADITIONAL CENTRIFUGATION AND COMMERCIALLIY AVAILABLE LIPOKIT AND PUREGRAFT® SYSTEMS</td>
<td>Min Zhu, MD</td>
<td>Cytori Therapeutics, Inc</td>
<td>Zhu M, Souverneva O, Prada A, Shanahan R, Hicok KC, Arm D</td>
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<td>10:30 am</td>
<td>109 DOES CELL-SUPPLEMENTED LIPOTRANSFER MAKE A DIFFERENCE IN COMPARISON TO CONVENTIONAL METHODS USED FOR FAT GRAFTING?</td>
<td>Alexandra Conde-Green, MD</td>
<td>Johns Hopkins Bayview Medical Center and University of Maryland Medical Center</td>
<td>Conde-Green A, Wu I, Graham I, Chae J, Singh DP, Holton LH, Slezak S, Elisseeff J</td>
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<td>10:40 am</td>
<td>110 COMPARISON OF STROMAL VASCULAR FRACTION CELLS OBTAINED FROM ENZYME DIGESTION AND NUTATIONAL INFRASONIC LIPOSUCTION</td>
<td>Robert E. Bowen, MD</td>
<td>The Center for Positive Aging</td>
<td>Bowen RE, Dihn B</td>
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<td>10:50 am</td>
<td>111 &quot;MINIMAL MANIPULATION&quot; OF HUMAN ADIPOSE-DERIVED STEM CELLS FROM LIPOSUCTION FOR CLINICAL APPLICATIONS</td>
<td>Yuan Liu, MD</td>
<td>Cooper University Hospital</td>
<td>Liu Y, Chang S, Jones R, Carpenter JP, Tulenko TN</td>
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11:00 am

112 ADIPOSE STEM CELLS: EFFECTS OF CRYOPRESERVATION AND DONOR AGE ON UTILITY IN REGENERATIVE MEDICINE

Presented: David T. Harris, PhD
Affiliation: University of Arizona
Authors: Harris DT, Muise A, Badowski M, Pierce J

113 ADIPOSE STROMAL VASCULAR FRACTION ISOLATION: A HEAD-TO-HEAD COMPARISON OF FOUR COMMERCIAL CELL SEPARATION SYSTEMS

Presented: Joel A. Aronowitz, MD
Affiliation: Cedars Sinai Medical Center
Authors: Aronowitz JA, Ellenhorn J

114 HYDROGEL MATRIX FOR SVF INJECTIONS: FINER APPLICATIONS

Presented: Isaac E. Erickson, PhD
Affiliation: BioTime, Inc
Authors: Erickson IE, Dos Anjos Vilaboa S, Zarembinski T, Llull R, Tew WP

10:00 - 11:20 am

CONCURRENT SESSION C4B
Musculoskeletal Regeneration
Moderators: Marco Helder, PhD & Benno Naaijkens, MSc

10:00 am

115 A 3-DIMENSIONAL OSTEOGENIC-LIKE STRUCTURE FROM HUMAN AUTOLOGOUS ADIPOSE MESENCHYMAL STEM CELLS: REPRODUCIBILITY, GENETIC STABILITY, CLINICAL SAFETY/EFFICACY

Presented: Denis Dufrane, MD, MSc, PhD
Affiliation: Saint-Luc-Université Catholique de Louvain
Authors: Dufrane D, Antoine-Poirel H, Docquier PL, Aouassar N, Ameye G, Verhaeghe L, Nonckreman S, Andre W, Delloye C

116 OSTEOGENIC PERFORMANCE OF DONOR MATCHED HUMAN ADIPOSE AND BONE MARROW MSCS UNDER DYNAMIC CULTURE

Presented: Miles Pfaff, MD
Affiliation: Yale University School of Medicine
Authors: Chang J, Mendez J, Pfaff M, Niklason L, Steinbacher D

117 ADIPOSE-DERIVED STEM CELLS IMPROVE COLLAGENASE-INDUCED TENDINOPATHY IN RAT MODEL

Presented: Takashi Oshita, MD
Affiliation: Juntendo University School of Medicine
Authors: Oshita T, Tobita M, Tajima S, Ishihara H, Nishimuta Y, Mizuno H

118 GROWTH FACTOR GENE EXPRESSION PROFILES OF BONE MORPHOGENETIC PROTEIN-2-TREATED HUMAN ADIPOSE STEM CELLS SEATED ON CALCIUM PHOSPHATE SCAFFOLDS IN VITRO

Presented: Astrid Bakker, PhD
Affiliation: VU University Medical Center
Authors: Overman JR, Helder MN, ten Bruggenkate CM, Schulten EA, Klein-Nulend J, Bakker AD
10:40 am

ADIPOSE-DERIVED STEM CELLS FROM BUCCAL FAT PAD FOR PERIODONTAL AND ORAL BONE REGENERATION: AN IN VITRO STUDY

WITHDRAWN

Presenter: Anna T. Brini, PhD
Affiliation: Department of Biomedical Surgical and Dental Sciences University of Milan
Authors: Brini AT, Arrigoni E, Broccaioli E, Niada S, Ferreira LM, Yenagi V, Rasperini G

10:50 am

STROMAL VASCULAR FRACTION CELLS FOR THERAPY OF 275 PATIENTS WITH OSTEOARTHRITIS

Presenter: Jaroslav Michalek, MD, PhD
Affiliation: Cellthera Ltd
Authors: Michalek J, Kristkova Z, Skopalik J, Cibulka M, Holek M, Moster R

11:00 am

REJUVENATION OF THE ARM THROUGH LIPOSUCTION AND FAT TRANSFER, AN INNOVATIVE NO SCAR BRACHIOPLASTY TECHNIQUE

Presenter: Saad Dibo, MD
Affiliation: MA Clinic
Authors: Dibo S, Abboud MH

11:10 am

SCALABLE BIOFABRICATION OF CHONDROSPHERES FROM HUMAN ADIPOSE STEM CELLS ISOLATED BY MECHANICAL DISSOCIATION

Presenter: Leandra S. Baptista, PhD
Affiliation: Federal University of Rio de Janeiro

10:00 - 11:50 am

CONCURRENT SESSION C4C

ASCs: Aging and Passage Effects
Moderators: Jacqueline Bliley, MS & Kacey Marra, PhD

10:00 am

CELL SURFACE MARKER PROFILING OF ADIPOSE-DERIVED STEM CELLS FROM HUMAN SUBCUTANEOUS AND VISCERAL FAT DEPOTS

Presenter: Shigeki Sugii, PhD
Affiliation: Singapore Bioimaging Consortium and Duke NUS Graduate Medical School
Authors: Chan E, Toh SA, Han W, Sugii S

10:10 am

AN IMMUNOPHENOTYPIC CHARACTERIZATION OF THE STROMAL VASCULAR FRACTION OF OBESE DONORS

Presenter: Jonathan Kenyon, PhD
Affiliation: Case Western Reserve University
Authors: Kenyon J, Sadeghi Z, Sramkoski M, Jacobberger J, Soltanian H, Hijaz A, Daneshgari F

10:20 am

THE FIBROGENETIC EFFECT OF BMI1 AND EZH2 PROTEINS ON ADIPOSE DERIVED STEM CELLS IN DIFFERENT AGE GROUPS

Presenter: Minsuk Kang, MD
Affiliation: Seoul National University Hospital
Authors: Kang M, Jin US, Kim SW
10:30 am

126
PASSAGE-DEPENDENT AND SERUM-DEPENDENT CHANGES OF ADIPOSE-DERIVED STEM CELLS IN VITRO – IS A STEM CELL THE SAME IN VITRO AS IN VIVO?
Presenter: Renata Sonnenfeld, BS
Affiliation: Medical School Hannover
Authors: Sonnenfeld R, Kuhbier J, Radtke C, Lazaridis A, Vogt PM, Reimers K

10:40 am

127
AGE-DEPENDENT CHANGE IN EXPRESSION OF GENERAL AGING MARKERS AND MARKERS OF CELLULAR SENESCENCE IN PRIMARY HUMAN ADIPOSE TISSUE
Presenter: Joshua Cornman-Homonoff, AB
Affiliation: Perelman School of Medicine at the University of Pennsylvania
Authors: Cornman-Homonoff J, Percec I, Dierov R

10:50 am

128
STEM CELL CONCENTRATION IN PEDIATRIC ADIPOSE TISSUE
WITHDRAWN
Presenter: Kevin S. Hopkins, MD, FACS
Affiliation: Driscoll Childrens Hospital

11:00 am

129
MESENCHYMAL STEM CELLS PRODUCED FROM DIFFERENT SOURCES OF ADULT ADIPOSE TISSUE DEMONSTRATE A SIGNIFICANT AND REPETITIVE DIFFERENCE IN THEIR LONG TERM PROPAGATION AND DIFFERENTIATION ABILITIES
Presenter: Nir Shani, PhD
Affiliation: Tel Aviv Sourasky Medical Center
Authors: Shani N, Tirza G, Sela M, Krelin Y, Friedman O, Gur E

11:10 am

130
SVF CELL COUNTING: COMPARISON OF DIFFERENT AUTOMATED CELL COUNTING DEVICES
Presenter: Severiano Dos Anjos Vilaboa Sr., PhD
Affiliation: Stem Center SL
Authors: Dos Anjos Vilaboa S, Cimino W, Llull R

11:20 am

131
HIGH THROUGHPUT CELL-BASED SCREENING ASSAY FOR ADIPOGENIC AGENTS IN SOFT TISSUE ENGINEERING
NEW Presenter: Trent Gause, MD
Affiliation: University of Pittsburgh
Authors: Kling RE, Gough AH, Gause T, Kokai L, Philips BJ, Ravuri SK, Fernstrom JD, Marra KG, Rubin JP

11:30 am

132
19F HOT SPOT MRI OF SVF AND MSCS FOR CLINICAL MONITORING OF STEM CELL THERAPY
NOT Presented
Presenter: Jeff Bulte, MD
Affiliation: Cosmeticsurgnet

11:40 am

133
POINT-OF-CARE INSTRUMENTATION FOR READOUT OF FLUORESCENT CELL YIELD AND VIABILITY ANALYSES
NEW Presenter: Michael Coleman, PhD
Affiliation: InGeneron Incorporated
Authors: Vykoukal J, Nazari-Shafti T, Bruno I, Martinez R, Stone G, Coleman M

12:00 pm
Adjourn
PAPER PRESENTATIONS
in numerical order
LONG-TERM ADIPOSE TISSUE RETENTION IN A MOUSE MODEL ENHANCED BY CONTROLLED DRUG DELIVERY

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

Purpose: Resection of tumors in the head and neck, and upper and lower extremities, as well as trauma and congenital abnormalities often result in contour defects due to loss of soft tissue, largely composed of subcutaneous adipose tissue. Standard care includes tissue flap transfer, or prosthetic components such as silicone or saline implants. However, the limitations following these procedures are numerous, with a special notice to short term prolongation of the treatment. 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: Dexamethasone was encapsulated in poly(lactic-co-glycolic acid), (PLGA) microspheres (MS) and mixed with human lipoaspirate to induce adipogenesis in vivo, using a slow drug delivery approach. Dexamethasone-loaded PLGA microspheres (Dex MS) were prepared using a double emulsion/solvent extraction technique, and release kinetics were determined. Two different doses of the drug were examined, 50 and 27 mg of Dex MS mixed with 0.3 mL of human lipoaspirate as well as 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, with the dexamethasone microsphere-containing samples demonstrating a twofold lipo 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: This study demonstrates that the controlled delivery of adipogenic factors such as dexamethasone via polymer microspheres can significantly enhance and retain tissue formation and vascularization and clinically relevant module of adipose retention.

ASSESSMENT OF ANGIOGENIC FACTORS IN DE-NOVO TISSUE ENGINEERED VASCULARIZED ADIPOSE TISSUE

Presenter: Juergen H. Dolderer, MD
Authors: Dolderer JH, Klein S, Schiller SM, Schroeder UH, Siegel-Axel D, Prantl L
Center of Plastic Hand and Reconstructive Surgery

WITHDRAWN
IDENTIFICATION OF THE MINIMUM CELL DOSE NECESSARY TO INCREASE FAT GRAFT RETENTION

Presenter: Jacqueline M. Bliley, BS, MS
Authors: Bliley JM, Grahovac TL, McLaughlin MM, Kling RE, Philips BJ, Day JR, Marra KG, Rubin JP

University of Pittsburgh

Abstract: While fat grafting is an increasingly popular practice, suboptimal volume retention remains an obstacle. The stromal vascular fraction (SVF) has gained attention as a method of increasing retention due to its ability to promote angiogenesis. However, many studies have used a high loading dose that is not clinically feasible. We aimed to examine the effects of cell dose on volume retention of fat grafts to determine the minimum effective dose and to establish a method of cell enrichment that provides homogeneous distribution of SVF throughout the grafts.

Methods: Lipoaspirate was prepared by Coleman processing. SVF was isolated from the same patient. The isolated SVF was resuspended in PBS and transferred to a syringe containing the processed fat graft. The cell-enriched graft was transferred between syringes to ensure thorough mixing. Escalating doses starting at 1 million cells/mL of processed lipoaspirate were prepared for injection. Athymic mice were injected bilaterally with a cell enriched fat graft and a standard fat graft control. Samples were explanted after 3 or 6 weeks and assessed for volume. Fluorescent tracer Vybrant DiR was used to label the SVF to track cell fate and dissemination throughout the graft. In vivo fluorescence of the SVF was quantified using the IVIS imaging system. Cellular proliferation and differentiation was also assessed in order to evaluate DiR dye toxicity.

Results: A minimal dose of 3 million cells/mL dose was necessary to cause a significant increase in volume retention (p<.05). No significant difference in differentiation nor proliferation was seen in DiR-labeled versus unlabelled cells (p>.05). A significant difference in fluorescence was seen in grafts with differing cell doses (p<.05). SVF fluorescence within the graft is still visible at 76 days post-operatively (ongoing imaging study) indicating viability of these cells within the graft.

Conclusions: Ongoing analysis will assess a full dose response curve and examine the distribution and viability of the labeled SVF. Our results demonstrate that SVF can be used to supplement fat grafts to improve volume retention. Identification of a minimum effective dose will be useful in the clinical application of cell-enriched fat grafting.

LIDOCAINE IMPACT IN FAT GRAFTING: AN IN VIVO STUDY

Presenter: AnneClaire Girard, PhD
Authors: Girard A, Atlan M, Mirbeau S, Delarue P, Hulard O, Festy F, Roche R

Stemcis

WITHDRAWN
Tissue Grafts

Introduction: Lipoaspirate grafting procedures for soft tissue reconstruction have shown loss of volume over time and patient-to-patient variability. Incorporating adipogenic agents into adipose tissue grafts has great potential to improve fat retention. Perfusion drug delivery systems such as indwelling catheters are an innovative solution for controlled and tunable drug delivery. This study focuses on the development and implementation of a novel biocompatible, biodegradable porous hollow silk catheter as a conduit for the direct infusion of adipogenic agents into adipose tissue grafts.

Materials and Methods: Silk perfusion catheters were prepared by gel spinning a highly concentrated silk solution generated from Bombyx mori silkworm cocoons onto micron-sized wires. Implantable slow-release osmotic pumps (ALZET) with a flow rate of 0.5 ul/hr were filled with dexamethasone or insulin and directly connected to the silk catheter to assess the drug release profile over 7 days in-vitro. Insulin was measured using microLowry protein assay, and dexamethasone was analyzed with UV spectrometry. An athymic nude mouse model of adipose tissue reconstruction was used to study the local delivery of 0, 30, 100, 200, 300 ug dexamethasone, a known adipogenic agent, to human lipoaspirate grafts via an indwelling silk catheter connected to ALZET pump (flow rate 0.11 ul/hr). After 28 days, the fat grafts were excised and analyzed for mass on a standard balance and volume using gas pycnometry (Accupyc II), followed by fixing and processing for histological analysis.

Results: Insulin and dexamethasone were released at a controlled rate from the pump through the porous silk fiber. In-vivo, mass and volume measurements of fat grafts that received silk catheter and 300ug or 200ug dexamethasone were higher than fat grafts containing silk catheter and vehicle only.

Conclusion: In-vitro cumulative release profiles and in-vivo volume retention study showed the feasibility of using silk catheters to locally deliver adipogenic agents directly into a fat graft. The potential application of this perfusion system allows for greater flexibility and ease-of-use for future studies involving pharmacologically active agents in creating an adipose tissue graft with stable volume retention.

DELIVERY OF ADIPOSE DERIVED STEM CELLS VIA HYALURONIC ACID HYDROGEL MICROCARRIERS

Introduction: Stem cell therapy holds promise, but injectable applications may be limited by poor cell engraftment and viability. Injecting cells attached to microcarriers (MCs) could improve viability and maximize the availability of cells and secreted factors to the recipient. Therefore, the objective of this work was to synthesize degradable hydrogel MCs and demonstrate their potential for cell expansion and seamless delivery.

Methods: The HyStem-C™ hydrogel (BioTime, Alameda, CA) was used within a water-in-oil emulsion to study the effects of stir rate and sterilization on fabricated MCs. Next, adipose derived stem cell (ADSC) seeding efficiency and growth kinetics in suspension culture were observed. Lastly, the viability of ADSCs on MCs was assessed after passing through needles of various gauge sizes.

Results: Hydrogel MCs were successfully fabricated and a linear relationship between the stir rate (RPM) and the resulting diameter of the MCs was established (Fig. 1A-C). Mean MC diameter ranged from 135 to 276 microns for mixing rates between 800 and 1141 RPM. Ethanol sterilization and gamma irradiation were effective in maintaining MC size, morphology, and ability to support stem cell culture (Fig. 2A-B). ADSC seeding efficiencies of 50-80% were observed with minimal cell loss (Fig. 3A-B). ADSC-colonized MCs were passed through 20 and 25 gauge needles with minimal cell loss (Fig. 3A-B).

Conclusions: In order to improve the delivery and engraftment of cultured stem cells, MCs were fabricated from a hydrogel matrix that is known to support stem cell attachment. The diameter of the resulting MCs could be controlled by variations in the stir rate (Fig. 1) and multiple sterilization techniques may be utilized to prepare them for stem cell culture. The ADSCs attached efficiently and proliferated with time, indicating that these hydrogel MCs retained the necessary surface factors to support stem cell culture and expansion (Fig. 2). To demonstrate that stem cell coated MCs could be seamlessly delivered, they were successfully passed through a 25 gauge needle (Fig. 3). Taken together, these data demonstrate a hydrogel based MC system with the potential to improve the successful delivery, localization, and engraftment of stem cells.
6 DELIVERY OF ADIPOSE DERIVED STEM CELLS VIA HYALURONIC ACID HYDROGEL MICROCARRIERS
Presenter: Thomas Zarembinski, PhD
Authors: Hsiung MC, Zarembinski T, Tew WP, Erickson IE
BioTime Inc

7 THE EFFECTS OF SYNTHETIC POLYMER BASED BIOMATERIALS ON THE BEHAVIOR OF ADIPOSE DERIVED PERI-VASCULAR STEM CELLS (PSC) IN VITRO
Presenter: Christopher C. West, MBChB, BMedSci, MRCS(Eng)
Authors: West CC, Murray IR, Jiang Z, Zhang R, Stewart KJ, Hay DC, Bradley M, Peault B
The University of Edinburgh

Introduction: Adipose derived stem cells show great promise for cell based therapies and tissue engineering. Before this potential can be realized, substrates that permit their safe and stable expansion whilst also supporting their ultimate therapeutic action need to be developed. We have identified 5 synthetic polymer substrates that selectively bind adipose derived PSC using a high throughput microarray screening platform (IFATS 2011 – Miami). In this current work we demonstrate the influence that these substrates have on PSC behavior in vitro.

Methods: PSC were isolated from human adipose tissue by fluorescence activated cell sorting. Polymers were dissolved in tetrahydrofuran (2% w/v) and spin coated on to the surface of standard glass coverslips. Cells were cultured in standard medium (DMEM+20%FBS), or differentiation medium (osteo, adipo, chondro). Maintenance of PSC phenotype was assessed using flow cytometry and immunocytochemistry. Growth kinetics was calculated by performing cell counts at defined intervals. Differentiation was assessed using histological stains (Oil red O, Alizarin red, Alcian Blue) and qPCR for specific genes associated with osteo, adipo and chondrogenesis.

Results: Polymers demonstrated the ability to support stable PSC expansion through extended periods of in vitro culture whilst maintaining their ability to differentiate. Specific polymers were identified that significantly enhanced the growth rate of PSC. Polymers were also identified that preferentially supported specific lineage differentiation.

Conclusions: Synthetic polymers are ideal candidates for scaffolds in tissue engineering and regenerative medicine and offer a number of advantages over natural alternatives. We have identified 5 distinct polymers that show high binding affinity for PSC and can enhance proliferation whilst maintaining a stable phenotype through long periods in vitro. Furthermore these polymers demonstrate the ability to maintain multipotency and facilitate subsequent differentiation of PSC. We are currently investigating the potential of these polymers as scaffolds for tissue engineering in vivo.
**Introduction:** The adipose stromal vascular fraction (SVF) is a readily available source of regenerative cells for cell-based therapies. While SVF cell suspensions have been directly injected into diseased tissues, hydrogel encapsulation of SVF cells may offer a more effective option for cell delivery. A hydrogel spheroid provides cellular localization and immobilization, dosage control, and protection of cells against inflammation and mechanical forces. This report describes the automated production of SVF-loaded alginate hydrogel spheroids utilizing a computer-aided 3D bioprinting system.

**Methods:** Stromal vascular fraction cells from human adipose were suspended at 3 x 10^6 cells/mL in 1.5% sodium alginate in culture medium. The cell suspension was loaded into a cell dispensing module fit with an 18-gauge pen. The dispensing module was incorporated into a bioprinting system known as the BioAssembly Tool (BAT). Commands and 3D coordinates were coded directly into the system to print alginate droplets containing SVF cells into a 1.1% CaCl2 solution. Resulting hydrogel spheroids were analyzed with phase contrast microscopy and nuclear staining. Additional spheroids were cultured in a microcarrier spinner flask. A live/dead fluorescence assay was performed on cultured spheroids.

**Results:** The 3D printer (BAT) supported the rapid dispensing of SVF-alginate droplets that formed gelled spheroids when immersed in CaCl2. Computer programming of the BAT resulted in automated, consecutive production of multiple spheroids. Phase contrast and epifluorescent microscopy revealed the presence of SVF cells distributed throughout the alginate spheroids (Figure 1A, 1B). A live/dead assay of cultured spheroids (Figure 1C, 1D) confirmed viability of encapsulated SVF cells.

**Conclusions:** Direct-write 3D bioprinting can be used to produce alginate hydrogel spheroids for therapeutic delivery of viable, encapsulated SVF cells. An automated 3D printing system may meet critical requirements for controlled, reproducible, high-throughput manufacturing of encapsulated regenerative cells for implantation.
COLORECTAL TISSUE ENGINEERING: COMPARATIVE IN VIVO AND IN VITRO STUDY FOR OPTIMAL SCAFFOLD SELECTION

Presenter: Quentin Denost, MD
Authors: Denost Q, Adam JP, Montembault A, Bareille R, Siadous R, Delmont S, Rullier E, David L, Bordenave L

INSERM Bioingenierie Tissulaire U1026

Background: Regenerative medicine technology may provide new surgical tool for alternative therapy to conventional colorectal surgery in elective indications. The aim of this study was to select an optimal bioscaffold for colorectal tissue engineering.

Methods: We compared two bioscaffolds with in vitro and in vivo experiments: porcine Small Intestinal Submucosa (SIS) vs. chitosan hydrogel matrix. Endpoints of in vitro experiments were rates of adhesion and proliferation, and capacity to maintain mesenchymal stem characteristics after seeding. In vitro, we assessed and compared adhesion, proliferation and characterisation of human Adiposed Derived Stem Cell (hADSC) in both bioscaffolds. In vivo, a 1x2 cm whole layer was excised on the anterior wall of the cecum in 16 rabbits. Animals were randomly divided into two groups: rabbits with cecum defects given porcine SIS graft (SIS group, n=8) and rabbits with cecum defect given with chitosan hydrogel graft (Chitosan group, n=8). Endpoints of in vivo experiments were animal survival, scaffold stability in situ and histologic analyses performed by hematoxylin–eosin staining (H&E) at 4 and 8 weeks.

Results: In vitro, rates of adhesion and proliferation were better with SIS scaffold with an optimal cells seeded density of 50000 per centimetre square. However, this difference between both scaffold was not significant. In vivo, one animal died in each group after postoperative occlusion in SIS group and after postoperative leakage with peritonitis in Chitosan group. At 8 weeks after implantation, weight gain was similar between the two groups. Specimen obtained from the Chitosan group showed lower infiltration of inflammatory cells and granulation tissue formation into the neocolon wall than in SIS group. Muscle layer regeneration was not complete but was more developed in Chitosan group.

Conclusion: Outcomes of in vitro experiments did not differ significantly between the 2 groups. However, macroscopic and histological findings revealed a better wound healing of the colonic wall in the Chitosan group. These results suggest that chitosan hydrogel matrix could be the scaffold of choice for colorectal tissue engineering probably due to its antimicrobial effect and to its specifically biodegradability in colonic location.

THE EFFECTS OF THE CHEMOTHERAPEUTIC AGENT TAMOXIFEN ON ADIPOSE-DERIVED STEM CELLS

Presenter: Steven Pike, MD

Cooper University Hospital and Cooper Medical School at Rowan University

Background: Adipose-derived stem cells (ASCs) have multipotent differentiation capacity which may play an important role in reconstructive surgery. Chemotherapy can impair the healing abilities of tissue. Accordingly, this project is directed by the hypothesis that chemotherapeutic agent tamoxifen (TAM) affects the viability and function of hASCs.

Methods: hASCs were harvested from consenting patients who were chemotherapy and radiation negative. hASCs were expanded in M199 media + 10% FBS and antibiotics and re-fed every 2-3 days for up to 3 weeks. Standard growth curves and cell doubling times were calculated. Cytotoxicity of TAM was determined after incubation of hASCs with TAM for 24 hours followed by counting living vs dead cells. In addition, cells were incubated in adipogenic and osteogenic induction media in the presence of TAM (1 x 10^-3 M) in order to evaluate differentiation capacity. Cells were incubated with TAM and harvested for analysis of gene expression by qPCR for the presence of estrogen alpha (ER-α) and beta (ER-β) receptor mRNA. Also, hASC's were analyzed for the presence of ER-α and ER-β receptors. Thirdly, Annexin V was used to evaluate cytotoxicity of TAM.

Results: ASC doubling time was determined to be approximately 48 hours. With the MTT assay, it was determined that a concentration of 2.0 x 10^-5 M killed cells completely within 24 hours, 2.0 x 10^-5 M allows >24 hour survival, and 1.0 x 10^-5 M allows survival for 3 weeks. At 1.0 x 10^-5 M, TAM inhibited cell proliferation as well as differentiation of hASCs into adipocytes and changed cell morphology for osteocyte differentiation. Immunofluorescence microscopy showed the presence of ER-β but not ER-α receptors on ASCs. Cell death from TAM was found to be attributable primarily to apoptosis (i.e., independent of ER receptors).

Conclusion: This study indicates that tamoxifen has cytotoxic effects against hASCs and appears to interfere with the ability of hASCs to differentiate. The cytotoxic effect of tamoxifen involves the combination of apoptosis and inhibition of proliferation.
LIDOCAINE EFFECTS ON HUMAN ADIPOSE-DERIVED STEM CELLS: THE PHANTOM MENACE?

Presenter: AnneClaire Girard, PhD
Authors: Girard A, Gunasekaran M, Roche R, Hoareau L, Festy F

Introduction: Adipose tissue is a reliable source of mesenchymal stem cells which can be easily obtained from lipoaspirates. However, lipoaspiration procedure often involves a local anaesthesia at the fat donor site that is commonly carried by lidocaine. While controversial studies have described the effect of this drug, there was a need for further research to clearly indentify the impact of lidocaine on adipose-derived stem cells. In this study, investigations were focused not only on lidocaine-induced toxicity, but also on cell cycle and on autophagy within adipose-derived stem cells.

Methods: Adipose-derived stem cells (ADSCs) from lipoaspirates were grown in culture before being treated with different clinical doses of lidocaine, for different times of exposure (from 1h to 24h). On the one hand, cytotoxicity was measured by flow cytometry with annexin V /propidium iodide staining and cell cycle analysis was performed in parallel, using BrdU. On the other hand, autophagy was investigated by immunofluorescence staining, western blot and electron microscopy.

Results: Lidocaine affected cell viability, particularly after 24h, but apoptosis was not involved in this cytotoxicity. In parallel, lidocaine led to a sudden cell cycle arrest in G0-G1 phase. Regarding cell morphology, lidocaine induced a massive vacuolization in the cytoplasm and autophagy was detected by immunostaining and western blot. Electron microscopy could confirm the presence of phagophores in early treatment (2h), and the presence of autophagosomes in late treatment (24h). Inhibition of autophagy increased lidocaine-cytotoxic effect.

Conclusion: Lidocaine clearly impairs ADSC viability and growth. Therefore, for the use of these cells in regenerative medicine, appropriate handling of adipose tissue should be considered in order to remove lidocaine and avoid its deleterious effects. In this way, ADSCs seem able to adapt in response to this drug, involving autophagy as a cytoprotective mechanism against lidocaine attack.

NEUROGENIC STEM CELL (NSC) AND MESENCHYMAL STEM CELL (MSC)-MEDIATED TUMOR-TARGETED GENE THERAPY BASED ON LARGE-SCALE TRANSFECTION OF PLASMID DNA INTO PRIMARY ADIPOCYTES

Presenter: Patricia Aubanel, MD
Authors: Soriano RA, Aubanel P, Haghighat N, Torfi H

Invitrix Therapeutics Inc

Cancer continues to be one of the leading causes of mortality and morbidity throughout the world. Current conventional cancer therapies are often symptomatic and passive in nature. It is believed that one of the major obstacles in developing effective cancer therapies their lack of sufficient specificity for tumors. Human mesenchymal stem cells (MSCs) are non-hematopoietic progenitor cells that can be obtained from adipose tissue, expanded and genetically modified in vitro, and subsequently used for therapeutic cancer strategies in vivo. MSCs are capable of communicating with other cells in the human body and even appear to target areas of injury in response to “homing” signals of cellular damage. It has been discovered that MSCs possess tumor-oriented homing capacities and are thus a promising option for use as cell therapy carriers in the delivery of therapeutic agents into tumor sites. These MSCs were isolated using human processed lipoaspirate (PLA) cells that are capable of differentiating into multiple mesenchymal lineages and can be induced to form neural stem cells (NSCs). In this study, a nonviral vector, pORF5-codA plasmid containing the E. coli cytosine deaminase, was used to introduce the therapeutic gene tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to MSCs derived from adipose tissue. Meanwhile, the characterization, transfection efficiency, cytotoxicity, cellular internalization, and mechanism used by the nonviral vector were evaluated. The in vitro expression of this plasmid from the MSCs was demonstrated with luciferase reporter assays and electroporation pulse tests. Additionally, the human NSCs in this study were also an ideal vehicle for cell replacement and gene transfer. The homing ability of the MSC-plasmid was further investigated in vivo in the targeting of brain tumors. The transfected gene targeted strategy is based on the MSC’s capability of tumor-directed migration and incorporation, as well as the in situ effectiveness of nonviral plasmids in transferring the therapeutic gene to MSCs. The strategy is also based on the feasibility of using MSCs as targeted gene delivery carriers, indicating that MSCs could be a promising tumor-targeting therapeutic tool in future cancer gene therapies.
ADMINISTRATION OF THE STROMAL VASCULAR FRACTION (SVF) AMELIORATES CHRONIC AUTOIMMUNE ENCEPHALOMYELITIS

Presenter: Bruce A. Bunnell, PhD
Authors: Bunnell BA, Semon J, Strong AL, Zhang X, Gimble JM

Tulane University School of Medicine

The administration of adipose-derived stem cells (ASCs) represents a promising therapeutic approach for the treatment of autoimmune diseases, as the cells have been demonstrated to have immunosuppressive properties. The uncultured, non-expanded counterpart of ASCs, the stromal vascular fraction (SVF), is composed of a heterogeneous mixture of cells. The immunosuppressive capabilities of SVF have not been thoroughly investigated for the treatment of autoimmune diseases. In this study, the ability of murine and human SVF cells compared to culture expanded ASCs for the treatment of myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalitis (EAE) in C57Bl/6 mice. SVF or ASCs were administered intraperitoneally either concomitantly with the induction of disease or after the disease was established. While the data indicate that the intraperitoneal administration of either SVF or ASCs effectively inhibited disease severity over the course of the disease, the results indicate that the SVF was statistically more effective than ASCs. Both cell types demonstrated a reduction in tissue damage, loss of myelin, decreased inflammatory infiltrates and reduced levels of IFN-gamma and IL-12 in the serum. Based on this data, SVF appears to be more effective at inhibiting or reducing disease severity than culture-expanded ASCs.

HYPOXIA INFLUENCES ADIPOKINE PRODUCTION; IMPLICATIONS FOR BREAST CANCER PREVENTION

Presenter: Irene Pien, BS, BA
Authors: Pien I, Fisher M, Bond J, Ibarra-Drendall C, Klitzman B, Seewaldt V, Hollenbeck ST

Duke University School of Medicine

Purpose: Obesity is associated with breast cancer formation. Cytokines secreted by adipose tissue (adipokines) may be drivers of ER+ breast cancer. Adipose tissue responds to hypoxia by secreting pro-inflammatory adipokines. We evaluated serum adipokines in relation to body mass index (BMI) in patients at high risk for breast cancer. Next, we tested the hypothesis that hypoxia acts as a mediator for altering preadipocyte and mature adipocyte adipokine release.

Methods: IRB Approved: Peripheral blood was drawn from 74 women followed in our high-risk breast clinic. Serum was evaluated using ELISA to quantify adipokine levels and this was correlated with BMI. In Vitro: Human preadipocytes (PA), mature adipocytes (MA) and dermal fibroblasts (DF) were kept in either normal or hypoxic (0.5% O2) conditions for 48 hours to generate conditioned media. Soluble IL6, Adiponectin (Adp) and Leptin (Lp) were quantified using ELISA.

Results: Serum IL6 and Adp did not correlate with BMI. However, elevated serum Lp was associated with obesity (p<0.05) in our patients (Fig 1A). At both normal and hypoxia, conditioned media from PA’s contained more IL-6 than conditioned media from MA’s and/or DF’s (p<0.05). Hypoxia resulted in a decrease in soluble IL6 from PA conditioned media (0.7-fold) and an increase in soluble IL6 from MA conditioned media (2.9-fold). At both normal and low oxygen conditions, MA’s produced significantly more soluble Adp and Lp than PA’s and DF’s (p<0.05). In MA’s, hypoxia decreased soluble Adp (24-fold) and increased soluble Lp levels (1.3-fold) (Fig 1B). Next, we evaluated the effect of BMI. In normal oxygen, media from MA’s from high BMI (>30) donors contained 3.1-fold more Lp than media from MA’s from low BMI (<25) donors (p<0.05). Hypoxia increased soluble Lp in media from MA’s from both high and low BMI donors (2.0-fold and 5.5-fold respectively).

Conclusion: Serum leptin is elevated in obese patients in comparison to normal weight and overweight patients. Mature adipocytes are a powerful source of leptin production and this may be enhanced by obesity and hypoxia. Targeting obesity and adipokine production may be a valuable approach to prevent the development of breast cancer.
HYPOXIA INFLUENCES ADIPOKINE PRODUCTION; IMPLICATIONS FOR BREAST CANCER PREVENTION
Presenter: Irene Pien, BS, BA
Authors: Pien I, Fisher M, Bond J, Ibarra-Drendall C, Klitzman B, Seewaldt V, Hollenbeck ST
Duke University School of Medicine

HUMAN METABOLICALLY ACTIVE BROWN ADIPOSE TISSUE DERIVED STEM CELLS
Presenter: Francisco Silva, BS
Authors: Silva F, Vargas V, Grainger D, Bull D, Patel A
BioRestorative Therapies

Background: Although multipotent adult progenitor cells have been described in various white adipose depots, metabolically active brown adipose tissue derived stem cells have not been identified in adult humans to date.

Methods: Mediastinal adipose tissue depots were collected and studied in situ to identify and characterize human brown adipose derived stem cells. We define their phenotype and metabolic function.

Results: Here we demonstrate a resident stem cell population within depots of brown adipose tissue from adult human mediastinum. Cells from this tissue exhibit multi-lineage potential with capacities to undergo osteogenesis, chondrogenesis and both brown and white adipogenesis. Directionally differentiated brown adipocytes exhibit a distinct morphology and gene expression profile, with functional properties characteristic of brown adipose tissue in vivo.

Conclusions: These results uniquely demonstrate a resident stem cell population within depots of brown adipose tissue from adult human mediastinum. Cells from this tissue exhibit multi-lineage potential. These brown adipose-derived stem cells may offer a new target to activate and restore energy homeostasis in vivo for the treatment of obesity and related metabolic disorders.
**DEVELOPMENT OF AN AUTOLOGOUS THERAPEUTIC COCKTAIL FROM SVF-DERIVED CONDITIONED MEDIUM**

**Presenter:** Brian Johnstone, PhD  
**Authors:** Johnstone BH, Van Natta B, March KL  
**Indiana University School of Medicine**

**Introduction:** Cell-free adipose stem cell-conditioned medium (ASC-CM) is comprised of many beneficial bioactive factors having angiogenic, anti-apoptotic and immunomodulatory properties that function synergistically to protect and repair at risk tissues in various rodent disease models of hypoxic-ischemic encephalopathy, stroke, amyotrophic lateral sclerosis and peripheral arterial disease. Previous studies used ASC-CM derived from passaged ASCs; however, no studies have evaluated the biological properties of CM derived from minimally-manipulated freshly isolated SVF (SVF-CM). The latter may have similar effects as CM from passaged ASCs and, furthermore, would require minimal infrastructure to produce and face fewer potential regulatory hurdles than cellular therapies or CM derived from culture expanded cells. It is not known, though, how the presence of other cell types in SVF, such as leukocytes and endothelial cells, would influence SVF-CM potency. Therefore this study assessed the biological properties of both uncultured and briefly cultured SVF-CM.

**Methods:** The bioactivity of SVF-CM prepared by three different methods was compared to CM from passage 3 ASC (p3 ASC-CM). Freshly isolated SVF was cultured in basal medium (BM) under three different conditions before collecting CM. These conditions were: (CM 1) culture directly in BM; (CM2) culture in complete medium overnight before exposing to BM; and (CM3) culturing to 480% confluence before exposing to BM. Each CM was evaluated for the ability to promote endothelial cell (EC) survival in growth factor-deficient minimal medium (MM).

**Result:** After 3 days, only 17±15% EC (mean ± SEM percent of plated cells) survived in MM, compared to 117±25% in complete medium (P<0.05). Addition of p3 ASC-CM to MM induced EC proliferation (193±25%; P<0.05). Survival of EC in MM+CM3 was no different than in complete medium (130±36 %), indicating total protection. Survival of EC exposed to CM1 (50±20%) and CM2 (23±15 %) was slightly enhanced over BM, but did not achieve significance.

**Conclusions and Future Directions:** SVF-CM promoted the survival of endothelial cells, which may be indicative of therapeutic potential. Ongoing studies include further optimizing CM production, characterizing bioactivity, determining composition.

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**EFFECT OF DOxorubicin ON AdIPOSE-Derived STEM CELLS AND BREAST Cancer CELL LINEs: CAN WE INCORPORATE CHEMOTHERAPY INTO OUR RECONSTRUCTIVE STRATEGIES?**

**Presenter:** Wakako Tsuji, MD, PhD  
**Authors:** Tsuji W, Chung CW, McLaughlin MM, Valentin JE, Marra KG, Rubin JP  
**University of Pittsburgh**

**Introduction:** Breast deformity after surgery is distressing for breast cancer patients. Autologous fat transfer is gaining acceptance for soft tissue repair but in the post-mastectomy or lumpectomy patient there is concern that adipose-derived stem cells (ASCs) within fat may have tumor promoting effects and increase the rate of cancer recurrence. Incorporating tumor-suppressing elements into the graft may be an ideal way to reconstruct a natural appearing breast while minimizing local recurrence risk. This study aimed to determine if doxorubicin could be used to inhibit breast cancer cells while maintaining the viability and functionality of ASCs in vitro.

**Materials and Methods:** Human ASCs were isolated from non-diabetic female patients between 35 and 60 years of age (n=3). For breast cancer cell lines, estrogen receptor-positive BT-474 and triple-negative MDA-MB-231 were used. Doxorubicin-HCl was added to ASCs in ASC maintenance media, ASCs in differentiation media, and breast cancer cells individually at concentrations of 0, 10, 30, 100, 300, 1000, 3000, or 10000 nM (n=4). Proliferation, viability, and differentiation capacity were assessed with commercially available CyQuant, MTT, and AdipoRed assay kits, respectively.

**Results:** Dose-dependent inhibition was observed in ASCs and both breast cancer cell lines. The IC50 of doxorubicin on ASCs, BT-474, and MDA-MB-231 were 901.3, 656.5, and 333 nM, respectively. Doxorubicin didn’t inhibit ASCs differentiation into mature adipocytes.

**Discussion:** In vitro cytotoxicity studies demonstrated greater doxorubicin sensitivity in BT-474 and MDA-MB-231 breast cancer cell lines than in ASCs. Furthermore, differentiation into mature adipocytes was not affected by doxorubicin presence. These findings suggest that incorporating doxorubicin in fat grafts for breast reconstruction following primary breast cancer surgery may be a viable option for reducing the risk of cancer recurrence.
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ROLE OF PATIENT DEMOGRAPHICS AND DEPOT SITE ON ADIPOSE STEM CELL FUNCTIONALITY AND TUMORIGENESIS
Presenter: Amy F. Lin, MPH
Authors: Lin AF, Strong TA, Rhodes LV, Semon JA, Zhang X, Shi Z, Zhang S, Gimble JM, Burow ME, Bunnell BA
Tulane University School of Medicine
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FAT GRAFTING FOR TREATMENT OF POSTOPERATIVE SOFT TISSUE CONTOUR DEFORMITIES AGGRAVATED BY LATE SIDE EFFECTS OF RADIATION THERAPY
Presenter: Viacheslav S. Vasilyev, PhD
Authors: Vasilyev VS, Vasilyev SA, Vasilyev YS, Vasilyev IS, Karpov IA, Kazachkov EL, Orlova SS, Migranova NV
South Ural State Medical University

Introduction: Treatment of postoperative deformities of soft tissues aggravated by ionizing irradiation is considered to be challenging problem. On one hand utilizing of artificial materials in such clinical situation is related to a high risk of implant exposure. On the other hand excision of radiodamaged tissues often require applying difficult reconstructive techniques. Fat grafting is an effective, simple and minimally invasive surgical procedure that allows both to create volume of soft tissues and to restore irradiated tissues.

Methods: Since 2010 overall number of 15 patients, who needed restoring of postoperative contour soft tissue deformities aggravated by late side effects of radiation treatment, was treated with fat grafting. Coleman technique of fat transfer was used. Outcomes were assessed by photographing, ultrasound, MRI, ultrastructural analysis. For grading of late side effects of radiotherapy LENT-SOMA scale was used.

Results: In all cases goals of treatment were achieved. Number of sessions ranged from 3 to 6, and depended on grade of radiation damage of soft tissues and volume needed to be restored. The more severe radiation damage was the more session needed to be done to create required volume. With every following procedure quality of soft tissues has become better. Furthermore we observed restoration of skin sensitivity within the irradiated area. According to our experience fat grafting could be method of choice in two clinical situations. First, zone of radio damaged tissues extends for several anatomic areas or unresectable. Second, treatment of contour deformities of aesthetically important areas (e.g. face).

Conclusions: Although treatment of severe soft tissue deformities aggravated by late side effects of radiotherapy with fat grafting usually requires several consecutive procedures and takes a long period of time, it could be successfully applied for correction of contour deformities despite of the grade of radio damaged soft tissues. In order to make procedure more effective further investigations concerning enrichment of lipoaspirate with stromal-vascular fraction or adipose derived stem cells are need to be done.
IS THERE AN IDEAL DONOR SITE OF FAT FOR SECONDARY BREAST RECONSTRUCTION?
Presenter: Kevin H. Small, MD
Authors: Small KH, Petruolo O, Choi M, Karp N
New York Presbyterian Hospital

Introduction: Previous work has documented the validity of 3D imaging to assess surgical outcomes of autologous fat grafting (FG). However, no study exists to delineate the ideal donor site of fat for secondary breast reconstruction. Millard argued in Principilization of Plastic Surgery that tissue losses should be replaced in kind; thus, theoretically, plastic surgeons should harvest adipose tissue from neighboring donor sites to optimize aesthetic results. The following study compares fat graft survival from two distinctive anatomical sites utilizing three-dimensional imaging.

Methods: All patients receiving fat grafting to the reconstructed breast from 2009-2012 were enrolled in the study. The patients were divided into two groups: Patients who had fat harvested from the abdomen and those who had fat harvested from the thighs. FG surgery was performed using a modified Coleman technique to achieve symmetry. 3D scans were obtained on all patients. 3D imaging was performed using the Canfield VECTRA system, and volumes were analyzed using Geomagic software.

Results: In the observed time period, a total of 73 patients (109 breasts) received autologous fat transfer and associated 3D images. 46 patients (66 breasts) averaged 101.17cc of fat injected from the abdomen, and 27 patients (43 breasts) averaged 101.98cc of fat injected from the thighs. The abdominal subset had 81.98% volume retention at 16 days, 63.15% at 49 days, and 44.63% at 140 days. The thigh subset had 85.80% at 16 days, 62.71% at 49 days, and 46.43% at 140 days. (p>0.05) Patients were also stratified by radiation exposure and amount of volume injected. Neither variable had any affect on donor site viability. (p>0.05)

Conclusions: Our data suggests that the thighs or abdomen fat graft donor sites have fat graft donor sites have no effect on percent fat graft volume retention. Furthermore, radiation or volume inject does not affect donor site viability. Longer-term studies are needed to assess the stability of the breast after autologous fat transfer.

IMMEDIATE LIPOFILLING OF LATISSIMUS DORSI (LD) FLAPS AND PRE-EMPTELI LIPOFILLING OF MASTECTOMY SKIN FLAPS IN BREAST RECONSTRUCTION
Presenter: Stephen J. Goldie, MBChB, MRCS, PhD
Authors: Goldie SJ, Raine C, Dixon JM
St. Johns Hospital

Introduction: Lipofilling has become a routine part of breast reconstructive and augmentation surgery. However, following flap reconstruction any lipofilling has been delayed until after the primary surgery and undertaken as a secondary procedure. Recent experience in our Unit suggests that immediate lipofilling of latissimus dorsi (LD) flaps is safe and advantageous in order to increase the volume of the reconstruction. Pre-emptively lipofilling the thin skin flaps left following mastectomy may also increase the volume and quality of the skin.

Methods: Six patients undergoing mastectomy and flap reconstruction have undergone a combination of either immediate lipofilling of the LD and/or lipofilling of the mastectomy skin flaps. Fat was harvested from the abdomen and thighs using syringes and liposuction cannulae. It was processed and injected using the standard Coleman technique.

Results: No immediate complications of either lipofilling procedure have been observed. Long-term follow up data is being accumulated. Initial findings suggest improved volume and skin quality of the reconstruction.

Conclusions: Lipofilling of mastectomy skin flaps combined with immediate lipofilling of LD flaps may enhance the overall volume and quality of the breast reconstruction.
IMMEDIATE MEGA VOLUME FAT GRAFTING TO THE BREAST FOLLOWING REMOVAL OF BREAST IMPLANTS

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

Introduction: The purpose is to share the authors experience with immediate mega volume fat grafting to the breast following removal of breast implants over a 2 years period.

Material and Methods: Following removal of the implants, capsulotomy is perfomed. The recipient site is then expanded by infiltration. This is followed by harvesting and preparation of the fat. Using the vibroliposuction machine, multidirectional and multilayered tunneling is performed in the recipient site, in a way to fashion a matrix for fat grafting. Injection is then carried out, using multiple access points, with a custom made V shaped 3 mm multihole cannula designed by the senior author, enabling simultaneous vibration of the recipient site during fat injection. A drain is inserted within the capsule before closure of the wound.

Results: The technique was applied for 45 patients, excluding cases with ruptured implants. The injected volumes per session ranged from 300 to 600 ml, taking into account a 1.5:1 ratio of transplanted fat to original size of implant. Scoring of the capsule, followed by placement of an intracapsular drain was performed in all the cases. The operative time ranged between 45 to 60 min. Only one injection session was required. The follow up period ranged between 6 and 24 months.

Discussion and Conclusion: Simultaneous fat injection and vibration following multidirectional multilayered tunneling improves diffusion of the injected fat in the created tunnels of the matrix, enabling larger fat volume injection in addition to reducing the operative time. Immediate mega volume grafting is achievable thanks to preexpanded breast skin and subcutaneous tissues following removal of the implant that provide a large third space for fat injection. Scoring of the capsule and placement of drains insure total collapse of the capsule and further expansion of the breast third space, increasing the possible volumes of fat transfer.

10 YEAR EXPERIENCE WITH MASSIVE FAT GRAFTING (>1000CC/PATIENT) TO BUTTOCKS: RESULTS, COMPLICATIONS, AND LESSONS LEARNED

Presenter:  Ricardo L. Rodriguez, MD
Authors:  Rodriguez RL, Conde Green A

Introduction: For the past 10 years the author has accumulated a large series of over 200 patients who received massive volume (>1000cc/patient) fat grafting to the buttocks with a very safe and reliable record. Surgical planning and esthetic goals have evolved with experience, while keeping the guidelines for harvesting, processing and injection of fat graft material constant. New insights have included: a) the predictability and reliability of massive fat grafting to the buttocks when adhering to careful fat graft processing techniques, b) the importance of the Lateral trochanteric fat pad as a column of support for the buttock, and c) Paradoxically, in a buttock augmentation reduction of the surface area of the buttock mass enhances the appearance of projection and lift.

Methods: 1) Anesthesia- TIVA (no intubation). 2) Tumescent solution 15cc lido 1% with epi, 10cc Marcaine 0.5% with epi per liter RL. 3) Liposuction cannulae- 3mm diameter (initially Mercedes tip, past 5 years Hunstead tip) 2) Aspiration pressure – Initially 60cc Toomey tip syringe, last 4 years -400 mmHg vacuum aspirator 3) Decanting 10-15 minutes 4) Centrifugation- IEC Medilite centrifuge 1228G X 2½ mins, past 3 years Beckman Coulter Alegra R8 centrifuge 800g X 2½ mins 4) Injection- 10cc syringe, 18 gage blunt injection needle. 5) Injection planes- intramuscular, subcutaneous 6) Post op regimen- 3 weeks no sitting.

Results: Complications:

1) DVT, PE- none
2) Seromas- none that required aspiration
3) Fat necrosis- none evident by clinical exam
4) Infections- 1 abscess in ant abdominal wall donor site requiring drainage.
5) Over 200 cases no return to OR for fat graft because of graft loss.

Conclusions and Lessons learned: A) Methods described provide an extremely safe and reliable technique for large volume fat graft to buttocks. B) The trochanteric fat pad is a column of support for the buttock mass. Liposuction in this area leads to ptosis of the buttock. Conversely, fat injection to this area reverses buttock ptosis. C) For any given buttock volume augmentation, the appearance of projection is enhanced by reduction of the surface area of the buttock mass. This is analogous to the case of a moderate vs high profile implant.
AESTHETIC GLUTEAL LIPOAUGMENTATION OR AESTHETIC GLUTEAL REMODELING BY FAT GRAFTING: THE “FRENCH TOUCH”

Presenter: Christophe Ho Quoc, MD
Authors: Ho Quoc C, Dlimi C, Delay E

Leon Berard Center

Introduction: The demand for aesthetic improvement of the gluteal region has been recently increasing among patients. The classic techniques used for gluteal augmentation based on prosthesis had a good result, but also presented important complications. Fat grafting is an interesting alternative to prostheses for gluteal aesthetic remodeling. Often associating liposuction in the areas with excess fat deposits, the gluteal lipomodeling or gluteal lipoaugmentation underlines the natural body contour. The purpose of this work is to describe our experience of gluteal modeling by fat grafting.

Material and Methods: A prospective study was undertaken including 34 patients desiring aesthetic gluteal remodeling by lipoaugmentation and liposuction of the areas with excess fatty tissue. The average fat tissue volume transferred was registered. The lipomodeling complications to the gluteal region were evaluated (infection, hemorrhage, fat embolism, seroma and dysesthesia). The result was evaluated a year after surgery by the surgeon and the patient using 4 marks: very good, good, average, insufficient.

Results: The average age was 38 years (27 - 65) and the body mass index was 21 (18 - 24.2). The average volume transferred was 320 cc (120 - 440). In our series there was no infection, hemorrhage or fat embolism. One case of seroma was registered and 30 cc drained by one puncture. The patients and the surgical team judged the result as good or very good in 85% of cases.

Conclusion: The aesthetic gluteal remodeling by fat transfer or aesthetic gluteal lipoaugmentation is a reliable and reproducible technique with very good results in the long run. A moderate and natural augmentation of the gluteal region is obtained, adapted to the patient’s desire. If it is associated to liposuction in the areas with excess fatty tissue, it redefines the body contour with minimal scaring. In conclusion, the aesthetic gluteal augmentation or aesthetic gluteal remodeling by fat transfer has met an important development in France because it corresponds to the French practice and to the increasing demand of gluteal improvement by augmentation without prosthesis.

NANOFAT GRAFTING: BASIC RESEARCH AND CLINICAL APPLICATIONS

Presenter: Patrick Tonnard, MD
Authors: Tonnard P, Verpaele A, Peeters G

Coupure Centrum Plastic Surgery Gent

Introduction: The indications for fat grafting are steadily increasing. Microfat grafting is typically carried out with thinner injection cannulas or needles up to 23 Gauge. We describe our experience of fat injection with even thinner injection needles up to 27 Gauge. The fat used for this purpose is processed into what we call ‘nanofat’. The cellular contents of nanofat are investigated, and clinical applications are described.

Material and Methods: Three fat samples are analyzed. The first fat sample is a classical lipoaspirate. The second sample is microfat, harvested with a multiport small-holed cannula. The third sample is microfat processed into nanofat. Processing consists of emulsification and filtering of the lipoaspirate. Emulsification is performed by repeated shifting between two connected 10 cc Luer-lok syringes. Fat samples are analyzed for adipocyte viability. Adipose derived stem cells (ASCs) are quantified using a CD34+ cell count. Stem cell quality is investigated by culturing the cells from the stromal vascular fraction (SVF) and the CD34+ subfraction in standard and adipogenic media.

Between May 2010 and September 2012, nanofat grafting has been performed in 67 clinical cases. Nanofat grafting was used for the correction of perioral and glabellar rhytids (81%), rhytids in the décolletage (11%), scars (6%) and dark lower eyelids (2%).

Results: The fat analysis showed that no viable adipocytes are left in the nanofat. Mesenchymal stem cells on the other hand are still richly present in the nanofat. Moreover, cell cultures show an equal proliferation and differentiation capacity of the stem cells from the three samples. Clinical applications show remarkable improvements in skin quality 6 months postoperatively. There were no major complications in this series. Infections, fat cysts, granulomas, or other unwanted side effects were not observed.

Conclusion: Emulsified and filtered microfat creates so called nanofat. Nanofat is injectable through 27 Gauge needles, opening a wide field of therapeutical indications. Research analysis shows that nanofat still contains a large amount of mesenchymal stem cells, which have kept their differentiation capacity. In clinical situations, nanofat seems suitable for skin rejuvenation purposes.
IN VIVO IMAGING OF APOPTOSIS AFTER
LIPOTRANSFER WITH/WITHOUT STEM CELLS
Presenter: Keisuke Takanari, MD, PhD
Authors: Takanari K, Toriyama K, Yagi S, Sato H, Yamamoto T, Funahashi Y, Gotoh M, Kamei Y
Nagoya University Graduate School of Medicine

WITHDRAWN

A PLEA FOR STANDARDIZATION IN FAT GRAFT REPORTING; A SIMPLE METHOD OF CREATING A STANDARD FAT VOLUME UNIT
Presenter: Jeffrey M. Hartog, MD
Author: Hartog JM
The Adrecyte Regenerative Medicine and Surgery Center

It is often extremely difficult to evaluate and compare results reported in publications on clinical fat grafting due to the variety of methods used to harvest and prepare the fat. This is particularly the case with large volume fat grafting. Clearly a report on grafting a given volume of decanted fat for example will, not be comparable to a report of a similar volume of fat centrifuged using the Coleman method. Even in decanted fat, the volume of actual fat will vary depending on the harvest method. Decanted at harvested with the Body-Jet method for example will have a significantly greater water content than other methods.

The author is proposing a simple method whereby a clinician can standardize the measured volume of actual fat grafted, irrespective of the method used to harvest and prepare the fat for grafting.

The method of grafting that provides the most compacted unit of fat is the Coleman Centrifugation method. It is suggested therefore that a clinician perform a Coleman method centrifugation of a sample of fat prepared by his usual method, in order to establish what proportion of his sample is actual compacted fat.

Our measurements in the operating room have established the following: With the Coleman centrifugation method being considered as a unit of 1, simple decanted fat harvested with standard tumescent technique and instrumentation provides 0.4 units of fat as compared to the Coleman method. Fat harvested and centrifuged using the Khouri technique provides 0.6 unit. In other words a practitioner who reports grafting 100cc of fat using the Coleman method, is grafting significantly more actual fat than a practitioner using the other methods mentioned. It is proposed that every practitioner evaluate his preferred method of harvest and preparation in this manner in order to provide such a ‘Coleman Unit’ of actual fat grafted for reporting and comparison purposes. The presentation will show examples of fat harvested and prepared by various methods, as well as washing techniques such as Puregraft.
A NOVEL THERAPY FOR CORRECTION OF POST-LIPOSUCTION CONTOUR DEFECTS: CASE SERIES AND REVIEW OF THE LITERATURE

Presenter: Som Kohanzadeh, MD
Authors: Kohanzadeh S, Martin MS, Collawn S
University of Alabama Birmingham

Introduction: Post lipectomy contour defects are a difficult problem. Despite many treatments described in the past, a good and reliable method has yet to be defined. We present a new method combining 3 modalities: traditional liposuction, laser-assisted liposuction and autologous fat-grafting to treat these contour problems.

Materials and Methods: 3 patients presented with various post-liposuction contour deformities. They all underwent combination therapies, including traditional liposuction, laser-assisted liposuction and autologous fat-grafting. For each treatment, Tumescent was injected directly before the procedure. No other anesthesia was used. Lipoaspirate was harvested from the available body sites, and injected into the associated depressions as needed. Laser-assisted liposuction was used with total energy delivered varying from 5,785 to 15,000 J.

Results: Case 1 - 34 yo female post liposuction and Lipodissolve on the upper lateral thighs, presented with skin atrophy, scar formation, telangiectasia, and concavities around the area of injection (Figure 1). 3 sessions of fat-grafting were performed with laser-assisted liposuction on the latter two treatments. Figure 2: 2 weeks post-treatment.

Case 2 - 43 yo female 1 year post liposuction, presented with the complaint of uneven abdominal contour (Figure 3). Laser-assisted liposuction and fat-grafting was used in one treatment session. Figure 4: 2 and 4 weeks post-treatment.

Case 3 - 46 yo female 1 year post liposuction of the inner thighs, presented with the complaint of abnormal contours of the inner thighs (Figure 5). Laser-assisted liposuction, traditional liposuction, and fat-grafting were used in one treatment session. Figure 6: 2 weeks and 8 months post-treatment.

All patients had correction of their contour irregularities and were very satisfied with their outcomes.

Conclusion: Liposuction has advanced as Illouz predicted with broad applications and use across medical fields. With this extensive usage, the complications associated with liposuction are also more apparent. Combined modalities for the treatment of complex deformities have been described, and here we demonstrate the efficacy of combined laser-assisted liposuction and fat grafting as an effective and reliable treatment.
HYBRID AUGMENTATION MAMMOPLASTY

Presenter: Sungsoo Park, MD
Author: Park S
Bong Bong Plastic Surgery Clinic

Natural look of breast after augmentation mammoplasty is a common end of both plastic surgeons and patients. Palpability and visible rippling after augmentation mammoplasty is not a simple complication to correct in relatively thin patients especially when there is not much soft tissues remain to cover the implant for smooth contour.

Asymmetry of the breast in accordance with the skeletal deformity, such as pectus excavatum, can not be easily reformed with insertion of silicone implant alone.

In the present study, volume enhancement was accomplished by silicone implant augmentation and contour management was improved with the help of fat graft technique. We named the technique “Hybrid Augmentation Mammoplasty”.

From May 2011 to Feb. 2013, the hybrid augmentation mammoplasty procedure were performed on 94 patients who expected to have palpable implants, visible rippling or asymmetry due to their soft tissue and skeletal condition.

Breast augmentation with silicone implant was done in regular pattern followed by grafting fat tissues utilizing water-jet device into the pre-pectoral, sub-glandular and subcutaneous layer.

During the follow-up period, any patient complained palpability of the implant and all of them were satisfied with the contour of their breast. Hereby we suggest that the hybrid augmentation mammoplasty can be an ideal, effective and useful option in management of thin skinned patients or patients with chest wall deformities.

THE IMPACT OF COLD STORAGE ON HUMAN LIPOAスピRATES

Presenter: Wei Z. Wang, MD
Authors: Wang WZ, Fang XH, Williams SJ, Stephenson LL, Baynosa RC, Jaeger N, Khiabani KT, Zamboni WA
University of Nevada School of Medicine

Purpose: Autologous fat grafting has gained popularity, particularly with the discovery of Adipose-derived Stem Cells (ASCs). The purpose for the present study was to determine whether human liposapirate can be stored in refrigerator for a few days after liposuction without affect the quality for later fat grafting.

Materials and Methods: Human lipoaspirates (n=16) were harvested using standard technique. Each of lipoaspirate samples was equally divided into 5 portions (5 ml of each). Fresh portion was processed immediately served as a control. Other portions were stored in the refrigerator (2-8°C) and processed at 24h, 48h, 72h or 96h later respectively. Three endpoints were assessed. The viability of ASCs was determined by the number of adherent ASCs after 24 hours culture of stromal vascular fraction. The viability of adipocytes was assessed by GPDH (glycerol-3-phosphate dehydrogenase) activity and measured by spectrophotometer at 340nm of OD in 96-well plates. The Infection of lipoaspirate was assessed by bacteria culture.

Results: Average age of the participants was 52±3.8 years (±SEM) and the average BMI was 26±1.6. The average number of adherent ASCs in fresh samples was 523000±88399. The average of GPDH activity (ΔOD 340 U/mL) in fresh samples was 0.22±0.02. Both GPDH activity and the number of adherent ASCs were gradually decreased in the cold stored samples as storage time prolonged. The statistically significant difference was found only in the samples stored in refrigerator for 96h as it compared to fresh samples. No bacteria were detected in any sample by bacteria culture.

Conclusions: Human liposapirates stored in refrigerator for 2-3 days after liposuction are still relatively good for later fat grafting.
**SIGNALING PATHWAYS TO ACTIVATE AND DIFFERENTIATE ADIPOSE-DERIVED STEM/STROMAL CELLS (ASCs) AFTER ADIPOSE TISSUE INJURY**

**Presenter:** Shinichiro Kuno, MD  
**Authors:** Kuno S, Doi K, Mineda K, Kinoshita K, Kato H, Yoshimura K  
*University of Tokyo School of Medicine*

**Introduction:** We had demonstrated that chemokines, such as I-TAC and SDF-1 (CXCR4 and CXCR7 ligands), and damage-associated molecular pattern molecules (DAMPs) activated hASCs in damaged adipose tissue (AT). We sought to further dissect ASC-activating mechanisms for establishing a therapeutic strategy controlling ASCs.

**Methods:** AT-damage-associated factors were collected as AT-soaked buffer (ATSB) by incubating fragmented ATs in buffered saline. We examined ASC response to ATSB under inhibition of CXCR4 or CXCR7. Similarly, we evaluated accelerating effects of ATSB on wound healing of skin ulcers in diabetic mice. As we found that HMGB1 was contained in ATSB, we examined the expression of HMGB1 receptors on hASC. We also performed microarray assay for ATSB-activated hASCs vs. non-activated hASCs.

**Results:** ATSB significantly promoted wound healing of diabetic ulcers. A CXCR7 inhibitor reduced migration of ASCs, while anti-CXCR4 neutralizing antibody inhibited network formation of ASCs (angiogenesis). Proliferative effects of ATSB on ASCs was also inhibited by blocking CXCR4 or CXCR7. Similarly, wound healing of diabetic ulcers was significantly impaired by CXCR4 or CXCR7 inhibition. Immunocytochemistry revealed that cultured ASCs expressed HMGB1 receptors such as RAGE, TLR2, and TLR4. Microarray assay results suggested that signaling pathways related to some upstream molecules such as IL-17 and TREM-1/DAP12 may be involved in ASC activation by ATSB and also that ATSB may contain inflammatory cytokine and growth factors such as TNF, IL-1α, IL-1β, and PDGF-BB. ELISA for ATSB detected low level of IL-17 in ATSB and immunocytochemistry clearly showed expression of TREM-1 and DAP12 by cultured ASCs.

**Discussions:** It was revealed that ASCs were activated in the wound healing process after AT damage in vitro and in vivo. Both CXCR7 and CXCR4 contributed to post-AT damage-ASCs activation and acceleration of diabetic wound healing. HMGB1 and TREM-1/DAP12 axis are also suggested to be involved in those. These findings suggested the substantial roles of damage-associated factors and inflammatory cytokines in ASC activation and gave insights into therapeutic strategies for impaired wound healing.

**Conclusions:** Overall, the adipose-derived bioscaffolds provide a uniquely inductive microenvironment that stimulates fat formation and tissue regeneration. Future work will focus on the further translation of the DAT as a clinical alternative or additive to autologous fat transfer for volume augmentation.

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**DECELLULARIZED ADIPOSE TISSUE AS A PLATFORM TECHNOLOGY FOR SOFT TISSUE RECONSTRUCTION AND AUGMENTATION**

**Presenter:** Lauren E. Flynn, PhD  
**Authors:** Flynn LE, Fuetterer L, Brown C, Yu C, Han T, Bianco J, Watkins JF  
*Queens University*

**Introduction:** With the goal of engineering new strategies for soft tissue augmentation and regeneration in plastic and reconstructive surgery, our group has developed a range of bioscaffolds derived from human decellularized adipose tissue (DAT) including implantable 3-D scaffolds and foams, as well as injectable micronized particulates, microcarriers, and gels [1,2,3]. The DAT technology holds great potential for clinical translation as an off-the-shelf tissue substitute for soft tissue reconstruction.

**Methods:** Human adipose tissue was decellularized via an optimized detergent-free protocol involving mechanical disruption, enzymatic treatment with trypsin-EDTA, and lipid separation using isopropanol. The DAT was further processed into the various scaffolding formats. In vitro studies with human adipose-derived stem cells (ASCs) were conducted to comparatively assess adipogenic gene and protein expression on the range of DAT-based bioscaffolds. The in vivo response was characterized over 16 weeks in an immunocompetent Wistar rat model, followed by a pilot large-animal study of the intact DAT (n=8) in a Gottingen mini-pig model, with assessment at 1, 3 and 6 months. All in vivo work complied with the Canadian Council on Animal Care (CCAC) guidelines and was approved by the UACC.

**Results:** Our studies have demonstrated that the adipose-derived matrices are adipo-inductive, and provide a uniquely supportive microenvironment for human ASC adipogenesis. Further, the histological and immunohistochemical in vivo results clearly indicate that the DAT-based bioscaffolds are non-immunogenic and integrate well into the host tissues, stimulating angiogenesis, adipogenesis, and soft tissue regeneration. Integration, angiogenesis and degradation rates can be tuned by varying the DAT processing methods and scaffold format.

DECELLULARIZED ADIPOSE TISSUE AS A PLATFORM TECHNOLOGY FOR SOFT TISSUE RECONSTRUCTION AND AUGMENTATION

Presenter: Lauren E. Flynn, PhD
Authors: Flynn LE, Fuetterer L, Brown C, Yu C, Han T, Bianco J, Watkins JF

Queens University

UNMODIFIED ADIPOSE TISSUE-DERIVED ECM INTRINSICALLY FACILITATES REMODELING OF ADIPOSE TISSUE CONSTRUCTS BUT NOT BONE

Presenter: Courtney Kim, PhD
Authors: Kim C, Lee JQ, Shimoda C, Paek HJ
Tissue Genesis Inc

Autologous extracellular matrix (ECM)-based scaffolds can play a pivotal role in tissue engineering as it supports cell adhesion and expansion, while avoiding adverse reactions upon transplantation. The adipose-derived stromal vascular fraction (SVF) embodies a rich source of adipose stem cells (ASCs) and growth factors that has the potential to create constructs to repair and replace damaged tissues.

SVF was isolated from lipoaspirated human adipose tissue using the fully automated Icellator™ Cell Isolation System and cultured to near confluency. Adipose tissue-derived ECM (ATEM) was procured from adipose tissue following a series of mechanical and biochemical treatments that allowed for complete decellularization while maintaining the structural integrity. ATEM was then placed into 24-well low binding plates, ethanol-sterilized, and rinsed with DPBS. ASCs were harvested and seeded directly onto ATEM, cultured in growth medium and allowed to adhere to ATEM for at least 48 hours. Growth medium was then replaced with adipogenic or osteogenic induction medium and cultured, with medium replaced every 2-3 days. Qualitative evaluation of lipid accumulation or calcium deposition was conducted histologically. Quantitative expression of adipogenic or osteogenic genetic markers was measured using real-time PCR.

Large, lipid-filled vacuoles were distinctly observed in adipogenic induced ASCs on ATEM compared to non-induced controls. Preliminary assessment of genes associated with early and mature adipocytes, including PPAR-γ, leptin, lipoprotein lipase, and adiponectin demonstrated markedly increased expression compared to non-induced controls. Calcium deposition was also abundant in osteogenic-induced ASCs on ATEM compared to non-induced controls. Interestingly however, quantitative expression of genes associated with an osteo-lineage, such as RUNX2, osteonectin, and osteocalcin were similar to non-induced controls. These results suggest ATEM may have the intrinsic capacity to serve as a robust scaffold for adipose tissue replacement, however, not as robustly for bone. ATEM may require supplementary structural material to augment the scaffold towards a more ossified architecture capable of supporting differentiation of ASCs into an osteogenic lineage.
Adipose tissue (AT) engineering is considered a promising alternative for soft tissue augmentation. The substitutes we produced are natural and composed of AT-derived stromal cells differentiated into adipocytes and their derived human matrix components. Engineered tissues are produced using culture media that can affect AT functions. We investigated variations in mRNA expression in order to assess the differences between in vitro 3D human model and native subcutaneous liposaspirated fat. Whole-genome gene expression was performed using the Illumina HumanWG-6 v3. The expression values were log2-transformed and quantile-normalized using the limma package in R. The Significance Analysis of Microarrays method was used to identify differentially expressed genes (DEG) with a false discovery rate <5% and fold change >2.0. In total, 2915 DEG were included as data set in Ingenuity pathway analysis 8.5 (IPA) to detect association with significant pathways. Then, IPA allowed us to connect those pathways with relevant biological functions. Fisher’s exact test with a Benjamini-Hochberg correction was used to examine cell adherence. Successfully treated ASC-seeded SIS samples were plated on 6-well plates and trypsinized every 2-3 days to monitor proliferation. Trypsinized SIS was evaluated under confocal microscopy for residual cells. ASC-seeded SIS, which was either pre-treated with vehicle (control) or fibronectin, were stained and transferred to slides. Confocal microscopy was used to examine cell adherence. Similarly treated ASC-seeded SIS samples were plated on 6-well plates and trypsinized every 2-3 days to monitor proliferation. Trypsinized SIS was evaluated under confocal microscopy for residual cells. ASC-seeded SIS samples were treated for 3-5 weeks using adipogenic, chondrogenic, osteogenic, or control media. Additional ASCs grown on SIS were compared to cells grown on plastic by flow cytometry.

Results: All test groups showed cell adherence and proliferation on SIS. Fibronectin-treated scaffolds showed more cells per high power field than those treated with vehicle (p<0.05). Fresh, non-cultured stromal vascular fraction (SVF) pellets injected onto SIS showed similar results to cultured ASCs. ASCs completely detached from SIS when trypsinized. Cells proliferated on SIS similarly to ASCs grown on plastic. Successful differentiation was confirmed by lineage-specific markers. ASCs grown on SIS displayed identical flow cytometry profiles. Conclusion: The use of ASCs to create “living” scaffolds allows for the advantage of being readily accessible while still offering the pluripotency of mesenchymal stem cells. Our pre-treatment of the scaffold with fibronectin offers a method to increase cell adhesion, and thus delivery. Importantly, seeding the freshly isolated SVF onto the scaffold resulted in characteristics similar to that observed for ASCs, demonstrating that minimally manipulated cells may be useful for point-of-service surgical applications wholly contained in the OR suite. In vivo studies are currently underway using the SIS scaffold ± ASCs in a rat model of hernia repair.
NEW FAT-DERIVED PRODUCTS FOR TREATING INDUCED-SKIN LESIONS OF SCLERODERMA IN NUDE MICE

Presenter: Nicolas Serratrice, MS
Authors: Serratrice N, Bruzzese L, Magalon J, Veran J, Daumas A, Andrac-Meyer L, Magalon G

Hôpital de la Conception

Background: Scleroderma is an auto immune disease characterized by an excessive fibrosis of the skin. We previously validated a murine model of scleroderma and showed an antifibrotic and a proangiogenic effect of fat micro-injection or micro-fat (MF). Fat is harvested with a 14 gauge, 2mm cannula and reinjection is performed with a 21 gauge cannula. In addition to the MF, we can also purify the stromal vascular fraction (SVF) of adipose tissue and platelet-rich plasma (PRP) from blood.

Objectives: Here we evaluated and compared the efficacy of MF, SVF, PRP, and mixtures of these products: MF + SVF and MF + PRP, in the murine model of skin-induced lesions of scleroderma.

Materials and Methods: This project was divided in three parts: Induction of skin sclerosis in nude mice by daily subcutaneous injections of bleomycin (BLM) during 4 weeks. Preparation and subcutaneous injections of the different cell therapy products of human origin. Skin biopsies and histological analyses 8 weeks post-injections. 66 nude mice were used in this study. Fat was harvested from the abdomen, to obtain MF and SVF, peripheral whole-blood was taken to prepare PRP. We injected 0.5 cc of the different cell therapy products containing respectively: 131,000 different cell therapy products containing respectively: 131,000 cells injected/mouse for the SVF and 0.64 million of platelets injected/mouse for PRP derived products.

Results: BLM skin-induced lesions were checked by histological analyses in control mice. BLM-treatment induced a 21.74% increase of the dermis thickness, a 40.28% increase of the epidermis thickness i.e. a 23.74% increase of the total skin thickness. Time did not affect skin-induced lesions of scleroderma and injections of the control solutions (chloride sodium or ringer lactate) did not reverse skin sclerosis. This work has demonstrated the effectiveness of these different restorative biotherapies on skin-induced lesions of scleroderma. MF, MF + SVF and MF + PRP completely reversed while SVF and PRP partially corrected skin sclerosis. A 13.7% decrease of the dermis thickness was observed with SVF, and a 20.7% decrease was observed with the PRP. Products containing MF, were still present 8 weeks post-injections, suggested the long-term potential effects of the MF. The number of visible vessels observed in the deep dermis was significantly increased in SVF or MF+SVF conditions, compared to others, showing the expecting SVF proangiogenic effects.

Conclusion: We highlighted the interest of mixtures MF+SVF and MF+PRP compared to the MF, SVF and PRP separately for their regenerative and proangiogenic properties to treat skin-induced lesions of scleroderma. These beneficial effects on the sclerotic skin should have potential clinical applications in the treatment of the SSC human disease.
LATTICE ACCELLULAR DERMAL MATRIX FOR STRUCTURAL SUPPORT IN A FAT GRAFT
Presenter: Joonkyu Park, MD
Authors: Park J, Kwon ST
Seoul National University Hospital

ANALYSES OF HUMAN ADIPOSE TISSUE-DERIVED BIOSCAFFOLDS
Presenter: Caasy Thomas-Porch, BS
Authors: Thomas-Porch C, Shah F, Frazier T, Hayes D, Scherp P, Flynn LE, Bunnell BA, Gimble JM
Tulane University

Human adipose tissue is a routine source of material for tissue grafts in repair and regeneration after injury and has promise as a biological scaffold. An ideal biological scaffold would be of human origin, and be suitable for both autologous and allogeneic transplantation. Furthermore, it would be composed of a functional extracellular matrix (ECM), free of donor DNA, biocompatible, bioactive, and would encourage cell growth. Although there has been success in the use of adipose tissue-derived ECM as a biological scaffold, work remains to be done in the areas of material absorption/degradation, rejection, and stem cell proliferation resulting in long-term, functional regeneration. This study addresses the hypothesis that a decellularized human adipose tissue-derived bioscaffold will exhibit properties of an ideal biological scaffold and will promote soft tissue regeneration alone or in combination with adipose tissue derived stem/stromal cells (ASCs). Human adipose tissue was obtained from consenting abdominoplasty patients under an IRB approved protocol. Preliminary work involving the optimization of a decellularization process yielded a final product with desired characteristics of a useful biological scaffold. The bioscaffolds were evaluated for DNA depletion, ECM composition, physical structure, influence on ASC differentiation, and protein content. Decellularization was confirmed by a lack of cell nuclei present upon H&E staining, and further supported by the absence of residual DNA found in spectrophotometric analysis and gel electrophoresis examinations. Extensive collagen composition was observed via trichrome staining. Scanning electron microscopy (SEM) revealed a complex fibrous physical constitution. Proteomic analysis identified 39 proteins as definitively present in the scaffolds, including collagen type I, III, IV, and VI. In vitro attempts to recellularize the scaffolds with ASCs, and concurrently induce differentiation of ASCs along the adipocyte and osteocyte lineages, displayed a possible preference for the adipocyte lineage. The results herein suggest that these bioscaffolds will serve as an environment which promotes ASC adhesion, proliferation, and differentiation. Future work will explore translational applications of the scaffolds.
Directed differentiation of adult multipotent stromal cells (MSC) is critical for developing effective treatment strategies. This study was designed to evaluate the capability of equine MSC from bone marrow (BMSC) and adipose tissue (ASC) on type I collagen (COL1) scaffolds to form chondrogenic, osteogenic and adipogenic neotissue in vitro. Following determination of surface antigen expression, MSC were loaded into scaffolds in a perfusion bioreactor and loading efficiency was quantified. Cell-scaffold constructs were assessed after loading and 7, 14 and 21 days of culture in stromal, or induction medium. Cell number was determined with DNA content, cell viability and spatial uniformity with confocal laser microscopy, and cell phenotype and matrix production with light and scanning electron microscopy and mRNA levels. Isolated ASC and BMSC expressed CD29, CD90 and CD105. Loading efficiencies were > 70%. Type of culture medium affected cell number in a manner dependent on the MSC source. Cells remained viable and uniformly distributed in scaffolds for up to 21 days and could be directed to differentiate or to maintain a stromal phenotype. Micro- and ultra-structure showed lineage-specific cell and extracellular matrix changes. The RNA levels remained stable in stromal medium while they changed with time and MSC source in induction media. Based on these results, equine MSC from adipose tissue and bone marrow differentiate into chondrogenic, osteogenic and adipogenic lineages and form neotissue when cultured on collagen scaffolds. The collected data supports the capacity of the cells to support diverse equine tissue formation for regeneration and controlled biological studies.

In the quest to enhance adipose tissue engineering, our institute has developed a thermosensitive adipose tissue derived matrix in gel-like form (Adipogel). Previous studies have confirmed its effectiveness in inducing adipogenic differentiation in adipose-derived stem cells, both in vitro and in vivo. Our rat chamber model study has also shown promising results. We performed further studies to evaluate its effects on different cell populations, in the hope of producing a more structurally sound tissue construct. For the in vitro study, we used both human and porcine Adipogels. The cells tested included human adipose-derived stem cells (ADSC), normal human dermal fibroblasts (NHDF), 3T3-L1 mouse preadipocytes, and L6 rat myoblasts. Each cell type was cultured in triplicates in 24-well plates using complete media, followed by addition of the matrix. Negative (media only) and positive (Matrigel, Zuk) controls were also prepared. Photographs were taken at different time points, followed by fixation with staining using Oil Red O and H&E. Direct cell count and real-time PCR were also performed. The in vivo study consists of 2 models. 1) rat groin subcutaneous injection of porcine Adipogel- single vs triplet injections; and 2) mouse chamber model. We tested the gel with either mouse ADSC or NHDF, with and without additives. The in vitro studies showed that both the human and porcine Adipogels were found to be adipogenic in all cells, including the NHDF and L6 cells. Cytology and RT-PCR results will be reviewed. The first in vivo model using subcutaneous injection showed persistence of gel and evidence of neo-adipogenesis and angiogenesis at 6 weeks and 3 months. We will also present results of the second model which is still progressing. We further confirmed the adipogenicity of both our porcine and human Adipogels. Interestingly, its adipogenic effects extend to other cell types. This may help augment engineered adipose tissue construct, with probable additional cellular support.
EVALUATION OF THE EFFECTS OF ADIPOGEL (ADIPOSE-DERIVED MATRIX) ON DIFFERENT CELL POPULATIONS, WITH OR WITHOUT THE USE OF ADDITIVES

Presenter: Beryl H. Tan, MD
Authors: Tan BH, Han XL, Morrison WA, Abberton KM

O’Brien Institute

TRANSPLANTATION OF MOUSE ADIPOSE DERIVED STEM CELLS IMPROVED CARDIAC FUNCTION IN RATS WITH ACUTE MYOCARDIAL INFARCTION AND MODULATED EXPRESSION OF INFLAMMATORY CYTOKINES AND IMMUNE CELLS

Presenter: JongHo Kim, MS
Authors: Kim JH, Park CY, Park JH, Choi SC, Choi JH, Hong SJ, Lim DS

Korea University

Background: Mouse adipose derived stem cells (mADSCs) immortalized with retroviruses harboring the hTERT-IRES-eGFP genes were transplanted into acute myocardial infarction (AMI) rats to investigate their effects on cardiac regeneration and immune response.

Methods: mADSCs were selected on the basis of their morphology, GFP intensity and phenotypic characterization. 500,000 cells per rat were transplanted into 3 groups (Control; Medium (n=16), CD34+/Sca-1+ mADSCs (n=14) and CD34-/Sca-1+ mADSCs (n=14) groups), and cardiac function and peripheral blood inflammatory cytokines and immune cells were analyzed at 1, 7, 14, 28 day(s) after cell transplantation.

Results: Significant improvements in ejection fraction value were observed in the CD34+/Sca-1+ mADSCs and CD34-/Sca-1+ mADSCs-transplanted groups compared with the Control group at 28 days (48.80±3.28%, 57.40±1.08%, 64.23±1.84%, P<0.005, respectively). MCP-1 was significantly decreased in the CD34-/Sca-1+ mADSCs-transplanted group at 1, 7 and 14 day(s) compared with the Control group (Fig.1.A). Peripheral blood NK cells were significantly decreased at 1 day in the CD34-/Sca-1+ mADSCs-transplanted group (2.87±0.36%, 2.71±0.42%, 1.43±0.21%, P<0.005, respectively). Furthermore, peripheral blood NKT cells were significantly increased in the CD34-/Sca-1+ mADSCs-transplanted group at 14 days. But, NKT cells were significantly decreased in the CD34-/Sca-1+ mADSCs-transplanted group at 28 days (Fig.1.B). Peripheral blood CD4T, CD8T and B cells were not affected by transplantation of mADSCs.

Conclusions: Transplantation of mADSCs into infarcted myocardium improved cardiac function in rat AMI model, and modulated expression of peripheral blood inflammatory cytokines and immune cells in a cell specific manner. The hTERT-immortalized mADSCs are very useful for the study of stem cell differentiation as well as for cell therapy in cardiovascular field.
**DIRECTING STEM CELLS TO THE INFARCTED HEART USING TARGETED MICROBUBBLES: DEVELOPMENT OF A NEW MOLECULAR THERAPEUTIC TECHNIQUE**

**Presenter:** Benno A. Naaijkens, MSc  
**Authors:** Naaijkens BA, Bogaards SJ, Krijnen PA, Kamp O, Musters RJ, Kokhuis TJ, de Jong N, Niessen HW, van Dijk A, Juffermans LJ  
**VU Medical Center Amsterdam**

Stem cell therapy is a promising tool to restore contractile function after myocardial infarction. Unfortunately, clinical trials still show disappointing results with only minor improvements in cardiac function. The major problem of cellular therapy is lack of persistence of sufficient stem cells at the site of injury, independent of administration route. We designed a novel technique to overcome this problem by directing stem cells to the infarcted area using targeted microbubbles (MBs) and ultrasound (US). For this we coupled adipose-derived stem cells (ASC) to MBs using an antibody against CD90 via biotin-avidin bridging. This stem cell-bubble complex was named ‘StemBell’. StemBells were targeted to the infarcted area via a second antibody on the MB: anti-CD54. US (1 MHz) was applied to exert acoustic radiation force on the StemBells. In vitro we demonstrated that the procedure to create StemBells, as well as exposure to US had no negative effect on cell viability, using flow cytometry. Binding of MBs to ASC did not affect their ability to attach to a culture dish, demonstrated by light microscopy. In a flow system we showed the ability of US to push StemBells from the main flow (0.2 dyne/cm²) to the side. In vivo, acute myocardial infarction was mimicked in a rat by ligating the left anterior descending coronary artery for 40 min, followed by reperfusion. 7 Days post-infarction 1 million DAPI-labeled StemBells (‘StB’) or ASC were injected intravenously. 3 Hours post-injection hearts were excised, stored in liquid N² and cryo-sections were made. By performing fluorescence microscopy we found significantly more cells (6-fold increase) specifically in the infarcted area in the StB group compared with ‘ASC alone’ group (n=6; p<0.01). Notably, retrieved stem cells coincided with CD54 positive areas.

In conclusion, we successfully demonstrated proof-of-principle of a novel technique to increase the number of stem cells at the site of injury. This holds great promise for stem cell therapies in general.

**PROLIFERATION OF ADIPOCYTE PROGENITORS FROM HUMAN ADIPOSE TISSUE CAPILLARY NETWORKS INDICATES A VASCULAR NICHE FOR HUMAN ADIPOCYTE STEM CELLS**

**Presenter:** Silvia Corvera, MD  
**Authors:** Corvera S, Min SY, Gealekman O, Lalikos J, Fudem G, Chouinard M  
**University of Massachusetts Medical School**

**Introduction:** Identifying adipocyte progenitors within human adipose tissue is crucial for understanding the basic mechanisms that regulate tissue expansion, and for developing new strategies to treat obesity and to improve the engrafting of transplanted fat. Studies in mouse models indicate that adipocyte progenitors reside within capillary walls and co-express pericyte markers, suggesting that the adipose tissue vasculature provides the niche for adipocyte stem cells. Moreover, lineage-tracing studies indicated that some adipocytes derive from cells expressing VE-cadherin, suggesting that certain progenitors can undergo endothelial or adipocyte cell fates. Whether adipocyte progenitors in human adipose tissue also originate from the vascular network remains to be established. To address this question we examined whether ex-vivo development of human adipose tissue capillaries would be accompanied by proliferation of adipocyte progenitors.

**Method:** Human adipose tissue explants were cultured in Matrigel under pro-angiogenic conditions (EGM-2 MV). After 14 days, adipocyte differentiation was induced by addition of dexamethasone, methylisobutylxanthine and insulin (DMI) for 72 h. Ten days later, adipocyte conversion was assessed by the presence of lipid droplets and by the expression levels of endothelial and adipocyte markers.

**Results:** Explants cultured under pro-angiogenic conditions formed well structured capillary networks, demonstrated by tight junctions between endothelial cells, primitive lumens, and pericyte-like cells (Tran et al. Cell Metabolism 2012 15(2):222-9). In response to DMI capillary growth decreased and numerous cells embedded along the capillaries differentiated into adipocytes. More adipocytes formed when pro-angiogenic media was replaced by non-angiogenic media (DMEM+10%FBS), suggesting a reciprocal relationship between vascular or adipocyte cell fate.

**Conclusion:** Ex-vivo expansion of human adipose tissue vasculature is accompanied by proliferation of human adipocyte progenitors, consistent with a vascular niche for human adipocyte stem cells. This finding will allow further identification and characterization of these cells, and their therapeutic potential.
DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS (ASCs) TO ENDOTHELium BY SPHINGOSINE-1-PHOSPHATE FOR SEEDING onto a BIOLOGICAL SCAFFOLD

Presenter: Ping Zhang, PhD

Cooper Medical School at Rowan University

Background: Sphingosine-1-phosphate (S1P) is a bioactive lipid with potent effects on stem cell proliferation and differentiation. This project was driven by the hypothesis that S1P differentiates ASC to endothelial-like cells and increases their proliferation and attachment to small intestinal submucosa (SIS) scaffold.

Methods and Results: ASCs were isolated from periumbilical fat tissue. To assess the effect of S1P on proliferation and EC differentiation, ASCs were cultured 2wk in Endothelial Growth Medium (EGM2) supplemented with FBS (2%) and S1P. After 14d, we observed increased proliferation (1.4 fold, p<0.05) in ASCs treated with S1P (1 µM) compared to ASCs in EGM2 without S1P. High concentrations of S1P (10 µM) decreased the proliferation of ASC. qRT-PCR showed that cultured ASC in EGM2 medium with S1P (1 µM) increased mRNA expression of the EC markers vWF (12.6-fold) and eNOS (2.2-fold) when compared to ASCs in EGM2 without S1P. To determine the effect of S1P on cell attachment, ASCs were seeded onto tissue culture plates and incubated for 2 hr on surfaces coated with EGM2, FBS (100%), fibronectin (10 µg/ml) or S1P (1 µM). Attachment was greatest for S1P surface coating than to any other coating materials using crystal violet staining for quantification. Similarly, ASCs seeded onto S1P-coated SIS scaffolds for 3d exhibited increased ASC attachment by nearly twofold compared with ASC grown without S1P. Finally, S1P treated tubular scaffolds without cells were implanted into the canine carotid artery. Duplex ultrasound demonstrated that the graft remained patent at 3 wk post-implantation, presumably by capturing host endothelial progenitor cells. In vivo surveillance is currently underway evaluating the ability of the S1P treated scaffolds and scaffolds luminally seeded with S1P-treated ASCs to form an endothelial lined neo-artery in vivo.

Conclusions: These results indicate that: 1) S1P promotes proliferation, attachment and endothelial differentiation of human ASCs on SIS scaffold, 2) S1P coating of a biological scaffold may provide a useful method to improve patency and functionality of tissue engineered vascular grafts.

ACTIVIN A IS KEY FACTOR RESPONSIBLE FOR ADIPOSE STROMAL CELLS ACQUISITION OF SMOOTH MUSCLE CELL PHENOTYPE AND FOR MODULATION OF THEIR ANGIOGENIC ACTIVITY AS A RESULT OF CONTACT WITH ENDOTHELIAL CELLS

Presenter: Dmitry O. Traktuev, PhD
Authors: Traktuev DO, Merfeld-Clauss S, Lupov IP, Lu H, Compton-Craig P, March KL

Indiana University

Adipose stromal cells (ASC) are therapeutically potent cells that possess properties of pericytes and produce a variety of angiogenic factors. Local or systemic delivery of ASC into animals with injuries has significant therapeutic effects, which mostly are attributed to angiogenic, anti-inflammatory, and tissue preservation factors secreted by ASC. Also, ASC together with endothelial cells (EC) are able to form functional multilayer vessels in vivo, in which ASC form the outer layer of the vessel and differentiate into mural cells. Previously, we have reported that EC-ASC heterotypical cell interaction led to diminishing or even inverting their angiogenic activity. The principal signaling mechanisms responsible for ASC differentiation towards smooth muscle cell (SMC) as a result of interaction with EC and for modification of their paracrine activity are unknown. Here, using an in vitro model of EC co-cultivation with ASC, we demonstrated that ASC migrated towards EC cords and up-regulated expression of αSMA, SM22α, and calponin. This effect was replicated by EC-ASC CM when applied on intact ASC. We found that EC, through to be defined mechanism, induced up-regulation of expression of activin A, but not TGFβ in ASC that were in direct proximity to EC. While exogenous TGFβ and activin A were each sufficient to stimulate expression of SMC antigens in ASC, only activin A IgG inhibited the inductive effect of EC-ASC CM. Incubation of EC-ASC co-cultures in the presence of activin A IgG or ALK4/5/7 receptor inhibitors blocked the expression of αSMA in ASC in the absence of direct contact with EC cords, but this inhibition was circumvented by EC contact. We hypothesize that activin A plays a key reprogramming role for ASC neighboring, but not directly adjacent to EC cords, by facilitating propagation of the SMC differentiation at distances beyond the reach of direct EC-ASC contact. Also, we found that activin A had anti-mitogenic effect on EC, and was responsible for inhibitory effect of EC-ASC CM on EC proliferation. Activin A played an important role in regulation of ASC vasculogenic potential: while blocking its expression in ASC by siRNA led to development of more dense vascular networks by EC in co-cultures, exogenously added activin A inhibited it.
EXAMINATION OF VASCULAR REMODELING EVENTS IN A MURINE FLAP DELAY MODEL

Presenter: Scott A. Seaman, BS
Authors: Seaman SA, Peirce SM
University of Virginia

Introduction: Flap delay is a common surgical pretreatment technique in which the vascular supply to the transferred tissue is interrupted prior to reconstructive soft tissue flap surgery. Empirical evidence reveals that delayed flaps have increased survival and are more likely to successfully engraft; however, the exact mechanism remains to be elucidated. Vascular adaptations within the flap are hypothesized to be responsible for the increased survival of delayed flaps.

Methods: Here we present a flap delay model adapted from a murine model of ischemia to the inguinal fat pad. We selectively ligate the vascular pedicle (epigastric artery) supplying the inguinal fat pad and observe vascular remodeling and inflammatory responses post-ligation. Whole-mounting of the immunofluorescently labeled tissue and confocal microscopy (Figure 1) allows the visualization of angiogenesis (growth of new blood vessels), arteriogenesis (expansion of preexisting blood vessels), and capillary arterialization (capillary diameter expansion and recruitment of smooth muscle cells). Further, we immunolabel other cell types (e.g. macrophages) to examine the potential importance of these cells during vascular remodeling events.

Results: Macrophage recruitment, one hallmark of active vascular remodeling, is increased in ligated/delayed inguinal fat pads as compared to the sham operated contralateral fat. Further work will examine vascular remodeling metrics (vascular length density, vessel diameter changes, vessel tortuosity) to quantify the associated angiogenesis and arteriogenesis in the fat pad following ligation.

Conclusions: Better understanding vascular adaptations within the delayed fat will provide insight into the mechanisms that enhance autologous fat grafting survival following flap delay. This research may allow potential advances and optimization of flap delay techniques to better clinical outcomes during autologous fat grafting.

Objective: We have previously investigated the use of ASCs differentiated towards an endothelial cell (EC) lineage in the creation of a tissue-engineered vascular graft (TEVG). In this study, we report progress towards adding a medial layer to the artificial artery, composed of the same autologous ASCs, but differentiated towards a smooth muscle cell (SMC) lineage. Such a layer may improve the strength, endothelial function, and anti-inflammatory properties of the conduit.

Methods: ASC isolated from vascular patients via periumbilical liposuction were differentiated toward SMC lineage using TGF-b. Phenotypic evaluation included PCR and immunoblot for SMC-specific markers (a-actin, calponin, myosin heavy chain (MHC)). Contractile function was evaluated using a collagen lattice gel. SIS vascular scaffolds (Cook Biotech, Inc.) seeded with ASC-SMC (outside surface) and ASC-EC (luminal surface) were flow conditioned over 5d within a bioreactor (up to 9 dynes, 120% stretch). Cell morphology and retention was evaluated using confocal microscopy.

Results: ASC newly acquired SMC message and protein markers following in vitro culture in TGF-b medium, although MHC expression was only variably expressed after 2 weeks of differentiation, perhaps suggesting an incomplete SMC differentiation in some cell lines. ASC-SMC demonstrated a 12% increase in contraction when plated onto a collagen gel (p=0.04). Cells seeded onto the SIS scaffold and flow conditioned for 5 days exhibited extensive cellular retention and orientation in the direction of flow on both the abluminal and luminal surfaces (FIGURE 1).

Conclusions: These data demonstrate: 1) the potential of autologous ASC to acquire SMC characteristics (molecular markers, contraction) in response to growth factor stimulation, and 2) the creation of a multi-layered, tissue-engineered vascular graft from a single, autologous adult stem cell source. In vivo investigation of the graft within a canine model to evaluate the addition of the medial layer is underway.
ANTI-INFLAMMATORY PROTEIN TSG-6 SECRETED BY ADIPOSE STROMAL CELLS INHIBITS NEUTROPHIL TRANSMIGRATION ACROSS ENDOTHELIAL MONOLAYER

Presenter: Jie Xie, MD
Authors: Xie J, Yi R, Feng D, Clauss MA, March KL
Indiana University School of Medicine

Introduction: The vascular wall formed by endothelial cells (EC) restricts passage of circulating molecules and inflammatory cells into the underlying tissues. Adipose stromal cells (ASC) reside in the perivascular niche and exhibit potent effects against inflammation. The effect of ASC on endothelial permeability and the key mediator remains unknown.

Methods: Transwell inserts were coated with confluent EC monolayer. Fluorescently labeled human neutrophils were induced to transmigrate across EC monolayer by human TNF± (20ng/ml). Human ASC were cocultured in the bottom compartment without contacting with inserts. Transmigrated neutrophils were quantified under fluorescent microscope. Secretion of protein TSG-6 from ASC was measured by enzyme-linked immunosorbent assay (ELISA).

Results: TNF± treatment for 3 hours led to transmigration of 4.0 ± 0.5% neutrophils across EC monolayer, which was reduced to 1.2 ± 0.3% (p<0.05, vs. TNF± only) by cocultured ASC in the bottom compartment. TNF± also highly activated secretion of TSG-6 protein from ASC (100-500ng/ml depending on the donor and passage). Interestingly, recombinant TSG-6 (100ng/ml) alone in the absence of ASC reduced neutrophil transmigration to 0.9 ± 0.3% (p<0.05, vs. TNF± only).

Conclusions: Inflammatory cytokine TNF± stimulates release of the anti-inflammatory protein TSG-6 from ASC. TSG-6 was able to reproduce the inhibitory effect of ASC on TNF±-induced neutrophil transmigration across EC monolayer.
USE OF ADIPOSE EXPLANTS TO TEST WOUND HEALING POTENTIAL OF LYMTHATIC OPTIMIZED ADIPOSE DERIVED SVF

Presenter: Catherine J. Baty, DVM, PhD
Authors: Baty CJ, Karlsson JM, Finegold DN, Acarturk TO, Futrell WJ

University of Pittsburgh

Introduction: To our knowledge, the benefit of optimizing lymphatic vascular expression within adipose derived stromal vascular fraction (ADSVF) has not been explored. We are particularly interested in the application of short term optimization prior to autologous transplant for potential applications for lymphedema reduction, fat grafting and wound repair. While a large amount of regenerative research has focused on stimulating an angiogenic milieu, we hypothesize that a mixed population of cells providing both angiogenic and lymphangiogenic stimuli may be more beneficial in some disease or wound conditions.

Methods: Discarded white adipose tissue from reduction surgeries were obtained according to IRB protocol and used to generate ADSVF as previously described. Small pieces (3-5 mm square) of adipose were also prepared for explant culture in endothelial cell optimized media (EGM-2MV, Lonza). ADSVF was cultured a total of 48 hr prior to transplant but cultured using three different medias in the last 24 hours: EGM, lymphatic endothelial cell (LEC) optimized EGM, and EGM supplemented with 10 ng/ml VEGF-C. After initial culture, cells were labeled with CellTracker Green (Invitrogen), pelleted in appropriate media, mixed with extracellular matrix and injected into fat explants. Controls included injections with matrix alone, LECs in endothelial media and LECs supplemented with VEGF-C. After initial culture, cells were labeled with CellTracker Green (Invitrogen), pelleted in appropriate media, mixed with extracellular matrix and injected into fat explants. Controls included injections with matrix alone, LECs in endothelial media and LECs supplemented with VEGF-C. Explants were routinely incubated in 5% CO2, then harvested and routinely fixed at 24 and 48 hrs. Explant identity was blinded prior to whole mount and/or thick section immunofluorescent confocal imaging. CellTracker Green identified injection sites, and lymphatic (LYVE-1 and Prox-1), panendothelial (CD31), and nuclear markers were used to evaluate the lymphangiogenic response.

Results: Preliminary results show the most cellular response to the VEGFC supplemented injection. Response will be quantified as a LYVE-1 or Prox-1 to CD31 (both lymphatic and blood vascular) ratio and cellularity (nuclei) for at least 5 random fields within the injection zone and in replicate samples to address lymphatic response and sample variability.

Conclusions: Our preliminary findings show that adipose explants can be used to evaluate lymphangiogenic response to lymphatic optimization of ADSVF.

COMPARISON OF BONE MARROW AND ADIPOSE TISSUE DERIVED STEM CELLS

Presenter: Ziya Saylan, MD
Author: Saylan Z
Office Dr. Saylan

Background: Adipose tissue derived stem cells (ADSCs) have been shown to differentiate into skin, fat, cartilage, or muscle. This study investigates whether or not ADSCs are equal to bone marrow derived stem cells (BMSCs) for their muscle, cartilage, and bone forming potential.

Methods: ADSCs were enzymatically released from aspirated fat from human donors. BMSCs were harvested from the iliac bone. Flow-cytometric analysis showed that ADSCs have a marker population which is almost similar to that of BMSCs, but the c-kit expression and HLA-DR were missing. These absent elements are pivotal for the survival of adult stem cells, because their fundamental role is to generate healthy and functioning cells during embryonic, fetal and adult life.

Results: The main differences between adipose stem cells and bone marrow stem cells include:
• Harvested ADSCs are phenotypically different than BMSCs: ADSCs excrete a different pattern of cytokines.
• Self-renewal: BMSCs have more capacity to renovate the host tissue. ADSCs have shown a limited and localized regeneration of the inserted region.
• Extensive capacity of proliferation: Only BMSCs and embryonic stem cells can build new tissues and organs. The proliferation capacity of the ADSCs is very limited.
• Migration to and homing at distant sites: This makes the BMSCs a gold standard in intravenous treatment of diseases. It is also an ideal anti-aging agent when given intravenously.
• Resistance to toxic agents: The stem cells in the fatty tissue are fragile and can be easily destroyed if mixed with chemicals (e.g. Lidocain etc.). BMSCs are more stable to external conditions.
• BMSCs were superior to ADSCs in respect to maintenance of proliferating ability of the osteoblast, adipocyte, hematogenic, myogenic and chondrogenic lineages.

Conclusion: This study is not in any way definitive and does not mean that one should stop using any stem cells derived from ADSCs. From this author’s perspective, it is important to realize that fat may contain stem cells, but it is not yet a true stem cell treatment. Their presence in the harvested fat is an “incidental” by-product of the process, and they might be used for aesthetic purposes.
LIPOSHIFTING, TREATMENT OF POSTLIPOSUCTION IRREGULARITIES

Presenter: Ziya Saylan, MD
Author: Saylan Z
Office Dr. Saylan
WITHDRAWN

A PHASE I CLINICAL TRIAL FOR MAXILLARY BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE SCAFFOLDS; AN INTRAOPERATIVE CONCEPT

Presenter: Marco N. Helder, PhD
Authors: Helder MN, Prins HJ, ten Bruggenkate CM, Overman JR, Klein-Nulend J, Schulten EA
VU University Medical Center

Patients with require maxillary sinus floor elevation (MSFE) prior to dental implant placement. Synthetic bone substitutes are used as an alternative for the ‘gold standard’, i.e. autologous bone. However, bone substitutes only allow osteoconduction, since viable osteogenic cells are lacking. Cell-based bone tissue engineering is a promising technique to improve the bone forming capacity of bone substitutes. We evaluated the feasibility, safety and efficacy of combining a calcium phosphate as bone substitute with freshly isolated adipose stem cells during a one-step surgical procedure for Maxillary Sinus Floor Elevation (MSFE).

Osteoinductive implants (calcium phosphate (CaP) carriers seeded with the freshly isolated stromal vascular fraction of adipose tissue) were generated in an intra-operative procedure within the OR-complex within hours, thereby avoiding costly stem cell expansions and a second intervention. Where possible, a ‘split mouth design’ (with only CaP scaffold at the contralateral control side) was applied to allow efficacy evaluation. Adverse events (AE) were monitored, and clinical, X-ray, and Cone-beam CT data are collected at regular intervals during follow-up. After six months biopsies are obtained during dental implant placement, and evaluated for bone formation by histomorphometry and µCT.

Sofar, we included 10 patients. All patients uneventfully underwent an MSFE procedure and no adverse effects were reported during 1 year follow-up. Bone as well as osteoid percentage were significantly higher in bone biopsies taken from study sides than control sides throughout the complete biopsies, suggesting that bone formation does not only occur from the pre-existing sinus floor, and that the adipose stem cells may stimulate bone formation.

This study demonstrated for the first time the feasibility, safety and potential efficacy of freshly isolated adipose stem cells with a calcium phosphate for MSFE, and provides the first step towards a novel treatment concept that might offer broad potential for cell-based regenerative medicine applications.
53 A PHASE I CLINICAL TRIAL FOR MAXILLARY BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE SCAFFOLDS; AN INTRA-OPERATIVE CONCEPT
Presenter: Marco N. Helder, PhD
Authors: Helder MN, Prins HJ, ten Bruggenkate CM, Overman JR, Klein-Nulend J, Schulten EA
VU University Medical Center

54 EXPANDED ALLOGENEIC ADIPOSE-DERIVED STEM CELLS (eASCs) FOR THE TREATMENT OF COMPLEX PERIANAL FISTULA IN CROHN’S DISEASE: RESULTS FROM A MULTICENTER PHASE I/IIA CLINICAL TRIAL
Presenter: Xavier X. Gonzalez-Argente MD
Authors: Gonzalez-Argente XX, De La Portilla F, Alba F, Garcia-Olmo D, Herrera JS, Galindo A
University Multicenter Study: Hospital Son Espases/Hospital Virgen del Rocio/Hospital San Juan de Dios/Hospital la Paz/Hospital Virgen de la Macarena/Hospital de Valme

Introduction: The management of perianal fistula in patients with Crohn’s disease is an extremely challenging medical problem as many fistulas do not respond to available treatments.

The objectives were to assess the safety and efficacy of a suspension of expanded adipose-derived allogeneic mesenchymal stem cells (eASCs) for the treatment of complex perianal fistula in Crohn’s disease.

Methods: An open-label, single-arm clinical trial was conducted at six Spanish hospitals. Twenty-four patients were administered intraleosionally with 20 million eASCs in one draining fistula tract. A subsequent administration of 40 million eASCs was performed if fistula closure was incomplete at week 12. Subjects were followed until week 24 after the initial administration.

Results: Treatment-related adverse events did not indicate any clinical safety concerns after 6 months follow-up. The full analysis of efficacy data at week 24 showed 69.2% of the patients with a reduction in the number of draining fistulas, 56.3% of the patients achieved complete closure of the treated fistula achieved, and 30% of the cases presenting complete closure of all existing fistula tracts. Of note, closure was strictly defined as: absence of suppuration through the external orifice and complete re-epithelization, plus absence of collections measured by magnetic resonance image scan (MRI). Furthermore, MRI Score of Severity showed statistically significant differences at week 12 with a marked reduction at week 24.

Conclusions: Locally injected eASCs appear to be a simple, safe, and beneficial therapy for perianal fistula in Crohn’s disease patients. Additional studies are needed to further confirm the efficacy of the eASCs.
AURICULAR TISSUE ENGINEERING USING ADSCS AND 3D SCAFFOLD
Presenter: Beatriz Nicaretta, MD
Authors: Sterodimas A, Nicaretta B
IASO General Hospital

NOT PRESENTED

FIVE YEARS EXPERIENCE OF HIGH SEPTAL FAT INJECTIONS VERSUS STEM CELL ENRICHED FAT INJECTIONS FOR FACIAL REJUVENATION: WHAT WORKS AND WHAT DOESN’T WORK!
Presenter: Tunc Tiryaki, MD
Authors: Tiryaki T, Aksungur E, Oymak O, Tiryaki D
Istanbul Academy of Plastic Surgery

Introduction: Autologous fat transplantation has become a major treatment for facial rejuvenation. However, the limitations of fat transplantation like the unpredictability of volume maintenance as well as the tremendous variations in aesthetic outcome continue to remain as fields to progress. Regenerative cell-based strategies such as autologous stem cell enrichment of the fat seem promising for facial rejuvenation. More importantly, a re-evaluation of the dogmas of facial fat transfer gives us some insights about what we can do better.

Methods: We present altogether 354 cases treated with conventional fat grafts and 86 cases with stem cell enriched tissue (SET) injections for facial rejuvenation between December 2007 and December 2012. Fat grafts ranged in volume from 12 to 36 cc, whereas cell-enriched grafts ranged from 10 to 46 cc per patient, obtained by a manual MACS cell separation system. In all cases, a high post-septal mid-facial fat injection was performed; in the enrichment cases the obtained ADSC solution was injected into the transplanted fat to help to increase the graft uptake, as well as intra-dermally for skin rejuvenation. The mean follow-up period was 28 months.

Results: Results were evaluated by clinical examination, patient photographs and the Vectra 3D Surface Imaging®. Postoperative atrophy of the injected tissue was minimal and did not changed after 8 weeks in both groups. All patients were satisfied with the results; particularly lower lid rejuvenation and blending of lid-cheek junction, as well as skin rejuvenation, with no need of any secondary session in both groups. The preoperative and postoperative volume differences between two groups were not statistically significant.

Conclusion: Our experience suggests that high through-septal fat injections to the lower lid are necessary in order to rejuvenate the lid cheek junction in contrast to the general hesitation of lower lid fat injections. The injection technique and location seem to be the most important factors in success. Our preliminary results of regenerative cell enrichment suggest that they might have significant comparative advantages to traditional fat transplantation only when grafting hostile recipient area conditions in the face.
ADVERSE EFFECTS OF SVF ON INTERVERTEBRAL DISC REGENERATION IN A LARGE ANIMAL MODEL
Presenter: Suzanne E. Detiger, MD
Authors: Detiger SE, Hoogendoorn RJ, Mevorat Kaplan K, van Royen BJ, Smit TH, Yayon A, Helder MN
VU University Medical Center

Introduction: Intervertebral disc (IVD) degeneration is an important target for regenerative strategies, including stem cell therapies. Adipose tissue-derived stem cells (ASCs) enable a one-step surgical procedure for IVD regeneration because of a high yield and easy accessibility of the tissue. Stromal vascular fraction (SVF) harvested from adipose tissue is a heterogeneous mixture containing ASCs, monocytes and erythrocytes, among others. SVF has been shown to promote IVD regeneration before in a dog nucleotomy model. The objective of this study was to assess safety and efficacy of SVF in a fibrin-HA gel carrier after injection into the IVD of our previously established goat model of disc degeneration.

Methods: In seven adult Dutch milk goats, lumbar IVDs were chemically degenerated during 3 months using 0.25 U/ml Chondroitinase ABC, after which subcutaneous adipose tissue was harvested and SVF isolated. Subsequently, IVDs were randomised to three intervention groups: hydrogel with SVF (10^6 nucleated cells), hydrogel only or no intervention. After sacrifice, at 3 months follow-up, lumbar spines were harvested for radiological, macroscopic and histological analysis.

Results: Injection of SVF induced an inflammatory response in 83%, followed by severe degenerative changes on all parameters. Radiographs showed holes in the involved endplates; on MRI the nucleus pulposus could not be distinguished from the surrounding annulus fibrosus. Macroscopically, massive scarring was observed, including endplate destruction and osteophyte formation. Histological scores deteriorated from 1.7 with hydrogel only and 2.1 in the no intervention group, to 4.1 in the SVF group (scale 0-6).

Conclusions: The observed inflammatory response in the SVF injected discs may be caused by the combination of different chemical agents and/or cells (e.g., monocytes and erythrocytes) in an avascular, cell-hostile environment. ASCs alone and residual enzyme levels are less likely the cause of inflammation, as tested and proved safe in previous studies. SVF was applied successfully before in articular cartilage and bone regeneration studies; however, we discourage clinical application of SVF in IVD degeneration as long as the cause of the adverse effects reported here remain to be elucidated.
Urinary incontinence is a common health problem affecting a large number of women. Approximately 35% of women over 18 years in Europe reported involuntary urine loss. Adipose stem cells (ASCs) provide a novel alternative to invasive surgical treatment of stress urinary incontinence (SUI). The objective of our study was to find out if transurethral injections of autologous ASCs in combination with injectable bulking agent are an effective and a safe treatment for female SUI.

Five SUI patients were treated with ASCs combined with bovine collagen gel and saline solution between. The subcutaneous fat from lower abdomen was collected under local anaesthesia. The ASCs were isolated and expanded for three weeks. Multipotency (adipogenic, osteogenic, chondrogenic and myogenic differentiation) and mesenchymal stem cell surface marker profile (CD14, CD19, CD34, CD45, CD73, CD90, CD105, HLA-ABC-PE, and HLA-DR) of ASCs was analysed. Moreover, chromosomal integrity, sterility, endotoxins and mycoplasma were determined before cell transplantation. Mixture of ASCs and collagen gel was injected transurethrally via cystoscope. The patients were followed at 3, 6 and 12 months after the injections. The primary end point was cough test to objectively measure the effect of the treatment. Validated questionnaires were used for determining the subjective cure rate.

At six months, one out of five patients displayed a negative cough test with full bladder filled with 500ml saline. At one year, the cough test was negative with three patients; two of them were satisfied with the treatment and didn’t wish for further treatment for SUI. There was some subjective improvement with all five patients.

This is the first study describing use of autologous ASCs in combination with collagen gel in treating SUI in women. So far the treatment with autologous ASCs has proven safe and well tolerated. However, feasibility and efficacy of the treatment were not optimal and more studies are needed to find a better carrier material for ASCs and the ideal technique for the transurethral injections.

BURN SCAR REGENERATION WITH THE “SUFA” (SUBCISION SCAR RELEASE AND FAT GRAFTING) TECHNIQUE. A PROSPECTIVE CLINICAL STUDY

Introduction: Challenges in burn reconstruction are often due to recurrent scar contractures. The use of fat grafting in thermal injury is reported only in small size clinical series and results are often biased by simultaneous surgical procedures and lack of scientific methods validation. The goal of our study is to prospectively evaluate outcomes in patients who underwent the “SUFA” technique (Subcision Scar Release and Fat Grafting) for the treatment of contracted burn scars.

Method: Our study is to our knowledge the first Institutional Review Board approved in the United States. Three patients with secondary burns were prospectively treated. Patient demographics show two male and one female, mean age 53 years, mean time since primary burn was 2 years and four months. All patients were initially treated with skin grafts and then with scar release and Z-plasties for secondary contractures. Scars were clinically evaluated with the Vancouver scale and range of motion of the head and neck; fat graft survival and skin remodeling were evaluated by high frequency 18MHz ultrasound preoperatively, at 1, 3 and 6 months postoperatively. Operation time, tumescent infusion, lipoaspirate and injected fat were recorded. Abdomen represented the donor site and fat was processed on telfa after ringer lactate wash out. The “SUFA” technique consists of subcision of the scars with V-shape cannulas through stab incisions far away from the band to be released and injection of fat. Treated areas were mainly face, neck and hands. Postoperative protocol included stretching exercises at 5 days, oral antibiotics for three days, sutures removal at 1 week.

Results: All patient showed improved contractures in the Vancouver scale and range of motion of the head and neck; fat graft survival and skin remodeling were evaluated by high frequency 18MHz ultrasound preoperatively, at 1, 3 and 6 months postoperatively. Operation time, tumescent infusion, lipoaspirate and injected fat were recorded. Abdomen represented the donor site and fat was processed on telfa after ringer lactate wash out. The “SUFA” technique consists of subcision of the scars with V-shape cannulas through stab incisions far away from the band to be released and injection of fat. Treated areas were mainly face, neck and hands. Postoperative protocol included stretching exercises at 5 days, oral antibiotics for three days, sutures removal at 1 week.

Conclusions: The “SUFA” technique represents a new promising approach in the era of “regeneration” of the connective tissues. Improvements in clinical and diagnostic outcomes suggest dermis regeneration promoted by adipose tissue-derived stem cells.
BURN SCAR REGENERATION WITH THE “SUFA” (SUBCISION SCAR RELEASE AND FAT GRAFTING) TECHNIQUE. A PROSPECTIVE CLINICAL STUDY

Presenter: Francesco Gargano, PhD, MD
Authors: Gargano F, Schmidt S, Zienowicz R, Guo Y, Evangelista P, Harrington DT, Liu P

Brown University

IMPROVEMENT OF SURVIVAL RATE OF FAT TRANSPLANTATION BY OXYGEN-RELEASING MICROSPHERES AND ADIPOSE-DERIVED STEM CELLS

Presenter: DongWoo Jung, MD, PhD
Authors: Jung DW, Kim YH, Lim JO, Kim TG, Lee JH

College of Medicine Yeungnam University Daegu Korea

Background: Free-fat grafting has become popular in plastic surgery. But, clinical results are variable and technique-dependent. In order to improve the outcomes, stem cells are added with fat grafting. The authors use adipose-derived stem cell (ASCs) and oxygen-releasing microspheres (Fig. 1) which are hydrogen peroxide microencapsulated materials to further improve the result of fat graft.

Methods: Twenty SD rats were performed autologous free fat graft (150~200 mg) from inguinal fat fad to back in following components: fat tissue only (Group I), fat tissue with microspheres (Group II), fat tissue with microspheres and low dose ASCs (5x10⁵ cell) (Group III), fat tissue with microspheres and high dose ASCs (1x10⁶ cell) (Group IV). ASCs were isolated human adipose tissue and microspheres were made by double emulsion method with hydrogen peroxide, poly (lactic-co-glycolic acid) (PLGA) and alginate. Rats were divided into two groups. Each group was harvested at 7 days or 14 days. The assessment was done by gross features and microscopy.

Results: In grossly, mean volume and weight are larger group II,III and IV than group I. Compared with group I, group III and IV are statistically increased in 14 days (p<0.05) (Fig. 2). But, there was no significantly difference between group III and IV. Under the microscopy, distance and area of survival adipocytes are checked. There was a statistically significant in 14 days. Mean distance from edge of graft tissue is as follows: 192 (Group I), 426 (Group II), 517 (Group III) and 683 (Group IV) (Fig. 3). Mean survival area ratio is as follows: 8.9 (Group I), 15.4 (Group II), 19.1 (Group III) and 22.6 (Group IV). Compared with group I, group II, III and IV are statistically increased (p<0.05). Also, distance and area of group III and IV are higher than group II (p<0.05).

Conclusions: To improve the outcomes of free fat graft, the stem cells are used and the effect is excellent. The authors use oxygen-releasing microspheres with ASCs in free fat graft. The oxygen-releasing microspheres could help fat survival and acting of ASCs through oxygen supply. So, oxygen-releasing microspheres and ASCs bring on synergic effect in free fat graft.
IMPROVEMENT OF SURVIVAL RATE OF FAT TRANSPLANTATION BY OXYGEN-RELEASING MICROSPHERES AND ADIPOSE-DERIVED STEM CELLS

Presenter: DongWoo Jung, MD, PhD
Authors: Jung DW, Kim YH, Lim JO, Kim TG, Lee JH
College of Medicine Yeungnam University Daegu Korea

ADIPOSE-DERIVED MESENCHYMAL STEM CELLS PROMOTE BREAST CANCER GROWTH AND METASTATIC SPREAD

Presenter: Pranitha Kamat, PhD
University of Zurich

Introduction: Stem-cell enriched fatgrafting has recently been proposed for reconstructive purposes on the breast level. This novel approach however has raised concerns about safety of stem cell-based therapies, especially in the post-cancer scenario. The aim of the present study was to investigate the interaction between human adipose-derived mesenchymal stem cells (AD-MSC) with human breast cancer cells (MCF-7- and MDA cell line) with a special focus on the tumor microenvironment, tumor growth and metastatic spread.

Methods: Human AD-MSC (CD34-CD73+CD90+CD105+) and MDA or MCF-7 breast cancer cell lines were used in this study. In vitro co-culture systems were utilized for assessment of cytokine analysis and viability assays. Using an in-vivo breast cancer model in nu/nu mice, cancer progression and metastasis was assessed. Different ratios of AD-MSCs and MDA/MCF-7 cells were investigated. Microdialysis was performed repeatedly for analysis of the microenvironment. Tumor and metastasis samples were analyzed with multiplex assays for oncogene expression, growth factors and metastatic phenotype markers.

Results: Metastatic spread (40% vs. 0% in controls) and mean tumor size (408±527mg vs. 38±99mg in controls, p<0.01) were both significantly increased by high AD-MSC aliquots in MDA tumors in vivo. These results were paralleled by up-regulation of RANTES, Eostatin and TNF-expression in vitro as markers of metastatic phenotype. In MCF-7 tumors epithelial-to-mesenchymal transition could be observed under the influence of high AD-MSC numbers with a switch in oncogene expression (HER2/neu). This was accompanied by follistatin and osteopontin expression indicating metastatic phenotype and poor outcome in the clinical scenario. In vivo tumor growth was significantly (p<0.05) promoted. Also metastatic spread was observed in 20% in comparison to 0% in cancer controls.

Conclusion: Our results suggest that human AD-MSCs bear potential to promote progression and metastatic spread in breast cancer. Epithelial-to-mesenchymal transition is associated with a development towards a highly aggressive phenotype.
UPSTREAM/PROMOTER ANALYSIS OF WNT SIGNALING ANTAGONIST, SFRP1 IN ADIPOSE DERIVED STEM CELL BIOLOGY: IMPLICATIONS FOR SOFT TISSUE RECONSTRUCTION

Presenter: Sudheer K. Ravuri, PhD
Authors: Ravuri SK, Philips BJ, Li X, Marra KG, Donnenberg VS, Donnenberg AD, Rubin JP
University of Pittsburgh

Introduction: Human adipose-derived stem cells (ASCs) are known to display differences in innate adipogenic potentials. Our studies demonstrated higher mRNA levels of Wnt signaling antagonist, Secreted Frizzled Related Protein1 (sFRP1) in mature or fully differentiated adipocytes, suggesting that over-expression of endogenous sFRP1 results in increased adipogenic potential of ASCs. However, hypermethylation and/or silencing of sFRP1 promoter often reduce and/or inhibit adipogenic potential of ASCs/pre-adipocytes. Little is known about the role of epigenetics in the regulation of ASCs differentiation. In this study, we focused on sFRP1 upstream/promoter analysis to determine its influence on adipogenic potential of ASCs.

Methods: ASCs isolated from 3 donors were cultured in both regular DMEM (undifferentiation) & adipogenic (differentiation) media for 14 days. RT-PCR was done on ASCs mRNA of 3 subjects with sFRP1 promoter specific primers and clones were constructed. At least five transformed bacterial colonies of each subject clone were sequence analyzed for sFRP1 promoter DNA. Corresponding mRNA levels of PPARy & FABP4 and lipid content in mature adipocytes were measured by respective qPCR & adipored assays.

Results: DNA sequence analysis of sFRP1 promoter showed 3-45% methylation in undifferentiated ASCs with hypermethylation of CpG motifs that correlated with reduced sFRP1 mRNA expression levels and intra-cellular lipid maturation with increased cytoplasmic beta-catenin mRNA. However, hypomethylated sFRP1 promoter in mature adipocytes showed increased but varying levels of sFRP1 mRNA expression among different subjects. Correlation between mRNA levels of sFRP1, PPARy (7-32 fold), FABP4 (6-25-fold) and intracellular lipid maturity in CD34+, CD31-pure pre-adipocyte sub-population was observed. Increased adipogenesis was found with sFRP1 augmentation under a strong promoter (CMV or Ef1a) without affecting the stemness of ASCs (CD34+).

Conclusions: CpG motifs and hypermethylation of sFRP1 promoter could negatively regulate adipogenic potential of ASCs, which has implications for soft tissue engineering. However, augmentation of endogenous sFRP1 expression under a strong promoter could greatly increase ASCs adipogenesis.

PREDICTABLE & DURABLE FAT GRAFTING BY TARGETED PROTECTION OF THE DONOR MICROVASCULATURE USING PHOSPHODIESTERASE 5 INHIBITORS

Presenter: Marc A. Soares, MD
Authors: Soares MA, Ezeamuzie O, Ojo C, Ham M, Saadeh PB, Ceradini DJ
New York University School of Medicine

Background: Autologous fat grafting is limited by unpredictable long-term retention of transplanted fat. We postulate that a functional donor graft microvasculature is critical for survival, and that vascular injury during harvest and the subsequent ischemic period may account for this clinical variability. Here we examine the use of the FDA-approved PDE5i sildenafil citrate to protect the donor graft microvasculature from ischemic injury, facilitating revascularization & long-term retention.

Methods: Inguinal fat from donor Tie2/LacZ FVB mice was infiltrated with sildenafil or saline 20min prior to harvest, and transplanted onto the dorsum of recipient FVB mice. Additional donors were perfused with intra-arterial trypsin to disrupt the fat graft microvasculature prior to harvest & transplantation. Graft revascularization, perfusion, volume of retention, and biochemical changes to the vasculature were assessed.

Results: Surviving fat grafts were characterized by exclusively donor-derived vasculature with peripheral inosculation to the recipient circulation. Disruption of the donor microvasculature with trypsin significantly decreased graft perfusion at 2 weeks vs. saline controls (47 FU vs. 97 FU, p<0.05) with complete graft loss by 8 weeks. Sildenafil markedly decreased ischemic injury to the donor vasculature (95% reduction in VCAM-1, 67% reduction in SDF-1 at 48hrs) and increased vascular survival at 2 weeks (400% increase in CD31 & LacZ expression) compared to saline controls (p<0.01 for all values). This improved early graft perfusion (167 FU vs. 97 FU at 2wks, p<0.05), and doubled the volume of retention at 12wks (80% vs. 40%, p<0.05) compared to saline-treated grafts.

Conclusions: Fat graft vascularization is critically dependent on maintenance of the donor fat vasculature during the ischemic period. Sildenafil protects the donor microvasculature during transfer and revascularization, markedly increasing long-term graft volume retention. These data demonstrate a rapidly-translatable, FDA-approved method to increase predictability and durability of autologous fat grafting in clinical practice.
THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE STROMAL CELLS IN RETINOPATHY OF PREMATURITY

Presenter: Rajashekhar Gangaraju, PhD
Authors: Gangaraju R, Callaghan B, Rogers P, Samuels B, March K
Indiana University School of Medicine

In USA, nearly 50% of low-birth weight infants develop Retinopathy of prematurity (ROP). Current strategies for the treatment of ROP including cryotherapy are not effective in all patients and there is unmet need for identification of new therapies. Cells derived from the stromal fraction of adipose tissue (ASC) are pluripotent stem cells, reside in perivascular niche, possess functional and phenotypic overlap with pericytes and improve ischemia reperfusion in-vivo. In this study, we hypothesized that ASC may stabilize vasculature and therapeutically rescue ROP features. To analyze the effect of ASC, postnatal day-7 neonatal immune compromised NSG mice along with nursing mothers were subjected to oxygen induced retinopathy (OIR). At p12 mice were randomized to receive human ASC (10,000 cells/eye/2μL) into the left eye and saline into the right eye. At p17, mice were euthanized; retinal wholemounts were imaged by confocal microscopy and mRNA expression by qRT-PCR. In-vitro human retinal endothelial cells (HREC) subjected to hypoxia were co-cultured with ASC and apoptosis was measured by Capase-3 and viability by WST-1 assay. Retinal wholemounts obtained from OIR mice, revealed a dramatic decrease in total tube length compared to age-matched control mice, was significantly increased in OIR mice that received ASC (p<0.001). In addition, OIR mice that received ASC demonstrated a 2-3 fold decrease in mouse specific ICAM-1, MCP-1 and an increase in VEGF and VEGFR2 compared to OIR mice that received saline. In-vitro, HREC formed capillary networks with ASC in direct contact co-culture, as evidenced by vWF staining. Immunostaining with alpha SMA suggested that HREC were able to direct differentiation of the ASC along a pericytic lineage potentially capable of stabilizing vasculature. Furthermore, HREC subjected to hypoxia demonstrated a significant decrease in cell viability and increased capase-3 staining, while those co-cultured with ASC alleviated apoptosis and improved cell viability. Our findings suggest that, ASC impart vasculoprotection under hypoxic/inflammatory conditions and may have therapeutic potential in treating ischemic conditions and pathological angiogenesis. Ongoing studies will provide vital information about vasculoprotective nature of these cells.

THE NOVEL APPROACH OF GENERATING SKIN FROM FAT

Presenter: Claudia Chavez-Munoz, MD, PhD
Authors: Chavez-Munoz C, Nguyen K, Xu W, Hong SJ, Mustoe TA, Galiano RD
Northwestern University

NOT PRESENTED
Unsegregated populations of adipose stromal cells (ASC) and pericytes (PC) are both readily and abundantly procured from adipose tissue with each showing promise for regenerative medicine. However, numerous studies now suggest that certain cellular subsets within each population may possess tissue-specific regenerative potentials. We sought to explore this hypothesis using single cell qPCR and clonal analysis. Previously, single cell qPCR analysis performed on the Biomark Fluidigm platform was used to examine the transcriptional signatures of ~430 genes in 30-40 single cells isolated from each of four subpopulations categorized by immunophenotype, both ASC (CD31-/CD45-/CD34+/CD146-) and PC (CD31-/CD45-/CD14-/CD146+), as well as ALDH1A activity, both Aldefluor–dim and Aldefluor–bright. Transcript profiling established transcriptional heterogeneity in all four subpopulation, and clearly discriminated ASCs from pericytes via the signatures of just nine dominant genes. Presently, we have performed whole transcriptome analysis (RNA-SEQ) on freshly sorted and passage 3 cultured Aldefluor–dim and Aldefluor–bright cells to uncover additional gene expression differences relevant to these four subpopulations, and changes that accompany tissue culture, to vet the suitability of clonal analysis to identify unique ASC and PC within SVF. Freshly sorted, Aldefluor–stained, single cells from two donors revealed that ~41% and ~38% of Aldefluor–mid and -bright cells, respectively, produced clones. Aldefluor–dim cells were not clonable. Interestingly, adipogenesis and osteogenesis occurred efficiently in only Aldefluor–mid and -dim stained ASC, when freshly sorted cell were induced to differentiate immediately post-sorting. Altogether twenty-four clones were examined with respect to proliferation rate, multipotency, paracrine properties, and immunomodulatory potential. Clones derived from Aldefluor–bright and -mid cells segregate into 3 groups based upon their proliferation rate, and morphological differences were also evident. Results from the tri-lineal differentiation of these clones will also be discussed as well as the possible implications of these results for selective applications in regenerative medicine.

**Introduction:** In adipose tissue engineering for reconstructive surgery, the generation of large vascularized constructs still remains a major challenge. Decellularized jejunal segments represent a potential scaffolding system providing the option of reconstructing pre-existing capillary structures and, thus, possibly of growing large tissue constructs. Here, utilizing a custom-made flow-through bioreactor system, we aim to a) reseed the capillary structures of decellularized porcine jejunal segments with microvascular endothelial cells (MVEC), and b) investigate adipogenic differentiation of adipose-derived stem cells (ASC) seeded in the jejunal lumen.

**Methods:** The capillary structures of decellularized porcine jejunum (segment length 8 cm) were seeded with 6*10^6 MVEC. After 14 days of culture within the flow-through bioreactor system, the jejunal lumen was seeded with 5*10^6 ASC. From day 21 on, adipogenic differentiation of ASC was induced applying a mixture of dexamethasone, IBMX, indomethacin, and insulin. Adipogenesis and vascular ingrowth was analyzed up to day 42 on the cellular and molecular level. For imaging, histology, immunohistochemistry and whole-mount staining was performed; furthermore, qRT-PCR was conducted.

**Results:** Two weeks after seeding of MVEC, successful repopulation of the capillary structures was shown by CD31 staining and MTT assay for living cells. In contrast to uninduced controls, adipogenesis was observed for induced ASC within the jejunal lumen over the course of the three week induction (day 21-42). Lipid droplets increasing in size were detected using histological Oil red O staining and whole mount staining employing a fluorescent BODIPY dye. Expression levels of the key transcription factors PPARgamma and C/EBPalpha and adipogenic marker genes such as aP2 and Glut4 increased concurrently. Remarkably, abundant vascular ingrowth occurred from the reseeded capillaries into the ASC-loaded jejunal lumen, as demonstrated by immunohistochemical and whole-mount staining for CD31.

**Conclusions:** Utilizing decellularized jejunal segments, a novel approach was established for the vascularization of adipogenic constructs in vitro. Currently, coculture conditions of ASC and MVEC are further investigated to optimize tissue development.
CELLULAR STRESS INCREASES TNF-ALPHA RESPONSE IN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS
Presenter: Tomas Robredo, BSc
Author: de Miguel F, Robredo T
La Paz University Hospital Research Institute

FAT GRAFTING: SKIN AND SCAR REGENERATIVE PROPERTIES
Presenter: Mohammad Nassimizadeh, MBChB, BSc
Authors: Nassimizadeh M, Nassimizadeh A, Dancey A
University Hospital Birmingham

A 40 year old female presented in Oct 2010 with a spike of cartilage eroding through a full thickness skin graft on her nose. She had a history of Basal Cell Carcinoma excised with Moh’s surgery in 1997. This was reconstructed with a full thickness graft and cartilage graft from her ear. She has had no recurrence to date. On examination she had a thin full thickness skin graft to the tip of her nose which had overlying telangiectasia and hypopigmentation. The cartilage framework was prominent and a small area of cartilage was close to eroding through the skin. Whilst shaving the spicule of cartilage would remove the immediate problem it would not improve the overall appearance of the nose. She had previously been offered a forehead flap reconstruction she had declined this on the basis that she did not want additional scars to her forehead. In February 2011 she underwent removal of the spicule of cartilage and fat grafting from the abdomen in order to disguise the framework and potentially revascularise the area. In March 2011 and Jan 2012 she was reviewed in clinic, and was delighted with the results. The overlying skin had improved in texture and quality, the colour had normalised and the telangiectasia had resolved. The cartilage framework was less obvious and the sharp contours hidden. This case illustrates the potential regenerative benefits of even small volumes of fat graft. This minimally invasive approach avoided the need for a complex reconstruction. From the pre and post fat transfer (pictures) results the regeneration of the overlying skin produced very satisfactory results.
FACIAL FAT GRAFT: A VARIETY OF SITE AND PURPOSE
Presenter: Marco Klinger, MD
Author: Klinger M
Universita Degli Studi di Milano Scuola di Specialit di Chirurgia Plastica Ricostruttiva ed Estetica

Introduction: Autologous fat tissue grafting is used for many clinical application from functional to aesthetic surgery. This is thanks to its properties both of filler and of regenerative tool. In particular, this function seems to be due to the mesenchymal stem cells reserve that is present in adipose tissue. In our experience autologous fat grafting improves mature scars and fibrotic tissues in terms of texture, colour, softness and quality of skin patterns. We decided to take advantage of this regenerative effects of fat grafting in order to treat peri-oral thickening and mouth opening limitation of patient with SSc.

Methods: The adipose tissue is harvested in local anaesthesia from the hips or the umbilical region given the easier access to abundant amounts of adipose tissue and it is subsequently centrifuged following Coleman's technique. The adipocyte fraction is injected using an 18-gauge angiographic needle with a snap-on wing at the dermal-hypodermal junction of facial skin areas needed to treat and a mild manipulation can be perform to refine the graft.

Results: In our experience with autologous fat grafting we obtained improvement in volume and skin texture. Almost all patients report a personal improvement in face appearance. In patients with Systemic Sclerosis we found that autologous fat grafting can improve mouth opening and function, induces a neovascularization, and seems to provide a sort of partial restoring of the skin structure.

Conclusions: Autologous fat tissue grafting can be used in surgery of the face to improve skin quality, to recreate a pleasant contour of the face, to fill soft tissue volume lost and to correct perioral fibrotic changes in sclerodermic patients. The versatility of this procedure make it useful not only as a filler of almost every part of the face, including nose and chin, but also as a rejuvenating-regenerative technique.

FAT GRAFTING AS THE LAST RESOURCE FOR FACIAL REJUVENATION IN PATIENTS WITH SEVERE CO-MORBIDITIES
Presenter: Marcos Sforza, MD
Authors: Sforza M, Andjelkov K, Zaccheddu R
Dola Park Hospital

Introduction: Facelift is undoubtedly the most effective treatment for facial rejuvenation despite the chosen technique. Patients seeking remarkable changes in their face, usually select facelift, the procedure of choice. These patients often present excess skin and an alteration on the facial volume distribution that reflects the loss of a youthful aspect. However, there are contraindications regarding this procedure, such as: severe heart conditions, COPD, heavy smokers and any other condition that speaks against a couple of hours under anesthesia for cosmetic reasons. Recently, thread lifts have been introduced to be applied to face lift which has proved to be rather effective. However, it should be noted that it has also systematically failed to improve the volume distribution. Many surgeons widely accept the use of fat as natural filler. The filling effect of fat has the potential to be used as a natural volumizing agent, improving the facial tissue distribution. Moreover, the anti-inflammatory properties of fat-derived stem cells are well recognized subsequent to the cell transfer to other areas, naturally giving the facial skin a better aspect. In this paper authors present retrospective study of 60 patients who had a facial “thread lift” associated with fat injections to enhance the deformities caused by the residual excess skin and aging.

Method: All sixty patients presented a formal indication for a facelift, but had co-morbidities that contradicted the procedure. The age ranged between 59 and 73 years. In all patients, the fat was harvested and processed using the Puregraft® system and the threads used were the Silhouette®. The fat was usually harvested from the legs and the volume of fat transferred ranged from 10cc to 30 cc, with average of 20cc per procedure. The procedures were performed under local anesthesia only.

Results/Complications: The results were evaluated by comparing before and after pictures and a satisfaction rate from the patients. Patient’s evaluation of results at 3 months were “excellent” in 100%. After 6 months the results were “excellent” in 70% of cases and “good” in 20% and fair in 10%.

Conclusion: The medical team was highly satisfied with the Silhouette® threads as the procedure was quick and pain free for the patient.
FAT GRAFTING AS THE LAST RESOURCE FOR FACIAL REJUVENATION IN PATIENTS WITH SEVERE CO-MORBIDITIES
Presenter: Marcos Sforza, MD
Authors: Sforza M, Andjelkov K, Zaccheddu R
Dola Park Hospital

EVALUATION OF THE HISTOLOGICAL CHANGES OF THE FAT GRAFTED FACIAL SKIN: CLINICAL TRIAL
Presenter: Patricio Covarrubias, MD
Authors: Covarrubias P, Cardenas-Camarena L, Guerrerosantos J, Valenzuela L, Espejo I, Robles JA, Gioia S
DIPRECA Hospital

WITHDRAWN
EVALUATION OF CLINICAL OUTCOME OF AUTOLOGOUS FAT TRANSPLANTATION WITH SMALL-NEEDLE-KNIFE IN RECONSTRUCTION OF BODY SURFACE CONCAVE DEFORMITY

Presenter: Jianhong Long, MD
Authors: Long J, Sun Y
Xiangya Hospital of Central South University

NOT PRESENTED
A PHASE ONE, OPEN LABEL, SINGLE ARM STUDY TO DEMONSTRATE THE SAFETY OF THE ANTRIA CELL PREPARATION PROCESS DURING FACIAL FAT GRAFTING ASSISTED WITH AUTOLOGOUS, ADIPOSE-DERIVED STROMAL VASCULAR FRACTION (SVF)

Presenter: Shah Rahimian, MD, PhD
Authors: Rahimian S, Maliver L, Johns F, Bizousky D, Gore R, Johnson T, McNitt D, Quist L

Antria Inc

Antria cell preparation process and its special reagent Adipolyx may display a safe method of supplementing traditional lipografts with adipose-derived stromal vascular fractions (SVFs), which can be utilized in cosmetic or therapeutic applications. Autologous transplantation of adipose tissue is a common treatment for facial lipoatrophy; however, treatment-result inconsistencies, regarding the sustainability of the adipose engraftment, require identification of a more efficacious treatment option according to Ersek et al. (1998) and Shiffman et al. (2001). In addition, facial lipoatrophy has been treated utilizing dermal fillers; however, dermal fillers are a less advantageous treatment option due to composite deterioration. Moreover, dermal fillers may induce allergic responses, skin depigmentation, and/or nasolabial folds according to Lowe et al. (2001).

Cellular components of the SVF have shown to secrete various growth factors that sustain the lipograft. Imperative to the function of SVF, in conjecture with lipoaspirate, is believed to be adipose-derived stem cells (ADSCs). According to Puglisi et al. (2010), Ichim et al. (2011), and Lu et al. (2011), ADSCs possess the ability to differentiate into various tissue types, inhibit inflammation, and stimulate angiogenesis. Thus, the proprietary reagent and the methodology of extracting and integrating the SVF with adipose tissue, utilized in transplantation, may enhance graft retention. Ergo, Antria will analyze the safety of SVF use in facial fat grafting via targeted physical examinations, laboratory assessments and long term follow ups concluding at 36 months post-op.

Antria has recently gained FDA and IRB approval to conduct a phase I study, within the United States, verifying the safety of SVF-enhanced lipografts within human subjects. Six subjects will be enrolled. Analysis of the resultant data, documenting whether Antria cell preparation process is a safe form of treatment, will be available at the time of presentation.

AESTHETIC AND FUNCTIONAL RECOVERY OF CONGENITAL MUSCULAR TORTICOLLIS TREATED WITH INTRAMUSCULAR FAT GRAFTING

Presenter: Juan Monreal, MD
Author: Monreal J
Hospital San Rafael

Congenital muscular torticollis is the third most common congenital musculoskeletal disorder. The condition usually presents as a result of contracture and shortening of the sternocleidomastoid muscle. Skull and facial asymmetry may occur in the presence of prolonged uncorrected head tilt. Treatments include observation, the use of orthotics, exercise programs, traction, and various forms of surgical techniques. These include open or endoscopic transection or release of one muscle insertion, bipolar release, and radical resection of a sternomastoid tumor or the sternocleidomastoid muscle. Some authors have stated that operative treatment is of little value after the age of five, and the results are even worse when the operation is done after puberty. All the best known surgical techniques rely on the fact that the muscle must be elongated in some form, but none of them (if succeeded) provide a good combination of aesthetic and functional improvement. Fat grafting has never been reported as a technique to treat congenital muscular torticollis, and has only been partially reported as surgical technique as a means of replacing volume after a complete sternocleidomastoid resection. The author reports a case of a 19 year old male that presented with severe sequel of congenital muscular torticollis unsuccessfully treated with open bi-polar release in his infancy. The patient presented with a severe retraction and thinning of his left sternocleidomastoid muscle associated with ipsilateral craniofacial deformity and scarring at the level of muscular release. Given that other muscle release, probably could not improve the condition, the plan was to conduct micromiotomies associated with fat grafting along the entire length and thickness of muscle. Two fat grafting sessions were performed over a period of six months. Eleven months after the first session the patient has recovered almost all of the aesthetics of the neck and muscle function without any adverse effects or loss of grafted volume. Due to the extremely good outcome of this patient, the author believes that fat grafting should be considered as a more logical and less invasive alternative for the treatment of congenital muscular torticollis particularly in failed or neglected cases.
Purpose: Complications from silicone injections have been frequent and difficult to manage. Our goal was to evaluate a surgical option for these desperate patients in order to minimize their risks of complications from these procedures.

Methods: All patients presenting to us with complications from "silicone injections" into their gluteal region were evaluated by CT scan examination and visual analogue scale for pain, ER visits, previous admissions, and cellulitis requiring antibiotics within the past year were recorded. Patients were treated with ultrasonic and suction assisted liposuction followed by lipotransfer into the gluteal musculature.

Results: Eight patients were evaluated. Pain evaluation using a 0-6 scale with 100% of patients presenting with intense pain and by 12th week 100% had remission. The student t-test for paired means in one year follow up for the following were evaluated: infections, 75% of our patients presented with infection preoperatively while 100% of patients (p 0.028) did not present with infections after the procedure; ER visits, 50% of patients appeared to the ER preoperatively while 100% of patients (p 0.058) did not visit the ER after the procedure. All patients presented a median of 1.5 hospitalizations preoperatively while 100% of patients (p 0.066) did not get hospitalized postoperatively. (Case Example: Figures 1a-c. showing 37 year old female who could not sit down secondary to pain and multiple hospital admissions for cellulitis, CT scan obtained preoperatively revealed silicone toxicosis and subsequently treated with SAL for fat harvest, UAL and SAL of buttocks to reduce silicone toxicosis burden and postoperative result at 6 months with no pain or hospital admissions since surgery at one year).

Conclusions: Ultrasound and suction-assisted liposuction with immediate intramuscular fat transfer for buttock augmentation, appears to be a surgical option for patients suffering from the effects of gluteal silicone toxicosis which preserves their aesthetic appearance.
Fat grafting is commonly used procedure for reconstructive, therapeutic & cosmetic purposes. It is regarded as low risk of complication procedure, which of absorption is most common.

**Aim:** To illustrate procedure related difficulties in correlation to complication rate.

**Material & Method:** This is overview of complications of fat grafts done over period of 2007-2012 for various clinical problems including treatment of scars, contractures, keloids, craniofacial & cleft, oculoplastic reconstructions & radionecrosis.

**Conclusions:** Analyzing above it is suggestive that higher fat absorption & higher infection rate occurs when:

- Recipient area contains foreign body like medpore or acrylic implants.
- There is bacterial colonization of chronic wounds
- Contour correction is difficult in periorbital area
Fat transferred to scars, keloids, contracture has very good take with low reabsorption rate.
FAT GRAFTING AS ADJUVANT TO REDUCE SCARS IN ARM LIFTS

Presenter: Katarina Andjelkov, PhD
Authors: Andjelkov K, Sforza M, Kuka G, Zaccheddu R
Private Clinic

Introduction: Upper arm deformities secondary to senile elastosis have led to an increased demand for aesthetic contouring procedures such as brachioplasty. These patients often present excess skin and a “wrinkly” skin aspect that deprives them of using short sleeve clothes, which would leave their arms exposed. The conventional brachioplasty technique in many cases, involves a long scar that precludes the patient from revealing the arm. Moreover, the conventional procedure is assumed to be associate with high percentage of revisions and undesirable scarring. Recently, short scars techniques (arm pit scars only) have been proved to be effective to a certain extent, however they systematically fail to improve the “wrinkly” area caused by non removed excess skin. In this paper, authors present retrospective study of 40 patients who had a “short scar” brachioplasty associate with fat injections in order to improve the deformities caused by the residual excess skin.

Method: All forty patients presented excess skin in their arms and expressed the desire for surgical improvement without the use of the extensive scars. The age ranged between 58 and 63 years. In all patients, the fat was harvested and processed using the Puregraft® system. The fat was usually harvested from the arms (or other parts of the body if arm surgery was associated with other surgeries) and the volume of fat transferred ranged from 60cc to 110 cc per arm, with average of 160cc per procedure.

Results/Complications: The results were evaluated by comparing before and after pictures and a satisfaction rates were obtained from the patients. Patients evaluation of results at 3 months were “excellent” in 90% of cases, “good” in 5%, and “fair” in 5%. After 6 months the results were “excellent” in 70% of cases and “good” in 20% and fair in 10%.

Conclusion: The medical team was highly satisfied with the Puregraft® system as we could process more fat, quicker. At 6 months, a percentage of the injected fat had been reabsorbed, but the results were very predictable and reproducible. This technique entails minimal associated risks and complications. Consequently, It has been shown to be very effective, in particular, in the areas where the “short scar” brachioplasty fail to produce improvements.
Wide facial scars are a frustrating problem that has challenged many plastic surgeons. To provide mechanical support to the skin edges during wound healing, the author published a surgical technique based on creating a scar flap that would bear tension of surrounding muscle pull rather than the skin edge (Wilson AM. Widening of Scars: Foe coaxed into friend? The Millard technique revisited. Plast. Reconstr. Surg. 2000, 106: 1488). The scar was de-epithelialized during revision surgery, and a tongue of scar tissue was created and buried as a double breasted flap to bear all tension on the wound rather than the suture line. Thus tough scar tissue is rendered a central beam, bearing all the tension of distracting forces and minimizing any tension on the skin edges. Another approach to reduce the effect of tensile forces distracting wounds during the healing process would be through weakening muscles pulling on the wound, thus gaining the crucial advantage of providing rest to the part in healing until collagen maturates. To achieve this goal, the author extrapolated the use of Botulinum toxin on human scars and improvement was noted in the outcome (Wilson AM. Use of Botulinum Toxin Type A to prevent widening of facial scars. Plast. Reconstr. Surg. 2006, 117: 1758). Since 2007, the author has been using a combination of both techniques in resistant scars. This double attack would provide ultimate rest to the wound edges since the tough beam of scar tissue flap would bear all tension on the wound, and simultaneously surrounding muscles were immobilized until collagen reaches maturation.

Since 2011, the author has stopped using those techniques and replaced them with grafting of stem cells to all wide facial scars presenting to his practice. In 15 wide facial scars, use of both surgery and botulinum toxin was omitted, and only ASC was grafted. The scars were followed up for 12 months. The outcome of scars treated by stem cell grafting were compared to results of similar scars previously treated by mechanical support and/or botulinum toxin techniques. Scars with similar length, orientation, site, skin type and pretreatment width were subjected to the comparison. Results of comparison performed retrogradely to these treatment modalities is to be presented.
ADIPOSE DERIVED STEM CELL THERAPY AMELIORATES DELAYED WOUND HEALING AFTER RADIATION BY MODULATING TGF-BETA EXPRESSION
Presenter: Alex K. Wong, MD
Authors: Ragina N, Hoang D, Hill C, Lee YS, Kim G, Han B, Hong Y, Chuong CM, Senagore A, Urata MM, Wong AK

Keck School of Medicine of USC

Background: Recent studies have reported that Adipose Derived Stem Cells (ADSCs) in lipoaspirated fat could significantly improve non-healing, radiation-damaged wounds. However the mechanism of action is not completely understood, and limited animal studies have restricted approval of this technique in human patients. Therefore, we have developed a murine model of chronic radiation injury to determine whether there is a beneficial effect of topical delivery of ADSCs on radiation-induced wound delay.

Methods: To generate a murine model of chronic radiation-induced wound delay, an 8 x 4 cm field on the dorsal, lateral surface of Balb/c mice was irradiated 10Gy of radiation. After 6-8 weeks a 0.8cm full thickness circular wound was created utilizing the splinted wound model. ADSCs were derived from inguinal fat pads were from 6-7 week GFP transgenic mice using previously described protocols. Different quantities of ADSCs were applied topically to the wounds in a transglutaminase gel matrix. Fibroblast cells in the gel or gel alone without cells were used as controls.

Results: Wounds were assessed on post operative day 18 and 26. Topical delivery of 1x10^5 ADSCs to radiated wounds resulted in 2.5 and 2 times greater wound closure compared to 1x10^5 fibroblasts or no cells, respectively [p=.008 and p<.005]. By day 26 the rate of wound closure post ADSCs application, resembled that of the wound closure in unirradiated animals. No difference in wound closure was observed in wounds that were not irradiated, with or without the application of ADSCs. Further analysis revealed that ADSC application reduced pathologic upregulated TGF-beta expression in healing irradiated wounds and decreased M2 Macrophage recruitment to the wound bed.

Conclusions: We have established a reproducible pre-clinical model of radiation-induced delayed wound healing in Balb/c mice. We demonstrated that purified ADSCs delivered topically in a biologic matrix significantly accelerated closure in wounds in chronically irradiated animals compared to the controls. This data can serve as a potential basis serve as a basis for IRB-approved clinical trial in patients with non-healing radiation induced wounds. Further studies are underway to better define the mechanism of action for this promising therapy.

A NOVEL KERATIN MICROSPHERE DELIVERY OF ADIPOSE-DERIVED STEM CELLS FOR ACCELERATED HEALING OF DIABETIC FOOT ULCERS
Presenter: Hilal Arnouk, MD, PhD
Authors: Arnouk H, Bharawaj S, Nagy T, Barrows TH
Cell Constructs Inc

CANCELLED
IMPROVEMENT OF THE FIBROTIC DERMAL TISSUE BY A COMPOSITE GRAFT MADE OF AUTOLOGOUS ADIPOSE MESENCHYMAL STEM CELLS (AMSCS) SEEDED ON HUMAN ACELLULAR COLLAGEN MATRIX (HACM): APPLICATION IN LARGE CRITICAL SIZE OF CHRONIC WOUNDS

Presenter: Aurore Lafosse, MD
Authors: Lafosse A, Aouassar N, Hanet MS, Andre W, Lengele B, Vanwijck R, Dufrane D

University Clinical Hospital Saint Luc UCL

Introduction: This work aims to develop a bioengineered graft made of autologous AMSCs/HACM to remodel the fibrotic dermal tissue in view to restore the quality of the chronic wound bed for an epidermal grafting.

Methods: 1) The biocompatibility of the scaffold was assessed after implantation in rats (n=32) to study the kinetic of HACM integration/remodelling. 2) Human AMSCs (n=8) were isolated/proliferated up to Passage 4th and characterized by a significant higher in vitro VEGF release for AMSCs incubated at 0.1% PO2 (9636 pg/ml) vs. 5/21% PO2 (<2500 pg/ml, p<0.001). 3) Human AMSCs adhesion/spreading on the HACM was studied in vitro by confocal laser scanning microscopy up to day 30 post-incubation (n=40). 4) AMSCs genetic stability was in vitro/in vivo assessed by Karyotyping/FISH and tumorogenicity in nude rats at Passage 1st/4th/>10th, respectively. Three patients with large untreatable chronic wounds were implanted with this composite graft to follow: angiogenesis, biological inflammatory reaction, microscopical tissue remodelling (for lymphocytes, macrophages, angiogenesis, epithelialization prior-/after transplantation) and the clinical outcome.

Results: HACM demonstrated an in vivo integration compatible with the AMSCs delivering in a chronic wound at day 30 post-implantation. The composite graft was implanted in patients when 90% of HACM was covered by AMSCs (58 000 cells/cm2) with a spindle-shape configuration of AMSCs (a significant reduction of the cellular shape factor: 0.93±0.13 vs. 0.31±0.03 at day 1 and 27, respectively, p<0.005). In the 3 patients, the graft was macroscopically integrated at day 28 post-implantation without any sign of inflammation. At day 53 post-implantation, the dermis was characterized by (i) a significant higher VEGF expression (+176%, p<0.001) and Fact VIII+ cells (+276%, p<0.05); (ii) a significant lower CD3 infiltration (-54%, p<0.005) and (iii) a decrease of fibrosis in dermis in comparison to the pre-implantation biopsies, allowing a stable epithelialization post-skin implantation. Currently, a maximum of complete healing is maintained after 22 months of follow-up.

Conclusion: This bioengineered graft made of autologous AMSCs potentiates angiogenesis and dermis remodelling to cure fibrotic area in chronic wounds.

OUTCOMES OF FAT GRAFTING IN NON-HEALING WOUNDS

Presenter: Kristen Aliano, MD
Authors: Aliano K, Bassiri-Tehrani B, Stavrides S, Mathews B, Davenport T

Long Island Plastic Surgical Group

Non-healing wounds are responsible for significant morbidity and hospital cost. Radiation and trauma are notable causes of chronic wounds. Such lesions develop when the chemical mediators responsible for tissue regeneration are not released in a timely manner. Advances in stem cell therapy have revolutionized the means by which plastic surgeons treat these wounds. Human adipose tissue, which can easily be harvested from the abdomen by liposuction, contains adult mesenchymal stem cells that not only have the potential to differentiate into a variety of different cell lineages, but they also have the capability of secreting angiogenic growth factors that can facilitate wound healing. Here, we review our experience with fat grafting for chronic traumatic and radiation-induced wounds.

We conducted a retrospective chart review of three patients with chronic wounds who underwent autologous fat grafting as part of their wound management. Two patients were female and one was male; they ranged in age from 52-63 years of age. Two patients developed wounds secondary to radiation therapy and one developed a sore that resulted from a minor traumatic accident.

Prior to fat grafting, the patients had been treated with other wound care therapies, such as debridement, hyperbaric oxygen therapy, and skin grafting. However, their wounds were not healing. The subjects then underwent between one and three fat grafting procedures in order to provide tissue bulk and stem cells to the affected areas. All of the patients’ wounds became smaller, they had accelerated wound healing, and they experienced improvements in their functional capabilities.

The adipose-derived stem cells facilitate wound healing and tissue regeneration by secreting the needed mediators that are absent from severely injured tissue. Fat grafting is a minimally-invasive procedure that can be a useful means of treating chronic, difficult-to-heal wounds.
DIABETIC ULCER TREATMENT BY INJECTABLE
COMMUNUTED ADIPOSE CONNECTIVE TISSUE (CAC):
A THERAPEUTIC TOOL OF ADIPOSE- DERIVED STEM
CELLS (ASCS) WITHOUT NEED OF CELL ISOLATION
Presenter: Kentaro Doi, MD
Authors: Doi K, Yoshimura K, Kato H, Kuno S, Mineda K, Kinoshita K
University of Tokyo

Introduction: ASCs are generally isolated from aspirated fat tissue through collagenase digestion, but isolated ASCs may not be functioning in the unphysiological condition. To maximize its therapeutic potency, ASC are frequently mixed with various growth factors and bioscaffolds. We experimentally used micro-fragmented adipose connective tissue as an injectable therapy for diabetic ulcer. The comminuted adipose connective tissue (CAC) contained functional ASCs in their physiological microenvironments.

Method: Mouse inguinal fat pad was manually micro-minced by surgical scissors for 5 minutes and suspended in PBS. After centrifugation, the minced fat tissue was separated into two portions; upper floating portion as minced fat tissue and sediment portion as CAC. Histological analyses for both were performed by scanning electron microscope and whole mount 3D imaging. ASC content and viability in CAC was checked by explant culture. Injection of CAC obtained from healthy mice was compared with PBS injection to treat skin ulcers in diabetic mice with silicone splints (n=4). The wound healing was evaluated by measuring ulcer size on days 0, 2, 4, 7, 9, 11 and 14.

Results: Histological analysis showed CAC was mainly fibrous structures containing small vasculatures with little mature adipocytes. ASC growth was detected after 3.3±0.8 (average±SEM; n=3) days of explant culture. CAC was injectable with a 29G needle. CAC injection to diabetic ulcers appeared to accelerate wound healing compared to PBS and significant ulcer size reduction was observed as early as on days 4 and 7.

Conclusion: We successfully prepared CAC that is supposed to contain substantial amount of ready-to-function ASCs as well as vascular endothelial cells and pericytes. Single CAC injection promoted the wound healing of diabetic ulcer in mice. The CAC preparation is simple and does not require any enzymatic digestion. Although CAC yield of current method is small and should be further optimized, CAC skips multiple processes in SVF isolation and may lead to more predictable therapeutic outcomes.
POINT-OF-CARE DERMAL REPLACEMENT CONSTRUCTS CONTAINING ADIPOSE-DERIVED SVF CELLS

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

Background: The formulation of autologous cell-seeded constructs in real-time at the “point-of-care” may engender translational and regulatory advantages. However, this strategy is also associated with various technical challenges – such as thorough and uniform seeding of a scaffold with cells. We hypothesized that freshly isolated human adipose-derived stromal vascular fraction (SVF) cells could survive homogenous seeding in “real time” into dermal scaffold materials; proliferate within these constructs; and secrete bioactive factors that are critical to tissue repair and effect the migration of wound-related cells.

Methods: Freshly uncultured human SVF cells were isolated and combined with dermal scaffold and ECM, and the resulting constructs placed into culture. Parallel control groups consisted of constructs without cells. During a 14-day period, cell viability was assessed using AlamarBlue staining (Invitrogen) and conditioned medium was collected for analysis of growth factor levels, and for evaluation of bioactivity in the context of cell migration assays. On day 14, constructs were processed for histology.

Results: Freshly isolated human SVF cells were seeded into constructs (30,000-60,000 cells/mm3). Cells were reproducibly and uniformly distributed within dermal constructs in “real time”, and remained viable and proliferative during 14 days of culture (average increase in cell number ~157%). A number of important angiogenic and immunomodulatory proteins were detected at elevated levels (compared to acellular controls) using Luminex® protein quantification assays, and conditioned media from cell-containing constructs enhanced the transwell migration of keratinocytes and endothelial cells. On histology, lumen-like structures with CD31+ staining suggest the formation of neo-capillaries.

Conclusion: Our results demonstrate that freshly isolated human cells can be reliably 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. This point-of-care strategy may provide a flexible platform for the translation of autologous cell-based therapies for a number of clinical challenges.

FAT GRAFTING A CURE FOR ALOPECIA?

Presenter: Abdul Karim Nassimizadeh
Authors: Nassimizadeh M, Nassimizadeh A, Dancey A
University Hospital Birmingham

A 56 year old male presented with a 4 cm parotid gland carcinoma and underwent a left parotidectomy and a neck dissection followed by radiotherapy. Following surgery he had a 20cm indented scar from his left temple down to his left neck with scar alopecia. He had a tight scar across his neck anteriorly causing a slight restriction of movement and scar contracture. The loss of soft tissue lead to a marked contour deformity of the face and neck. He underwent 5 episodes of fat grafting from the abdomen ranging from 30cc to 80cc with riggotomies to release the scar tissue. On final review & after the last episode of fat grafting he had good facial symmetry with softening of the scar and improvent in the quality and colour of the skin. Most interestingly the hair had regrown within the scar alopecia in his temple.

This case illustrates the potential regenerative benefits of even small volumes of fat grafting to radiotherapied facial scar in resolving the soft tissue deficits and restoring facial symmetry. From the pre and post fat transfer (pictures) results the regeneration of the overlying skin produced outstanding results as well as interestingly the regrowth of hair.
EFFICACY OF ENDOSCOPIC SUBMUCOSAL INJECTION OF HUMAN ASC FOR THE TREATMENT OF EXPERIMENTAL COLITIS IN RATS

Presenter: Fernando de Miguel, PhD
Authors: de Miguel F, Martin Arranz E, Robredo T, Mancheno P, Menta R, Diez J, Lombardo E
La Paz University Hospital Research Institute

Introduction: Mesenchymal stem cells, due to their immunomodulatory properties, have a potential role in inflammatory bowel disease (IBD) therapy, having shown promising effects through i.v. or i.p. injections both in animal models and in patients treated for other concomitant diseases. We aimed at evaluating feasibility, safety and efficacy of endoscopic administration of human ASC in a colitis model in rats.

Methods: Colitis was induced in SD-OFA male rats (375-400 g) by rectal enema of 0.5 ml 2,4,6 trinitrobenzensulfonic acid 30 mg/ml in 50% ethanol. On day +1 a colonoscopy was performed with a 5.9 mm endoscope (GIF-XP160, Olympus Optical Co). Rats were randomly assigned to treatment group (ASC: 10^7 cells, endoscopically injected divided in 4 spots in the colonic submucosa using a 22 G endoscopic needle) or control (phosphate buffered saline, PBS). hASC were isolated and cultured from healthy donors and characterized as usual (Tigenix SAU, Tres Cantos, Spain). On day +11 another colonoscopy was performed and rats were sacrificed. We analyzed: a)Daily weight (% from day 0); b)Endoscopic score (0-30) at day 1 and 11; c)Colon length (mm); d)Macroscopic appearance at necropsy (0-9).

Results: Colitis was induced in 46 rats: 25 hASC/21 PBS (26 rats healthy controls). Endoscopic injection was successful in all rats, with visible submucosal bleb formation. No significant adverse events and no mortality due to the procedure occurred. Weight recovery was significantly better in ASC group on day 9 (-8.45% vs -3.66%, p 0.034), 10 (-8.42% vs -1.94%, p 0.013) and 11 (-6.72% vs -0.84%, p 0.037). Colon length significantly recovered (ASC 222 mm vs PBS 198 mm vs healthy controls 235 mm) ASC vs PBS p<0.001. Endoscopic score change between day 1 and 11 improved in ASC group -6.65 vs -3.53 in PBS group p 0.011. There was a high correlation between endoscopic score and weight loss on day 1 (r=0.75, p<0.001) and on day 11 (r=0.78, p<0.001). Stenosis was more frequent in PBS group (41.2% vs 4.8%) p 0.01. Macroscopic necropsy score showed a trend to less inflammation.

Conclusion: hASC submucosal endoscopic injection is feasible, safe and ameliorates TNBS-induced colitis in rats. We have initiated a clinical trial with ASC for mild ulcerative colitis (EudraCT 2010-023798-20).

ADIPOSE STEM CELL IN LIMITATION OF ACUTE LUNG INJURY

Presenter: Natalia V. Bogatcheva, PhD
Authors: Bogatcheva NV, Lu H, Poirier C, Traktuev DO, Cook T, Merfeld-Clauss S, Petrace I, March KL
Indiana University

The accumulating body of evidence shows that Mesenchymal Stem Cell application can alleviate Acute Lung Injury (ALI) in vivo. Here we tested the ability of Adipose Stem Cells (ASC) to suppress endotoxin (LPS)-induced ALI, and compare therapeutic properties of ASC to the properties of cell-free media enriched with stem cell secreted factors. To induce ALI, we instilled LPS into the lungs of C57BL/6 mice. At the peak of hypothermic response to LPS (4h post LPS administration), we injected mice intravenously with cultured murine ASC (3rd passage) or ASC conditioned media (ASC-CM). 48h later, mice were sacrificed for the analysis. 1h prior sacrifice, mice were injected with the tracer of vascular permeability (Evans Blue Dye). Upon sacrifice, bronchoalveolar lavage fluid (BALF) was collected. Lungs were flushed free of blood and analyzed for the content of Evans Blue Dye. LPS-induced ALI was associated with the accumulation of neutrophils and protein in BALF, increase in proinflammatory cytokine TNF alpha, and the accumulation of Evans Blue Dye in lungs tissue. Our data show that ASC application significantly reduced BALF white blood cell content, whereas ASC-CM application showed similar trend with no significant level of reduction. LPS-induced vascular leak was also stronger attenuated by ASC than by ASC-CM. On the contrary, protein content in BALF was significantly decreased by ASC-CM, but not by ASC application. Both ASC and ASC-CM decreased the level of pro-inflammatory TNF alpha in BALF. Surprisingly, anti-inflammatory cytokine IL 10 was only increased in ASC/LPS lungs, but not in the ASC-CM/LPS lungs. These data show that both ASC and ASC-CM suppress the development of ALI in LPS-induced model; however, the targeted components of the edemagenic response and the key suppressing mechanisms are different for ASC and ASC-CM. Further studies are needed to develop ASC-based therapy for the possible application for the treatment of Acute Lung Injury and Adult Respiratory Distress Syndrome in patients. This work was supported by VC-CAST and Indiana University Health – Indiana University School of Medicine Strategic Research Initiative.
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**INTRALYMPHATIC ADMINISTRATION OF ADIPOSE MESENCHYMAL STEM CELLS SHOWS THERAPEUTIC EFFECTS IN EXPERIMENTAL COLITIS AND ARTHRITIS**

**Presenter:** Eleuterio Lombardo, PhD  
**Authors:** Escolano A, Garin M, Lopez-Santalla M, Menta R, DelaRosa O, Redondo JM, Dalemans W, Lombardo E

*TiGenix*

**Background and Aims:** Rheumatoid arthritis and inflammatory bowel disease are both systemic chronic inflammatory diseases with a great impact on the patient's quality of life. Mesenchymal stem cells (MSCs) of allogeneic origin have been reported to reduce inflammatory processes and could therefore have therapeutic effects in immune disorders. The aim of our work was to determine potential anti-inflammatory and therapeutic effects of human adipose-derived MSCs (hASCs) in well-established models of rheumatoid arthritis and inflammatory bowel disease. In this study, we did compare the intralymphatic (IL) route of administration, an innovative route for ASC administration developed by us, with other routes: intraperitoneal (IP) and intravenous (IV).

**Methods:** We administered hASCs in the inguinal lymph nodes of mice and compared the distribution of Luciferase-expressing hASCs in healthy and diseased mice using IL, IV and IP routes of administration. We examined the therapeutic action of hASCs in colitis induced by administration of trinitrobenzene sulfonic acid or dextran sodium sulphate (using IP, IV and IL routes), and in arthritis induced by administration of chicken collagen II (using IV and IL routes) in immunocompetent mice. Efficacy was determined by clinical signs of the disease, inflammatory markers and imaging techniques.

**Results:** For the first time to our knowledge, we report that IL administration of hASCs ameliorates the severity of colitis and arthritis. Distribution of hASCs varied depending on the route of administration and the inflammatory status, and a different mobilization throughout the lymphatic system and main organs was observed depending on the experimental conditions.

**Conclusions:** The direct administration of adult human ASCs to the lymphatic system is an effective alternative for cell-based therapy for the treatment of inflammatory and autoimmune disorders.

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**COMPARISON OF THE THERAPEUTIC EFFECTS OF HUMAN AND MOUSE ADIPOSE STEM CELLS IN A MURINE MODEL OF ACUTE LUNG INJURY**

**Presenter:** Trivia Frazier, MD  
**Authors:** Frazier T, Bunnell BA, Zhang S, Danchuk S, Gimble J, Betancourt A, Sullivan D

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**Introduction:** Adipose-derived stem cells (ASCs) have emerged as important regulators of inflammatory/immune responses in vitro and in vivo and represent attractive candidates for cell-based therapies for diseases involving excessive inflammation. Acute lung injury (ALI) is an inflammatory condition for which treatment is mainly supportive due to the lack of effective therapies. In this study, the therapeutic effects of ASC-based therapy were assessed in vivo by comparison of the anti-inflammatory properties of both human and murine ASCs in a mouse model of lipopolysaccharide (LPS)-induced ALI.

**Methods:** Human ASCs (hASCs) or mouse ASCs (mASCs) were delivered to C57Bl/6 mice by oropharyngeal aspiration (OA) four hours after the animals were challenged with LPS. Mice were sacrificed 24 and 72 hours after LPS exposure, and lung histology examined for evaluation of inflammation and injury. Bronchoalveolar lavage fluid (BALF) was analyzed to determine total and differential cell counts, total protein and albumin concentrations, as well as myeloperoxidase (MPO) activity. Cytokine expression in the injured lungs was measured at the steady-state mRNA and protein levels for assessment of the degree of lung inflammation.

**Results:** Treatment with both human and mouse ASC provided protective anti-inflammatory responses. Both cell types resulted in decreased levels of leukocyte migration into the alveoli, total protein and albumin concentrations in BALF and MPO activity after the induction of ALI. Additionally, cell therapy with both cell types effectively suppressed the expression of pro-inflammatory cytokines and increased expression of the anti-inflammatory cytokine interleukin 10 (IL-10). Overall, the syngeneic mASC therapy had a more potent therapeutic effect than the xenogeneic hASC therapy in this model.

**Conclusions:** Treatment with hASCs or mASCs significantly attenuated LPS-induced ALI. These results suggest a potential benefit for using an ASC-based therapy to treat clinical ALI and may possibly prevent development of acute respiratory distress syndrome (ARDS).
EARLY PASSAGE ADIPOSE-DERIVED STROMAL CELLS ACCELERATE HEALING IN WOUNDED 3-D CULTURES

Presenter: Sherry S. Collawn, MD, PhD
Authors: Collawn SS, Chow LT, Banerjee NS
UAB

Introduction: Early passage adipose-derived stromal cells (ADSC) accelerate wound re-epithelialization in injured 3-D skin cultures.

Method: Adipose tissue is harvested during abdominal liposuction, collagenase digested, centrifuged, and expanded in culture. The early passage ADSC are incorporated into the collagen bed of 3-D organotypic cultures. Primary keratinocytes are then cultured at the air-medium interface on these dermal equivalents to create a stratifying skin equivalent culture.

Results: Wounded 3-D skin cultures re-epithelialize laser wounds much faster in the raft cultures containing early passage ADSC with fibroblasts. The cultures only containing fibroblasts in the collagen bed healed more slowly. These results are statistically significant with a p-value less than 0.05 (bar graph 1). Survival of the ADSC has been determined using antibody probes that only recognize human markers in the collagen bed (Fig 2). This antibody does not recognize mouse mesenchymal cells in the collagen bed of fibroblast control cultures (Fig 3).

Conclusion: Early passage ADSC survive when added to 3-D skin organotypic cultures and accelerate re-epithelialization in these wounded 3-D skin equivalent cultures.

Figure Legends:
1. Bar graph 1 shows wound diameter in rafts 48 hours after injury. Y-axis shows burns in microns. X-axis shows A: rafts with ADSC and Fibroblasts, and B: rafts with fibroblasts.
2. Figure 2 shows Fibroblast and ADSC raft cultures demonstrating vimentin (red) in the human mesenchymal cells in the collagen bed. DAPI stain (blue) is for nuclei. (20x magnification).
3. Figure 3 shows Fibroblast raft culture control with no vimentin (no red) in collagen bed that contains no human mesenchymal cells. DAPI stain (blue) is for nuclei. (20x magnification).
THE COMBINED USE OF ENHANCED STROMAL VASCULAR FRACTION AND PLATELET-RICH PLASMA IMPROVES FAT GRAFTING MAINTENANCE IN BREAST RECONSTRUCTION: CLINICAL AND INSTRUMENTAL EVALUATION

Presenter: Pietro Gentile, MD, PhD
Authors: Gentile P, Cervelli V
University of Rome Tor Vergata

Introduction: The use of autologous fat grafting is ideal in breast reconstruction. However, published data on long-term outcomes and instrumental results of fat grafting to the breast are lacking. The purpose of this study was to review the authors’ experience of fat grafting, evaluating the effects related to the use of enhanced stromal vascular fraction (e-SVF) and fat grafting with platelet-rich plasma (PRP) in the maintenance of fat volume in breast reconstruction, comparing the results with a control group.

Methods: Twenty-three patients aged 19-60 years affected by breast soft tissue defects were analyzed at the Plastic and Reconstructive Department of the University of Rome Tor Vergata. Ten patients were treated with SVF-enhanced autologous fat grafts, and 13 patients were treated with fat grafting and platelet-rich plasma. The patients in the control group (n=10) were treated with centrifuged fat grafting injection according to Coleman’s procedure.

Results: The patients treated with SVF-enhanced autologous fat grafts showed a 63% maintenance of the contour restoring and of three-dimensional volume after 1 year compared with the patients of the control group treated with centrifuged fat graft, who showed a 39% maintenance. In those patients who were treated with fat grafting and PRP, we observed a 69% maintenance of contour restoring and of three-dimensional volume after 1 year.

Conclusion: As reported, the use of either e-SVF or PRP mixed with fat grafting produced an improvement in maintenance of breast volume in patients affected by breast soft tissue defect.

EFFECT OF PLATELET-RICH PLASMA ON ADIPOGENIC AND ANGIOGENIC GENE EXPRESSION IN ADIPOSE-DERIVED STEM CELLS

Presenter: Han Tsung Liao, PhD
Authors: Liao HT, Ravuri SK, Kokai LE, Marra KG, Rubin JP
University of Pittsburgh

Introduction: Platelet-rich plasma (PRP) containing multiple growth factors has been documented to enhance bone regeneration, wound healing and muscle or tendon healing. Recently, PRP-assisted therapies have been extended to increase fat graft survival. Adipose-derived stem cells (ASCs) were believed to play an important role on the survival of fat grafts, and PRP has been shown to enhance ASC proliferation. We hypothesized that PRP would also enhance adipogenesis and secretion of angiogenic growth factors from ASCs.

Materials and Methods: Human Platelets were purchased from HEMOCARE, fitting the definition of PRP. Human adipose-derived stem cells were isolated as per laboratory protocol. The experiments were divided into 4 groups: 1). ASCs were cultured in standard medium, 2). ASCs were cultured in standard medium + 5% PRP, 3). ASCs were cultured in adipogenic medium (supplemented with IBMX, dexamethasone, and insulin) 4). ASCs were cultured in adipogenic medium + 5% PRP. The m-RNA expression of adipogenic and angiogenic genes among groups was measured by qPCR and protein expression by Western-blot.

Results: The results demonstrated that PRP inhibited expression of key adipogenic genes in ASCs both in standard and adipogenic medium. PPAR-gamma mRNA expression decreased by 2-fold and correlated with Western-blot data. In contrast, the angiogenic gene, VEGF, was expressed at a 2-fold increase in m-RNA level after treating with PRP in both standard and adipogenic medium.

Conclusions: While PRP is known to enhance proliferation of ASCs, we show that PRP also increases the expression of angiogenic gene and downregulates adipogenesis. These findings suggest that PRP would be a good carrier agent for ASC therapies to induce neo-vascularization of tissues, but perhaps not as useful for engineering adipocytes from ASCs.
AUTOLOGOUS PLATELET RICH PLASMA (PRP) IMPROVES ADIPOSE-DERIVED MESENCHYMYAL STEM CELLS PROLIFERATION

Presenter: Ali Modarressi, MD
Authors: Modarressi A, Atashi F, Pittet B
University Hospitals of Geneva

Adipose-derived mesenchymal stem cells (AMSCs) are in focus regarding their clinical potential for tissue regeneration and engineering. These techniques require either an important fat harvesting, which could be impossible in some patients, or in vitro cell expansion. Currently, the media used for cell culture are xenogenic (e.g. bovine or fetal calf serum (FCS)) or allogenic. However, these media are not suitable for clinical applications, because 1) it contains non-autologous proteins, with a potential infection risk or immunological reactions, and 2) it achieves a slow and low cell proliferation rate. Finding a suitable autologous medium for cell culture is therefore a critical key point. In this study we aimed to investigate the effects of autologous platelet-rich plasma (PRP) on AMSCs culture, as a FCS substitute.

Fat was harvested (3mm cannula connected to 10ml LuerLok© syringe) and purified (3 minutes centrifugation at 3000rpm) by Coleman technique from 8 patients after local ethical committee approvement. PRP was obtained from same patient’s blood after 3 min centrifugation in RegenLab© tubes. AMSC were isolated after collagenase activity and cultured for 10 days in serum-free DMEM supplemented with 1%, 5%, 10%, 20%, 40% and 60% PRP, or with 10% FCS as control media. Cell proliferation and differentiation were assessed with hemocytometer chamber and FACS respectively.

PRP had a dose dependent effect on AMSC: cell proliferation increased significantly (vs. control media (FCS), p<0.01) in parallel with PRP concentration of 5, 10 and 20% (4x vs. control). However 40% and 60% PRP was less effective (p<0.05) but remained higher than control. The cell differentiation was not affected by PRP, and AMSC remained pluripotent similarly to those cultured in FCS media.

Our results demonstrated that autologous PRP enhances dose-dependently AMSC proliferation. For future clinical applications, like stem-cell-enriched fat grafting, PRP could be considered as a simple, cost-effective and safe substitute for allogenic or xenogenic media currently used in AMSC culture. Furthermore in clinic, PRP addition could also be considered effective for fat grafting survival at PRP concentration of 20%.

PLASMA ENRICHED LIPOFILLING (PEL): TRANSLATIONAL RESEARCH OR JUST ANOTHER IDEA?

Presenter: Filip B. Stillaert, MD
Authors: Stillaert FB, Roche N, Van Landuyt K, Blondeel P, Monstrey S, Doornaert M, De Pypere B
University Hospital Gent

Purpose: The major issue of the lipofilling technique is the unpredictable resorption rate. To obtain a homeostatic tissue construct in vivo, four basic principles need to be considered: (1) space for tissue expansion, (2) cells to build up the tissue, (3) vascular input to feed the cells, and (4) a matrix that directs cellular interactions. Lipofilling is the free transplantation of cells without any structural or nutritional support. Those cells need to survive initially through plasmatic imbibition. In our search for an autologous supportive matrix, plasma is the only “tissue” available. It is easily accessible, can be stored and processed easily, shows no cytotoxicity, does not inflict any morbidity or immune response, and is available in large quantities.

Materials and Methods: An in vitro study was performed to observe the potential nutritional support of plasma when added to liposaspirate samples. Plasma was compared to other standard culture media (DMEM, fetal calf serum, glucose 5% and NaCl 0.9%). Alamar blue staining and spectometry for leptin expression to analyse cell viability were performed. Clinically, patients have been treated with the PEL-technique. Histological analysis and MRI studies of the grafted areas were performed.

Results: Statistical analysis confirmed a nutritional support of plasma for liposaspirate (LA) material. Leptin expression peaked at day 6 showing possible terminal differentiation of adipocyte-precursor cells. LA cells stayed viable for up to 7 days without adding additional media. No viability was observed in the control groups containing glucose 5% and NaCl 0.9%. The clinical outcome in PEL-treated patients significantly showed stable results with better graft volume retention, less repetitive procedures and no formation of lipid cysts or indurations. MRI analysis confirmed the viability of the grafted material as well as histological analysis of grafted areas.

Conclusion: Grafted LA material initially “drinks plasma”, a process called plasmatic imbibition. To overcome the post graft ischemic period we have been “seeding” the recipient site with plasma prior to the lipofilling procedure in order to obtain a homeostatic adipose tissue construct at the long-term.
THE EFFECT OF PLATELET-RICH PLASMA FOR BONE REGENERATION WITH ADIPOSE-DERIVED STEM CELLS
Presenter: Morikuni Tobita, DDS, PhD
Authors: Tobita M, Tajima S, Mizuno H
Juntendo University School of Medicine

Introduction: We have previously reported the effect of the admixture of adipose-derived stem cells (ASCs) and platelet-rich plasma (PRP) for bone regeneration in a rat calvarial defect model. The results showed that the implantation of the admixture of ASCs with PRP was significantly promoted the bone regeneration after 8 weeks implantation. However the complementary effect of ASCs and PRP is not clearly understood. Therefore, to evaluate the effect of PRP for ASCs implantation, the several growth factor levels, which are secreted from PRP and ASCs, were investigated with Enzyme-Linked Immune Sorbent Assay (ELISA).

Materials and Methods: ASCs were isolated from inguinal fat pads of F344 rat, cultured with control medium (CM; DMEM+10%FBS+1%ABAM). PRP was prepared from the whole blood of the inbred rats with the double spin preparation method. ASCs were cultured with CM which were adding 5% PRP or 10% FBS. The levels of TGF-ß1, VEGF, IGF-1, HGF and PDGF-AB were analysed in whole blood plasma, the pre-activated PRP and the activated PRP (adding 2% CaCl2) by ELISA. Furthermore, the levels of TGF-ß1, VEGF, IGF-1, HGF and PDGF-AB in the supernatant of CM were investigated after 7 days cultivation of ASCs (passage 3) to compare the CM adding 5% PRP or 10% FBS.

Results: In comparative analysis for the concentration ratio of the growth factors in whole blood, the pre-activated PRP and the activated PRP, the growth factors in the activated PRP were significantly secreted compared with the other groups (IGF-1 was 1.3 times, TGF-ß1 was 5.3 times, HGF was 6.5 times, VEGF was 48.9 times and PDGF-AB was 6.7 times). The growth factors levels of the supernatant in CM adding 5% PRP were significantly secreted to compared with the supernatant of CM adding 10% FBS (IGF-1 was 5.7 times, TGF-ß1 was 5.2 times, HGF was 3.6 times and VEGF was 1.1 times).

Conclusion: Activated PRP was significantly secreted the growth factors to compared with the pre-activated PRP and whole blood. These secreted growth factor levels might relate with the tissue regeneration/repair. Furthermore, ASCs cultivation with CM adding PRP has secreted a large quantity of growth factors. These results supported the efficacy of admixture implantation ASCs and PRP for tissue regeneration such as bone.

IS THE COMBINATION OF FAT GRAFTS AND PLATELET RICH PLASMA EFFECTIVE AND SAFE? AN EXPERIMENTAL STUDY IN RATS
Presenter: Alexandre Blumenschein, MS
Authors: Blumenschein A, Freitas R, Moreira MA, Cysneiros MA, Tufanin AT
Universidade Federal de Goias
WITHDRAWN
Capsule formation around a breast implant is a well-known clinical entity in breast surgery. However, its exact reason is still unclear. Because the fibrosis is the basic pathophysiologic factor for the development of capsule, the prevention of fibrosis has been investigated. Recent studies regarding the application of adipose-derived stem cells (ADSCs) around implanted biomaterials have shown encouraging results based on the speculated roles of ADSCs on wound healing: 1. ADSCs inhibit fibrogenesis by hepatocyte growth factor, which is induced by fibroblast growth factor-2 in radiated tissues, 2. ADSCs promote angiogenesis by angiogenic growth factors, 3. ADSCs can differentiate into adipocytes and angiogenic progenitor cells. However, the effects of the adipose-derived stromal vascular fraction (SVF) with the addition of platelet-rich plasma (PRP) on radiation-induced capsular contracture have not been investigated. In this study, the effects of SVF combined with PRP on capsule formation around silicone implants were investigated in irradiated rats. On day 0, rat's dorsum were implanted bilaterally with silicone, and both the treated and non-treated sides were irradiated with 10 Gy as a single fraction electron beam. After irradiation, the stromal vascular fraction combined with platelet-rich plasma was injected into right sides of the animals. The other side was injected with saline as a control group. On day 28, both the treated and the non-treated sides were imaged to evaluate the thickness of the capsular contracture by ultrasonography, and histopathologic and immunohistochemical evaluations were performed. The results demonstrated that there was no statistically significant difference in terms of capsule thickness between the experimental and control sides on ultrasonographic (393 µm and 363 µm, p: 0.731 respectively), histopathologic (1.5 mm and 1.4 mm, p: 0.190 respectively) and immunohistochemical evaluations although our macroscopic observations indicated that the capsule formation was reduced on the treated side. These preliminary results suggest that ADSCs need a suitable environment for their survival and functioning and further studies on SVF and PRP combined with liposubaspirated fat tissue are needed to reduce capsule around the implants.
**EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS TREATMENT IN A MOUSE MODEL OF NEUROPATHIC PAIN**

**Presenter:** Anna T. Brini, PhD  
**Authors:** Brini AT, Niada S, Rossi A, Arrigoni E, Franchi S, Panerai AE, Sacerdote P  
**Department of Biomedical Surgical and Dental Sciences**  
**University of Milan**

**Introduction:** Adipose-derived Stem Cells (ASCs) are multipotent, able of self-renewal, low immunogenic and with immunosuppressive properties. Additionally, ASCs have shown the capacity of limiting neuronal damage through an anti-apoptotic effect and releasing neurotrophic molecules. These features make ASCs an attractive tool for the treatment of pathology involving neuronal-tissue damage and inflammation, such as neuropathic pain. Therefore we studied the effect of human ASCs in a mouse sciatic nerve chronic constriction injury (CCI) model.

**Methods:** hASC were isolated from subcutaneous adipose tissue of 5 healthy women (age 37±12); all the populations were phenotypically characterized and their clonogenic and differentiative potential evaluated to verify their stemness. 10^6 hASC were e.v. injected into C57BL/6 mice 7 days after CCI of the sciatic nerve, and, at day 1, 3, 7, 14, 21 and 28 post injection, we assessed their effect on mechanical allodynia and thermal hyperalgesia and correlated it with pro- and anti-inflammatory cytokines levels. We have also injected 10^6 or 5X10^5 cells twice, and their effects were also monitored.

**Results:** hASCs have been characterized for their stemness by standard assays while growing them for the animals’ treatment. All the injected animals survive to the treatment and no abnormal behaviors were observed. Already 24 hours after injection, hASCs were able to completely reverse hyperalgesia and to reduce alldynia; the intensity of this effect was correlated to the number of injected cells, beginning to fade 21 days after cell treatment, and it could be restored by a new treatment. In addition, the anti-hyperalgesic effect was hASCs-specific, since treatment with murine fibroblast was un-effective. At the level of the lesion site, we have also noticed cytokines balance both for pro- (IL-1β) and anti-inflammatory ones (IL-10) was restored by hASCs.

**Conclusions:** Here we have shown that expanded hASCs reduce neuropathic pain symptoms in a CCI mouse model. The mechanism needs to be elucidated, however we have shown that the cytokine balance might be re-established in the lesioned tissue, confirming an anti-inflammatory role of these cells which could be exploited for the treatment of other inflammatory pathologies.

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**PREVENTION OF FAT GRAFT ABSORPTION BY EPINEURAL SHEATH TUBE - A PRELIMINARY REPORT**

**Presenter:** Maria Siemionow, MD  
**Authors:** Siemionow M, Uygur S, Kwiecien G, Bobkiewicz A, Madajka M  
**Cleveland Clinic**

**Introduction:** The mechanism of fat graft survival is still not known. The goal of this project was to develop and test the epineural sheath as a new protective material for fat volume maintenance. We have confirmed the presence of proangiogenic markers and semipermeability of the epineural sheath. We hypothesize that implantation of fat graft into epineural sheath tube will maintain fat volume.

**Method:** The efficacy of epineural sheath in fat volume maintenance was tested in Lewis rat model. Three experimental groups were created (n=16): Group 1: Fat graft without coverage (control) (Figure 1); Group 2: Epineural tube filled with fat graft (Figure 2); Group 3: Fat graft mixed with minced epineural sheath (Figure 3). All grafts were implanted into the dorsal subcutaneous region of the rat and followed for 1, 3, 6 and 12 weeks.

**Surgical Procedure:** A 3 cm long segment of sciatic nerve was harvested and empty epineural tube was created. The branches of sciatic nerve were used to create minced epineural sheath. The adipose tissue was extracted from the gluteal region of the rat. Each graft was measured and weighed to compare fat graft volume before implantation and at the end of each follow-up period. Samples were obtained for histological evaluation and morphometric analyses.

**Results:** In groups 1 and 3, grafts lost over 25% of volume at 1-st week, over 50% of volume at 3-rd week and 100% of graft volume was lost at 6-th week post-implantation. The weight of fat graft within the epineural sheath tube (Group 2) was maintained up to 6 weeks post-implantation. Epineural sheath tube was intact and no leakage of fat graft was observed.

**Conclusion:** The epineural tubes filled with fat graft maintained the volume up to 6 weeks after implantation (Group2). In contrast, fat implanted without any coverage (Group 1-Control) and fat graft mixed with epineural sheath (Group 3) showed similar fat volume loss. The final analysis of fat graft volume maintenance and histological analysis at 12 weeks is in progress.

**Figure legends:** Figure 1. Fat graft without coverage. Figure 2. Epineural tube filled with fat graft. Figure 3. Fat graft mixed with epineural sheath.
PREVENTION OF FAT GRAFT ABSORPTION BY EPINEURAL SHEATH TUBE - A PRELIMINARY REPORT
Presenter: Maria Siemionow, MD
Authors: Siemionow M, Uygur S, Kwiecien G, Bobkiewicz A, Madajka M
Cleveland Clinic

PERIPHERAL NERVE REPAIR: MULTIMODAL COMPARISON OF THE REGENERATIVE POTENTIAL OF ADIPOSE TISSUE DERIVED CELLS IN A BIODEGRADABLE CONDUIT
Presenter: Elizabeth Kappos, MD
Authors: Kappos E, Engels PE, Meyer zu Schwabedissen M, Tremp M, Fischmann A, Schaefer DJ, Kalbermatten DF
University Hospital Basel

Introduction: Tissue engineering is a popular topic in peripheral nerve repair. Combining a nerve conduit with supporting cells appears to offer an opportunity for improved clinical outcomes, which have been poor to date. The aim of this study was to provide a broad overview over the most interesting and promising transplantable cells under equal experimental conditions over a long term period.

Methods: 1 Mio. of each of the following cell types were introduced into biodegradable fibrin conduits: rat adipose-derived stem cells (rASCs), Schwann cell (SC)-like differentiated rASC (drASC), rat SCs (rSCs), human (h)-ASCs from the superficial and deep abdominal layer as well as human stromal vascular fraction (SVF). The sciatic nerve injury model was used creating a 10mm gap in the left nerve of female Sprague Dawley rats (7 groups of 7 animals, 8 weeks old) and was bridged through this conduit. As a control we re-sutured a 10mm cut nerve segment backwards as an autograft. Long-term evaluation was carried out after 12 weeks in a multimodal manner comprising walking track and morphometric, as well as MRI analysis. The Sciatic Function Index (SFI) was calculated with the help of a functional evaluation tool. Moreover, cross sections of the nerve proximal, distal and in between the two sutures, corresponding to the former gap, were analysed. Furthermore gastrocnemius muscle weights between groups were compared and in addition imaging analysis (MRI) was performed.

Results: MRI revealed muscle atrophy across all groups and proved biodegradation of the fibrin conduit. Correlating trends throughout the different evaluation techniques could be shown: Superficial human ASC supported regeneration better than deep, in line with published in vitro data. SC-like drASC had the best regeneration potential when compared to the other adipose tissue derived cells.

Conclusion: We compared the most interesting transplantable cells in peripheral nerve repair, analysing them in a multimodal manner comprising functional and morphometric, as well as MRI analysis. In conclusion, particularly differentiated ASCs could be a clinically translatable route towards new methods to enhance peripheral nerve repair.
Introduction: Tissue engineering using a combination of nerve conduits and cell based therapies represents a new but as yet unproven approach to nerve repair. The aim of this study was to monitor enhanced early nerve regeneration by MRI of fibrin conduits seeded with different cell types.

Material and Methods: The sciatic nerve injury model in female Sprague Dawley rats (7 groups of 7 animals, 8 weeks old) was applied and a 10mm gap created by using a fibrin conduit seeded with the following cell types: rat adipose-derived stem cells (rASCs), Schwann cell (SC)-like cells from rASC, rat SCs (rSCs), human (h)-ASCs from the superficial and deep abdominal layer as well as human stromal vascular fraction (SVF) (1 x 10^6 cells). As a negative control group culture medium only was used. After two weeks, nerve regeneration was assessed by immunohistochemistry. Furthermore, imaging analysis (MRI) was performed after two and four weeks to monitor enhanced nerve regeneration.

Results: By using a clinical 3T MRI scanner with human wrist coils, we were able to visualize the graft as a small black outline (white arrowhead), distal and proximal sutures (white arrow) and small hyperintensity indicating the growth cone (arrow outline) (Figure 1). Furthermore, a good correlation was found between the length of growth cone measured by MRI and length of the growth cone measured by immunohistochemistry. Nerve growth as evaluated by radiology was significantly higher in animals four weeks post implantation (p < 0.01). Human and murine ASCs as well as SC-like cells promote and accelerate nerve regeneration at the tip of the growth cone whereas human SVF does not improve nerve regeneration (Figure 2 & 3).

Conclusions: To monitor enhanced early nerve regeneration we reliably implemented the clinical 3T MRI scanner for all cell types.
Since 2009, we have treated systemic sclerosis patients. Systemic scleroderma is an autoimmune disease characterized by varying degrees of fibrosis in the skin and other tissues. First, we treated patient faces using Coleman’s technique for its volumetric and trophic effects. After several trials, we have switched to microfat grafting. We also had to deal with hands on which we aimed at an angiogenic and anti-fibrotic effects which was achieved only thanks to Stromal Vascular Fraction.

Methods: As far as the face is concerned, we treated 12 patients using 16 to 22 cc of fat with a minimally invasive technique. The fat was harvested with 14 gauge or 2mm cannulae and reinjected with 21 gauge or 0.8mm cannulae. The procedures were performed under local anaesthesia. As far as the hands are concerned we treated 24 hands and used from 135 to 270g of fat which allowed us to get 5 cc of stromal vascular fraction with the Celution system. We got on average 61 x 10^6 cells which have been divided into 10 doses of 1 cc. A subcutaneous injection was performed in the patient’s every finger with 25 gauge or 0.5mm cannulae.

Results: On the face, we observed a continuous improvement process. The pain was reduced in the temporomandibular joints, the tissues softened, the buccal aperture was improved with special consideration to the aesthetic enhancement. The improvement was immediately assessed. Some patients underwent a second injection procedure, 2 years after the first one. As far as the hands are concerned, the results were spectacular with a very rapid improvement of the vascularisation of the fingers and later of trophic disorders that allowed a functional enhancement and a better quality of life. No complications were observed.

Conclusion: Microfat grafting on the face is efficient to treat functional and aesthetic disorders. The injection of stromal vascular fraction in fingers triggers an obvious functional improvement in every day life activities. These safe and minimally invasive techniques provide an important benefit in terms of aesthetic and functional improvements.

Centrifugation of lipoaspirate for fat graft preparation reduces the number of mesenchymal stromal cells (MSC) remaining in the graft. We have evaluated a new device (Stromacell®, Microaire) which is designed to recover these “lost” MSC in less than 15 minutes using a minimal manipulation procedure. The Stromacell is a single use, sterile, non-pyrogenic device with dual functionality as both a suction canister and a centrifugal cell separator. The goal of this study was to compare the yield of MSC in Stromacell processed lipoaspirate versus the number of MSC in fresh human bone marrow aspirates (BMA). BMA was selected as a comparative source of MSC for regenerative medicine applications.

Methods: Under informed consent, lipoaspirate samples (200 to 450 mL) were processed from 16 healthy patients, ages 22 to 55 years having a body mass index of less than 32 undergoing first time lipoplasty. Power assisted liposuction was employed. Lipoaspirate was harvested into the Stromacell suction canister(s) by standard aspiration. Up to 4 filled canisters were centrifuged per spin at 1,000xg for 10 minutes to pellet unbound adipose and blood derived cells (stromal vascular fraction, SVF). The Stromacell device design enabled simple syringe harvesting of the SVF fraction without risk of intermingling with the tumescent, adipose or free oil fractions. MSC concentration was determined by 10 day CFU-F culture assay for SVF and BMA (N=20, Lonza). Cell counts were made using a NC-3000 Nucleocounter.

Results: The Stromacell process generated 15 mL of SVF consistently. The SVF fraction had 51,971 + 9,607 and BMA had 1,133 + 261 CFU-F/ml, respectively (mean + SE). The MSC yield divided by volume of lipoaspirate processed = 2,010 + 342 CFU-F/ml. The percentage of viable cells found to be CFU-F was 1.5 + 0.6%.

Conclusions: A simple and rapid method for the preparation of MSC concentrates from lipoaspirate at the point of care is disclosed. The concentration of MSC present in the SVF fraction was on average 46 fold greater than that observed in BMA samples. To match the average number of MSC present in 15 mL of Stromacell SVF would require >500 mL of BMA to be collected. The Stromacell was found to provide a simple and rapid means for preparing MSC cell concentrates.
PROCESSING TECHNIQUE INFLUENCES ADIPOSE DERIVED STEM CELL CONCENTRATION AND CELL VIABILITY IN LIPOASPIRATE

Presenter: Elizabeth Zellner
Authors: Wu W, Zellner E, Steinbacher D
Yale University School of Medicine

Background: Autologous fat grafting is a highly utilized technique in plastic and reconstructive surgery. Several fat processing techniques have been described, with centrifugation frequently touted as the optimal method. Processing is one factor important to maximize cell viability and adipose-derived mesenchymal stem cell (ADSC) concentrations. This study compares two methods of fat preparation, centrifugation versus Telfa-rolling to determine which method results in the greatest degree of cell viability and ADSC concentrations.

Methods: Abdominal fat was harvested from five patients. Equal aliquots were divided and processed by both centrifugation and Telfa-rolling. Samples were analyzed for ADSC proportions and cell viability via flow cytometry and methylene blue-based cell counting, respectively. Paired t-tests were performed on all samples with a P < 0.05 considered statistically significant.

Results: Telfa-rolling processing resulted in a higher percentage of isolated ADSCs (P < 0.5 in 3 of 4 parameters) and a significantly higher number of viable cells (P < 0.05).

Conclusion: Telfa-rolling results in a higher proportion of ADSCs and greater cell viability compared to centrifugation for donor adipose graft preparation. Further studies are necessary to confirm if optimal preparation translates to improved augmentation and cell take at the recipient site.

IN VITRO TISSUE QUALITY ASSESSMENT OF AUTOLOGOUS FAT GRAFT PREPARED USING TRADITIONAL CENTRIFUGATION AND COMMERCIALLY AVAILABLE LIPOKIT AND PUREGRAFT® SYSTEMS

Presenter: Min Zhu, MD
Authors: Zhu M, Souverneva O, Prada A, Shanahan R, Hicok KC, Arm D
Cytori Therapeutics Inc

Background: Standardization and optimization of fat graft preparation is critical to achieve the highest level of clinical success. Gift processing methods are highly individualized as many surgeons prefer to develop their own techniques for removing nonessential “contaminants” from the tissue. Commercially available systems have emerged to address the issue of process standardization and optimization of graft quality. This study compared the ability of traditional centrifuge method and two commercial tissue washing based systems to remove residual tumescent solution, free lipid, red blood cells (RBCs), and leukocytes.

Methods: Subcutaneous adipose tissue from 6 donors was divided and processed using various graft preparation methods: 1) a “no manipulation” negative control, 2) centrifugation, 3) the commercially available Lipokit™ System, and 4) a commercially available Puregraft® System. Fat graft was examined for free lipid and aqueous liquid content, viable tissue metabolic activity, and blood cell content. Viable activity of the fat graft was determined measuring glycerol release after agonist induction of lipolysis.

Results: Graft prepared by all 3 processing methods contain significantly less aqueous fluid content than control (p<0.0001). There was no significant difference among the three different methods in the removal of aqueous content. Graft tissue prepared using the Puregraft System had the lowest free-lipid content (p<0.004 for all comparisons). Similarly, Puregraft processed tissue contained less blood cell contamination than tissue prepared using the other processing methods (p<0.0001 for all comparisons). Finally, lipolytic activity (a measure of adipocyte viability) was significantly higher in Puregraft compared to the control and other processing methods (p<0.0001 for all comparisons). Figure 1. Macroscopic evaluation of fat grafts (from left to right): Control, Centrifugation, Lipokit, and Puregraft.

Conclusion: All tested methods removed aqueous content equally, whereas the Puregraft System removed lipid and blood more efficiently than the other methods, and the remaining graft tissue was more metabolically active. Clinical follow-up studies will be required to confirm the relevance of these differences on patient outcomes.
IN VITRO TISSUE QUALITY ASSESSMENT OF AUTOLOGOUS FAT GRAFT PREPARED USING TRADITIONAL CENTRIFUGATION AND COMMERCIALLY AVAILABLE LIPOKIT AND PUREGRAFT® SYSTEMS

Presenter: Min Zhu, MD
Authors: Zhu M, Souverneva O, Prada A, Shanahan R, Hicok KC, Arm D
Cytori Therapeutics Inc

DOES CELL-SUPPLEMENTED LIPOTRANSFER MAKE A DIFFERENCE IN COMPARISON TO CONVENTIONAL METHODS USED FOR FAT GRAFTING?

Presenter: Alexandra Conde-Green, MD
Johns Hopkins Bayview Medical Center and University of Maryland Medical Center

Introduction: Given the wide application of autologous fat grafting and the growing interest in regenerative medicine, approaches to supplement fat grafts with adipose-derived stem cells (ASCs) are evolving in hopes of promoting vascularization and neoadipogenesis. This new emphasis on fat processing techniques has emerged in an effort to use fat grafting in challenging cases such as delayed wound healing, burn scars, contractures and radiation fibrosis. Therefore we aimed to evaluate the outcomes of four fat processing methods to determine the one that leads to a higher percentage of graft retention and better quality of the skin.

Methods: Adipose tissue was prepared using four techniques: decantation, washing, high-speed centrifugation and cell-enrichment. The morphology and quantity of adipocytes with each method were determined by histological analysis. The viability and number of ASCs’ were obtained by flow cytometry. Subsequently, a total of 32 subcutaneous injections of processed human lipoaspirate were carried out in eight athymic rats receiving four different samples, followed for 12 weeks.

Results: Cell count per high-powered field of intact nucleated adipocytes was significantly greater in decanted lipoaspirates, whereas centrifuged samples showed a greater majority of altered adipocytes. ASCs concentration was significantly higher in washed lipoaspirates compared to decanted and centrifuged samples taken from the middle layer. However, the pellet collected at the bottom of the centrifuged samples showed the highest concentration of ASCs. At 12 weeks, cell-enriched and centrifuged grafts showed consistent volume maintenance. Based on histological analysis, cell-enriched and washed grafts had higher scores of viability and vascularity, with the former presenting fewer cystic necrosis, minimal inflammation and least calcification.

Conclusions: Graft retention is a strong indicator of long term survival of fat grafts. When using fat grafting in chronic wounds and burn scars, viability and vascularity are also critical indicators of long term survival. Therefore, cell-enriched lipotransfer might be a better choice to further improve the quality of the skin in these challenging cases.
**COMPARISON OF STROMAL VASCULAR FRACTION CELLS OBTAINED FROM ENZYME DIGESTION AND NUTATIONAL INFRASONIC LIPOSUCTION**

**Presenter:** Robert E. Bowen, MD  
**Authors:** Bowen RE, Dihn B  
**The Center for Positive Aging**

**Introduction:** Stromal vascular fraction (SVF) cells can be isolated from lipoaspirate using enzyme digestion and centrifugation. These cells can be used immediately in a point of care procedure or as a source of cells to grow in culture. An alternative approach to obtain SVF without the use of an enzyme by using nutational infrasonic liposuction (NIL) was studied. NIL employs 3d motion of a canula vibrating at 10-15 Hz to obtain lipoaspirate. Our group has reported that similar numbers of cells can be obtained by either method. We have now compared yield and cellular composition of cells obtained from NIL and enzyme digestion.

**Method:** Lipoaspirate was collected during routine liposuction by 2 methods: 1) Manual aspiration via a 3mm Mercedes canulla at 1/2 atm. suction and 2) NIL using a 3.5mm “super G” canulla at 1/2 atm. The lipoaspirate was decanted for 10 min. in syringes or in a sterile aspiration canister, the infranatant was discarded and 60 ml of adipocytes was processed for each arm of the study. The specimens obtained from manual aspiration were washed and treated with an enzyme (collagenase I, II and neutral protease-Cizyme/Vitacyte) and centrifuged at 300g for 5 min. The specimens obtained from NIL were centrifuged at 300g for 5 min and the SVF fraction was resuspended in HBSS and incubated with ACK buffer for 15 min. The paired specimens were subjected to cell counting, viability, flow cytometry and cell culture.

**Results:** n=8, mean cell counts: 1) manual with enzyme =1.68 million+/-.97 /1ml adipose, 26% of cells were CD45+ and 74% were CD45-. 2) NIL=1.84 million+/-.11 /1ml adipose, 18% were CD45+ and 82% were CD45-. The CD45- cell populations of both contained CD 31, 34, 73, 90, 146+ cells. There was no consistent difference in doubling times or differentiation potential between the groups.

**Conclusion:** SVF obtained by NIL and enzymatic digestion of lipoaspirate yield similar numbers and poulation of cells as determined by surface markers and differentiation potential. If further study confirms these findings the regenerative effects of SVF cells could be obtained by an alternative method. This approach may be particularly attractive when processing larger aliquots of lipoaspirate.

**“MINIMAL MANIPULATION” OF HUMAN ADIPOSE-DERIVED STEM CELLS FROM LIPOSUCTION FOR CLINICAL APPLICATIONS**

**Presenter:** Yuan Liu, MD  
**Authors:** Liu Y, Chang S, Jones R, Carpenter JP, Tulenko TN  
**Cooper University Hospital**

**Introduction:** Adipose-derived stem cells (ASCs) are an ideal source of stem cells for regenerative medicine. ASCs obtained from the stromal vascular fraction (SVF) of human adipose tissue can be differentiated into adipocytes, osteoblasts, chondrocytes, endothelial cells and cardiomyocytes. However, an important issue in stem cell therapy is how to meet the stringent FDA regulatory requirements in place to protect patient safety. Therefore, the goal of this study was to evaluate a quick collagenase-free ASC isolation method compared to ASCs isolated using a common, unapproved collagenase (Worthington I) and an uncommon but FDA approved collagenase (Xiaflex).

**Methods:** Liposuction fluid drained from the lipoaspirate was processed separately for ASCs isolation. The fluid was centrifuged directly for the SVF collection and plating of ASCs. The lipoaspirate was then digested using either Worthington collagenase type I or Xiaflex. After overnight culture, the attached cells were counted as ASCs yield. The differentiation capacity of ASCs from each group was evaluated by adipogenic and osteogenic differentiation using oil red O staining, alizarin red staining and real-time PCR.

**Results:** The ASC yield from 10 ml liposuction fluid without collagenase is 1.5 ± 0.25 x 10^4 which is about 3% of the ASCs yield from 1 gram of collagenase digested lipoaspirate. There is no difference in ASCs yield between Xiaflex and Worthington collagenases. Oil red-O and alizarin red staining show multipotent capacity of liposuction fluid ASCs. Real-time PCR further demonstrates a similar differentiation capacity between the three ASC isolation groups by the expression of lipoprotein lipase and PPARγ for adipogenic differentiation and alkaline phosphatase and osteocalcin for osteogenic differentiation.

**Conclusion:** Our results demonstrate a rapid collagenase-free method to isolate ASCs contained in the liposuction fluid for clinical application which involves minimal manipulation. We also show that the use of Xiaflex, an FDA approved collagenase, for ASC isolation results in much larger ASC yield than without collagenase. Our findings reveal “FDA friendly” methods of isolating ASCs for their clinical application in regenerative medicine.
ADIPOSE STEM CELLS: EFFECTS OF CRYOPRESERVATION AND DONOR AGE ON UTILITY IN REGENERATIVE MEDICINE

Presenter: David T. Harris, PhD
Authors: Harris DT, Muise A, Badowski M, Pierce J

University of Arizona

NOT PRESENTED

ADIPOSE STROMAL VASCULAR FRACTION ISOLATION: A HEAD-TO-HEAD COMPARISON OF FOUR COMMERCIAL CELL SEPARATION SYSTEMS

Presenter: Joel A. Aronowitz, MD
Authors: Aronowitz JA, Ellenhorn J

Cedars Sinai Medical Center

Background: Supplementation of fat grafts with stromal vascular fraction (SVF) cells is an emerging technique used to improve graft reliability. A variety of systems for isolating SVF are commercially available. The lack of performance data obtained operating the systems in a standardized environment prevents objective assessment of performance. This prospective, blinded study compared performance of four commercially available SVF isolation systems when operated in a clinical outpatient surgery environment.

Methods: Four different systems were compared: 1) PNC’s Multi-Station, 2) CHA Biotech Cha Station™, 3) Cytori Celution®800/CRS System, and 4) Medi-Khan’s Lipokit™ with MaxStem (Lipokit). Identical lipoaspirate samples from 5 separate volunteer donors were used to evaluate system process time, viable cell yield, composition, residual enzyme and operating costs.

Results: The mean processing time ranged from 88 to 115 minutes. The highest mean number of viable nucleated cells was obtained using the Celution System (2.41 x 10⁵ cells/gram) followed by the Multi-Station (1.07 x 10⁵ cells/gram). Lipokit and Cha Station systems yielded nearly a log fewer nucleated cells (0.35 x 10⁵ cells/gram and 0.05 x 10⁵ cells/gram, respectively). The Celution System also yielded a significantly greater yield of endothelial cells, CD₃₄+/CD₃₁- cells, and adipose derived stem cells when measured using a colony forming unit biologic assay. Residual enzyme levels observed with the Multi-Station, Cha Station, and Lipokit respectively averaged 5.1, 13.0, and 57-fold higher than that observed with the Celution system.

Conclusions: While all systems generated measurable amounts of SVF, significant variability exists in the number, identity, and safety profiles of recovered viable cells. Side-by-side clinical trials will be required to establish the relevance of these differences.
Fat grafting is a widely accepted technique for soft tissue augmentation. Enriching fat graft with cells of the stromal vascular fraction (SVF), or cell-assisted lipotransfer (CAL) is gaining popularity as a method for improving engraftment. Many have reported that CAL results in better engraftment or permanence than traditional fat grafting. While the mechanism behind this effect has not been fully elucidated, it is evident that SVF cells have an intrinsic regenerative potential.

An alternative approach to delivering the therapeutic effects of SVF is the use of a biosynthetic matrix to encapsulate and support cells upon implantation. Renevia (BioTime, Inc.) is a hyaluronan and collagen hydrogel that can be mixed with any cell fraction before in situ gelation. It has been reported that this hydrogel improves cell localization and survival. Further, experiments with adipose-derived cells have indicated its ability to promote adipogenesis in vitro and in vivo.

A biosynthetic matrix may be of particular utility for augmentations of small volume or injections requiring a finer gauge needle than is possible with fat grafting or CAL due to needle fouling by tissue fragments. Therefore, the objective of this study was to determine the potential for fine needle injections of SVF associated with the Renevia hydrogel.

Towards this end, freshly isolated SVF was combined with the Renevia hydrogel ($5 \times 10^6$ cells/cc) and expressed through needles of various gauge sizes to assess feasibility by observing cell viability. There were no notable differences in the ratios of live (calcein AM) vs dead (propidium iodide) cells that had been expressed from needles as large as 14G and as small as 30G (Fig. 1). This experiment was repeated to increase the challenge to cell viability, by allowing the cell-laden hydrogel to completely form within the syringe before being extruded from 18G and 30G needles. Cell viability was followed for 2 days and no obvious differences or changes in viability were observed (Fig. 2).

These data indicate that Renevia encapsulated SVF cells remain viable after needle injection and together with future clinical data, may lead to a more versatile and consistent method for clinical applications that require finer needles and smaller volumes.
Introduction: This work studied the potential of a 3-dimensional osteogenic autologous in term of human AMSCs differentiation in 3D, genetic stability and clinical safety/efficacy to cure a oncogenic/congenital large bone defect.

Methods: AMSCs isolation/differentiation into a 3 Dimensional «bone-like» structure were performed: (i) Five patients with bone tumour characterized by several clonal cytogenetic alterations (study of tumor suppressor gene loci such as TP53,CDKN2,CDKN2,CDKN2,CDKN2,CDKN2,CDKN2 for the original tumour and (ii) Three patients with congenital pseudarthrosis. Graft characterization and genetic analysis (Karyotype/FISH) were performed on AMSCs proliferation/differentiation phases. Microarrays analysis studied the gene expression for osteogenic (RUNX2, BMP2, OPN3, FGF2, ALPL, SP7, FGF23, SMAD9, MEN1) and senescence/tumorogenic (c-Myc, TP53, NFKB, Cyclin D) development between un-/and osteogenic-differentiated states of AMSCs. The clinical safety/efficacy of the 3D was clinically followed biologically/radiologically post-transplantation.

Results: A mean of 65 ± 22 days (proliferation phase) was required to obtain a pure population of AMSCs in view to achieve the osteogenic 3-D (after 59 ± 17 days of differentiation). The microarrays analysis demonstrated the up-regulation of osteogenic genes for differentiated cells (p<0.05) and no sign of upregulation for TP53/NFKB/Cyclin D/c-Myc for both un-/differentiated AMSCs. In Group 1, no native tumour anomalies were found prior/after osteogenic differentiation of AMSCs. However, AMSCs culture can induced, in both Group 1 and 2, tri-/tetraploidies (0.5-14% of cells), recurrent clonal alterations as trisomy 7 (in 6-20% of cells for 3 patients) and chromosomal breakage cht(3)(q13.3) (for 4 patients) for undifferentiated AMSCs in proliferation phase. Interestingly, the osteogenic differentiation reduced significantly anomalies found in proliferation state (trisomy 7: <2-5.5% of cells). A total bone fusion was found after a mean of 12 months in all tumour and congenital pseudarthrosis contexts, respectively.

Conclusion: These preliminary results demonstrated: (i) the reproducibility to obtain the 3D structure from all autologous AMSCs and (ii) the safety and efficacy of this clinical procedure.

Tissue engineered bone holds translational promise for myriad applications in reconstructive surgery. Both adipose-derived and bone marrow-derived stem cells (ADSCs and BMSCs) have been used for bone regeneration, and can be seeded on a variety of rigid scaffolds. This study aims to compare ADSCs and BMSCs from the same donor in three distinct bioreactor settings to create the most viable osseous engineered construct. We hypothesize that physiologic flow dynamics will optimize osteogenic cell viability and function, which are prerequisites to successful human tissue implantation. Human ADSCs and BMSCs were isolated from the same donor, then cultured and seeded on decellularized porcine bone constructs. The constructs were then subjected to either static or dynamic (stirring or perfusion bioreactor) culture conditions for 7 to 21 days. Afterwards, the constructs were analyzed for cell adhesion and distribution using histology and electron scanning microscopy. Proliferation and osteogenic differentiation were further gauged using DNA quantification, alkaline phosphatase (ALP) assay, immunostaining for osteocalcin and real-time-PCR, and calcium deposition assay. hADSCs demonstrated higher seeding efficiency and proliferative potential in static culture than hBMSCs. However, dynamic culture, driven by stirring or perfusion flow, significantly increased BMSCs proliferation more than ADSCs proliferation. The highest cellularity was seen in the stirring bioreactor. In all conditions, BMSCs demonstrated stronger osteogenic activity compared to ADSCs, in ALP activity assay and gene expression for various bony markers. Conversely, ADSCs expressed more collagen I. In all constructs (ADSC and BMSC), dynamic conditions (stirring and perfusing bioreactors) enhance overall osteogenic gene expression. BMSCs in the stirring bioreactor exhibited the greatest calcium production, likely secondary to the greater cell proliferation and osteogenic function. To conclude, scaffolds seeded with BMSCs in dynamic conditions exhibit the greatest osteogenic proliferation and function. In particular, the stirring bioreactor optimizes the bone engineered construct, and may portend clinical success.
ADIPOSE-DERIVED STEM CELLS IMPROVE COLLAGENASE-INDUCED TENDINOPATHY IN RAT MODEL

Presenter: Takashi Oshita, MD
Authors: Oshita T, Tobita M, Tajima S, Ishihara H, Nishimuta Y, Mizuno H

Juntendo University School of Medicine

Introduction: Tendinopathy is a common and highly prevalent musculoskeletal disorder characterized by repetitive activity-related pain and focal tendon tenderness. Histopathologically, tendinopathic tissue shows a degenerative change with little inflammation. Therefore, existing anti-inflammatory therapy is insufficient, and novel approach including stem cell-based therapy is needed to treat tendinopathy. The purpose of this study is to evaluate the effects of adipose-derived stem cells (ASCs) on tendon healing in a rat tendinopathy model.

Materials and Methods: 250 unit of type I collagenase in 25 \( \mu \)L phosphate-buffered saline (PBS) was injected into the Achilles tendon of F344/NSlc rat intratendinously. The rats were divided into two randomly assigned groups: group A (ASCs treated tendons; \( n = 16 \)), and group B (placebo treated tendons; \( n = 16 \)). One week after the injection, either 50 \( \mu \)L of PBS containing the ASCs (5x10^5) or 50 \( \mu \)L of PBS alone was injected into the collagenase-induced lesion. At 4 and 12 weeks after the treatment, the Achilles tendons were harvested for evaluation (\( n = 8 \) tendons for each time point in each group). The sections were stained with hematoxylin and eosin (H.E.) staining and Alcian blue staining. The semiquantitative analysis with Bonar histopathological scale was performed on the histological sections. Furthermore, microstructure of healing tendons was observed under Scanning Electron Microscopy (SEM).

Results: Group B has shown a highly degenerative change characterized by disrupted collagen fiber, an increase in cellularity, hypervascularity, and ground substance deposition. The average score of the Bonar scale was 4.5, and that lasted until twelve weeks after treatment. In contrast, group A has shown a decrease number of tenocytes, lined up collagen fiber, lost of vascular and ground substance deposition. The average score of Bonar scale was 1.5, and the improvement of degeneration occurred in early stage after ASCs transplantation. In the SEM observation, the group A has shown the representative of high density of collagen fiber compared to group B.

Conclusions: These findings suggest that transplantation of ASCs improve collagenase-induced tendinopathy in rat model.

GROWTH FACTOR GENE EXPRESSION PROFILES OF BONE MORPHOGENETIC PROTEIN-2-TREATED HUMAN ADIPOSE STEM CELLS SEEDED ON CALCIUM PHOSPHATE SCAFFOLDS IN VITRO

Presenter: Astrid Bakker, PhD
Authors: Overman JR, Helder MN, ten Bruggenkate CM, Schulten EA, klein-Nulend J, Bakker AD

VU University Medical Center

The secretome of stem cells strongly determines the outcome of tissue engineering strategies. We investigated how the secretome of human adipose stem cells (hASCs) is affected by substrate, BMP-2 treatment, and degree of differentiation. We hypothesized that as differentiation progresses, hASCs produce increasingly more factors associated with processes such as angiogenesis and bone remodeling. Human ASCs were treated for 15 min with BMP-2 (10 ng/ml) to enhance osteogenic differentiation, or with vehicle. Subsequently, hASCs were seeded on plastic or on biphasic calcium phosphate (BCP) consisting of 60\% hydroxyapatite and 40\% fl-tricalcium phosphate. A PCR array for \( \sim \)150 trophic factors and differentiation-related genes was performed at day 21 of culture. A limited set of factors was quantified by qPCR at days 0, 4, 14 and 21. Compared to plastic, hASCs cultured on BCP showed \( \sim \)2-fold higher expression of \( \sim \)20 factors, amongst which cytokines such as IL-6, growth factors such as FGF7 and adhesion molecules such as VCAM1. However, expression of another \( \sim \)50 genes was decreased \( \sim \)2-fold on BCP compared to plastic, even though hASCs differentiate better on BCP than on plastic. BMP-2-treatment increased the expression of \( \sim \)30 factors by hASCs seeded on BCP, while it decreased the expression of only PGF, PPARG and PTN. No clear association between the degree of osteogenic differentiation of hASCs and the pattern of trophic factor production was observed. Considering our observed lack of association between the degree of differentiation and the production of factors associated with angiogenesis and bone remodeling by hASCs, future bone regeneration studies should focus more on systematically orchestrating the secretome of stem cells, rather than on inducing osteogenic differentiation of stem cells only. Short incubation with BMP-2 may be a promising treatment to enhance both osteogenic differentiation and environmental modulation.
ADIPOSE-DERIVED STEM CELLS FROM BUCCAL FAT PAD FOR PERIODONTAL AND ORAL BONE REGENERATION: AN IN VITRO STUDY
Presenter: Anna T. Brini, PhD
Authors: Brini AT, Arrigoni E, Broccaioli E, Niada S, Ferreira LM, Yenagi V, Rasperini G

WITHDRAWN

STROMAL VASCULAR FRACTION CELLS FOR THERAPY OF 275 PATIENTS WITH OSTEOARTHRITIS
Presenter: Jaroslav Michalek, MD, PhD
Authors: Michalek J, Kristkova Z, Skopalik J, Cibulka M, Holek M, Moster R

Cellthera Ltd

Therapy of osteoarthritis relies on non-steroid analgesics, chondroprotectives and in late stages total joint replacement is considered a standard of care. We performed a pilot study using novel stem cell therapy approach that was performed during one surgical procedure. It relies on abdominal lipoaspiration and processing of connective tissue to stromal vascular fraction (SVF) cells that typically contain relatively large amounts of mesenchymal stromal and stem cells. SVF cells are injected immediately to the target joint or to the connective tissue of the target joint. Since 2011, total of 275 patients have been recruited and followed for up to 24 months to demonstrate the therapeutical potential of freshly isolated SVF cells. At the same time, one to four joints (knees and hips) were injected with SVF cells per patient. A total number of 433 joints were treated. Semiquantitative clinical scale evaluation and non-steroid analgesics dependence was used as measurement of the clinical effect, all patients were diagnosed with stage II-IV osteoarthritis using X-ray and ultrasound, in some cases MRI was also performed to monitor the changes before and after stem cell therapy. After 3 months from SVF therapy, at least 50% clinical improvement was recognized in 95%, at least 75% clinical improvement in 68%, and complete remission in 54% of patients, respectively. Within 1-2 weeks from SVF therapy 85% of patients were off the non-steroid analgesics and remain such for at least 6 months. No serious side effects, infection or cancer was associated with SVF cell therapy. In conclusion, here we report a novel and promising therapeutical approach that is safe, cost effective, and relying only on autologous cells.

This work was supported in part by the International Consortium for Cell Therapy and Immunotherapy (www.iccti.eu) and Czech Ministry of Education Grant No. CZ.1.07/2.3.00/20.0012.
REJUVENATION OF THE ARM THROUGH LIPOSUCTION AND FAT TRANSFER, AN INNOVATIVE NO SCAR BRACHIoplastY TECHNIQUE

Presenter: Saad Dibo, MD
Authors: Dibo S, Abboud MH

MA Clinic

Introduction: A new no scar brachioplasty technique to rejuvenate and reshape the arm through combined liposuction and fat transfer has been successfully practiced over a three year period. The technique employs a custom made V shaped cannula designed for this innovative procedure.

Material and Methods: All cases are performed under general anesthesia with the patient in the supine position. Tumescent infiltration of the arm is achieved using Klein’s solution. Liposuction of the excess fat deposits of the arm, between the underarm and the elbow, was achieved with a vibroliposuction machine using a 3- and 4-mm multiple hole blunt cannula until the area is deflated. The harvested fat is prepared for injection by allowing it to decant and then filled into large syringes. Fat transfer is performed to the medial and anterior aspects of the arm to fill the empty zones between the biceps and triceps muscles. Fat transfer is achieved using a vibroliposuction machine detached from suction system with the hand piece connected to a custom made V shaped cannulae, allowing simultaneous vibration and fat injection, while performing multilayered tunnelization of the zone of injection. Resection of the excess skin was not performed in any of the cases and the patients were left with no scars.

Discussion: By deflating the ptotic region and filling the medial and anterior aspect of the arm, the ptotic skin is pulled and redraped upward, reshaping and reestablishing the rejuvenated aspect of the arm without the need for resection of the skin excess. The combination of the vibroliposuction machine and custom V shaped cannulae achieved simultaneous tunnelization and vibration as the fat was injected. Simultaneous tunnelization and vibration optimizes filling and diffusion of the fat in the desired area.

Conclusion: The presented technique rejuvenates and reshapes the arms by combining simultaneous liposuction and fat transfer, without the need of skin resection. This innovative non-scarring brachioplasty technique resulted in high patient satisfaction rates.

SCALABLE BIOFABRICATION OF CHONDROSHERES FROM HUMAN ADIPOSE STEM CELLS ISOLATED BY MECHANICAL DISSOCIATION

Presenter: Leandra S. Baptista, PhD

Federal University of Rio de Janeiro

Introduction: Our group is responsible for an innovative method of human adipose stem cells (ASCs) isolation, based on mechanical dissociation of liposapirates samples. ASCs are in fact multipotent, however, there is no consensus to date regarding the most efficient three-dimensional cell culture model for biofabrication of chordrospheres. The objective of our study was to investigate cartilage tissue formation using two scaffold-free approaches: traditional pellet culture or micromolded scalable technology.

Methods: Lipoaspirates were harvested from healthy female donors, according to the research ethics committee of the University Hospital, Brazil. Stromal-vascular fraction (SVF) isolated by our mechanical dissociation method was characterized by flow cytometry. ASCs were obtained under standard cell culture conditions. Tissue spheroids formed under pellet culture or micromolded sections of non-adhesive hydrogel (Agarose) were subsequently cultivated for 3 weeks under chondrogenic stimulus (Humanzyme, USA). Diameter and shape of tissue spheroids were investigated. Cell viability was estimated using live/dead assay. Chondrogenic differentiation was evaluated using safranin-O staining and immunofluorescence for collagen type II. We also performed a semi-quantitative histological analysis to better evaluate cartilage constructs, suggested by our group (submitted article).

Results: SVF isolated by our mechanical method reveals the same three major populations (Figure 1) as enzymatic protocols do. It was possible to fabricate ASCs tissue spheroids using pellet culture or micromolded technology. Using molded non-adhesive hydrogel tissue spheroids of three different sizes have been fabricated. The biofabricated tissue spheroids demonstrated high level of cell viability. ASCs responded to chondrogenic stimulus, as seen by a majority of Safranin-O areas rising from blue (no stain) to orange (moderate staining) (p<0.0001) and collagen II expression (Figure 2).

Conclusions: Our data demonstrated that molded non-adhesive hydrogel technology is a scalable effective method of chordrospheres fabrication with standard shape and controlled size. Improvements on culture conditions are necessary to produce more mature chordrospheres from ASCs.
SCALABLE BIOFABRICATION OF CHONDROSPHERES FROM HUMAN ADIPOSE STEM CELLS ISOLATED BY MECHANICAL DISSOCIATION

Presenter: Leandra S. Baptista, PhD

Federal University of Rio de Janeiro

CELL SURFACE MARKER PROFILING OF ADIPOSE-DERIVED STEM CELLS FROM HUMAN SUBCUTANEOUS AND VISCERAL FAT DEPOTS

Presenter: Shigeki Sugii, PhD
Authors: Sugii S, Chan E, Toh SA, Han W, Sugii S

Singapore Bioimaging Consortium and Duke NUS Graduate Medical School

White adipose tissues of subcutaneous and visceral depots differ in their pathophysiological contribution to metabolic homeostasis. The subcutaneous fat depot physiologically stores excess lipids thus preventing their deposition into other organs. Visceral fat accumulation, on the other hand, leads to pathological metabolic profile due to dysfunction in lipid storage. Increasing evidence suggests that this can be attributed to difference in inherent properties of the adipose-derived stem cells (ASCs) from the two fat depots. Currently, little is known about the molecular difference in identity of ASCs from the two fat depots. We isolated and cultured subcutaneous and visceral (omental region) ASCs from human subjects. As expected, ASCs from subcutaneous fat differentiate better into mature adipocytes than those from visceral fat by the standard adipogenesis protocol. High content screening assay of over 200 human cell surface markers was performed to identify potential depot-specific cell surface markers of ASCs. Several candidates that showed differential immunofluorescence signals in terms of signal intensity and cell percentage were selected for further study. Among these, CD10 was found to be subcutaneous specific whereas CD200 was selective for visceral ASCs. Furthermore, we found that these markers can distinguish different populations by their adipogenic capabilities; CD10hi and CD200lo ASCs differentiate into adipocytes more than CD10lo and CD200hi cells that are derived from subcutaneous and visceral depots, respectively. Collectively, identification of such markers would allow us to differentially isolate, visualize and characterize ASCs in the depot-specific manner.
AN IMMUNOPHENOTYPIC CHARACTERIZATION OF THE STROMAL VASCULAR FRACTION OF OBESE DONORS
Presenter: Jonothon Kenyon, PhD
Authors: Kenyon J, Sadeghi Z, Sramkoski M, Jacobberger J, Soltanian H, Hijaz A, Daneshgari F

Introduction: The stromal vascular fraction (SVF) is a reservoir of adipose derived stem cells with potent regenerative properties. The SVF therefore, is a promising candidate for therapeutic intervention in stress urinary incontinence (SUI), however, comorbid factors associated with SUI may effect SVF in unknown ways. We hypothesized cells of the SVF, obtained from obese donors have a different immunophenotypic cell subpopulation distribution than observed in non obese individuals. We determine cell subpopulations by multicolor cell surface immunostaining flow cytometry of the SVF obtained from obese individuals and assessed the distribution of cells with mesenchymal cell surface immunostaining flow cytometry of the SVF obtained from obese individuals and assessed the distribution of cells with mesenchymal cell surface markers.

Methods: Fat from liposuction donors was obtained with informed consent (n=6) (with BMI scores > 25), macerated, incubated with collagenase, stained for the cell surface markers fluorescent conjugated antibodies against CD31, CD34, CD45, CD73, CD90, CD105, and CD176, and then quantified on a BD LSR II™ flow cytometer. Data was analyzed on Winlist™, by Verity Software House©.

Results: Flow cytometry data were gated to identify hematopoietic, endothelial, pericyte, and possible stromal cell populations. Endothelial cell populations were the most variant subpopulation, while stromal cells varied the least (10%-25%) between donors. Linear regression identified a modest (R²=0.75) linear association with BMI and the frequency of cells with a marker of hematopoietic lineage differentiation, CD45+. The CD45+ cell subpopulation revealed an increasing linear association between BMI and probable endothelial progenitor cells of hematopoietic origin (CD45+/CD31+/CD90+) (R²=0.90), a modest association between BMI and a subset of activated T-cells (CD45+/CD31+/CD46+) (R²=0.46) but no linear association with (CD45+/CD31+/CD105+) probable macrophages and/or endothelial progenitors (R²=0.02).

Conclusions: Reduced CD34+ cells with BMI is indicative of increased inflammatory potential and reduced adipose derived stem cells. The SVF derived from obese donors is therefore, potential risk of inducing an inflammatory response. How the SVF obtained from obese donors allows us to now determine if these inter-donor differences alter efficacy and necessitate new exclusion criteria based on BMI.

THE FIBROGENETIC EFFECT OF BMI1 AND EZH2 PROTEINS ON ADIPOSE DERIVED STEM CELLS IN DIFFERENT AGE GROUPS
Presenter: Minsuk Kang, MD
Authors: Kang M, Jin US, Kim SW
Seoul National University Hospital

Adipose-derived stem cell is on focus currently in many medical fields because of its easy-accessibility. Many researches are on progress about the issue of the effect of ADSC on fibroblast and the aging process of stem cells. Recently, epigenetic regulation is attracting attention among the several factors that regulate differentiation of stem cells. Chromatin modifiers such as BMI1, EZH2 proteins are known to be important factors of the issue of epigenetic regulation. The authors focused on the different level of chromatin modifiers in ADSC among different age groups, and the effect of these proteins on fibrogenetic activity of ADSC.

ADSCs were extracted from fat tissue of 6 premenopausal & 6 postmenopausal women. BMI1, and EZH2 proteins were tagged with antibodies and were quantified by western blot method. Additionally, to find out if there is a significant difference in fibrogenetic potential when chromatin modifiers are added to ADSCs from postmenopausal women, ADSCs were co-cultured with human dermal fibroblasts with BMI1, and EZH2 proteins, and fiber materials such as collagen type1, 3, fibronectin, MMP-1, beta-actins. They were quantified by western blot and compared with each other to find out if there were significant differences between groups.

From the experiment, we could access the relationship between the fibroblastic activity of ADSCs and BMI1, EZH2 protens. This can give us clues for rejuvenating old ADSCs, and for maximized effect of ADSCs in clinical field.
PASSAGE – DEPENDENT AND SERUM – DEPENDENT CHANGES OF ADIPOSE-DERIVED STEM CELLS IN VITRO – IS A STEM CELL THE SAME IN VITRO AS IN VIVO?

Presenters: Renata Sonnenfeld, BS
Authors: Sonnenfeld R, Kuhbier J, Radtke C, Lazaridis A, Vogt PM, Reimers K

Medical School Hannover

Purpose: The aim of this study was to investigate passage-dependent quantitative changes in expression of stemness-related genes and the influence of the origin of the supplement serum.

Background: Stemness-related properties (SRP) of Adipose-derived Stem Cells (ASC) in vivo are considered to be dependent to the micro environment, the so-called stem cell niche. As a consequence, phenotypical changes in ASC, in particular the loss of SRP, must happen in vitro, which might be a disadvantage for the biomedical use of ASC. The aim of this study was to investigate the changes of stemness-related genes (SRG) in ASC cultures in vitro using either fetal calf serum (FCS) or human serum (HS) as supplements to cell culture media.

Methods: Human ASC (hASC) were isolated from adipose tissue collected by elective dermolipectomy (N=5) and cultivated under standard conditions until passage 5 (P5) using either FCS or HS as medium supplement. Cells were characterized via flow cytometry with CD73 and CD90 as positive markers and CD11b and CD31 as negative markers. Changes in the transcriptome were determined with quantitative RT-PCR.

Results: Isolated hASC showed typical growth morphology and phenotype regarding ASC surface markers. However, STRO1 showed a distinct increase after one passage. The expression of SRG, in particular MCAM and cKit, was down-regulated during cultivation in a time-dependent manner. Cultivation in HS-supplemented medium resulted in significantly higher cell doubling time than in FCS.

Discussion & Conclusion: A possible explanation for higher cell doubling time rates in media supplemented with allogene serum (HS) compared to xenogene serum (FCS) might be the superior binding rates of growth factors due to allogene epitopes. The passage-dependent decrease of SRG as well as the initial increase of STRO1 occurred after transfer of ASC to in vitro conditions. Therefore, an environmental shift caused by dissociation of ASC from the stem cell niche might lead to a loss of cell-cell- and especially cell-matrix-contacts and thus change of intracellular pathways. Consequently, some markers regarded as typical stem cell marker might reflect a stem cell phenotype altered from in vivo conditions as they were measured in an in vitro-e.

AGE-DEPENDENT CHANGE IN EXPRESSION OF GENERAL AGING MARKERS AND MARKERS OF CELLULAR SENERCENCE IN PRIMARY HUMAN ADIPOSE TISSUE

Presenters: Joshua Cornman-Homonoff, AB
Authors: Cornman-Homonoff J, Percec I, Dierov R

Perelman School of Medicine at the University of Pennsylvania

Introduction: Challenges surrounding in vivo study of human aging necessitated use of model systems of questionable validity. Use of primary human tissue is superior, but few tissues are sufficiently abundant, accessible, robust, and easily manipulated for use as a model. Adipose tissue may be unique in its fulfillment of these criteria. The goal of this study is to demonstrate the utility of human adipose tissue as a model for the study of aging through the assessment of changes in expression of general aging-associated markers as well as specific measures of cellular senescence in primary adipose tissue from patients of varying ages.

Methods: Subcutaneous abdominal adipose samples were obtained from healthy patients undergoing elective surgical procedures. A tissue bank was constructed and provided samples utilized in this study. Adipocytes (AD) and stromal vascular fraction (SVF) were separated and analyzed in parallel for expression of aging and senescence markers by Southern blot, Western blot, and qRT-PCR. Adipose-derived stem cells (ASCs) were analyzed via staining and visualization. Patients were grouped by age <50 or age ≥50 for comparison of mRNA and protein levels.

Results: Telomere length decreased with increasing age in both AD and SVF. Expression of aging marker gamma-H2AX increased with age in AD and SVF, though this change was significant in AD only. mRNA of aging marker eEF1A1 declined significantly with age in both cell fractions. mRNA levels of senescence markers p14ARF, p16INK4a, p21, and Lamin B1 did not demonstrate age-dependent changes in either cell fraction. Senescence-associated beta-galactosidase (SA-beta-gal) staining in ASCs demonstrated a larger percentage of SA-beta-gal positivity in cells from older patients. Senescence-associated heterochromatin foci were not visible upon staining in ASCs. Assessment of Lamin B1 protein in ASCs suggested a decrease in expression with age.

Conclusions: General aging-associated markers demonstrated expected changes in both AD and SVF. Senescent cells did not appear to accumulate in AD or SVF, though they may in ASCs. This suggests that cellular senescence may not be an appropriate model for aging research. Rather primary human adipose should be considered for use in future studies of human aging.
STEM CELL CONCENTRATION IN PEDIATRIC ADIPOSE TISSUE

Presenter: Kevin S. Hopkins, MD, FACS

Driscoll Childrens Hospital

WITHDRAWN
MESENCHYMYAL STEM CELLS PRODUCED FROM DIFFERENT SOURCES OF ADULT ADIPOSE TISSUE DEMONSTRATE A SIGNIFICANT AND REPETITIVE DIFFERENCE IN THEIR LONG TERM PROPAGATION AND DIFFERENTIATION ABILITIES

Presenter: Nir Shani, PhD
Authors: Shani N, Tirza G, Sela M, Krelin Y, Friedman O, Gur E
Tel Aviv Sourasky Medical Center

Introduction: Mesenchymal stem cells (MSCs) are multipotent progenitors, which manifest an ability to differentiate into several mesodermal lineages, especially adipocytes, chondrocytes and osteoblasts. Although MSCs were initially isolated from bone marrow, it is now evident that these cells can be isolated from many adult tissues. The most available and abundant source of MSCs for clinical use is adipose tissue. Although defined as a single entity through their characterization by surface markers and differentiation potential, it has been demonstrated that MSCs from different tissues may vary in their properties. Thus, the source of cells that are used for clinical application can markedly influence their clinical efficacy. The realization that adipose derived MSCs are the most adequate candidates for clinical autologous treatment has promoted us to try and define whether there are differences among MSCs produced from different fat tissue sources, in rats.

Methods: Subcutaneous and abdominal fat was extracted from rats. MSCs were extracted from the fat following standard procedures using collagenase and were propagated in culture. Long term propagation, doubling time, senescence and differentiation were examined.

Results: We found a dramatic difference in the long-term propagation and replication time between abdominal and subcutaneous fat. The difference repeated itself in repetitive independent MSCs extractions. The inability of abdominal fat MSCs for long-term propagation translated into a stage of senescence at a very early passage of their propagation. This did not occur in subcutaneous fat that was propagated long-term in culture (passage 15). A significant and repetitive superior fat differentiation of subcutaneous fat MSCs over abdominal fat MSCs was observed.

Conclusions: Our findings indicate that the source of fat tissue from which MSCs are produced may be critical to their efficacy in the clinical settings. Moreover, independent subcutaneous and abdominal fat MSCs behaved in a very similar manner indicating the homogeneity of fat derived MSC preparation. This has important bearings for their use for semi-industrial and clinical purposes.

SVF CELL COUNTING: COMPARISON OF DIFFERENT AUTOMATED CELL COUNTING DEVICES

Presenter: Severiano Dos Anjos Vilaboa Sr., PhD
Authors: Dos Anjos Vilaboa S, Cimino W, Llull R
Stem Center SL

Introduction: The stromal vascular fraction (SVF) of adipose tissue can be isolated after adipose tissue dissociation and centrifugation. The SVF is composed of all supporting cells from the adipose tissue stroma (non adipocytes) obtained after this processing. This fraction includes a heterogeneous cell population composed of different cell types such as preadipocytes, endothelial cells, pericytes, macrophages, mesenchymal stem cells, and others.

There has been a great interest in developing platforms and devices to be used for SVF isolation at the point of care to treat human patients. The quality and safety of the cell inoculum obtained is pivotal to the patient’s care, as well as the number of cells obtained (therapeutic dose) for clinical studies.

The technology used to measure the real cell concentration and viability in the SVF cell inoculum isolated from adipose is critical for assuring cell quality. The aim of this study was to determine whether different automated cell counting methods could yield different results.

Methods: Human adipose tissue liposaspirates were obtained after signing informed consent from healthy female donors aged 20 to 45 years (n=5). SVF isolation was carried out in the OR using the GID SVF-1 device. Briefly, the adipose tissue was washed using lactated Ringer, digested using GIDzyme-2 and centrifuged at 800 g for 10 minutes to obtain the SVF pellet. After cell pellet resuspension using a long spinal needle an aliquot was taken for cell counting and viability analysis using various methods, including different image citometry devices, as well as devices using Coulter principle.

Results and Conclusions: The cell concentration and viability values obtained indicate that both image cytometry devices analyzed give consistent and similar results (referred only to nucleated cells), although they use different fluorescent dyes and technology. The Coulter technology yields more variable results, and the cell concentration mean values obtained were higher. A reliable cell counting method is essential to precisely determine the dosage, what is critical for succeeding in clinical studies. This is also very important when calculating cell yields and comparing different technologies for adipose SVF isolation.
HIGH THROUGHPUT CELL-BASED SCREENING ASSAY FOR ADIPOGENIC AGENTS IN SOFT TISSUE ENGINEERING

Presenter: Russell E. Kling, BA
Authors: Kling RE, Gough AH, Kokai L, Philips BJ, Ravuri SK, Fernstrom JD, Marra KG, Rubin JP

University of Pittsburgh

Introduction: Autologous fat grafting is a promising technique for soft tissue reconstruction of the breast post-mastectomy, but graft loss remains an unresolved obstacle to widespread clinical implementation. The paradoxical use of adipogenic agents to enhance the conversion of adipose derived stem cells to mature adipocytes may be an effective strategy to overcome graft resorption. To date, few compounds are known to increase de novo adipogenesis. Therefore, we sought to screen the commercially available Library of Pharmacologically Active Compounds (LOPAC) to identify previously unknown adipogenic agents as a way to enhance long term fat graft retention.

Methods: Adipogenic stimulation of murine 3T3-L1 cells was assessed over 14 days in a validated 96-well plate rapid-screening protocol that allows for simultaneous confocal imaging and fluorescent measurements. In brief, differentiation was quantified as the fraction of cells with intracellular lipid content, measured with LipidTOX (Invitrogen), significantly above background (0.5% differentiation media). Cell number was measured with the Hoechst stain. 1280 different agents were tested from the LOPAC library. Test compounds were dosed every 96 hours at 10 µM and 2 µM. To track overall assay performance a positive control (10 µM insulin) was employed on each plate. Compounds were considered adipogenic when the percent differentiated cells >3 standard deviations above the mean background level of differentiation.

Results: There were 40 hits. 34 agents were positive at 1 concentration and 6 were positive at both concentrations. The 6 non-concentration dependent hits were: 1) Brefeldin A (antibiotic), 2) Amsacrine (anti-neoplastic), 3) Cytarabine (anti-neoplastic), 4) Colchicine (gout), 5) Calcimycin (antibiotic), 6) Ancitabine (anti-neoplastic).

Conclusions: We have developed a rapid screening protocol that is able to identify compounds with adipogenic potential that may be useful for regenerating soft tissue. The compounds identified included antibiotics, anti-neoplastics, chemotherapeutics, and compounds from other drug classes, which may prove beneficial in post-mastectomy breast reconstruction with autologous fat. Future work includes confirmation, further characterization and safety analysis.
Introduction: Hemocytometer counting is the gold standard method for readily quantifying yield and viability of therapeutic cell preparations derived from adipose or other stromal tissues. Fluorescent staining methods enhance assay sensitivity and specificity compared to colorimetric counterparts as they offer improved visualization, higher S/N ratios, and more rapid enumeration of cell subpopulations and characteristics. As adipose-derived therapeutics are advanced into practice, in-clinic tissue processing is increasingly being utilized to yield regenerative cells from autologous sources. A concurrent need exists for instrumentation that facilitates analyses of these prepared cells at the point-of-care. We have developed an efficient, handheld fluorescence excitation system to enable readout of fluorescent assays with a standard microscope. We have implemented various fluorescent cell counting and viability assays with the system on a low-cost single objective microscope and validated counting performance against a laboratory epifluorescence microscope.

Method: SVF was isolated from lipoaspirate with Matrase™ reagent and automated cell recovery instrumentation from InGeneron, Inc. AdMSC were obtained by culturing SVF in DMEM+FBS. Cells were labeled with 5µM SYTO13, 1µM CalceinAM, and 1µM SYTOX Green respective nucleic acid, vitality, and viability stains (Invitrogen). Samples were aliquoted into disposable hemocytometer chambers and viewed at 200x.

Results: System performance was evaluated by four different operators using fresh SVF and 72h cultured AdMSC. Nuclear-localized SYTO13 fluorescence was apparent in all samples regardless of excitation source. Nucleated cell counts were obtained for 1 mm² subdivisions of a Neubauer grid and the same aliquot was counted using the two fluorescence excitation systems. Data matched to aliquot, subdivision, and operator reveals robust agreement (r=0.9949) between counts obtained using the handheld excitation system compared to standard epifluorescence.

Conclusion: A handheld fluorescence excitation instrument paired with a standard optical microscope yields equal accuracy and precision compared to a laboratory microscope with epifluorescence for cell enumeration and viability assays of fresh SVF and cultured AdMSC.
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