IFATS QUÉBEC 2012 CONFERENCE

10TH ANNUAL MEETING

IFATS

International Federation for
Adipose Therapeutics and Science

October 5-7, 2012

Loews Hôtel Le Concorde • Québec City, Québec, Canada

MARK YOUR CALENDAR

International Federation for
Adipose Therapeutics and Science

11th Annual Meeting

IFATS NEW YORK 2013

November 21-23, 2013
Conrad New York Hotel
New York City, NY USA

Abstract Deadline:

Midnight EST, Tuesday, June 1, 2013

The Call for Abstracts will be sent this winter. All members of IFATS and all registered attendees of the 2012 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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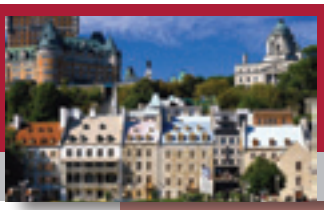

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IFATS
International Federation for Adipose Therapeutics and Science

Welcome to the 10th Anniversary Meeting of the International Federation for Adipose Therapeutics and Science (IFATS). This is a special year for IFATS and I am honored to host this meeting for the first time in Canada - in my hometown of Québec City.

IFATS is the world's only multi-disciplinary society that focuses on the use of adipose-derived stromal/stem cells (ASC) for regenerative purposes. Our IFATS founders' pioneering efforts now result in a vibrant community sharing knowledge and joining forces to push further ASC basic science and applications.

Highlights of this year's conference include hot topics to be addressed during the Keynote and Invited presentations featuring the latest information in the fields of bone tissue engineering and brown fat differentiation respectively. Renowned panelists will share their vision and stimulate discussion and reflection on many important current issues.

I am also pleased to introduce for the first time a session highlighting the outstanding work performed by our young investigators (graduate students, postdoctoral fellows and residents). An entire plenary session is dedicated to fifteen finalists in the Best Paper Award Session based on the quality of their submitted abstract. Following their oral presentations, three of them will receive a $1,000 award. Thank you to the Cercle des Ambassadeurs and Université Laval for their generous contribution that catalyzed this initiative.

Finally, thanks to those of you who contributed original results that shaped our program. In addition to great basic and applied science topics, many presentations will address therapeutic research and updates on human clinical trials reflecting the great strides achieved in translational research with ASCs.

Québec City, the capital of the Province of Québec, is famous for its hospitality and French-Canadian charm. The colorful fall landscapes, natural wonders and historical sites are sure to seduce you – especially at the Montmorency Falls, the site of our Saturday evening banquet. Québec City features two major poles of excellence most relevant to IFATS interests: the Laval University tissue engineering center LOEX (Laboratoire d'Organogenèse Expérimentale) as well as important metabolic and obesity-related research recognized through Canadian Research Chairs in Obesity, Adipose tissue, and Bariatric surgery, respectively.

We are very grateful to our exhibiting companies and sponsors for their support. We could not provide this educational program without their contribution.

On behalf of the IFATS Board of Directors, I thank you for joining us for IFATS Québec 2012 and supporting the field of adipose science, technologies and therapeutics.

Julie Fradette, PhD
IFATS 2012 President

FOUNDERS' MESSAGE

To IFATS members, colleagues, and interested others:

Wow! Ten years and who would have "thunk it"---a world class, scientific organization, now even the premier entity in the field, based on a waste product with predominantly negative connotations, but with a membership that is energized, growing, and clearly developing new basic science knowledge and clinical applications for direct patient benefit from "all things adipose". Today, "Fat is hot!" and IFATS has played a proud but humble role in that recognition. There is tremendous global interest in what is known now and what is to come from the "Adipose World" of stem cells, adipocytes, and the entire associated regenerative niche. Much, or even most, of that interest has evolved from the contributions of members here today. The IFATS Founders say, simply, thanks for being here, enjoy the meeting, and let's keep pushing the "adipose envelope". It's all in the name: THERAPY and SCIENCE! A,P,R,&B

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Adjunct Professor of Bioengineering, Carnegie-Mellon University
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SPECIAL THANKS TO:

IFATS Board of Directors

Local Organization: André Tchernof, PhD

Office du Tourisme de Québec

All members who participated as abstract reviewers and oral presentation award judges

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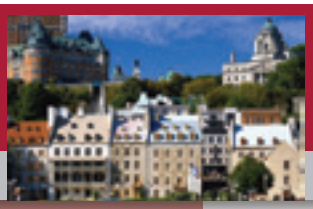

INVITED SPEAKERS AND SESSION MODERATORS

Torsten Blunk, PhD
Stéphane Bolduc, MD
Philippe Bourin, MD, PhD
Spencer Brown, PhD
Bruce Bunnell, PhD
Louis Casteilla, PhD
Mary Ann Chirba , JD, DSc, MPH
Sydney Coleman, MD, FACS
Alexandra Conde-Green, MD
Daniel Del Vecchio, MD
Paul DiMuzio, MD
Nathalie Faucheux, PhD
Lauren Flynn, PhD
Julie Fradette, PhD
William Futrell, MD
Lucie Germain, PhD
Jeffrey Gimble, MD, PhD
Guillaume Grenier, PhD
Marco Helder, PhD
Kevin Hopkins, MD, FACS
Jae-Ho Jeong, MD, PhD
Brian Johnstone, PhD
Adam Katz, MD, FACS
Ramon Llull Cerda, MD, PhD
Keith March, MD, PhD
Kacey Marra, PhD
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Kevin Small, MD
Henry Spinelli, MD, FACS
André Tchernof, PhD
Dmitry Traktuev, PhD
Gordana Vunjak-Novakovic, PhD
Stuart Williams, PhD
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

Gordana Vunjak-Novakovic is the Mikati Foundation Professor of Biomedical Engineering, and a Professor of Medical Sciences at Columbia University. She directs the Laboratory for Stem Cells and Tissue Engineering, and the Stem Cell Imaging Core, and co-directs the NIH Tissue Engineering Center, and the Craniofacial Regeneration Center. She is the lead for bioengineering for the Columbia Stem Cell Initiative. She obtained a Ph.D. in chemical engineering at the University of Belgrade in Serbia where she stayed on faculty and became Full Professor in 1993. Upon moving to the USA, she spent twelve years at MIT, to join Columbia University in 2005. The focus of her research is on engineering functional human tissues using stem cells, biomaterials and bioreactors, for regenerative medicine and study of development and disease.

Gordana published 2 books, >50 chapters and >300 journal articles (cited ~11,000 times, h=63 on ISI Web of Science; ~16,000 times, h=73 on Google Scholar), has 55 patents, and gave >250 keynote and plenary lectures. She is a frequent advisor to government and industry, has been a study section chair and distinguished editor for NIH, and is serving on editorial boards of 12 scientific journals and numerous advisory boards and councils. She is an active member of TERMIS from its inception, serving on the Continental Council for North America, European Advisory Board, and the Advisory Boards of the TERMIS meetings, including the 3rd World Congress in September 2012 in Vienna.

In 2000, she was elected Fellow of the American Institute for Medical and Biological Engineering. In 2007, she gave the Director's lecture at the NIH, as the first woman engineer to receive this distinction. In 2008, she was inducted into the Women in Technology International Hall of Fame "for developing biological substitutes to restore, maintain or improve tissue function". In 2009, she was elected to the New York Academy of Sciences. In 2010, she received the Clemson Award of the Biomaterials Society "for significant contributions to the literature on biomaterials". In 2012 she was elected to the National Academy of Engineering "for bioreactor systems and modeling approaches for tissue engineering and regenerative medicine"

Lecture Title: Tissue Engineering of Bone Using Adipose Derived Stem Cells
When: Friday, October 5th – 10:15 to 10:45 am

The increasing availability of functional human tissues, engineered to meet the needs of a specific patient and clinical situation, is changing the way we currently treat tissue loss due to trauma, disease or congenital defects. Tissue engineering is addressing this major challenge by directing the assembly of stem cells into functional tissue structures through a combined use of biomaterial scaffolds (cell-instructive templates for tissue formation) and bioreactors (controllable environments providing molecular and physical signals). Human tissues of high biological fidelity are also of great interest for studies of disease, drug development and "human in a dish" toxicology screening platforms. While the specific requirements for regenerative medicine and screening technologies are different in many respects, the utility of engineered tissues in all cases depends on our ability to predictably guide cell fate and function.

Adipose derived stem cells have emerged as a cell source of great clinical relevance due to their ease of harvesting, abundance, and ability to differentiate into various mesenchymal lineages, including bone and vascular cells. This talk reviews the utilization of adipose derived human stem cells for engineering living bone grafts for reconstructing head and face, tailored to restore normal anatomy and function for complex bone defects. Clinical scans of the affected area were used to make precisely shaped scaffolds and the matching perfusion bioreactors that were used in conjunction with adipose derived stem cells to grow in vitro anatomically shaped bone grafts. We discuss the bioreactor cultivation and a 6-month animal study (in pig) of one of the most complex and needed bone grafts – temporomandibular joint condyle, in the context of translating this tissue engineering modality towards clinical application.

INVITED SPEAKER

Patrick Seale obtained his Ph.D. from McMaster University in Hamilton, ON, Canada in the laboratory of Dr. Michael Rudnicki. During his graduate training, he studied regenerative processes in adult skeletal muscle and demonstrated a key requirement for Pax7 in the development of skeletal muscle stem cells. He then trained as a postdoctoral fellow with Dr. Bruce Spiegelman at Harvard Medical School and the Dana-Farber Cancer Institute. His research there focused on the development and differentiation of adipose lineages. In particular, he identified PRDM16 as an important cell-autonomous regulator of brown adipose cell fate. His studies also revealed a developmental connection between brown adipocytes and skeletal muscle cells.

He was appointed as an assistant professor in the School of Medicine at the University of Pennsylvania in September 2009.

Lecture Title: Transcriptional Control of Brown and Beige Adipocyte Fate
When : Sunday, October 7th – 8:05 to 8:30 am

Our research is aimed at identifying and examining the pathways that control the development, differentiation and function of brown adipose cells. Brown fat cells in brown adipose tissue depots arise from cellular precursors that also give rise to skeletal muscle cells but not white fat cells. In addition to the classic brown fat depots, “brown-like” fat (a.k.a. beige or “brown-in-white” [brite]) cells also develop in white fat tissue in response to beta-adrenergic signaling. These beige adipocytes are not part of the cellular lineage that gives rise to muscle and brown fat tissues. Interestingly, the zinc-finger transcriptional regulator, Prdm16 is a key driver of brown fat-specific gene expression in beige adipocytes analogous to its function in the classic brown fat lineage. To gain insight into the physiological role of Prdm16 in adipose lineages, we have engineered mice that lack Prdm16 expression specifically in brown or beige fat cells. Metabolic and molecular analyses of these mice will be presented. Recent studies addressing the mechanism of Prdm16 action in the activation of a brown fat-specific gene program and repression of alternative genetic programs will also be discussed.

PANEL DESCRIPTIONS

Panel 1: Regulatory Pathways for Adipose Technology

Friday, October 5, 2012
3:00 pm - 4:00 pm

Panel Chairs:
Stuart Williams, PhD - *Executive and Scientific Director, Cardiovascular Innovation Institute, Louisville, KY*
Keith March, MD, PhD - *Professor of Medicine, Physiology and Biomedical Engineering; Director, Vascular and Cardiac Center for Adult Stem Cell Therapy (VCCAST); Indiana University*

Panelist:
Mary Ann Chirba, JD, DSc, MPH - *Professor, Boston College of Law*

Synopsis: This panel will highlight key concepts and recent evolution in regulatory aspects and practices relating to the movement of adipose derived technology into clinical programs.

Panel 2: Do's and Don'ts In Translational Research: Importance of Choosing Adequate Preclinical Models

Friday, October 5, 2012
4:30 pm – 5:30 pm

Panel Chairs:
Marco Helder, PhD - *Department of Orthopedics, VU University Medical Center, Amsterdam, The Netherlands*
Lucie Germain, PhD - *Centre LOEX (Experimental Organogenesis Laboratory) of Université Laval, Québec City, Canada; Director of the Cell and Tissue Therapy Network (Thécell) of Fonds Recherche Québec - Santé (FRQS)*

Panelist:
Gordana Vunjak-Novakovic, PhD - *Professor of Biomedical Engineering and Professor of Medical Sciences at Columbia University, Director of the Laboratory for Stem Cells and Tissue Engineering, Columbia University, New York, NY, USA. TERMIS -Continental Council for North America*

Synopsis: Within translational research, it is pivotal to match the choice of your in vitro and/or in vivo model system(s) with your research questions and required steps to come to clinical implementation eventually. Topics that will be shortly introduced in this panel session and subsequently discussed in an interactive manner will be, among others:
- Parameters to be considered in in vitro models to best mimick in vivo conditions
- Importance of the microenvironment for cell behaviour and functional outcome
- Sense and non-sense of ectopic models in skeletal research
- Comparison of in vitro and animal models in dermatology-related research
- Ex vivo organ culture models: valuable intermediates between in vitro and in vivo models?
- Translational scientists and clinical practitioners; how to match their perspectives?

Panel 3: Clinical Applications of Fat and Fat-derived Cells: A Global Perspective

Saturday, October 6, 2012
3:20 pm - 5:00 pm

Panel Chairs:
Adam Katz, MD - *University of Florida, Gainesville, FL*
William Futrell, MD - *Clinical Professor of Surgery, University of Pittsburgh; Adjunct Professor of Bioengineering, Carnegie-Mellon University, Pittsburgh, PA*

Panelists:
Sydney Coleman, MD, FACS - *Tribeca Plastic Surgery, New York, NY*
How Does Grafted Fat Heal Damaged Tissue into Which it is Transplanted?

J. Peter Rubin, MD, FACS - *University of Pittsburgh, Pittsburgh, PA*
Translating Adipose Therapies for Craniofacial Reconstruction

Henry Spinelli, MD, FACS - *Clinical Professor of Surgery and Neurological Surgery; Weill Medical College of Cornell University; Attending, New York Presbyterian Hospital; Editor in Chief, Aesthetic Plastic Surgery*
Reality or Fantasy: Evidence Based Medicine in Fat Grafting, Aesthetic Medicine and Surgery

Kotaro Yoshimura, MD - *Associate Professor, Department of Plastic Surgery, University of Tokyo*
Functional Roles of ASCs in Fat Grafting for Tissue Volumization and Revitalization

Daniel Del Vecchio, MD - *Associate Clinical Staff, Massachusetts General Hospital, Harvard Medical School, Boston, MA*
FAT VS FICTION: The Truth About Unadulterated Fat vs Stem Cell Enriched Fat

Ramon Llull Cerda, MD, PhD - *Director of Stem Center, Mallorca, Spain*
Long term Follow up on Cell-Enhanced Fat Grafting in Breast Surgery

Synopsis:
This panel will present the most recent findings and experience related to the regenerative and reconstructive applications of adipose tissue and/or adipose-derived cells, as presented by global leaders in the field of Plastic Surgery. The presentations will include clinical cases and provide a forum for interactive discussion and debate.

NOTES

PROGRAM IN BRIEF

Friday, October 5, 2012

6:30 am - 5:00 pm	Registration
7:00 - 8:00 am	Continental Breakfast - *Exhibit Hall*
8:00 - 8:15 am	Welcome and Introduction *Julie Fradette, PhD - IFATS President*
8:15 - 8:30 am	**A Brief History of IFATS** *J. Peter Rubin, MD, FACS - IFATS Chairman*
8:30 - 9:45 am	**PLENARY SESSION 1** **Assessing Safety and Therapeutic Potency of Adipose Derived Stem Cells (ASCs)** Moderators: *Louis Casteilla, PhD & Kacey Marra, PhD*
9:45 - 10:15 am	Coffee Break/Exhibits
10:15 - 10:45 am	**KEYNOTE SPEAKER** **Tissue Engineering of Bone Using Adipose Derived Stem Cells** *Gordana Vunjak-Novakovic, PhD - Columbia University, New York, NY; Member, TERMIS-Americas Council*
10:45 am - 12:00 pm	**PLENARY SESSION 2** **Advances in Musculoskeletal Repair and Regeneration** Moderators: *Jeffrey Gimble, MD, PhD & Ramon Llull Cerda, MD, PhD*
12:00 - 1:30 pm	Lunch and Exhibits
1:30 - 2:40 pm	**CONCURRENT SESSION C1A** **Clinical Fat Grafting** Moderators: *Kevin Hopkins, MD, FACS & Kevin Small, MD*
1:30 - 2:40 pm	**CONCURRENT SESSION C1B** **Basic Science: Biological Challenges and Cell Functions** Moderators: *Anthony Scimè, PhD & Ivona Percec, MD, PhD*
1:30 - 2:40 pm	**CONCURRENT SESSION C1C** **Applied Science: Cell Processing and Amplification Methods** Moderators: *Dmitry Traktuev, PhD & Lauren Flynn, PhD*
2:40 - 3:00 pm	Coffee Break/Exhibits
3:00 - 4:00 pm	**CONCURRENT SESSION C2A** **Cell Harvesting Methods Under Investigation** Moderators: *Alexandra Conde-Green, MD & Maryse Proulx, MSc*
3:00 - 4:00 pm	**CONCURRENT SESSION C2B** **Tissue Engineering Innovations based on ASCs** Moderators: *Guillaume Grenier, PhD & Nathalie Faucheux, PhD*
3:00 - 4:00 pm	**PANEL 1** **Regulatory Pathways for Adipose Technology** Panel Chairs: *Stuart Williams, PhD & Keith March, MD, PhD* Panelist: *Mary Ann Chirba, JD, DSc, MPH*

4:00 - 4:30 pm	Coffee Break/Exhibits
4:30 - 5:30 pm	**CONCURRENT SESSION C3A** **Cell Isolation and Processing: Recent Advances** Moderators: *William Futrell, MD & Sammy Sliwin, MD, FRCSC*
4:30 - 5:30 pm	**PANEL 2** **Do's and Don'ts in Translational Research: Importance of Choosing Adequate Preclinical Models** Panel Chairs: *Marco Helder, PhD & Lucie Germain, PhD* Panelist: *Gordana Vunjak-Novakovic, PhD*
5:30 - 7:00 pm	**RECEPTION -** *Exhibit Area, Loews Hotel*

Saturday, October 6, 2012

6:30 am - 5:00 pm	Registration
7:00 - 8:00 am	Continental Breakfast - *Exhibit Hall*
7:50 - 8:00 am	**Introductory Remarks** *Julie Fradette, PhD*
8:00 - 9:45 am	**PLENARY SESSION 3** **Best Paper Award Finalists I** Moderators: *Spencer Brown, PhD & Kotaro Yoshimura, MD*
9:45 - 10:15 am	Coffee Break/Exhibits
10:15 - 12:00 pm	**PLENARY SESSION 4** **Best Paper Award Finalists II** Moderators: *Jae-Ho Jeong, MD, PhD & Keith March, MD, PhD*
12:00 - 1:30 pm	Lunch and Exhibits
1:30 - 2:30 pm	**CONCURRENT SESSION C4A** **Clinical Fat Grafting** Moderators: *Sydney Coleman, MD, FACS & Daniel Del Vecchio, MD*
1:30 - 2:30 pm	**CONCURRENT SESSION C4B** **Tissue Engineering Strategies: Matrix and Cells for Regenerative Medicine** Moderators: *Torsten Blunk, PhD & Bruce Bunnell, PhD*
2:30 - 2:50 pm	Coffee Break/Exhibits
2:50 - 3:20 pm	**CONCURRENT SESSION C5A** **Clinical Fat Grafting** Moderators: *J. Peter Rubin, MD, FACS & Henry Spinelli, MD, FACS*

2:50 - 4:10 pm	**CONCURRENT SESSION C5B** **Translational Science: In vivo Transplantation Studies of Cells and Tissues** Moderators: *Brian Johnstone, PhD & Stéphane Bolduc, MD*
3:20 - 5:00 pm	**PANEL 3** **Clinical Applications of Fat and Fat-derived Cells: A Global Perspective** Panel Chairs: *Adam Katz, MD, FACS & William Futrell, MD* Panelists: *Sydney Coleman, MD, FACS; J. Peter Rubin, MD, FACS; Henry Spinelli, MD, FACS; Kotaro Yoshimura, MD; Daniel Del Vecchio, MD; Ramon Llull Cerda, MD, PhD*
5:30 pm	Buses leave Loews Hotel
6:00 - 10:00 pm	**RECEPTION & DINNER** *Manoir Montmorency*
10:00 pm	Buses return to hotel

Sunday, October 7, 2012

6:30 am - 12:00 pm	Registration
7:00 - 8:00 am	Continental Breakfast - *Exhibit Hall*
8:00 - 8:05 am	**Introductory Remarks** *Julie Fradette, PhD & André Tchernof, PhD*
8:05 - 8:30 am	**INVITED SPEAKER** **Transcriptional Control of Brown and Beige Adipocyte Fate** *Patrick Seale, PhD - University of Pennsylvania, Philadelphia, PA*
8:30 - 9:30 am	**PLENARY SESSION 5** **Probing ASCs Biological Properties and Behavior** Moderators: *Valérie Planat-Benard, PhD & André Tchernof, PhD*
9:30 - 10:00 am	Coffee Break/Exhibits
10:00 - 10:20 am	**Announcement of Best Paper Awards** *Julie Fradette, PhD*
10:20 - 11:20 am	**PLENARY SESSION 6** **The Multiple Facets of Adipose Tissue as a Cell Source for Regenerative Medicine** Moderators: *Stuart Williams, PhD & Philippe Bourin, MD, PhD*
11:30 am - 12:30 pm	**PLENARY SESSION 7** **ASCs and Vasculature** Moderators: *Paul DiMuzio, MD & Adam Katz, MD, FACS*
12:30 pm	**Concluding Remarks and Farewell** *Julie Fradette, PhD*

NOTES

PROGRAM SCHEDULE

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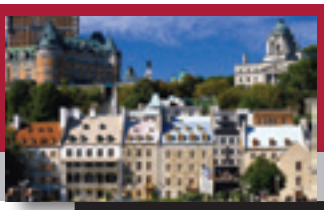

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8:30 - 9:45 am	**PLENARY SESSION 1** **Assessing Safety and Therapeutic Potency of Adipose Derived Stem Cells (ASCs)** Moderators: *Louis Casteilla, PhD & Kacey Marra, PhD*
8:30 am	**1** **EFFICACY AND CHRONIC SAFETY OF SVF ISOLATED WITH THE TISSUE GENESIS CELL ISOLATION SYSTEM IN A MOUSE HINDLIMB ISCHEMIA MODEL** Presenter: Brian Johnstone, PhD Affiliation: Indiana University School of Medicine Authors: Johnstone B, Cook TG, Feng D, Lupov IP, Merfeld-Clauss S, Randolph ML, Van Natta B, Lye KD, Williams SK, Kosnik P, March KL
8:42 am	**2** **MULTIPLE INTRAVENOUS ADMINISTRATIONS OF HUMAN ADIPOSE MESENCHYMAL STEM CELLS ARE SAFE AND DO NOT INDUCE TUMOR DEVELOPMENT** Presenter: JeongChan Ra, PhD Affiliation: RNLBio Authors: Ra JC, Chung MK, Shin IS, Ko MS, Kang SK, Kim YJ, Kwon E, Kang BC
8:54 am	**3** **MULTIPOTENT ADIPOSE STROMAL CELLS IN DIABETIC RETINOPATHY** Presenter: Rajashekhar Gangaraju, PhD Affiliation: Indiana University School of Medicine Authors: Gangaraju R, Abburi C, Kern TS, March KL
9:06 am	**4** **A MULTICENTER TRIAL TO ASSESS THE SAFETY AND EFFICACY OF ADIPOSE CELLS IN CONGESTIVE HEART FAILURE AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE** Presenter: Kristin Comella, MS Affiliation: Bioheart Authors: Comella K, McQuillan S, Lopez J, Perez J, Parcero J
9:18 am	**5** **SAFETY, EFFICACY AND MODE OF DELIVERY OF AUTOLOGOUS ADIPOSE-STROMAL VASCULAR FRACTIONS CELLS IN OSTEOARTHRITIS PATIENTS** Presenter: Ralph T. Bright, MD Affiliation: Macquarie Stem Cells Authors: Bright RT, Bright P, Ilhan E, Thomas W

9:30 am	6 **A PHASE I TRIAL (ACELLDREAM), USE OF AUTOLOGOUS ADIPOSE DERIVED STROMA/STEM CELLS TO TREAT CRITICAL LIMB ISCHEMIA (CLI)** Presenter: Louis Casteilla, PhD Affiliation: University of Toulouse Authors: Bura-rivière A, Léobon B, Bourin, Gross F, Grolleau Saleb B, Peyrafitte J, Fleury S, Planat-Benard V, Casteilla L
9:45 - 10:15 am	Coffee Break/Exhibits
10:15 - 10:45 am	**KEYNOTE SPEAKER** **Tissue Engineering of Bone Using Adipose Derived Stem Cells** *Gordana Vunjak-Novakovic, PhD - Member, Columbia University, New York, NY; TERMIS-Americas Council*
10:45 am - 12:00 pm	**PLENARY SESSION 2** **Advances in Musculoskeletal Repair and Regeneration** Moderators: *Jeffrey Gimble, MD, PhD & Ramon Llull Cerda, MD, PhD*
10:45 am	7 **ADIPOSE-DERIVED STEM CELLS COMBINED WITH DEMINERALIZED BONE MATRIX IN CRITICAL-SIZED SEGMENTAL BONE DEFECTS** Presenter: Nicole P. Ehrhart, DVM, MS Affiliation: Colorado State University Authors: Ehrhart NP, Chubb LS, Webb TL
10:57 am	8 **EFFECTS OF BIOMATERIALS AND GROWTH FACTORS ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS - IN VITRO AND IN VIVO STUDIES** Presenter: Bettina I. Mannerstrom, PhD Affiliation: BioMediTech Authors: Mannerstrom BI, Waselau M, Patrikainen M, von Rechenberg B, Miettinen S
11:09 am	9 **BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE CARRIERS FOR HUMAN MAXILLARY SINUS FLOOR ELEVATION: UPDATE ON A PHASE I CLINICAL TRIAL** Presenter: Marco N. Helder, PhD Affiliation: VU University Medical Center Authors: Helder MN, Prins HJ, Overman JR, ten Bruggenkate CM, Klein Nulend J, Schulten EA
11:21 am	10 **TREATMENT OF OSTEOARTHIRTIS OF THE KNEE WITH INTRAARTICULAR INJECTION OF AUTOLOGOUS ADIPOSE TISSUE DERIVED STEM CELLS: PHASE I & II CLINICAL TRIAL** Presenter: Kang Yoon, MD, PhD Affiliation: SMGSNU Boramae Medical Center Authors: Yoon K, Jo C

11:33 am	**11** **TREATMENT OF LUMBAR DEGENERATIVE DISC DISEASE WITH ADIPOSE DERIVED STROMAL VASCULAR FRACTION, POINT OF CARE** Presenter: Carlos J. Garcia, MD Affiliation: Premier Pain Care Author: Garcia CJ
11:45 am	**12** **CELL-BASED FLUORESCENCE IMAGING OF CRYOPRESERVED ANIMAL: LAVACELL® FLUOROPHORE DYE FOR EVALUATING THE FATE OF HUMAN MESENCHYMAL STEM CELLS (HMSCS) IN-SITU** Presenter: Zhina Sadeghi, MD Affiliation: Case Western Reserve University & University Hospitals Case Medical Center Authors: Molter J, Lennon D, Kavran M, Grimberg KO, Daneshgari F, Caplan AI, Flask CA, Hijaz AK
12:00 - 1:30 pm	Lunch and Exhibits
1:30 - 2:40 pm	**CONCURRENT SESSION C1A** **Clinical Fat Grafting** Moderators: *Kevin Hopkins, MD, FACS & Kevin Small, MD*
1:30 pm	**13** **CLINICAL EXPERIENCE WITH AUTOLOGOUS FAT GRAFTING IN THE PEDIATRIC PATIENT: AN UPDATE** Presenter: Kevin S. Hopkins, MD,FACS Affiliation: Driscoll Childrens Hospital Authors: Hopkins KS, Taylor BT
1:40 pm	**14** **REGENERATION OF HEAD AND NECK IRRADIATED TISSUE WITH AUTOLOGOUS FAT GRAFT: A RETROSPECTIVE STUDY** Presenter: Aurora Almadori, MD Affiliation: UCSC Universita Cattolica del Sacro Cuore Authors: Almadori A, Salgarello M, Fetoni A, Paludetti G
1:50 pm	**15** **CHANGES IN TISSUE PERFUSION DURING APPLICATION OF EXTERNAL VOLUME EXPANSION (EVE) SYSTEMS FOR FAT GRAFT SITE PREPARATION** Presenter: Luca Lancerotto, MD Affiliation: Brigham and Women's Hospital Authors: Lancerotto L, Chin MS, Freniere B, Lujan-Hernandez JR, Del Vecchio DA, Lalikos JF, Bassetto F, Orgill DP
2:00 pm	**16** **IRRADDIATED BREAST RECONSTRUCTION: UPPER EXTREMITY FUNCTIONAL IMPROVEMENT AFTER FAT INJECTION** Presenter: Nho V. Tran, MD Affiliation: Mayo Clinic Rochester Authors: Tran NV, Convery PA

~~2:10 pm~~

DID NOT PRESENT AT THE MEETING

17
~~TEMPERAMENTAL AND PERSONALITY TRAITS IN OVERWEIGHT/OBESE PATIENTS WHO SEEK PLASTIC SURGERY TREATMENT~~
~~Presenter: Mariafrancesca Azzi, MD~~
~~Affiliation: University of Padova~~
~~Authors: Azzi M, Vindigni V, Lancerotto L, Marini M, Bassetto F, Pavan C~~

2:20 pm

18
A FAT GRAFT OF CELL ENRICHED MATRIX DERIVED FROM LIPOASPIRATE SHOWS INCREASED VASCULARIZATION AND PROLIFERATION
Presenter: Carl Friddle, PhD
Affiliation: InGeneron
Authors: Friddle C, Husfeld R, Sanchez A, Martinez R, Coleman M

2:30 pm

19
INTERCOMMUNICATION WITH RNA TRANSFER FROM DAMAGED CELLS TO TARGED ADIPOCYTES IN SKIN TRAUMA, THE SURVIVAL CAPSULES
Presenter: Marco Aurelio Pellon, MD
Affiliation: Clinica Sao Vicente
Author: Pellon MA

1:30 - 2:40 pm

CONCURRENT SESSION C1B
Basic Science: Biological Challenges and Cell Functions
Moderators: *Anthony Scimè, PhD & Ivona Percec, MD, PhD*

1:30 pm

20
BIOLOGICAL EFFECTS OF NEUROPEPTIDE Y ON PRIMARY CULTURED HUMAN ADIPOSE-DERIVED STEM CELLS: IN VITRO AND IN VIVO STUDIES
Presenter: Brian J. Philips, PhD
Affiliation: University of Pittsburgh
Authors: Philips BJ, Kling RE, Valentin JE, Kelmendi-Doko A, Grahovac TL, Ravuri SK, Marra KG, Fernstrom JD, Rubin JP

1:40 pm

21
IMPACT OF AN INFLAMMATORY-LIKE CONTEXT ON THE CAPILLARY NETWORK WITHIN HUMAN TISSUE-ENGINEERED ADIPOSE TISSUES
Presenter: Maryse Proulx, MSc
Affiliation: Centre LOEX de l'Université Laval
Authors: Proulx M, Mayrand D, Aubin K, Audet-Casgrain MA, Fradette J

1:50 pm

22
A NOVEL ROLE FOR SIRTUIN 7 PROTEIN DEACETYLASE IN AGING ADIPOSE TISSUE
Presenter: Brian L. Chang, BA, BS
Affiliation: University of Pennsylvania
Authors: Chang BL, Dierova R, Percec I

2:00 pm

23
THE EFFECTS OF RADIATION THERAPY ON THE PROLIFERATION AND POTENCY OF ADIPOSE-DERIVED STEM CELLS
Presenter: Rachel L. Slotcavage, MD
Affiliation: Cooper Medical School of Rowan University
Authors: Slotcavage RL, Crutchfield M, Colacino A, Chang S, Liu Y, Matthews M, Carpenter J, DiSanto M, DiMuzio P, Tulenko T

2:10 pm

24
ADIPOSE-DERIVED STEM CELL TO EPITHELIAL STEM CELL TRANSDIFFERENTIATION: A MECHANISM TO IMPROVE UNDERSTANDING OF FAT GRAFTS SKIN REGENERATIVE POTENTIAL
Presenter: Brian M. Derby, MD
Affiliation: Southern Illinois University School of Medicine
Authors: Derby BM, Dai H, Reichensperger J, Cox L, Harrison C, Bueno RA, Neumeister MW

2:20 pm

25
HYPOXIA ALTERS MUSCLE RESIDENT STROMAL CELL PHENOTYPE AND IS A KEY REGULATOR IN TRAUMATIC HETEROTOPIC OSSIFICATION
Presenter: Guillaume Grenier, PhD
Affiliation: University of Sherbrooke
Authors: Drouin G, Couture V, Palidwor G, Perkins T, Faucheux N, Grenier G

2:30 pm

26
EFFECTS OF CURCUMIN ON HUMAN ADIPOSE DERIVED STEM CELLS
Presenter: Russell E. Kling, BA
Affiliation: University of Pittsburgh
Authors: Kling RE, Narayanan K, Ravuri SK, Philips BJ, Marra KG, Rubin JP

1:30 - 2:40 pm

CONCURRENT SESSION C1C
Applied Science: Cell Processing and Amplification Methods
Moderators: *Dmitry Traktuev, PhD & Lauren Flynn, PhD*

1:30 pm

27
COLLECTION, PROCESSING, TESTING AND RELEASE CRITERIA FOR GMP MANUFACTURED ADSC PRODUCTS
Presenter: Mary Pat Moyer, PhD
Affiliation: INCELL Corporation LLC
Author: Moyer MP

1:40 pm

28
HOW PATIENT DEMOGRAPHICS AND ANATOMIC SITE SELECTION AFFECT YIELD AND DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS
Presenter: Melanie R. Crutchfield, MD
Affiliation: Cooper Medical School of Rowan University
Authors: Crutchfield MR, Slotcavage RL, Colacino A, Chang S, Matthews M, Liu Y, DeSanto M, Carpenter J, Park SS, DiMuzio P, Tulenko T

1:50 pm

29
WHAT'S IN A NAME? INTRODUCING NON-ADHERENT PROGENITORS FROM ADIPOSE-DERIVED STEM CELLS (NAPASCS)
Presenter: Angelo A. Leto Barone, MD
Affiliation: Universita degli Studi di Palermo
Authors: Leto Barone AA, Giunta G, Carmisciano M, Toia F, Carollo R, Iovino F, Todaro M, Cordova A, Moschella F

2:00 pm

30
ADIPOSE STROMAL CELL ISOLATION AND EXPANSION FOR CLINICAL APPLICATIONS USING XENO- AND SERUM-FREE MEDIA
Presenter: Dmitry O. Traktuev, PhD
Affiliation: Indiana University
Authors: Traktuev DO, Merfeld-Clauss S, Cook T, Compton-Craig P, Lupov IP, Johnstone BH, March KL

2:10 pm

31
A NOVEL MICRORNA-OMIC APPROACH FOR CHARACTERIZING OF ADIPOSE-DERIVED REGENERATIVE CELL SAFETY AND EFFICACY
Presenter: Kevin Hicok, MD
Affiliation: Cytori Therapeutics Inc
Authors: Mallinson DJ, OBrien V, Olijnyk D, Zhu M, Fraser JK, Arm D

2:20 pm

32
SUCCESSFUL CULTURE OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS IN A FUNCTIONALLY CLOSED AUTOMATED CELL EXPANSION SYSTEM
Presenter: Kim T. Nguyen, PhD
Affiliation: Terumo BCT
Authors: Nguyen KT, Baila S

2:30 pm

33
AUTOLOGOUS CRYOPRESERVATION OF HUMAN ADULT ADIPOSE DERIVED STEM CELLS
Presenter: Ilana Platt, PhD
Affiliation: Adisave
Authors: Niapour M, Sliwin SJ, Platt I, Rice S

2:40 - 3:00 pm

Coffee Break/Exhibits

3:00 - 4:00 pm

CONCURRENT SESSION C2A
Cell Harvesting Methods Under Investigation
Moderators: *Alexandra Conde-Green, MD & Maryse Proulx, MSc*

3:00 pm

34
CHARACTERIZATION OF STROMAL VASCULAR CELLS FOLLOWING ENZYMATIC DIGESTION OR MECHANICAL PROCESSING OF ASPIRATED ADIPOSE TISSUE
Presenter: Alexandra Conde-Green, MD
Affiliation: University of Maryland Medical Center
Authors: Conde-Green A, Rodriguez RL, Vail SR, Ivo BG, Slezak S, McLenithan JC

3:10 pm

35
ISOLATION OF STROMAL VASCULAR FRACTION FROM NUTATIONAL INFRASONIC LIPOASPIRATE
Presenter: Robert E. Bowen, MD
Affiliation: The Center for Positive Aging
Authors: Bowen RE, McQuillan S, Comella K

3:20 pm

36
OPTIMIZING HARVESTING TECHNIQUES OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS (HADSCS): A MULTIVARIABLE STUDY COMPARING SIZES OF CANNULA AND POWER SUCTION, WATER-JET AND SYRINGE SUCTION LIPOSUCTION TECHNIQUES
Presenter: Sammy Sliwin, MD, FRCSC
Affiliation: Adisave
Authors: Sliwin S, Niapour M, Rice S

3:30 pm

37
NON-ENZYMATIC METHODS TO OBTAIN REGENERATIVE CELLS FROM ADIPOSE: IS IT PRACTICAL OR EVEN POSSIBLE?
Presenter: Min Zhu, MD
Affiliation: Cytori Therapeutics Inc
Authors: Zhu M, Hicok KC, Shanahan R, Yu J, Souverneva O, Fornace G, Alfonso Z, Arm D, Fraser JK

3:40 pm

38
CHARACTERIZATION OF HUMAN ADIPOSE DERIVED MESENCHYMAL STEM CELLS USING DIFFERENT SEPARATION TECHNIQUES
Presenter: Maryam Niapour, PhD
Affiliation: Adisave
Authors: Niapour M, Sliwin SJ, Rice S

~~3:50 pm~~

DID NOT PRESENT AT THE MEETING

39
VIABILITY OF ISOLATED ADIPOSE-DERIVED STEM CELLS VIA ULTRASONIC SEPARATION
Presenter: Joseph A. Broujerdi, ~~MD, DMD~~
Affiliation: Private Practice
Authors: Broujerdi JA, Schendel SA, Jacobson RL

3:00 - 4:00 pm

CONCURRENT SESSION C2B
Tissue Engineering Innovations based on ASCs
Moderators: *Guillaume Grenier, PhD & Nathalie Faucheux, PhD*

3:00 pm

40
ADIPOSE-DERIVED STROMAL CELLS FOR THE RECONSTRUCTION OF A HUMAN VESICAL EQUIVALENT
Presenter: Alexandre Rousseau, MSc
Affiliation: Centre LOEX de l'Université Laval
Author: Rousseau A, Bernard G, Marceau Fortier G, Bouhout S, Fradette J, Bolduc S

3:10 pm

41
PRE-VASCULARIZED CELL SHEETS COMPRISED OF HUMAN FIBROBLASTS, ENDOTHELIAL CELLS AND ADIPOSE-DERIVED STEM CELLS
Presenter: YenChih Lin, PhD
Affiliation: University of Pittsburgh
Authors: Lin YC, Grahovac T, Oh SJ, Rubin JP, Marra KG

3:20 pm

42
EVALUATION OF HUMAN ADIPOSE TISSUE STROMAL/STEM CELLS FOR BLOOD VESSEL TISSUE ENGINEERING
Presenter: Maxime Tondreau, MS
Affiliation: Laval University, LOEX
Authors: Tondreau M, Vallieres K, Laterreur V, Bourget JM, Germain L, Fradette J, Auger FA

~~3:30 pm~~

DID NOT PRESENT AT THE MEETING

43
EFFECT OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 AND ADIPOSE TISSUE-DERIVED STEM CELL ON NEW BONE FORMATION IN HIGHSPEED DISTRACTION OSTEOGENESIS OF ADULT RABBIT CRANIUM
Presenter: ByeongKyu Kim, PhD
Affiliation: Seoul National University College of Medicine
Authors: Kim BK, Lee SJ, Choi TH, Kim SH

3:40 pm

44
A SHORT BMP-2 STIMULUS SUFFICES FOR OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS SEEDED ON CALCIUM PHOSPHATE SCAFFOLDS
Presenter: Janice Overman, PhD
Affiliation: VU University Medical Center
Authors: Farre-Guasch E, ten Bruggenkate CM, Schulten EA, Klein-Nulend J, Helder MN

3:50 pm

45
IN VITRO RECONSTRUCTION OF A VASCULARISED TENDON-LIKE STRUCTURE WITH ADIPOSE DERIVED STEM CELLS (ADSCS)
Presenter: Franco Bassetto, MD
Affiliation: University of Padova
Authors: Bassetto F, Lancerotto L, Tonello C, Abatangelo G, Cortivo R, Zavan B, Vindigni V

3:00 - 4:00 pm

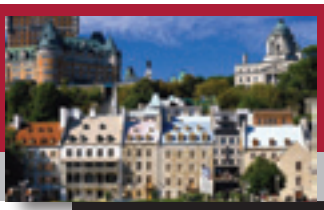
PANEL 1
Regulatory Pathways for Adipose Technology
Panel Chairs: *Stuart Williams, PhD & Keith March, MD, PhD*
Panelist: *Mary Ann Chirba, JD, DSc, MPH*

4:00 - 4:30 pm

Coffee Break/Exhibits

4:30 - 5:30 pm	**CONCURRENT SESSION C3A** **Cell Isolation and Processing: Recent Advances** Moderators: *William Futrell, MD & Sammy Sliwin, MD, FRCSC*
4:30 pm	46 **SELECTIVE ISOLATION OF ADIPOSE-DERIVED STEM/STROMAL CELLS FROM LIQUID PORTION OF LIPOSUCTION ASPIRATES USING AN ADHERENT COLUMN** Presenter: Kentaro Doi, MD Affiliation: University of Tokyo Authors: Doi K, Kato H, Kuno S, Mineda K, Kinoshita K, Yang S, Yoshimura K
4:40 pm	47 **DIFFERENTIAL DIGESTION OF ADIPOSE TISSUE. ROLE OF SPECIFIC PROTEASES ON CELL YIELD. IMMUNOMODULATORY AND ANGIOGENIC PROPERTIES OF STROMAL VASCULAR FRACTION OBTAINED** Presenter: Severiano Dos Anjos Vilaboa Sr., PhD Affiliation: Stem Center SL Authors: Dos Anjos Vilaboa S, Mercader J, Llull R, Katz A, Futrell W
4:50 pm	48 **EXTRACTION OF EQUINE STEM CELL RICH FAT TISSUE IN A MINIMALLY INVASIVE LIPOASPIRATE PROCEDURE AND CLINICAL APPLICATIONS** Presenter: Sharon McQuillan, MD Authors: McQuillan S, Comella K
5:00 pm	49 **STROMAL VASCULAR CELL THERAPEUTICS: SORTING OUT FACT FROM FICTION** Presenter: John Fraser, MD Affiliation: Cytori Therapeutics Inc Authors: Zhu M, Shanahan R, Hicok KC, Fraser JK, Arm D
5:10 pm	50 **SERVA COLLAGENASE NB 6 GMP GRADE FOR ADIPOSE TISSUE DIGESTION** Presenter: Rowena A. Soriano, BS Affiliation: Invitrx Therapeutics Inc Authors: Soriano RA, Torfi H
~~5:20 pm~~ **DID NOT PRESENT AT THE MEETING**	51 **CASE REPORT: OPTIMIZATION OF ROCHE LIBERASE IN THE ENZYMATIC DIGESTION OF HUMAN ADIPOSE TISSUE FOR THE ISOLATION OF STEM & REGENERATIVE CELLS** Presenter: Habib Torfi, MD Affiliation: Invitrx Therapeutics Inc Authors: Soriano RA, Lamblet H, Mohammadi SA, Torfi H

4:30 - 5:30 pm	**PANEL 2** **Do's and Don'ts in Translational Research: Importance of Choosing Adequate Preclinical Models** Panel Chairs: *Marco Helder, PhD & Lucie Germain, PhD* Panelist: *Gordana Vunjak-Novakovic, PhD*
5:30 - 7:00 pm	Reception - Exhibit Area, Loews Hôtel Le Concorde



Saturday, October 6, 2012

6:30 am - 5:00 pm	Registration
7:00 - 8:00 am	Continental Breakfast - *Exhibit Hall*
7:50 - 8:00 am	**Introductory Remarks** *Julie Fradette, PhD*
8:00 - 9:45 am	**PLENARY SESSION 3** **Best Paper Award Finalists I** Moderators: *Spencer Brown, PhD & Kotaro Yoshimura, MD*
8:00 am	52 **ADIPOSE STROMAL CELLS PREVENT AND RESCUE ACUTE HEMATOPOIETIC TOXICITY OF CIGARETTE SMOKE THROUGH SECRETION OF THE ANTI-INFLAMMATORY CYTOKINE TSG6** Presenter: Jie Xie, MD Affiliation: Indiana University School of Medicine Authors: Xie J, Feng D, Cook TG, Van Demark M, Schweitzer K, Johnstone BH, Petrache I, Broxmeyer HE, March KL
8: 12 am	53 **HUMAN ADIPOSE DERIVED STROMAL CELLS IN A NOVEL 3D CULTURE SYSTEM FOR OSTEOGENIC DIFFERENTIATION: AN IN-VITRO AND IN-VIVO INVESTIGATION** Presenter: Brian C. Werner, MD Affiliation: University of Virginia Authors: Werner BC, Shen FH, Liang H, Shang H, Katz AJ
8:24 am	54 **VOLUMETRIC EVALUATION OF FAT GRAFT SURVIVAL IN THE RADIATED BREAST** Presenter: Kevin H. Small, MD Affiliation: New York Presbyterian Hospital Authors: Small KH, Karp N, Choi M, Lee C, Levovitz C
8:36 am	55 **POROUS DECELLULARIZED ADIPOSE TISSUE FOAMS FOR SOFT TISSUE REGENERATION** Presenter: Claire Yu, BASc Affiliation: Queens University Authors: Yu C, Bianco J, Brown C, Watkins JF, Flynn LE
8:48 am	56 **SENESCENT CELLS COMPROMISE FAT TISSUE FUNCTION** Presenter: Ming Xu, PhD Affiliation: Mayo Clinic Authors: Xu M, Zhu Y, Pirtskhalava T, Giorgadze N, Baker DJ, Jensen MD, van Deursen J, Tchkonia T, Kirkland JL

9:00 am

57
CHARACTERIZATION OF HUMAN ADIPOSE TISSUE-RESIDENT HEMATOPOIETIC CELL POPULATIONS: A NOVEL MACROPHAGE SUBPOPULATION WITH CD34 EXPRESSION AND MESENCHYMAL MULTIPOTENCY
Presenter: Kahori Kinoshita, MD
Affiliation: University of Tokyo
Authors: Doi K, Eto H, Kato H, Kuno S, Mineda K, Kinoshita K, Yang S, Yoshimura K

9:12 am

DID NOT PRESENT AT THE MEETING

58
REGENERATION OF CRITICAL OSTEOCHONDRAL DEFECTS IN MINIPIG BY ADIPOSE-DERIVED STEM CELLS (ASCS) ON HYDROGEL OF OLIGO (POLYETHYLENE GLYCOL) FUMARATE
Presenter: Elena Arrigoni, PhD
Affiliation: University of Milan
Authors: Arrigoni E, de Girolamo L, Niada S, Di Giancamillo A, Domeneghini C, Dadsetan M, Yaszemski M, Vena P, Peretti GM, Brini AT

9:24 am

59
TRANSPLANTATION OF A CHIMERIC STEM CELL GRAFT: THE EX-VIVO ASSEMBLY OF A HYBRID STEM CELL GRAFT USING THE LGR6+ EPITHELIAL STEM CELL AND ADSC TO AUGMENT CELLULAR MASS AND ANGIOGENESIS ON ACELLULAR MATRICES
Presenter: Denver M. Lough, MD, PhD
Affiliation: Southern Illinois University School of Medicine
Authors: Lough DM, Dai H, Yang M, Reichensperger J, Wetter N, Cox L, Harrison C, Neumeister MW

9:45 - 10:15 am

Coffee Break/Exhibits

10:15 - 12:00 pm

PLENARY SESSION 4
Best Paper Award Finalists II
Moderators: *Jae-Ho Jeong, MD, PhD & Keith March, MD, PhD*

10:15 am

60
OPTIMIZING HYPOXIC PRECONDITIONING OF MESENCHYMAL STEM CELLS FOR ANGIOGENIC THERAPIES
Presenter: Julie Beegle
Affiliation: University of California Davis
Authors: Fierro FA, Beegle JR, Stewart H, Nolta JA

10:27 am

61
MULTI DRUG RESISTANCE PROTEIN BCRP PROTECTS ADIPOSE DERIVED STEM CELLS AGAINST ISCHEMIC DAMAGE
Presenter: Benno A. Naaijkens, MSc
Affiliation: VU Medical Center Amsterdam
Authors: Naaijkens BA, van Dijk A, Jurgens WF, Oerlemans R, Scheffer G, Visser FC, Schuurmans GJ, Juffermans LJ, van Milligen FJ, Niessen HW

10:39 am

62
ENGRAFTMENT OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS AS PERIVASCULAR CELLS OF BIOENGINEERED MICROVESSELS ENHANCES ADIPOSE TISSUE FORMATION
Presenter: RueiZeng Lin, PhD
Affiliation: Childrens Hospital Boston and Harvard Medical School
Authors: Lin RZ, Greene AK, Melero-Martin JM

~~10:51 am~~

63
EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS TREATMENT IN A MOUSE MODEL OF ~~NEUROPATHIC~~ PAIN
Presenter: Stefania Niada, MD
Affiliation: University of Milan
Authors: Niada S, Rossi A, Arrigoni E, Franchi S, Panerai AE, Sacerdote P, ~~Brini~~ AT

DID NOT PRESENT AT THE MEETING

11:03 am

64
ACTIVATION OF ADIPOSE-DERIVED STEM/STROMAL CELLS (ASCS) BY ADIPOSE TISSUE-DAMAGE ASSOCIATED FACTORS AND CHEMOKINES
Presenter: Shinichiro Kuno, MD
Affiliation: University of Tokyo School of Medicine
Authors: Kuno S, Doi K, Mineda K, Kinoshita K, Yang S, Yoshimura K

11:15 am

65
WNT PATHWAY ANTAGONIST, SECRETED FRIZZLEDRELATED PROTEIN1 (SFRP1) AS AN INDICATOR OF INNATE ADIPOGENESIS
Presenter: Sudheer K. Ravuri, PhD
Affiliation: University of Pittsburgh
Authors: Ravuri SK, Philips BJ, McArdle NL, Opene BA, Meyer EM, Pfeifer ME, Zimmerlin L, Donnenberg VS, Donnenberg AD, Marra KG, Rubin JP

11:27 am

66
SINGLE-CELL TRANSCRIPTION STATE ANALYSIS OF ALDEFLUOR-BRIGHT AND -DIM ADIPOSE STROMAL CELLS AND PERICYTES USING FLUIDIGM MICROFLUIDIC ARRAYS
Presenter: Winters R. Hardy, PhD
Affiliation: IUPUI
Authors: Hardy WR, Datta K, Livak K, Lupov I, Traktuev D, Corselli M, Peault B, Srour E, March K

11:40 am - 12:00 pm

Housekeeping & IFATS 2013 Announcements
Julie Fradette, PhD

12:00 - 1:30 pm

Lunch and Exhibits

1:30 - 2:30 pm	**CONCURRENT SESSION C4A** **Clinical Fat Grafting** Moderators: *Sydney Coleman, MD, FACS & Daniel Del Vecchio, MD*
1:30 pm	67 **THE USE OF A HYALURONATE-BASED INJECTABLE HYDROGEL AS A DELIVERY VEHICLE OF STROMAL VASCULAR FRACTION FOR ADIPOSE TISSUE REPAIR: PRELIMINARY RESULTS** Presenter: Thomas Zarembinski, PhD Affiliation: BioTime Inc Authors: Zarembinshi T, Atzet SK, Doty N, Tandeski T, Tew WP
1:40 pm	68 **TEAR TROUGH DEFORMITY TREATMENT: FAT GRAFTING X HYALURONIC ACID. UNRAVELING BENEFITS AND PITFALLS** Presenter: Katarina Andjelkov, PhD Affiliation: Private Clinic Authors: Andjelkov K, Sforza M, Zaccheddu R
1:50 pm	69 **HIGH DEFINITION ULTRASOUND MONITORING OF CRYOPRESERVED AND FRESH FAT GRAFTS IN THE BREASTS** Presenter: Jeffrey M. Hartog, MD Affiliation: The Adrecyte Regenerative Medicine and Surgery Center Author: Hartog JM
2:00 pm	70 **CORRECTING DEFORMITIES AFTER BREAST AUGMENTATION WITH SILICONE IMPLANTS: DOES FAT GRAFTING HAVE THE X FACTOR?** Presenter: Marcos Sforza, MD Affiliation: Dola Park Hospital Authors: Sforza M, Andjelkov K, Zaccheddu R
2:10 pm	71 **WRITING AN INVESTIGATIONAL REVIEW BOARD PROPOSAL FOR FAT GRAFTING TO THE BREAST: WHY IT SHOULD BE DONE** Presenter: Brannon R. Claytor, MD Affiliation: Atlantic Plastic Surgery Author: Claytor BR
~~2:20 pm~~ **DID NOT PRESENT AT THE MEETING**	72 **FAT GRAFTING IN AESTHETIC SURGERY OF THE FACE. GOOD FILLER MATERIAL** Presenter: Gennadiy Patlazhan, ~~MD, PhD~~ Affiliation: Institute of Plastic Surgery Virtus Author: Patlazhan G

1:30 - 2:30 pm	**CONCURRENT SESSION C4B** **Tissue Engineering Strategies: Matrix and Cells for Regenerative Medicine** Moderators: *Torsten Blunk, PhD & Bruce Bunnell, PhD*
1:30 pm	73 **COMPOSITE TISSUE-SPECIFIC BIOSCAFFOLDS FOR ADIPOSE TISSUE REGENERATION** Presenter: Lauren E. Flynn, PhD Affiliation: Queens University Authors: Flynn LE, Cheung HK, Watkins JF, Amsden BG
1:40 pm	74 **ADIPOSE TISSUE-DERIVED ECM AND SVF CELLS AS BUILDING BLOCKS FOR TISSUE ENGINEERED CONSTRUCTS** Presenter: Hyun J. Paek, PhD Affiliation: Tissue Genesis Inc Authors: Iwami S, Shimoda C, Lee JQ, Kim C, Paek HJ
1:50 pm	75 **3D ASC SPHEROIDS AS TOOL FOR BASIC RESEARCH AND BUILDING BLOCKS FOR ADIPOSE TISSUE ENGINEERING** Presenter: Torsten Blunk, PhD Affiliation: University of Wuerzburg Authors: Blunk T, Muhr C, Dietl S, Goepferich A, Winnefeld M, Bauer-Kreisel P
2:00 pm	76 **TRANSCRIPTOME ANALYSIS OF RECONSTRUCTED ADIPOSE TISSUES ENGINEERED FROM HUMAN STEM CELLS COMPARED TO NATIVE ADIPOSE TISSUES** Presenter: Marie-Ève Ouellette, MSc Affiliation: Genie tissulaire et regeneration LOEX; Centre de recherche FRSQ du CHA universitaire de Quebec Universite Laval Authors: Ouellette M, Vallée M, Bérubé J, Bossé Y, Fradette J
2:10 pm	77 **DIABETIC ADIPOSE TISSUE WITHIN A THREE-DIMENSIONAL HOLLOW FIBER-BASED BIOREACTOR** Presenter: Danielle M. Minteer, BS Affiliation: University of Pittsburgh Authors: Minteer DM, Lin YC, Young M, Over P, Gerlach JC, Rubin JP, Marra KG
2:20 pm	78 **ADIPOSE-DERIVED STEM CELLS SEEDED ONTO ACELLULAR DERMAL AND PERITONEAL EXTRACELLULAR MATRICES AS INJECTABLE CONSTRUCTS FOR SOFT TISSUE RECONSTRUCTION** Presenter: Jolene E. Valentin, PhD Affiliation: University of Pittsburgh Authors: Valentin JE, Bechtel J, McLaughlin MM, Hoffman DF, Bowley MR, Goldman S, Marra KG, Rubin JP
2:30 - 2:50 pm	Coffee Break/Exhibits

2:50 - 3:20 pm	**CONCURRENT SESSION C5A** **Clinical Fat Grafting** Moderators: *J. Peter Rubin, MD, FACS & Henry Spinelli, MD, FACS*
2:50 pm	79 **MEGA VOLUME FAT GRAFTING FOLLOWING AUTOLOGOUS BREAST RECONSTRUCTION** Presenter: Marwan H. Abboud, MD Affiliation: CHU Tivoli Authors: Abboud MH, Dibo SA
3:00 pm	80 **BEAUTY AND THE DIEP: IMPROVED AESTHETIC RESULTS WITH AUTOLOGOUS FAT GRAFTING TO THE RECONSTRUCTED BREAST** Presenter: Sybile Val, MD Affiliation: Louisiana State University Authors: Val S, Sadeghi A
3:10 pm	81 **A COMPARISON OF CELL ENRICHED FAT TRANSFER TO CONVENTIONAL FAT GRAFTING AFTER AESTHETIC PROCEDURES USING A PATIENT SATISFACTION SURVEY** Presenter: Brian Mailey, MD Affiliation: University of California San Diego Authors: Cohen SR, Mailey B, Wallace AM
2:50 - 4:10 pm	**CONCURRENT SESSION C5B** **Translational Science: In vivo Transplantation Studies of Cells and Tissues** Moderators: *Brian Johnstone, PhD & Stéphane Bolduc, MD*
2:50 pm	82 **ADIPOSE MATRIX-BASED SCAFFOLDS AS AN ALTERNATIVE TO FAT GRAFTING** Presenter: Iwen Wu, MS Affiliation: Johns Hopkins University Authors: Wu I, Conde-Green A, Graham I, Chae J, Elisseeff J
3:00 pm	83 **ENGRAFTMENT OF HUMAN ADIPOSE DERIVED STEM CELLS DELIVERED IN A HYALURONIC ACID PREPARATION IN MICE** Presenter: Isa Dietrich, MD, PhD Affiliation: Sao Paulo University Medical School Authors: Dietrich I, Cochet O, Villageois P, Rodrigues CJ
3:10 pm	84 **FATE OF STEM CELLS IN INJURY: IN-VIVO REAL TIME TRACKING OF MESENCHYMAL STEM CELLS (MSCS) IN A RAT MODEL OF STRESS URINARY INCONTINENCE (SUI)** Presenter: Kerry O. Grimberg, PhD Affiliation: Case Western Reserve University & University Hospitals Case Medical Center Authors: Molter J, Lennon D, Kavran M, Grimberg KO, Daneshgari F, Caplan AI, Lee Z, Flask CA, Hijaz AK

3:20 pm

85

THE CHEMOKINE, STROMAL DERIVED FACTOR-1 ALPHA, PROMOTES ENDOTHELIAL PROGENITOR CELL-MEDIATED NEOVASCULARIZATION OF HUMAN TRANSPLANTED FAT TISSUE IN DIABETIC IMMUNOCOMPROMISED MICE

DID NOT PRESENT AT THE MEETING

Presenter: Saher Hamed, MD, PhD
Affiliation: Technion Israel Institute of Technology
Authors: Hamed S, Egozi D, Malyarova N, Keren A, Kruchevsky D, Dawood H, Ben-Nun O, Gilhar A, Brenner B, Ullmann Y

3:30 pm

86

THE EFFECT FOR BONE REGENERATION WITH COMBINATION OF ADIPOSE-DERIVED STEM CELLS AND PLATELET-RICH PLASMA

Presenter: Morikuni Tobita, DDS, PhD
Affiliation: Juntendo University School of Medicine
Authors: Tobita M, Orbay H, Hyakusoku H, Mizuno H

3:40 pm

87

EXPERIMENTAL MODEL OF ISCHEMIA-REPERFUSION INJURY OF A MUSCULAR FREE FLAP OF THE MUSCULUS LATISSIMUS DORSI OF DOMESTIC SWINE

Presenter: Patrik Richtr, MD
Affiliation: Medical School and Teaching Hospital Plzen Czech Republic
Authors: Richtr P, Liska V, Racek J, Trefil L, Lavicka P

3:50 pm

88

ERYTHROPOIETIN IMPROVES FAT GRAFTING IN NUDE MICE MODEL

Presenter: Yehuda Ullmann, MD
Affiliation: Rambam Healthcare Campus
Authors: Ullmann Y, Fishelzon O, Hamed S, Kruchevsky D, Sliman L, Gilhar A

4:00 pm

89

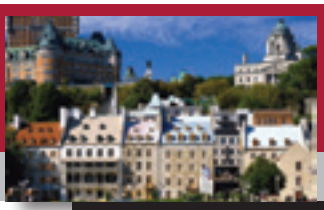
THE LIPOINJECTION OF DEFECTIVE VOCAL FOLD: THE GROWTH KINETIC OF THE ADIPOSE TISSUE STEM CELLS (HATSC) AND THE VOICE OUTCOME

DID NOT PRESENT AT THE MEETING

Presenter: Maria R. Marchese, MD, PhD
Affiliation: Catholic Univeristy of the Sacred Heart
Authors: Marchese MR, Fetoni AR, Lattanzi W, Almadori A, Salgarello M

Morency Falls

3:20 - 5:00 pm	**PANEL 3** **Clinical Applications of Fat and Fat-derived Cells: A Global Perspective** Panel Chairs: *Adam Katz, MD, FACS & William Futrell, MD* Panelists: *Sydney Coleman, MD, FACS* How Does Grafted Fat Heal Damaged Tissue into Which it is Transplanted? *J. Peter Rubin, MD, FACS* Translating Adipose Therapies for Craniofacial Reconstruction *Henry Spinelli, MD, FACS* Reality or Fantasy: Evidence Based Medicine in Fat Grafting, Aesthetic Medicine and Surgery *Kotaro Yoshimura, MD* Functional Roles of ASCs in Fat Grafting for Tissue Volumization and Revitalization *Daniel Del Vecchio, MD* FAT VS FICTION: The Truth About Unadulterated Fat vs Stem Cell Enriched Fat *Ramon Llull Cerda, MD, PhD* Long term Follow up on Cell-Enhanced Fat Grafting in Breast Surgery
5:30 pm	Buses leave hotel
6:00 - 10:00 pm	**RECEPTION & DINNER** *Manoir Montmorency*
10:00 pm	Buses return to hotel



Sunday, October 7, 2012

6:30 am - 12:00 pm	Registration
7:00 - 8:00 am	Continental Breakfast - *Exhibit Hall*
8:00 - 8:05 am	**Introductory Remarks** *Julie Fradette, PhD & André Tchernof, PhD*
8:05 - 8:30 am	**INVITED SPEAKER** **Transcriptional Control of Brown and Beige Adipocyte Fate** *Patrick Seale, PhD - University of Pennsylvania, Philadelphia, PA*
8:30 - 9:30 am	**PLENARY SESSION 5** **Probing ASCs Biological Properties and Behavior** Moderators: *Valérie Planat-Benard, PhD & André Tchernof, PhD*
8:30 am	**90** **INVESTIGATION OF P107 DOWN REGULATION IN DETERMINING THE BROWN ADIPOCYTE LINEAGE** Presenter: Anthony Scimè, PhD Affiliation: York University Authors: Isse M, Scimè A
8:42 am	**91** **SIRTUIN REGULATION OF HUMAN AGING IN PRIMARY ADIPOSE TISSUE** Presenter: Ivona Percec, MD PhD Affiliation: University of Pennsylvania Authors: Percec I, Dierov R, Auman D, Chang B
8:54 am	**92** **OBESITY-ASSOCIATED DYSREGULATION OF ADIPOSE STEM CELL BIOLOGY INFLUENCES BREAST CANCER TUMORIGENESIS AND PROGRESSION** Presenter: Amy F. Lin, MPH Affiliation: Tulane University School of Medicine Authors: Lin AF, Semon JA, Strong TT, Rhodes LV, Shi Z, Santoke TT, Zhang X, Zhang S, McFerrin HE, Burow ME, Gimble JM, Bunnell BA
~~9:06 am~~ **DID NOT PRESENT AT THE MEETING**	**93** **COMPARING THE IMMUNOREGULATORY EFFECTS OF BONE MARROW- AND ADIPOSE-DERIVED MESENCHYMAL STEM CELLS** Presenter: Lehao W. Wu, MD Affiliation: Johns Hopkins Medical Institution Authors: Wu LW, Yuan N, Rubin JP, Lee WP, Brandacher G, Cooney DS
9:18 am	**94** **AGE-DEPENDENT ALTERATIONS IN MIRNA PROFILES REVEALS DIFFERENTIAL EXPRESSION OF NF-KB AND MAPK IN ADIPOSE STEM CELLS** Presenter: Bruce A. Bunnell, PhD Affiliation: Tulane University School of Medicine Authors: Bunnell BA, Pandey AC, Semon JA, Kaushal D, OSullivan RP, Glowacki J, Gimble JM

9:30 - 10:00 am	Coffee Break/Exhibits
10:00 - 10:20 am	**Announcement of Best Paper Awards** *Julie Fradette, PhD*
10:20 - 11:20 am	**PLENARY SESSION 6** **The Multiple Facets of Adipose Tissue as a Cell Source for Regenerative Medicine** Moderators: *Stuart Williams, PhD & Philippe Bourin, MD, PhD*
10:20 am	95 **COMPARISON OF HUMAN ADIPOSE-DERIVED STEM CELLS ISOLATED FROM DIFFERENT DEPOTS FOR APPLICATIONS IN CELL-BASED REGENERATION** Presenter: Valerio Russo, MS Affiliation: Queens University Authors: Russo V, Belliveau P, Watkins JF, Hamilton A, Flynn LE
10:32 am	96 **CORRELATING BODY MASS INDEX TO ADIPOSE STEM CELL FUNCTIONALITY AND FAT GRAFT RETENTION** Presenter: Jacqueline Bliley, BS, MS Affiliation: University of Pittsburgh Authors: Bliley J, Grahovac TL, Nayar HS, Philips BJ, Courcoulas AP, Marra KG, Rubin JP
10:44 am	97 **THE ROLE AND THERAPEUTIC POTENTIAL OF THE ADSC ISOLATED FROM HUMAN BURN TISSUE** Presenter: Hui Dai, MD, PhD Affiliation: Southern Illinois University School of Medicine Authors: Dai H, Lough DM, Derby B, Wetter N, Reichensperger J, Cox L, Harrison C, Bueno R, Neumeister MW
10:56 am	98 **UNDERSTANDING THE DEDIFFERENTIATION PROCESS OF HUMAN MATURE ADIPOCYTES FOR OPTIMAL UTILIZATION OF THEIR STEM CELL CAPACITIES** Presenter: Julie Lessard, PhD Affiliation: Centre de Recherche de l'Institut de Cardiologie et Pneumologie de Québec Laval University Authors: Lessard J, Pelletier M, Biertho L, Biron S, Moustarah F, Marceau P, Tchernof A
11:08 am	99 **COMPARATIVE STUDY OF ADIPOSE DERIVED STROMAL CELLS CHARACTERISTICS FROM PEDIATRIC TO SENIOR HEALTHY DONORS** Presenter: Valérie Planat-Benard, PhD Affiliation: UMR5273 CNRS UPS EFS Inserm U1031 Authors: Planat-Benard V, Abbo O, de Barros S, Arnaud E, Casteilla L

11:30 am - 12:30 pm	**PLENARY SESSION 7** **ASCs and Vasculature** Moderators: *Paul DiMuzio, MD & Adam Katz, MD, FACS*
11:30 am	**100** **DIFFERENTIAL PROPERTIES OF STROMAL VASCULAR CELLS DERIVED FROM ARTERIES, VEINS, SMALL VESSELS AND ADIPOSE TISSUE** Presenter: Santsun Yang, MD, Msc Affiliation: Taipei Veterans General Hospital Authors: Yang S, Eto H, Doi K, Kuno S, Kinoshita K, Yoshimura K
11:42 am	**101** **LYMPHATIC POTENTIAL OF ADIPOSE DERIVED STROMAL VASCULAR FRACTION IN WOUND HEALING** Presenter: Catherine J. Baty, DVM, PhD Affiliation: University of Pittsburgh Authors: Baty CJ, Karlsson JM, Acarturk TO, Futrell WJ, Finegold DN
11:54 am	**102** **ADIPOSE STEM CELLS ALLEVIATE BARRIER DYSFUNCTION OF ENDOTHELIAL MONOLAYERS** Presenter: Natalia V. Bogatcheva, PhD Affiliation: IUPUI Authors: Bogatcheva NV, Merfeld-Clauss S, March KL
12:06 pm	**103** **INCREASED ANGIOGENISIS IN URETHRAL TISSUES AFTER TREATMENT WITH MESENCHYMAL STEM CELLS (MSCS) IN A RAT MODEL OF STRESS URINARY INCONTINENCE (SUI)** Presenter: Adonis Hijaz Affiliation: Case Western Reserve University & University Hospitals Case Medical Center Authors: Izgi K, Isariyawongse J, Tasdemir S, Tasdemir C, Kavran M, Grimberg KO, Daneshgari F, Caplan AI, Hijaz AK
12:18 pm	**104** **LONG-TERM REMODELING AND STABILIZATION OF ADIPOSE TISSUE AFTER NON-VASCULARIZED GRAFTING** Presenter: Kotaro Yoshimura, MD Affiliation: University of Tokyo School of Medicine Authors: Kuno S, Doi K, Kato H, Mineda K, Kinoshita K, Yang S, Yoshimura K
12:30 pm	**Concluding Remarks and Farewell** *Julie Fradette, PhD*

PAPER PRESENTATIONS

in numerical order

1

EFFICACY AND CHRONIC SAFETY OF SVF ISOLATED WITH THE TISSUE GENESIS CELL ISOLATION SYSTEM IN A MOUSE HINDLIMB ISCHEMIA MODEL

Presenter: Brian Johnstone, PhD
Authors: Johnstone B, Cook TG, Feng D, Lupov IP, Merfeld-Clauss S, Randolph ML, Van Natta B, Lye KD, Williams SK, Kosnik P, March KL
Indiana University School of Medicine

Introduction: Autologous adipose stem cells (ASC) are currently under evaluation for treatment of a variety of clinical indications. The Tissue Genesis, Inc. (TGI) Cell Isolation System (CIS) is an automated, point-of-care device for isolating minimally manipulated ASC contained in the stromal vascular fraction (SVF). To support FDA approval for human clinical trials in peripheral arterial disease (PAD) patients, a study was conducted to evaluate efficacy of SVF produced with the CIS device in a mouse model of PAD as well as to establish safety in the context of chronic ischemia.

Methods: Lipoaspirates from 6 unrelated male and female human donors were processed to SVF with the TGI CIS device. Each preparation was evaluated for viable cell numbers, potency, cell identity, purity, and sterility. The concentration of cells was adjusted to deliver one of 3 doses (10^5, 1.4x10^6 or 3x10^6 cells) in a fixed volume. Immunotolerant NSG mice of both sexes were randomly assigned to groups (N = 12 mice/sex) before unilateral hindlimb ischemia surgery. On each of the 6 days that adipose was processed, the ischemic limbs of 2 mice/sex from each group were injected intramuscularly (IM) with cell product or vehicle alone. Serial laser Doppler perfusion imaging (LDPI) was performed to monitor blood reperfusion during the 13 week study. An additional group of mice (N = 7/sex) received IM injections of tumorigenic HT1080 cells as a control for tumorigenicity. Safety evaluations were performed over entire study period.

Results and Discussion: Each dose was well tolerated by the study animals. Although delayed morbidity and two early deaths occurred, none of these events was associated with SVF administration. Blood flow restoration was significantly improved ($p<0.01$) compared to controls in both males and females receiving the middle and high doses, but not the low dose. Body weights, blood chemistry, and hematology values varied slightly between controls and treatment groups. No significant pathological differences were observed in treated or distal tissues.

Conclusion: The highest dose of TGI CIS product, which is 2-fold more concentrated than is obtainable with the present device configuration, proved safe and (along with the middle dose) was effective in the mouse PAD model.

2

MULTIPLE INTRAVENOUS ADMINISTRATIONS OF HUMAN ADIPOSE MESENCHYMAL STEM CELLS ARE SAFE AND DO NOT INDUCE TUMOR DEVELOPMENT

Presenter: JeongChan Ra, PhD
Authors: Ra JC, Chung MK, Shin IS, Ko MS, Kang SK, Kim YJ, Kwon E, Kang BC
RNLBio

The therapeutic use of human adipose mesenchymal stem cells (ASCs) has drawn keen attention. Intravenous applications of ASCs to various diseases have increased due to their homing property, however, there are still some worries remained in multiple, long-term use of ASCs. So we investigated the safety and the possibility of tumor development by multiple systemic injections of ASCs.

In the preclinical toxicity study, SCID mice were given three different doses of hASCs or saline intravenously once a week for 13 consecutive weeks. There were no adverse effects at low-dose (2.5x10^7/kg) and middle-dose (5x10^7/kg) which are 5-times and 10-times higher level than tentative clinical dosage (5x10^6/Kg), respectively. Only in high-dose (1x10^8/kg), some mice began to die after 8th consecutive injections. Pulmonary embolism was thought to be the cause of death at necropsies. These results indicated that no-observed-effect level for the developmental toxicity of hASCs for multiple injections are 5x10^7/kg per injection.

Next, we compared level of tumor markers between the current and retrospective data from 93 recipients who had received cumulative dose of more than one billion autologous hASCs intravenously for various reasons. The average number of cumulative cells was 26.0±1.63x10^8 (maximum 52.5) with average 9.44 times in male and 23.4 ± 1.31x10^8 (maximum 47.5) with average 9.19 times in female. Average duration from the first injection is 16.69±0.98 months (maximum 34) in male and 18.40±1.07 (maximum 38) months in female. In blood and serological test results, the differences were not detected between pre- and post-injection of stem cells. All serum tumor markers (AFP, CEA, CA19-9, CA125, CA15-3, or PSA) slightly declined but did not reach statistical significance. PET scan for 40 participants who agreed with the test showed no aberrant results.

In conclusion, multiple intravenous administrations of hASC is safe unless be given more 10-times over the tentative clinical dosage of 5x10^6/kg and long-term follow-up in humans showed no evidence of possibility to develop tumor by stem cells. These findings provide valuable milestone to investigators who trying to design clinical applications with mesenchymal stem cells for multiple systemic transplantation.

3
MULTIPOTENT ADIPOSE STROMAL CELLS IN DIABETIC RETINOPATHY

Presenter: Rajashekhar Gangaraju, PhD
Authors: Gangaraju R, Abburi C, Kern TS, March KL
Indiana University School of Medicine

Diabetic retinopathy (DR) is the leading cause of blindness in working-age adults. Early stage DR involves inflammation, vascular leakage, apoptosis of vascular cells and loss of pericytes. In this study, we hypothesized that adipose stromal cells (ASC) can rescue early stage DR features. Furthermore, we hypothesized that ASC can withstand hyperglycemic stress and can prove beneficial for transplantation studies. Streptozotocin (STZ) induced diabetic athymic nude rats were intravitreally injected with GFP labeled ASC into the right eye and the contralateral eye served as control with an equal volume of saline. After 7 days, rats were euthanized. Two months post STZ-induced diabetes, athymic nude rats demonstrated increased vascular leakage evidence by FITC-albumin extravasation, TUNEL positive cells in the ganglion cell layer as well as around vessels, increased acellular capillaries and pericyte ghosts as assessed by trypsin digest, and upregulated inflammatory genes such as ICAM-1, Edn2, Ccl2, Timp1, TGFbeta, TNFalpha and Stat3 (>2 fold) by realtime RT-PCR compared to non-diabetic rats. Diabetic rats that received ASC injection demonstrated a significant decrease in all the parameters above. In vitro ASC displayed sustained proliferation (MTT assay), decreased apoptosis (caspase-3 assay) and increased endothelial survival and vascular network formation subjected to hyperglycemic stress. This is the first demonstration of the use of ASC in the treatment of DR. We have shown that a novel model of the athymic nude rat develops early stage DR and that a single intravitreal injection of ASC limits the features of DR. Further to this, preliminary studies in an immune competent diabetic rat, ASC are well tolerated and assumed perivascular pericyte position after 21 days. Future studies in diabetes models will address the effects of intravitreal transplantation of ASC on the retinal integrity and function. By evaluating this approach in rodent models, we will be in a better position to determine whether such an approach should be tested in humans. The diabetic rat studies will provide valuable insights to guide the design of our future clinical studies in human patients.

4
A MULTICENTER TRIAL TO ASSESS THE SAFETY AND EFFICACY OF ADIPOSE CELLS IN CONGESTIVE HEART FAILURE AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Presenter: Kristin Comella, MS
Authors: Comella K, McQuillan S, Lopez J, Perez J, Parcero J
Bioheart

The Regenerative Medicine Institute (RMI) of Tijuana, Mexico has partnered with both the Ageless Regenerative Institute (ARI) and Bioheart to offer a variety of clinical protocols to patients suffering from degenerative diseases.

In one protocol, developed and executed by RMI and ARI, patients diagnosed with chronic obstructive pulmonary disease (COPD) are being treated with adipose or fat derived stem cells. To date, over 24 patients with COPD have received an intravenous delivery of the stem cells.

The trial has established a solid safety profile as well as some early evidence of efficacy. More than 83% of the patients have demonstrated a statistically significant improvement in their quality of life. Some of this data includes follow up greater than 6 months. The average improvement in the St. George's Respiratory Questionnaire was 23 points. This represents a potential new breakthrough for COPD patients who often suffer from a rapid decline in health after diagnosis. Existing therapies are designed to target acute symptoms and do not reverse the effects of the disease or improve the underlying issues.

In addition to improving the patient's quality of life, the treatments showed a marked improvement in their exercise capacity. Three months after receiving the therapy, the patients were able to walk on average an additional seventy meters in their six minute walk test.

RMI and ARI have also partnered with Bioheart to bring regenerative therapies to cardiac patients. In a protocol focused on congestive heart failure, more than 15 patients have been successfully treated using adipose derived stem cells. The cells are delivered directly into the heart muscle using a catheter. These patients have demonstrated on average, an absolute improvement of 13 percentage points in ejection fraction and an increase of 100 meters in their 6 minute walk distance at their 6 month follow up. This means that the patient's hearts appear to be functioning better on echocardiogram. In addition, the patients have improved their exercise capacity which allows for a more active and normal lifestyle.

5

SAFETY, EFFICACY AND MODE OF DELIVERY OF AUTOLOGOUS ADIPOSE-STROMAL VASCULAR FRACTIONS CELLS IN OSTEOARTHRITIS PATIENTS

Presenter: Ralph T. Bright, MD
Authors: Bright RT, Bright P, Ilhan E, Thomas W
Macquarie Stem Cells

Autologous adipose-derived stromal vascular fraction (SVF) cells may be an effective and safe tool for relieving osteoarthritic pain and improving joint mobility. We evaluated the safety and efficacy of different modes of delivery of SVF cells in patients with hip and knee osteoarthritis (OA).

From December 2011 until December 2012 twenty nine consecutive patients with OA of the knees and hips completed either the Hip Disability and Osteoarthritis Outcome (HOOS) measure (hips) or the Western Ontario and McMaster Universities Arthritis Index (WOMAC) measure (knees). Scores were standardised into percentage scores in order to combine hip and knee patients into a single analysis. Patients were randomised into three groups, intravenous (IV), intra-articular (IA), and intravenous plus intra-articular (IV + IA), with baseline pre-operative scores of 56.33 (n = 9), 56.48 (n = 9), and 61.21 (n = 11), respectively. Patients completed a WOMAC/HOOS monthly for 6 months post-transplantation.

There were no differences in baseline WOMAC/HOOS scores between groups. All groups scored significantly better at 6 months (IV = 30.33; IA = 26.19; IV + IA = 5.09) compared to baseline ($p < .05$). At 6 months, the IV + IA group scored significantly better than the other groups ($p < .05$), whereas there were no differences between the IV and IA group ($p > .05$).

There was no significant correlation between improvement and age after SVF treatment ($p > .05$).

Sixty nine patients treated since April 2009 and 2012 completed a safety survey. No major adverse events were observed. Minor side effects were observed such as bruising and abdominal pain which were due to the liposuction immediately after treatment.

Transplantation of autologous adipose-derived SVF cells in patients with OA of the knees and hips is a clinically efficacious and safe procedure. Intravenous plus intra-articular injections increase the effectiveness of SVF cells.

6

A PHASE I TRIAL (ACELLDREAM), USE OF AUTOLOGOUS ADIPOSE DERIVED STROMA/STEM CELLS TO TREAT CRITICAL LIMB ISCHEMIA (CLI)

Presenter: Louis Casteilla, PhD
Authors: Bura-rivière A, Léobon B, Bourin, Gross F, Grolleau Saleb B, Peyrafitte J, Fleury S, Planat-Benard V, Casteilla L
STROMALab

This phase I trial was designed to assess the feasibility and safety of autologous adipose derived stroma/stem cells (ASC) transplantation in patients with non revascularizable critical limb ischemia.

Preclinical data in animal models reported by independent groups demonstrated that ASC transplantation strongly improves neo-angiogenesis and subcutaneous blood flow. However, the same cells promote wound healing.

On twenty enrolled patients, seven patients were injected. A small amount of adipose tissue (30/60g) were harvested and after selection of cells by plastic adhesion, they were grown for 2 weeks with one passage. Their phenotype was checked by facs and Pcr analysis. One hundred thousands of these cells were implanted uneventfully No significant change was observed in TcPO2 but evaluation of ulcer evolution and wound healing strongly suggested a significant benefit. These preliminary data suggest the feasibility and safety of autologous ASC transplantation in critical ischemic limb. Furthermore, improvement of wound healing suggests a putative functional efficacy on second aim that requires confirmation by randomized studies but with a modified design.

7

ADIPOSE-DERIVED STEM CELLS COMBINED WITH DEMINERALIZED BONE MATRIX IN CRITICAL-SIZED SEGMENTAL BONE DEFECTS

Presenter: Nicole P. Ehrhart, DVM, MS
Authors: Ehrhart NP, Chubb LS, Webb TL
Colorado State University

Objectives/Hypotheses: The management of critical-sized segmental bone defects caused by trauma or tumor resection continues to be a major clinical challenge. The objectives of this study were: 1) to quantify and compare new bone formation in critical-sized athymic rat femur bone defects following implantation of adipose-derived stem cells (ADSCS) on demineralized bone matrix (DBM) and 3 other treatments and 2) to characterize the localization of stromal stem cells following implantation within the defect. We hypothesized: 1) ADSCs on DBM will result in greater bone formation than ADSCs alone or DBM alone and 2) ADSCs on DBM will remain localized to the femur defect and would not be detected in distant organs.

Methods: Twelve athymic rats had a 5mm defect created in the right femur stabilized with a bone plate. Four treatment groups (n=3) were: human ADSCs seeded on DBM, ADSCs alone, DBM and no treatment. Femurs were radiographed at 0, 21 and 42 days. Rats were sacrificed at 42 days and femurs were harvested for μCT and histology. Eight additional rats had identical femur defects created and GFP-expressing ADSCs seeded on DBM were placed in the defect. In vivo imaging was performed at 0,3 7,14,21 days and 2 rats were sacrificed at each imaging time point. Lungs, spleen, liver, kidneys and operated femurs were harvested and examined using immunohistochemistry for detection of GFP.

Results: Quantitative radiographic and μCT analysis showed that femur defects treated with human ADSCs on DBM had the greatest amount of new bone formation at 42 days post-implantation compared with the other treatment groups. Femur defects treated with ADSCs alone showed little to no new bone formation. GFP-expressing ADSCs persisted within the femur defect for up to 21 days and were not detected in distant organs at any time point.

Conclusions: ADSCs seeded on DBM regenerated bone in critical-sized femur defects. ADSC's alone were ineffective at regenerating bone in this model. ADSCs persisted within the defect for up to 21 days and did not appear to migrate to distant organs following local implantation. Confirmatory studies with a larger sample size and longer time points are in progress.

8

EFFECTS OF BIOMATERIALS AND GROWTH FACTORS ON THE OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS - IN VITRO AND IN VIVO STUDIES

Presenter: Bettina I. Mannerstrom, PhD
Authors: Mannerstrom BI, Waselau M, Patrikainen M, von Rechenberg B, Miettinen S
BioMediTech

Healing of large bone defects after reconstructive surgery remains challenging and the limited availability of autologous bone grafts has resulted in the exploration of new cell based therapies. Human adipose stem cells (hASCs) have therefore emerged as an attractive cell source due to the ease harvest and processing while offering osteogenic differentiation capacity. hASCs can be osteogenically induced using supplements such as bone morphogenic protein 2 and 7 (BMP-2/-7) to promote differentiation and new bone formation in vivo, however this potential requires further investigation. Furthermore, biomaterials such as bioactive glass (BAG) and beta tricalcium phosphate (beta-TCP) may serve as initial scaffolds owing to their osteoinductive activity and proven biocompatibility. De facto, the suitability of such composite grafts have been widely reported in vitro, however, in vivo studies confirming the safety and efficacy are needed.

In the in vitro study, the osteogenic response of hASCs to BMP-2/-7 and BAG or beta-TCP was investigated in vitro. Briefly, hASCs were seeded onto BAG and beta-TCP granules and maintained in basic medium (BM), osteogenic medium (OM) or BMP-2, BMP-7 or BMP-2/-7 supplemented OM. The cell attachment and viability, proliferation, osteogenic differentiation and angiogenic potential were evaluated. The data revealed good cell attachment and viability for both biomaterials; however, supplementation with BMP-2 and/or -7 had an overall negative impact on the cell proliferation, osteogenic differentiation and angiogenic potential of hASCs independent of biomaterial evaluated. The osteogenic effects were subsequently evaluated further in vivo. Briefly, the cellular response, osteogenic potential and tissue reactions of hASC, hASC + BMP-2, BMP-2 and plain BAG/beta-TCP granules was evaluated and compared when subcutaneously implanted into immunocompromised rats.

Overall, the results indicated that BAG and beta-TCP granules can be utilized safely and but require hASC and/or BMP-2 supplementation for efficacy to induce osteoblastic activity and calcification. Further studies are required and are underway; nevertheless, this study paves way for clinical studies using ASCs for bone reconstructive surgery.

9

BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE CARRIERS FOR HUMAN MAXILLARY SINUS FLOOR ELEVATION: UPDATE ON A PHASE I CLINICAL TRIAL

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

For patients with maxillary atrophy, transplantation of autologous bone into the maxillary sinus is the 'golden standard' to achieve sufficient alveolar bone volume ('sinus floor elevation') for dental implants. However, autografting has disadvantages, such as limited graft availability, patient discomfort, and donor-site morbidity. Synthetic bone substitutes are used as an alternative strategy, but only allow osteoconduction, since viable osteogenic cells are lacking.

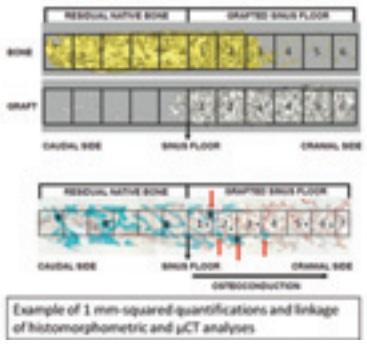
Last year, we presented the outline and some initial findings of our phase I trial, in which osteoinductive implants consisting of a calcium phosphate (CaP) carrier seeded with freshly isolated adipose tissue-derived stem cells are generated during a one-step surgical procedure. This novel concept is performed within hours in the operating theatre using a Celution® 800/CRS device, thereby avoiding costly GMP stem cell expansions and a second intervention. Where possible, we use a "split mouth design" with CaP + stem cells on the test side and CaP only on the contralateral control side (allowing efficacy evaluation as well).

Occurrence of any adverse events related to the product and/or procedure are monitored, and from preoperative phase until one year after implant placement clinical, X-ray, and Cone-beam CT data are collected. After six months biopsies are obtained during the preparation for dental implant placement, and evaluated for bone formation by histomorphometry and µCT.

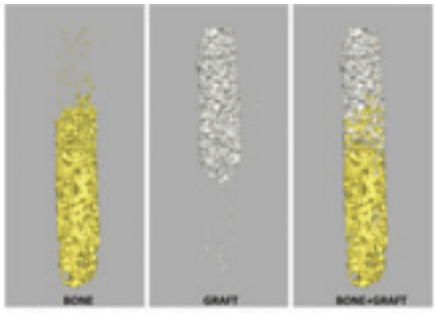
Currently (May 1), we have included 10 patients. No adverse effects have been observed and/or reported by the patients sofar. Biopsies have been collected from 5 patients, which are now being analyzed with µCT and histomorphometry. Preliminary outcomes show some unexpected but promising efficacy data. We will be able to report on the full outcomes of at least these five patients in the October meeting.

9

BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE CARRIERS FOR HUMAN MAXILLARY SINUS FLOOR ELEVATION: UPDATE ON A PHASE I CLINICAL TRIAL



Example of 1 mm-squared quantifications and linkage of histomorphometric and µCT analyses



Example of µCT analysis. By using appropriate threshold settings, bone and graft materials can be distinguished from each other

10

TREATMENT OF OSTEOARTHIRTIS OF THE KNEE WITH INTRAARTICULAR INJECTION OF AUTOLOGOUS ADIPOSE TISSUE DERIVED STEM CELLS: PHASE I & II CLINICAL TRIAL

Presenter: Kang Yoon, MD, PhD
Authors: Yoon K, Jo C
SMGSNU Boramae Medical Center

Introduction: The purpose of the present study was to evaluate the clinical and radiological effects of intra-articular injection of autologous adipose tissue derived mesenchymal stem cells for treatment of osteoarthritisof the knee.

Methods: Eighteen patients suffering from osteoarthritis of the knee was involved in the study. There were three men and fifteen women. Average age was 62.1 years old (range, 52 - 72). Autologous adipose tissue derived mesenchymal stem cells were isolated from the subcutaneous fat tissue of patients by liposuction technique. After 3 weeks of culture, 1x107 cells (n=3), 5x107 cells (n=3), and 1x108 cells (n=12) were injected in the knee joint under arthroscopic monitoring. Patients were allowed immediate range of motion exercise, quadriceps setting exercise and non-weight bearing crutch walking till 2 months postoperatively. Clinical and radiological variables were collected, measured and evaluated preoperatively and 6 months postoperatively. Clinical variables were range of motion, quadriceps circumference and power, visual analogue pain scale, and Korean Western Ontario and MacMaster University (K-WOMAC) score. Radiological variables were cartilage defect size, thickness and quality of the medial femoral condyle in magnetic resonance imaging. Biopsy of regenerated cartilage was obtained at the 2nd look arthrocopy.

Results: There were no postoperative complications such as infection, allergic reaction, injection site reaction and etc. Range of motion, quadriceps circumference, quadriceps isokinetic power, visual analogue pain scale, and K-WOMAC improved significantly after injection. MRI and histology analysis demonstrated regenerated articular cartilage with shiny, white, smooth, and firm appearance. However, the quality of regenerated cartilage was not exactly identical with the hyaline cartilage in the histologic examination.

Conclusions: This is the first Phase I/II clinical trial of treatment of osteoarthritis with mesenchymal stem cells. Autologous adipose derived mesenchymal stem cells regenerated cartilage with smooth and firm surface. Preliminary clinical results of this study are encouraging. However, further researches for identification of clinical and radiological results of more patients would be necessary.

11

TREATMENT OF LUMBAR DEGENERATIVE DISC DISEASE WITH ADIPOSE DERIVED STROMAL VASCULAR FRACTION, POINT OF CARE

Presenter: Carlos J. Garcia, MD
Author: Garcia CJ
Premier Pain Care

Introduction: Lumbar Degenerative Disc Disease (LDDD) is a common and potentially incapacitating condition. LDDD, in the advanced stage, is usually non responsive to conservative therapy and frequently requires aggressive and complex spine surgery. Hence, we present a case in which intra-discal SVF was used to treat a patient with advanced LDDD.

Methods: A 45 y/o female patient with intractable low back and lower extremity radicular symptoms due to advance LDDD at L-5, was evaluated with lumbar MRI and lumbar discography. Disc morphology was consistent with a herniated disc and central absence of nuclear material. 50cc of lipoaspirate was obtained and processed into SVF using standard procedures via lecithin based emulsification and centrifugation, yielding 93% viability. The SVF (approximately 10 x 10 x 6 MSC) was combined with 4:1 with platelet rich plasma/1.5gm of micronized collagen. This was injected through a 22g needle in the L5 disc nucleus and annulus.

Results: The patient was followed at 1, 3, 6 months and evaluated for potential complications, medication use, pain relief, presence adjacent level degeneration and anatomical changes in the treated lumbar disc. No significant adverse reactions were noted in the immediate post operative period. The pain relief was 30% at month 3 and 90% at month 6. Repeat lumbar MRI at month 6 was positive for increase T2 signal in the L5 nucleus consistent with production of ECM and proliferation of chondrocytes. Furthermore, there was preservation of disc height at the treated level and the adjacent discs level.

Conclusions: By utilizing a combination of point of care, growth factors (PRP), collagen scaffolding (micronized) and Adipose derived SVF, we were able to regenerate the intervertebral nucleus and provide curative pain relief. To our knowledge, this is the first case in the US, where non-expanded cell therapy was used to treat advance LDDD successfully. Further biologic scaffolding research is needed in order to optimize cell therapy.

12

CELL-BASED FLUORESCENCE IMAGING OF CRYOPRESERVED ANIMAL: LAVACELL® FLUOROPHORE DYE FOR EVALUATING THE FATE OF HUMAN MESENCHYMAL STEM CELLS (HMSCS) IN-SITU

Presenter: Zhina Sadeghi, MD
Authors: Molter J, Lennon D, Kavran M, Grimberg KO, Daneshgari F, Caplan AI, Flask CA, Hijaz AK
Case Western Reserve University and University Hospitals Case Medical Center

We explored the use of LavaCell®, a naturally occurring compound that fluorescently stains cell membranes or lipophilic organelles but not nucleic acids, as a novel fluorophore dye in cell-based fluorescence imaging of intact hMSCs in situ via cryofluorescence imaging, and the impact on MSC differentiation. Lavacell-labeled hMSCs (1.5 million cells/0.1ml PBS) were injected periurethrally through a vaginal approach in one control Sprague-Dawley rat and another rat that underwent 4hr of vaginal distention (VD). Animals were cryopreserved 24hr after injections and then scanned with a CryoViz (Bioinvision Inc) cryo-fluorescence imaging system. A high intensity orange signal was identified in the connective tissue near the urethra. There was a weak and dispersed fluorescent signal detected in the rat with no VD. The weak signals corresponded to the needle tracks from hMSC injections. To investigate the impact on hMSC differentiation, LavaCell®-labeled hMSCs were separately loaded into fibronectin-coated ceramic cubes and suspended in a chondrogenic-permissive pellet medium to induce differentiation into bone and cartilage, respectively. The differentiation potential was compared to non-labeled hMSCs. Calcium phosphate porous ceramic 3mm cubes were implanted in nude mice, then harvested at 6wk and scored for bone-fill. The differentiation potential was compared to non-labeled hMSCs in cubes 6wk after loading and in pellets at 3wk. A high intensity orange signal in the connective tissue near the urethra was identified by cryofluoroescent imaging, indicative of hMSC homing following VD and local injection (Figure). The average cube score for LavaCell®-stained cell-containing cubes was higher than for the control cells, although not significant. Chondrogenic differentiation and expression was lower in pellet cultures than is usually seen using unlabeled hMSC. Pellets formed from LavaCell®-stained cells appeared to include more chondrocytes and cartilaginous matrix than did the control pellets. LavaCell®-fluorophore dye keeps the multi potential differentiation characteristics of hMSCs almost intact and can be utilized in cell-based fluorescence imaging to evaluate the fate of stem cells in-situ.

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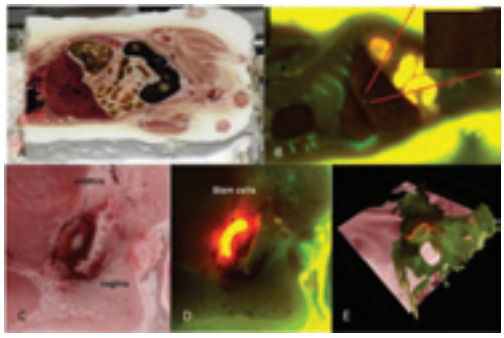
CELL-BASED FLUORESCENCE IMAGING OF CRYOPRESERVED ANIMAL: LAVACELL® FLUOROPHORE DYE FOR EVALUATING THE FATE OF HUMAN MESENCHYMAL STEM CELLS (HMSCS) IN-SITU



13
CLINICAL EXPERIENCE WITH AUTOLOGOUS FAT GRAFTING IN THE PEDIATRIC PATIENT: AN UPDATE

Presenter: Kevin S. Hopkins, MD,FACS
Authors: Hopkins KS, Taylor BT
Driscoll Childrens Hospital

Introduction: Autologous fat grafting (AFT) is very successful in soft tissue augmentation and contouring in the adult population. It is also a viable tool for correcting soft tissue congenital and traumatic defects in the pediatric patient. This paper is a one year follow-up and update on AFT for restoring soft tissue in the pediatric patient for congenital and traumatic defects.

Methods: 34 patients (21 female, 13 male) ages 2-18 years (average age 10.6) presented with 23 congenital defects (20 cleft lip/palate, 1 hemifacial microsomia, 1 hemangioma, 1 frontonasal dysplasia) and 11 traumatic defects (6 MVA, 4 burns, 1 dog bite) with follow up time ranging from 7 weeks to 26 months. The cleft lip and palate defects included palatal fistula with severe scarring and fibrosis (7); velopharyngeal insufficiency (5); a asymmetric soft tissue volume (8) and scarred, contracted nasal tip (2). Three congenital defects were hemifacial microsomia (1), malar deformation from an involuted hemangioma (1), and frontonasal dysplasia (1). The traumatic deformities involved the face (4), upper extremity (5), trunk (1), and lower extremity (3). Three patients underwent treatment of multiple deformities (3). Seven patients underwent serial AFT. (7). Fat was harvested using the Coleman technique and a 12-hole Khouri cannula, The volume of fat transferred ranged from 0.5 ml to 10 ml per site. Modifications were made to standard hypodermic and spinal needles to facilitate fat transfer.

Results and Conclusions: Autologous fat may be safely harvested and effectively grafted in pediatric patients. We have significantly improved hypernasality in our patients with VPI following autologous fat grafting. Direct observation and serial photographs demonstrate increased soft tissue volume and enhanced tissue quality of traumatic scars. It is speculated that there is a higher density of stem cells in this population that may significantly augment healing. This may explain the benefit we have seen. Care must be taken to safely harvest fat in these children so as to not create a contour deformity or perforation. More studies are warranted.

14
REGENERATION OF HEAD AND NECK IRRADIATED TISSUE WITH AUTOLOGOUS FAT GRAFT: A RETROSPECTIVE STUDY

Presenter: Aurora Almadori, MD
Authors: Almadori A, Salgarello M, Fetoni A, Paludetti G
UCSC Universita Cattolica del Sacro Cuore

Introduction: Therapeutic irradiation on healthy tissues induces injuries that may persist for decades after radiotherapy as results of a self-maintained process. A possible etiology is the radiation-induced endothelium dysfunction and pathological wound healing, inducing hypoxia, acute inflammatory response, granulation tissue formation, uncontrolled extracellular matrix (ECM) deposition and fibrosis. Autologous fat graft might represent a valid treatment as source of ASCs stimulating restoration of vascularity, immunomodulation and remodeling of ECM components. The aim of this study was to evaluate clinical efficacy and safety of fat injection and to demonstrate the presence of ASCs in the lipoaspirate.

Materials and Methods: From December 2009 to December 2011, fifteen patients presenting head and neck radiation sequelae were treated with fat graft. After atraumatic liposuction and gentle centrifugation, fat was injected in small quantities. Severe fibrotic and scar tissues were treated with nokor needle to allow skin release, and the new tunnels were filled with fat. Aesthetic and functional score (0 to 4) and LENT-SOMA scale, that evaluates late effects of radiotherapy, assessed the outcomes. Moreover, the lipoaspirate was analyzed with cell culture and flow cytometry.

Results: Improvement in overall aesthetic, functional and LENT-SOMA scores were observed. Outcomes were graded excellent to very good in all patients who undergone two or more sessions of fat injection, and good to poor when only one fat graft was performed due to poor patient compliance or late inclusion in the study. Patient satisfaction was reported high to very high. The average follow-up was 17 months (range 29-6 months). No short-term complications either in the donor or in the recipient site occurred. ASCs were identified and flow cytometry proved their positivity for typical endothelian and mesenchymal-like immunophenotypes.

Conclusions: Autologous fat graft is a simple and effective surgical treatment for late radiation injuries. The cellular mechanisms responsible for the observed therapeutic effects still need to be clarified, hence larger studies and longer follow-up are needed.

15

CHANGES IN TISSUE PERFUSION DURING APPLICATION OF EXTERNAL VOLUME EXPANSION (EVE) SYSTEMS FOR FAT GRAFT SITE PREPARATION

Presenter: Luca Lancerotto, MD
Authors: Lancerotto L, Chin MS, Freniere B, Lujan-Hernandez JR, Del Vecchio DA, Lalikos JF, Bassetto F, Orgill DP
Brigham and Women's Hospital

Introduction: External volume expansion (EVE) is clinically used to prepare fat grafting sites in autologous breast augmentation. Recently, we developed a murine model of EVE and demonstrated that 28 days continuous EVE increases subcutaneous tissue thickness and adipocyte number, induces cell proliferation, promotes vascular remodeling, and augments vessel density. Hypothesizing that these effects may be due to edema, ischemia, and inflammation induced by micro- and macro-mechanical forces, we studied EVE effects on tissue perfusion using hyperspectral imaging.

Methods: A miniaturized EVE device was applied to the dorsum of mice (n=18) for 6 hours at -25mmHg. Local oxyhemoglobin (OxyHb) and deoxyhemoglobin (DeoxyHb) were assessed by hyperspectral imaging pre-treatment, immediately post-treatment, and at multiple time points for to 2 days.

Results: EVE induced macroscopic tissue swelling. Upon release, OxyHb increased over baseline peaking at 130($p<0.01$), then decreased to a steady-state higher than baseline ($p<0.01$) by 6 minutes. A second, higher ($p<0.05$) peak was observed at 4 hours, and normalized by day 1. DeoxyHb decreased in the first minute post-treatment, but stabilized at a level 40% higher than baseline in the following hours ($p<0.01$). DeoxyHb remained elevated at day 1 and returned to baseline by day 2. Total hemoglobin peaked at 1 post treatment and remained elevated for upto 1 day ($p<0.01$).

Discussion: EVE, through mechanical stretch and resulting edema, establishes persistent tissue ischemia that is known to induce pro-proliferative and pro-angiogenetic factors. Upon release of suction, an elevated blood influx may sustain increased metabolic requirements, while the late peak in OxyHb suggests an inflammatory response, potentially stimulating proliferation and angiogenesis. These changes resolve within two days, suggesting that extending cyclical application may promote even further vascular development.

16

IRRADDIATED BREAST RECONSTRUCTION: UPPER EXTREMITY FUNCTIONAL IMPROVEMENT AFTER FAT INJECTION

Presenter: Nho V. Tran, MD
Authors: Tran NV, Convery PA
Mayo Clinic Rochester

Purpose: Irradiation poses a significant challenge in breast reconstruction, since progressive fibrosis impairs and degenerates result from implant reconstruction. It also worsens lymphedema and shoulder range of motion. This study evaluates the shoulder range of motion (ROM) in patients with irradiated breast reconstruction with implant and microfat transfer.

Materials and Method: Retrospective study at Mayo Clinic Rochester identified 20 patients with breast cancer underwent implant reconstruction and subsequent microfat transfer. Fat was harvested from bilateral flanks by conventional liposuction with maximal vacuum of 20 mm Hg with the Lipivage filter. Twenty to 40 cc of fat was injected with blunt canula into the fibrotic pectoralis major muscle. Pre and postoperative shoulder ROM was measured.

Results: All patients showed clinically improvement in shoulder ROM after a single fat transfer. ROM improved from 50 -100%. No complication occurred.

Conclusions: Restrictive shoulder ROM from irradiation benefits from microfat injection in patients with implant breast reconstruction.

17
TEMPERAMENTAL AND PERSONALITY TRAITS IN OVERWEIGHT/OBESE PATIENTS WHO SEEK PLASTIC SURGERY TREATMENT

Presenter: Mariafrancesca Azzi, MD
Authors: Azzi M, Vindigni V, Lancerotto L, Marini M, Bassetto F, Pavan C
University of Padova

DID NOT PRESENT AT THE MEETING

18
A FAT GRAFT OF CELL ENRICHED MATRIX DERIVED FROM LIPOASPIRATE SHOWS INCREASED VASCULARIZATION AND PROLIFERATION

Presenter: Carl Friddle, PhD
Authors: Friddle C, Husfeld R, Sanchez A, Martinez R, Coleman M
InGeneron

We have developed a custom processing system for the production of Cell Enriched Matrix (CEM) from lipoaspirate. Autologous lipoaspirate is an attractive option for graft material when performing cosmetic or reconstructive procedures. However, traditional fat grafts experience significant necrosis and resorption, leading some practitioners to overgraft the patient in an effort to compensate for this phenomenon. Clinical outcomes may be improved through centrifugation to remove lipids and aqueous fluid from the graft material, which increases the number of vital cells per gram tissue and improves short term retention of mass (Coleman 1995, Kurita et al 2008). However, it does not change the histological character of the graft. In contrast, fat grafts supplemented with adipose-derived SVF demonstrate better long term retention, and improved vascularization (Zhu et al 2010).

Eto et al (2012) demonstrated the importance of cell regeneration for survival of transplanted fat grafts and replacement of dying adipocytes. To enhance graft survival we have developed a mechanical method for producing a fat graft enriched in regenerative cells. Extrusion of lipoaspirate multiple times in combination with centrifugation at 1200 x g disrupts mature adipocytes and enriches for pre-adipocytes and other regenerative cells. The number of viable cells per gram of graft is increased 3-fold and the number of plastic-adherent cells per gram is increased 4 fold.

One month after engrafting processed human lipoaspirate into nude mice, the periphery of the traditional graft showed positive staining for Ki-67 and human collagen IA, but it had minimal vascularization. The interior of the traditional graft showed numerous lipid lakes and was devoid of staining for nuclei, Ki-67 and human collagen IA. In stark contrast, both the periphery and the interior of the CEM graft showed Ki-67 positive cells embedded in human collagen and numerous blood vessels with distinct epithelial cells.

Cell Enriched Matrix provides a simple, mechanical means of increasing regenerative cell content of lipoaspirate derived grafts resulting in greater and more uniform graft viability than traditional methods.

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INTERCOMMUNICATION WITH RNA TRANSFER FROM DAMAGED CELLS TO TARGED ADIPOCYTES IN SKIN TRAUMA, THE SURVIVAL CAPSULES

Presenter: Marco Aurelio Pellon, MD
Author: Pellon MA
Clinica Sao Vicente

Introduction: The author describes the changes in protein production of the intradermic and subcutaneous adipocytes induced by the arrival of mRNA and miRNA from the skin, following the cytokine storm in patients after burns. Nanotubes, exosomes, and nucleic acid-binding peptides are the recently described mechanisms of intercellular communication (Belting M., Wittrup A. - 2008). It has been demonstrated that the content and function of exosomes depend on the originating cell and the conditions under which they are produced (Losser C., Eldh M., Latval J. - 2012). After a burn, damaged cells and tissues that are dying or suffering, release a great number of molecules. Many of these molecules are released into exosomas carrying its RNA which will be transferred to another neighbor cell and subsequently affecting the recipient cell's protein production.

Method: The author conducted a clinical trial in burnt patients, chronologically registering the vascular and morphological changes that started in intradermic and subcutaneous adipocytes stimulated by the damaged cells and tissues from the skin, until the wound healing.

Results: The author observed the significant changes in the tissues' morphology below the burn wound due to genetic communication between the damaged cells and the intradermic, perivascular and subcutaneous adipocytes.

Conclusion: When a damage of the skins structures occurs, that information gets to the dermal and subcutaneous adipocytes via paracrine signaling, extracellular components, and cell-cell interactions for RNA transfer (probably by exosomes), carrying gene-regulatory function from the damaged to the target cells resulting in a change of adipokines production and expression. This process works like a survival shuttled capsule from the dying cells, bringing to the recipient cells the information about what type of cells were damaged by the trauma and how to recover them.

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BIOLOGICAL EFFECTS OF NEUROPEPTIDE Y ON PRIMARY CULTURED HUMAN ADIPOSE-DERIVED STEM CELLS: IN VITRO AND IN VIVO STUDIES

Presenter: Brian J. Philips, PhD
Authors: Philips BJ, Kling RE, Valentin JE, Kelmendi-Doko A, Grahovac TL, Ravuri SK, Marra KG, Fernstrom JD, Rubin JP
University of Pittsburgh

Introduction: The use of pharmacologically-active agents that promote adipose-derived stem cell (ASC) growth and differentiation, as well as adequate vascularization of the transplant, offers tremendous application for plastic surgery. Neuropeptide Y (NPY) is a promising candidate as a promoter of pre-adipocyte proliferation and differentiation though its effects have not been studied in primary cultured human ASCs. Consequently, we examined: 1). proliferative and adipogenic effects of NPY using primary cultures of human ASCs, and 2). dose-dependent effects of NPY on fat xenograft survival using an athymic nude mouse model.

Methods: ASCs were isolated from abdominal fat obtained from 20 human, non-diabetic female patients (Age: 30-68, BMI: 22-50) and cultured. The cultures were treated under low-serum conditions with 10-14M-10-6M NPY and assessed for (1) proliferation (3 day) and (2) differentiation (12 day). Expression of NPY receptor mRNA was analyzed using quantitative Real-Time PCR analyses. For in vivo studies, processed human lipoaspirate was injected subcutaneously into the flanks of 6-week-old BALB/c-nu mice. Simultaneously, NPY-infused mini-pumps (~1ug, 10ug and 100ug NPY/100uL 1X PBS) were implanted subcutaneously into the dorsum of BALB/c-nu mice. Graft volumes were measured at 4 weeks post-transplantation.

Results: NPY treatment significantly increased proliferation and differentiation in 30% and 50% of ASC patient sample cultures, respectively ($p < 0.05$). Quantitative PCR analyses indicated variable NPY receptor mRNA expression both within and among primary cultured ASC samples. Average volumes of human fat xenografts supplemented with NPY mini-pumps were not significantly different from average volumes of vehicle-treated xenografts ($p > 0.05$).

Conclusions: Our results demonstrate that in vitro proliferative and adipogenic effects of NPY on primary cultured human ASC do not appear to translate in vivo regarding human xenograft retention. Differences in vascularity (CD31 immuno-staining) between NPY-treated and control xenografts are currently being evaluated. Additional studies with human ASCs, as well as term-term xenograft studies, are necessary to help define the putative clinical role of NPY in soft tissue grafting.

21

IMPACT OF AN INFLAMMATORY-LIKE CONTEXT ON THE CAPILLARY NETWORK WITHIN HUMAN TISSUE-ENGINEERED ADIPOSE TISSUES

Presenter: Maryse Proulx, MSc
Authors: Proulx M, Mayrand D, Aubin K, Audet-Casgrain MA, Fradette J
Centre LOEX de l Université Laval

Reconstructed human adipose tissues (rhAT) produced by tissue-engineering represent a promising alternative to autologous fat grafts for reconstructive surgeries. Inflammation is a normal phase of the wound healing process that may also be present following fat grafting. It is therefore important to determine its impact on tissues in order to better control the fate of the grafts. We reconstructed tissues using a combination of adipose-derived stem/stromal cells (ASCs) and naturally secreted human matrix components. We used these human 3D tissues to investigate in vitro the impact of major inflammation mediators such as TNF± and IL-1x. Oil Red O staining confirmed the inhibition of adipogenic differentiation by 10 ng/ml TNF±. ELISA analyses of culture supernatants from rhAT treated with 10 and 100 ng/ml TNF± revealed a dose-dependent increase in secretion of MCP-1 (3.3 ± 0.3 and 5.0 ± 0.7 fold, respectively). The growth factors NGF (1.6 ± 0.1 and 2.4 ± 0.5 fold) and HGF (1.6 ± 0.3 and 1.6 ± 0.3 fold) were also increased following a 24h treatment while no differences were observed for VEGF or leptin expression (n=6 per group). Since incorporation of endothelial cells (EC) during rhAT production results in the formation of an in vitro capillary network, we then evaluated the impact of these pro-inflammatory cytokines on the developing network. Detection of the EC marker PECAM-1 on in toto tissue samples revealed morphological alterations in the organization of the vascular network. While TNF±-treated tissues (10 ng/ml) did not mediate statistically significant differences, IL-1x alone (10 ng/ml) and in combination with 10 ng/ml TNF± increased the proportion of capillary structures of smaller total volume (1.6 fold, $p<0.05$, Imaris software). IL-1x treatment also tended to decrease the percentage of complex structures featuring many branching points (57% reduction) with a more drastic effect observed in combination with TNF± (84%, $p<0.01$). In conclusion, studies using tissue-engineered rhAT possessing a capillary network in vitro can help predict the effect of an inflammatory context on both the adipocytes and the vascular network of fat grafts. This could lead to a better understanding of the mechanisms promoting or hindering graft survival. Supported by CIHR.

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A NOVEL ROLE FOR SIRTUIN 7 PROTEIN DEACETYLASE IN AGING ADIPOSE TISSUE

Presenter: Brian L. Chang , BA, BS
Authors: Chang BL, Dierova R, Percec I
University of Pennsylvania

Aging is a decline in physical, and sometimes mental, function that is often accompanied by the development of system-specific pathologies. Fat grafting and adipose derived stem cell-based therapies are promising interventions for the prevention and treatment of aging. However, these interventions are hindered by adipose tissue senescence in the older population who would benefit most from such treatments. Therefore, an understanding of normal adipose aging could have significant therapeutic implications. The sirtuin (SIRT) gene family of protein deacetylases has been shown through the caloric restriction pathway to regulate molecular mechanism of aging. Studies demonstrated that SIRT7 is associated with rDNA transcription and RNA polymerase I, but its exact role in adipose tissue aging is unclear. This study investigates SIRT7 in human adipose tissue aging.

Subcutaneous abdominal adipose tissue is collected from patients undergoing surgery at UPenn. Adipocytes, stromal vascular fractions (SVFs), and adipocyte-derived stem cells (ASCs) are isolated from each specimen. Protein and RNA are isolated to perform Western blot and qRT-PCR analyses to determine the expression of SIRT7 and associated factors in a cell- and age-specific manner.

Our data indicate SIRT7 protein expression increases in adipocytes, SVFs and ASCs with advancing age. In contrast, SIRT7 and target factors RNA expression is correlated and appears to decrease with age in adipocytes, and remain unchanged in SVFs. These data are the first to suggest that there is a role for SIRT7 in human tissue aging and that its function may be actively regulated at the posttranslational level in a cell-specific manner.

Our data support a role for SIRT7 in human adipose tissue aging perhaps through chromatin remodeling or regulation of RNA Pol I transcription. SIRT7 activity is regulated in a cell-specific manner at the posttranslational level. Future studies will examine SIRT7 chromatin binding in aging adipose tissue in a genome-wide manner to determine the pathways involved in adipose senescence. The discovery of epigenetic modifiers of normal adipose human tissue aging through our work may have implications for regenerative medicine surgery and advance the treatment and prevention of human aging.

23

THE EFFECTS OF RADIATION THERAPY ON THE PROLIFERATION AND POTENCY OF ADIPOSE-DERIVED STEM CELLS

Presenter: Rachel L. Slotcavage, MD
Authors: Slotcavage RL, Crutchfield M, Colacino A, Chang S, Liu Y, Matthews M, Carpenter J, DiSanto M, DiMuzio P, Tulenko T
Cooper Medical School of Rowan University

Background: The use of radiation therapy in cancer treatment is sometimes limited by its side effect profile. Chronic effects include necrosis, fibrosis, and non-healing wounds, thought to be the result of radiation-dependent depletion or alteration of tissue-specific stem cells. We hypothesize that adipose-derived stem cells (ASCs) which have been exposed to radiation during cancer treatment will be fewer in number, proliferate less rapidly, and exhibit diminished differentiation capacity than non-radiated cells.

Methods: During reconstructive surgery, two adipose tissue samples were harvested from each subject: one sample from within the radiated field (R+), and one from a non-radiated area (R-). The stromal vascular fraction (SVF), containing both ASCs and hematopoietic and other stromal cells, was isolated using collagenase and centrifugation, counted, and plated for cell culture (1x10^6 cells/75 cm^2, M-199 media changed every 3 days). At 24 hours, ASC's were removed from flasks and counted to determine the ASC/SVF ratio. After reaching confluence, cell aliquots were a) plated in 12-well plates for growth curves, b) prepared for EDU staining (to quantify proliferation), and c) plated in endothelial differentiation medium to assess differentiation capability by examining differential gene expression via RT-PCR. Differences in 1) yield and proliferation were determined using the paired T-test and 2) gene expression were identified using the ??Ct method.

Results: No difference was seen in SVF or ASC yield between R+ and R- cells, though yields were significantly lower than in samples from healthy subjects. No difference was seen in proliferation rate based on growth curves. A slight difference in proliferation may exist on EDU staining, but more samples are required. A difference was seen, however, in endothelial differentiation capacity based on expression of CD 31 and vWF, but more samples are required.

Conclusion: These results suggest that while radiated ASCs do not behave differently in controlled in vitro conditions, their ability to differentiate to an endothelial phenotype and thus form microvascular networks in vivo may be impaired, perhaps clarifying a mechanism of chronic radiation damage.

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ADIPOSE-DERIVED STEM CELL TO EPITHELIAL STEM CELL TRANSDIFFERENTIATION: A MECHANISM TO IMPROVE UNDERSTANDING OF FAT GRAFTS' SKIN REGENERATIVE POTENTIAL

Presenter: Brian M. Derby, MD
Authors: Derby BM, Dai H, Reichensperger J, Cox L, Harrison C, Bueno RA, Neumeister MW
Southern Illinois University School of Medicine

Introduction: Facial and breast lipofilling reportedly improves skin composition and appearance. Adipose-derived stem cells (ADSCs) have been implicated. In vivo evidence of ADSC differentiation into epithelial cells is sparse and focuses only on ADSC expression of epithelial cell surface markers after engraftment. Instead, we aim to identify ADSC differentiation into epithelial stem cells, through colocalization of engrafted ADSCs with the epithelial stem cell marker p63.

Methods: Six male, GFP+ (green fluorescent protein positive) mice served as adipose tissue donors. Twelve nude mice served as recipients. Recipients were subdivided into 2 arms (6 mice/each arm), and received either whole fat specimen (Group 1) or isolated and purified ADSCs encapsulated in a peptide hydrogel (Group 2) grafted into a 1cm2 left parascapular subdermal plane (Figure 1). The right parascapular subdermal plane served as control (phosphate buffered saline or sham). Skin flaps were harvested at 8 weeks, and subjected to (1) gross analysis, (2)fluorescent microscopy, and (3) reverse transcriptase polymerase chain reaction (RT-PCR) for p63 mRNA expression levels. Statistical significance of group mean values differences was determined using the Student's t-test. *p<0.05;** p<0.01

Results: At tissue harvest, gross morphology demonstrated angiogenesis present within skin flaps surrounding fat/ADSC specimens (Figure 2). Fluorescent microscopy demonstrated GFP+ cell migration into overlying dermal architecture with viable/proliferating ADSCs present within the peptide hydrogel. The epithelial stem cell marker, p63, co-localized to GFP+ ADSCs seen migrating through the dermis (Figure 3). RT-PCR demonstrated significantly increased levels of p63 expression in the ADSC + hydrogel skin flaps, compared to those of control and whole fat groups.

Conclusion: We offer direct evidence that GFP+ ADSCs can migrate into the dermis, and express the epithelial stem cell marker p63, suggesting that ADSCs can become epithelial stem cells after fat grafting. These findings may complement current insights into how fat grafts rejuvenate overlying skin, refine techniques for skin tissue engineering, and validate clinical/basic science studies aimed at using cellular therapies to treat skin pathologies.

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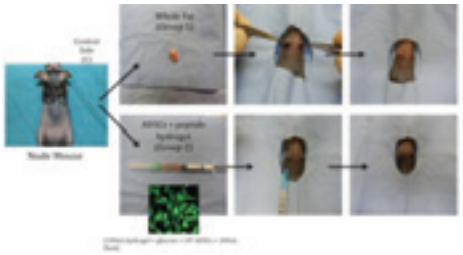
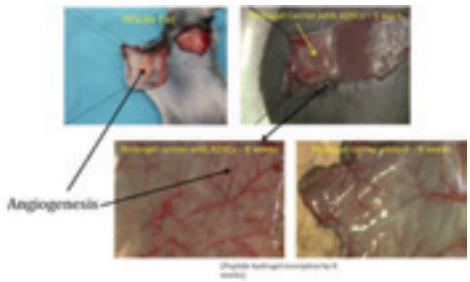
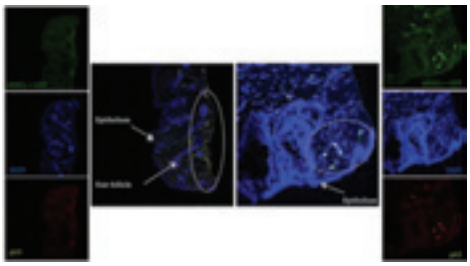
ADIPOSE-DERIVED STEM CELL TO EPITHELIAL STEM CELL TRANSDIFFERENTIATION: A MECHANISM TO IMPROVE UNDERSTANDING OF FAT GRAFTS' SKIN REGENERATIVE POTENTIAL







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HYPOXIA ALTERS MUSCLE RESIDENT STROMAL CELL PHENOTYPE AND IS A KEY REGULATOR IN TRAUMATIC HETEROTOPIC OSSIFICATION

Presenter: Guillaume Grenier, PhD
Authors: Drouin G, Couture V, Palidwor G, Perkins T, Faucheux N, Grenier G
University of Sherbrooke

Heterotopic ossification (HO) is debilitating condition occurring frequently after a severe muscle injury. HO is formed through a process known as endochondral ossification. In this process, progenitor cells first differentiate into chondrocytes, which hypertrophy and ultimately die. Then progenitor cells invade the cartilage matrix and differentiate into osteoblasts, which elaborate specialized matrix that mineralized to give rise to bone. We recently show that the muscle resident stromal cell (mrSC) population was involved in HO. However, the mechanism(s) underlying the alteration of mrSC phenotype is unknown. Because muscle trauma results in blood vessel breakdown, we hypothesized that the transient hypoxic state might influence mrSC's HO potential. Flow cytometry data from damaged muscle indicate that the state of the mrSC population is both augmented and in a proliferative state. In vitro, colony-forming assay of mrSCs reveal that the number and the size of colonies formed in hypoxic conditions (0.7% O2) were significantly higher compared to normoxia (21% O2). Moreover, mrSCs cultured under hypoxia revealed efficient differentiation into chondrocytes. Together, our results suggest that the transient hypoxic state found in damaged muscle increases the mrSC population that differentiates preferentially into chondrocytes, the first step of endochondral ossification. Therefore, hypoxia should be considered as a key factor involved in HO.

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EFFECTS OF CURCUMIN ON HUMAN ADIPOSE DERIVED STEM CELLS

Presenter: Russell E. Kling, BA
Authors: Kling RE, Narayanan K, Ravuri SK, Philips BJ, Marra KG, Rubin JP
University of Pittsburgh

Introduction: The worldwide obesity epidemic has increased morbidity and mortality for countless patients, while consuming large sums of health care funds. Consequently, any effort to minimize obesity would have tremendous clinical implications. Our group has examined curcumin, a derivative of the Indian spice turmeric, using primary derived human adipose cells. Curcumin has well-established anti-cancer and immune-modulary properties. Previous studies with curcumin have focused on established cell lines. Importantly, turmeric is widely consumed as a safe food additive.

Methods: Primary adipose stem cells (ASCs) were isolated from a healthy donor using a standardized laboratory protocol. Curcumin was dissolved 1mg/1mL with 100% DMSO. All tests were performed in the presence and absence of curcumin (27μM, 13.5 μM, 6.75 μM, 1.69 μM, 1nM, 1pM, 0). ASC proliferation was measured using the CyQuant assay at 48 and 96 hours. Lipid accumulation after 14 days was assessed using the AdipoRed assay. Glycerol and free fatty acid release were quantified in differentiated adipocytes by Lipolysis Assay. RNA from pre-adipocytes and differentiated adipocytes was isolated and cDNA was synthesized. qPCR was performed for PPAR-gamma, FABP4.

Results: ASC proliferation after incubation with 13.5 and 27μM curcumin was significantly lower than control (ASC media only) and vehicle control (1% DMSO) at 48 and 96 hours. Differentiated adipocytes treated with 13.5 and 27μM curcumin showed less differentiation than control (100% differentiation alone) and vehicle (1% DMSO). Glycerol and free fatty acid release increased dose-dependently as the concentration of curcumin increased, and the 13.5 and 27μM treatment groups showed increased release relative to the vehicle control (1% DMSO). qPCR showed down-regulation of PPAR-gamma, FABP4 in the presence of curcumin.

Conclusion: This study suggests that curcumin has lipolytic, anti-adipogenic and anti-proliferative properties in primary derived human adipose cells. Curcumin could potentially be used for the treatment of obesity. Continuing studies will focus on the parent compound, turmeric, as well as an assessment of toxicity.

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COLLECTION, PROCESSING, TESTING AND RELEASE CRITERIA FOR GMP MANUFACTURED ADSC PRODUCTS

Presenter: MaryPat Moyer, PhD
Author: Moyer MP
INCELL Corporation LLC

Adipose-derived stromal/stem cells (ADSCs) are being collected, isolated, packaged and stored using current Good Manufacturing Practices (cGMP). Brief case studies will be presented as examples to provide guidance on "translational" product design and development for clinical use. Case studies will also represent applications of cGMP to Human Cell and Tissue Products (HCT/Ps) under 21CFR 1271 FDA regulatory guidelines for use under section 361 and "more than minimal manipulation" or "combination" products that are being pursued through IRB-approved clinical trials and/or Investigational New Drug (IND) applications. Evaluations and actions done to meet cGMP and quality assurance standards, as well as the CMC (chemistry-manufacturing-control) sections of IND submissions include processing and dissociation reagents, collection kits, raw materials, and cryostorage materials. Similarly, sources and types of plastics used are reviewed not only for safety and compatibility with ADSCs, but in combination products such as those with growth factors, cytokines or specialized scaffolds. Closed or open systems, with disposable components for transfer and processing, may be off-the-shelf options or customized. Testing requirements are product-specific and consider whether or not the ADSCs are autologous or allogeneic, the route of transfer or delivery to the patient, packaging, shelf life, and other features important to product safety and efficacy. Sets of quality control criteria relevant for product release, such as potency and identity, will summarize assessments that would comprise a Certificate of Analysis or product insert in the case study examples. Outcomes of pre-clinical in vitro analyses (e.g., biomarkers, growth) and animal model studies of product utility (e.g., wound healing, tissue repair) will be reviewed. Product summaries will also define design history files, supportive FDA Master Files, risk assessments, and needs for validations and qualifications of personnel, equipment and materials. Collectively, these data and summaries will demonstrate important regulatory considerations, practical approaches and best practices to move promising ADSC products from laboratory research to the clinic.

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HOW PATIENT DEMOGRAPHICS AND ANATOMIC SITE SELECTION AFFECT YIELD AND DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS

Presenter: Melanie R. Crutchfield, MD
Author: Crutchfield MR, Slotcavage RL, Colacino A, Chang S, Matthews M, Liu Y, DeSanto M, Carpenter J, Park SS, DiMuzio P, Tulenko T
Cooper Medical School of Rowan University

Background: Adipose-derived stem cells (ASCs) are emerging as a method to harvest adult stem cells. Stromal vascular fraction (SVF) derived from adipose tissue contains an abundance of ASCs that can differentiate into fat, bone, cartilage, muscle and endothelial cells. This study is directed by the hypothesis that the SVF cellular yield and differentiation potential is affected by demographics and harvest site.

Methods: Forty-five adipose samples from 28 subjects were collected from subjects arm, thigh, abdomen, or flank. Cells were also cultured in osteogenic, adipogenic and endothelial cell differentiation media for 14-21 days. qRT-PCR was used with osteocalcin, lipoprotein lipase; CD31 as markers for differentiated osteocytes, adipocytes, and endothelial cells, respectively. Abdomen and arm ASCs were incorporated with 5-ethynyl-2-deoxyuridine (EdU) with subsequent florescent imaging to detect proliferating cells.

Results: There was no difference in gender or age in regards to SVF yield. Obese subjects and those with a history of smoking trended towards a decreased SVF yield. There was a statistically significant difference in total cell yield based on anatomic site; the highest yield from the upper arm (table 1). In addition, ASCs isolated from the arm have greater osteogenic differentiation ability than the abdomen, flank and thigh (figure 1). ASCs differentiated into adipocytes, osteocytes, and endothelial cells expressed appropriate markers using qRT-PCR. There was no difference in proliferation based upon EdU staining (figure 2).

Conclusions: Our results show that gender or age does not affect SVF yield. Smoking and obesity show a trend toward decreasing SVF yield. Cells harvested from the arm have shown a higher SVF cellular yield and better differentiation ability.

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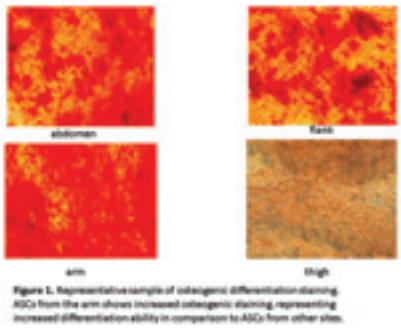

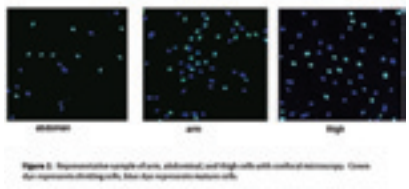

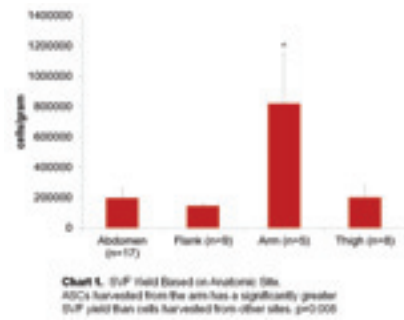

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WHATS IN A NAME? INTRODUCING NON-ADHERENT PROGENITORS FROM ADIPOSE-DERIVED STEM CELLS (NAPASCS)

Presenter: Angelo A. Leto Barone, MD
Authors: Leto Barone AA, Giunta G, Carmisciano M, Toia F, Carollo R, Iovino F, Todaro M, Cordova A, Moschella F
Universita degli Studi di Palermo

Introduction: The isolation of Mesenchymal Stromal Cells (MSCs) from adipose tissue has been investigated extensively. These cells grow as adherent, fibroblast-like cell colonies capable of differentiation toward different mesenchymal cell lineages and, as such, have been labeled as adipose-derived stem cells (ASCs). In the past, however, there has been debate on whether MSCs can be appropriately defined stem cells, as true stem cells grow in suspension maintaining their stemness conditions, as shown by their asymmetric division and clonogenesis capability.In this study, we investigate an upstream stem cell type derived from adipose tissue we denominated non-adherent progenitors from Adipose-derived Stem Cells (n.a.p.A.S.C.s) and its similarities to non-adherent cancer stem cells spheres from other tissues.

Materials and Methods: Lipoaspirate samples were harvested from 23 healthy donors following patients written consent. Mechanical and enzymatic digestion of the samples was obtained using sharp scissors and collagenase. Cells were plated in stem cell-specific enriched media and in no-adhesion culturing conditions. Clonal expansion and PKH26 staining were used to assess stemness. Medium containing ascorbic acid, dexamethasone and beta-glycerophosphate promoted differentiation toward the osteogenic lineage.

Results: NapASCs clusters defined as spheroids (polyclonal) and spheres (monoclonal) are visible in suspension 7-21 days after plating and display expansion patterns similar to colon, thyroid or breast stem cells. NapASCs stemness was confirmed in vitro by stem cell-specific biological behaviors such as lack of anoikis due to no-adhesion conditions, clonal expansion, asymmetric division and differentiation toward mesenchymal lineages.

Conclusions: We propose napASCs as an upstream line of mesenchymal progenitors compared to the more differentiated, adherent MSCs. NapASCs display expansion and division patterns typical of true stem cells and we hypothesize that the identification of napASCs may dissipate the doubts on the stem-cell origin of the more differentiated and commonly used adherent mesenchymal precursors. Ongoing studies aim to assess differentiation toward other mesenchymal cell lineages and use on synthetic scaffolds for regenerative purposes.

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ADIPOSE STROMAL CELL ISOLATION AND EXPANSION FOR CLINICAL APPLICATIONS USING XENO- AND SERUM-FREE MEDIA

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

Multiple animal models of human diseases have demonstrated that in vitro expanded adipose stromal cells (ASC) have strong therapeutic effects. Such studies have built a foundation for ASC evaluation in clinical trials. For ASC to be acceptable for clinical use, several aspects of cell manipulation must be considered in light of regulatory compliance. Recently, multiple potentially clinically acceptable collagenase preparations and serum-free media for mesenchymal cell expansion have been brought to the market.

In this study, cell preparations were compared with respect to collagenase and expansion medium. Cells were isolated with either Worthington Collagenase I or Roche Liberase MNP-S and then cultured in several test media, with EGM-2mv (Lonza) chosen as control. Cells were incubated for 5-7 days, harvested and counted. Expanded cells were screened for adipogenic and vasculogenic potency and for therapeutic effect in a murine hindlimb ischemia model.

Cell isolation using both enzymes gave a similar total cell yield and degree of cell adhesion. Analysis of the media revealed that neither StemPro (Invitrogen) nor MSCGM-CD (Lonza) were adequate to support ASC adhesion and proliferation. Concurrently, MesenCult-XF (Stem Cell) and Mosaic (BD Biosciences) were able to induce ASC proliferation up to 50% compared to EGM-2mv. Comparative analysis of several basal media revealed that EBM-2, DMEM/F12, and CellGenix MSC were optimal for ASC cultivation. Interestingly, replacement of 5% FBS with 5% human serum (HS) in EGM-2mv did not jeopardize mitogenic activity. However, PLTMax (Mill Creek), a platelet lysate tested as an alternative to serum, demonstrated only 50% of effect shown by FBS or HS supplemented media. Importantly, cells expanded in EGM-2mv or MesenCult-XF were indistinguishable based on the expression of surface markers, while the vasculo- and adipogenic potencies of MesenCult-XF expanded cells were 50% lower than the cells cultured in EGM-2mv. Moreover, MesenCult-XF expanded cells were unable to restore blood flow in the ischemic hindlimb, quite in contrast to the positive effect of EGM-2mv expanded cells. Specific cell potencies vary significantly based on conditions of expansion, and in vivo outcomes may not be well-predicted by cell surface phenotype.

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A NOVEL MICRORNA-OMIC APPROACH FOR CHARACTERIZING OF ADIPOSE-DERIVED REGENERATIVE CELL SAFETY AND EFFICACY

Presenter: Kevin Hicok, MD
Authors: Mallinson DJ, OBrien V, Olijnyk D, Zhu M, Fraser JK, Arm D
Cytori Therapeutics Inc

Background: As adipose-derived stem and regenerative cell (ADRC) based therapeutics have come of age, so has the impetus to characterize the cellular identity and purity of these cells to a level both appropriate and required for their regulation and adoption by the clinical community. Because excessive manipulation alters the phenotype of ADRCs, historical characterization has been limited to viable cell number determination and occasionally CD marker protein analysis by flow cytometry. Furthermore, no specific characteristics have yet been correlated to successful therapeutic outcome. Inappropriate application or poor methodology has further blurred the picture of ADRC composition. Here we describe a new characterization strategy using miRNA-omics to identify ADRC subpopulations and potential mechanisms of their therapeutic efficacy.

Methods: Adipose lipoaspirate from three healthy female donors was enzymatically processed to obtain stromal vascular cell populations. Viable cell number was determined using a NucleoCounter? (ChemoMetec A/S). Total RNA was isolated using the miRCURY RNA Isolation Kit (Exiquon Corp.) and quantified and assessed for quality using an Agilent 2100 Bioanalyzer. RNA samples were analyzed using SurePrint G3 Human v16 miRNA 8-60K microarray slides and miRBase version 16.0.

Results: 228 miRNAs were found to be expressed in all three donor ADRC samples using proprietary SistemQC statistical workflow methodologies. SistemQC analyses demonstrated that the ADRC samples were more similar to each other compared to a HeLa cell line comparator as well as the level of donor variability. Candidate miRNA molecules were identified for further bioinformatic analysis to identify cell-identity and mechanisms of action associations for regulation of angiogenesis, apoptosis, and immunomodulatory genes.

Conclusion: We have applied a novel, powerful approach, based on the miRNA profiles of the cell populations, which can be used for ADRC characterization to better define cellular composition and mechanisms of action. Application of this information may enable the identification of ADRC efficacy and safety biomarkers in upcoming clinical trials.

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SUCCESSFUL CULTURE OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS IN A FUNCTIONALLY CLOSED AUTOMATED CELL EXPANSION SYSTEM

Presenter: Kim T. Nguyen, PhD
Authors: Nguyen KT, Baila S
Terumo BCT

The large numbers of ex vivo expanded cells that are required in many clinical cell therapy protocols (>200 million per patient) make standard culture conditions problematic and expensive, resulting from the need for extensive personnel and facilities resources and the high potential of contamination. To meet such clinical demand, a robust, scalable, automated and closed cell expansion method is optimal. The Quantum Cell Expansion System is a functionally closed, automated hollow fiber bioreactor system designed to reproducibly grow both adherent and suspension cells in either GMP or research laboratory environments. The Quantum System has successfully been used by Terumo BCT for the ex vivo expansion of clinical-scale quantities of adult bone marrow-derived mesenchymal stem cells (BM-MSC) and adult normal human dermal fibroblasts (NHDF). It has now been demonstrated that a third adherent cell type of clinical interest, adult human adipose-derived mesenchymal stromal cells (ASC) are ex vivo expandable with the Quantum System.

This study was a proof-of-concept that clinical-scale doses of ASC, utilizing either pre-cultured ASC or the stromal vascular fraction (SVF) as starting products, may be expanded on the Quantum System. Two different bioreactor membrane coating reagents were deployed, human fibronectin (FN, BD Biosciences) and human cryoprecipitate (CPPT, Bonfils), as well as xeno-free media conditions utilizing pooled human platelet lysate as a culture media protein source. Bioreactor coating with FN or CPPT, cell loading, attachment, feeding, and harvest followed the standard Terumo BCT protocol a developed for the culture of BM-MSC. The initial seeding density for pre-cultured ASC for the bioreactor and the control flasks were 500 cells/cm2, or approximately 1 x 107 cells per bioreactor and 1.25 x 103 cells per T25 control flask. The initial product load size for experiments utilizing SVF as a starting product is SVF processed from 80g of adipose tissue. Experimental results demonstrate that robust ASC ex vivo expansion is possible using an automated hollow fiber-based bioreactor system to reach clinically relevant cell yields starting from either pre-cultured ASC or SVF.

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AUTOLOGOUS CRYOPRESERVATION OF HUMAN ADULT ADIPOSE DERIVED STEM CELLS

Presenter: Ilana Platt, PhD
Authors: Niapour M, Sliwin SJ, Platt I, Rice S
Adisave

Introduction: Cryopreservation of adult stem cells has been discussed frequently in the literature. It has been demonstrated that storage temperature, freezing rate, cell concentration, and cryoprotectant variables play major roles in post-thaw recovery, viability and functionality of human adult adipose derived stem cells (ADSCs). In this abstract we discuss how freezing rate, cell concentration and choice of cryoprotectants affect recovery, viability and functionality of ADSCs for the first time, in a completely autologous environment.

Methods: SVFs were processed as previously described by our group and frozen at different cell concentrations, using different freezing programs in different freezing media. ADSCs were frozen in 10% DMSO, 40% DMEM and 50% FBS or autologous plasma. DMSO concentrations varied between 2.5% to 10% with and without Dextran. To assess the effect of cell concentration on post-thaw viability, different concentrations of ADSCs per ml of freezing media were used ranging from 1x106 to 10 x 106.

Results: All post-thaw samples were tested for cell quantification, viability, immuno-phenotype and functional assays. We observed that cryopreservation of ADSCs in 50% FBS or 50% autologous plasma was equivalent in terms of cell recovery, cell viability and functionality. We also found that cryopreservation of ADSCs in 10% DMSO yields the highest post-thaw cell viability and functionality. We found that ADSCs have the highest recovery and viability when frozen with less than 2.5x106/ml. We also found that the presence of Dextran increases ADSCs viability and functionality compared to DMSO alone. We also observed that different freezing programs affect recovery, viability and functionality of ADSCs.

Conclusion: We described a method for autologous cryopreservation of ADSCs that facilitates their clinical use. The ADSCs functional status and morphologic characteristics were not changed by the autologous cryopreservation procedure described by our group. We also demonstrated that ADSCs are relatively resistant to exposure to DMSO.

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CHARACTERIZATION OF STROMAL VASCULAR CELLS FOLLOWING ENZYMATIC DIGESTION OR MECHANICAL PROCESSING OF ASPIRATED ADIPOSE TISSUE

Presenter: Alexandra Conde-Green, MD
Authors: Conde-Green A, Rodriguez RL, Vail SR, Ivo BG, Slezak S, McLenithan JC
University of Maryland Medical Center

Background: Adipose stromal vascular fraction (SVF) is an important source of adipose-derived mesenchymal stem cells (ADMSCs) that are used in regenerative medicine. Numerous procedures including both mechanical and enzymatic methods have been used to obtain stromal vascular cells (SVCs) from adipose tissue. The varying cellular composition of SVF obtained from these methods may have an effect on its regenerative capabilities. Therefore, we have compared the yield and cellular composition of SVCs obtained from adipose tissue lipoaspirates by centrifugation, vortexing/ centrifugation and collagenase digestion.

Methods: Subcutaneous adipose tissue was obtained from healthy adult patients (n=9) aged 21-55 years with an average BMI of 28, by vacuum-assisted (-400 mmHg) liposuction. SVCs were isolated from lipoaspirates by centrifugation (1256xg), vortexing for 3 minutes followed by centrifugation or collagenase digestion. After red blood cell lysis, SVCs were subjected to cell counting, viability measurements and flow cytometry. The relative percentages of ADMSCs (CD45- CD73+ CD90+), endothelial cells (CD45- CD31+) and monocytes/macrophages (CD45+ CD14+) were quantitated.

Results: SVC yields differed between enzymatic and mechanical methods with the highest yield obtained from enzymatic isolation (2.3 x105 cells/ml lipoaspirate), 10 fold fewer cells from centifugation and 20 fold fewer from vortex/centrifugation. Cell viability as estimated by trypan blue exclusion was similar between the methods (80-90%). The cellular composition of SVF was potentially the most significant variation between the methods. Collagenase digested SVF contained fewer cells of hematopoetic origin (32%) than mechanically isolated SVF (70-85%) and greater numbers of ADMSCs (60%) than mechanically isolated SVF (6-13%). In addition, collagenase digested SVF contained fewer inflammatory monocytes/ macrophages and greater numbers of endothelial cells than mechanically isolated SVF.

Conclusion: Although regenerative cells were isolated from all three methods, the increased hematopoetic and inflammatory cells as well as decreased ADMSCs and endothelial cells in the mechanically isolated cell populations may contribute to reduced therapeutic potential when used in regenerative medicine.

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ISOLATION OF STROMAL VASCULAR FRACTION FROM NUTATIONAL INFRASONIC LIPOASPIRATE

Presenter: Robert E. Bowen, MD
Authors: Bowen RE, McQuillan S, Comella K
The Center For Positive Aging

Introduction: Adipose tissue has been identified as an abundant source of adult MSCs. These adipose derived stem cells (ADSCs) can be isolated from adipose tissue using enzyme digestion and by separation of stromal vascular fraction(SVF) by centrifugation. This SVF can be used immediately during a same day procedure or as a source of cells to be grown in culture. Theraputic use of these progenitor cells has been limited by the expensive and labor intensive nature of using an enzyme as well as ambiguities in current govermental regulation. An alternative approach to obtain SVF without the use of an enzyme was studied. Nutational infrasonic liposuction (NIL) employs 3D motion (yaw,pitch,roll) of a canula vibrating at 10 hz to assist in obtaining a lipoaspirate.

Methods: 60ml aspirate was collected during routine liposuction procedure from syringe aspiration (x2) at approximately 1/2 atm. and from NIL and a vacuum aspirator at -15mm Hg on each of 10 subjects. SVF was obtained from: 1)syringe aspirate and processing with a commercial preparation (Liberase-Roche or CIzyme-Vitacyte), 2)NIL without enzyme and centrifugation at 1200g x 10 minutes, 3)syringe aspiration at 1/2 atm. without enzyme and centrifugation at 1200g x 10 minutes. Cell yield and viability was estimated using trypan blue stain and a hemocytometer.

Results: Viable cells counted: manual syringe and enzyme=1.54 million +/-0.95 /ml; NIL=1.34 million +/- 0.53/ml; manual syringe and centrifugation only= .25 million+/-.04. This was not a statistically significant difference between enzyme and NIL collection.

Conclusions: Centrifugation of NIL aspirate yields viable cells in the same order of magnitude as processing with an enzyme preparation (collagenase I, II, neutral protease), centrifugation of manual lipoaspirate without enyme also yields cells but at 1 lower order of magnitude. If further study confirms the validity of this approach (including characterization of these cell's surface markers and biologic activity), the regenerative effects of the SVF could be obtained by a less time consuming and more cost effective method.

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OPTIMIZING HARVESTING TECHNIQUES OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS (HADSCS): A MULTIVARIABLE STUDY COMPARING SIZES OF CANNULA AND POWER SUCTION, WATER-JET AND SYRINGE SUCTION LIPOSUCTION TECHNIQUES

Presenter: Sammy Sliwin, MD, FRCSC
Authors: Sliwin S, Niapour M, Rice S
Adisave

Introduction: Adipose-derived stem cells (ADSCs) hold a great promise for regeneration in vivo and tissue engineering. In this study for the first time we comparatively analyze viability, sterility, cell surface molecules, colony forming ability, differentiation potentials and genetic stability of ADSCs. We discuss the impact of liposuction techniques on the outcomes of fat grafting.

Methods: Lipoaspirates were collected from three different techniques, manual liposuction with syringe, power suction liposuction and water-jet liposuction. We compared cell number per gram of lipoaspirate, apoptosis, colony forming unit capacity, cell surface markers, and senescence index between groups and within groups. We analyzed data using ANOVA-PAST statistical program.

Results: We did not find any statistically significant differences between liposuction techniques in cell number, viability and apoptosis. We found a statistically significant difference between cannula, the 3mm pyramid tip producing the best harvesting results in cell number, viability, apoptosis and CFU-F. Freshly plated cells looked identical under converted microscope. The expression patterns of cell surface molecules for hematopoietic and mesenchymal stem cell markers were identical. Surprisingly, cells harvested by water-jet liposuction were not or had few plastic adherent cells, formed few colonies (ranged from 0 to 8 colonies/dish). In contrast, cells utilized by power suction or syringe suction liposuction were plastic adherent, formed colonies (ranged from 34 to 167 colonies/dish).

Conclusion: Autologous fat grafting has been used to treat soft tissue anomalies since 1893 with variable results. The effectiveness of fat grafting may lie in the transfer of ADSCs with the fat with the regenerative capacity to exit their quiescent state and enter active cell cycle in response to hypoxic conditions generated by the surrounding dying fat. Our study shows that the ADSCs separated from lipoaspirates harvested by water-jet liposuction technique lose properties of stem-ness and therefore might affect outcomes of fat grafting procedures.

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NON-ENZYMATIC METHODS TO OBTAIN REGENERATIVE CELLS FROM ADIPOSE: IS IT PRACTICAL OR EVEN POSSIBLE?

Presenter: Min Zhu, MD
Authors: Zhu M, Hicok KC, Shanahan R, Yu J, Souverneva O, Fornace G, Alfonso Z, Arm D, Fraser JK
Cytori Therapeutics Inc

Recently, non-enzymatic methods to obtain stromal vascular cells (SVF) from fat have been proposed. We report our findings for the evaluation of these two methods; one using an emulsifier and the other utilizing ultrasonic energy.

Methods: Intra-donor sample comparison studies were performed. Human adipose was processed using the gold standard enzymatic process or using either a commercially available emulsifier-based cell isolation kit (Adistem®) or exposure to ultrasonic energy varying in power and amplitude. The resultant cells obtained by the different methods were assessed for cell yield and viability, the frequency of CFU-F, and cellular composition based on CD marker protein expression.

Results: Compared to the enzymatic approach, the emulsifier-based method yielded primarily red blood cells and 10 times less nucleated cells than the enzymatic method. The majority of these nucleated cells were blood-derived granulocytes. No adherent colonies were detected in the CFU-F assay, whereas the CFU-F frequency of enzyme-derived SVF ranged from 0.5-2% of plated cells.

High energy ultrasound destroyed fat and yielded mainly cellular debris. The viable nucleated cell numbers obtained with control (zero-power), low, and medium acoustic energy settings were only 13.7 ± 4.9%, 13.0 ± 6.3% and 11.2 ± 6.5% of that obtained enzymatically (n=5, $p<0.02$). Nucleated cells in ultrasonically treated samples were primarily white blood cells, regardless of power setting. CD34+ cell content was between 14.5 to 22.2 times less within ultrasound samples and exhibited a CFU-F frequency that was 6- to 15-fold less than enzyme-derived samples ($p<0.05$) for low and medium energy, respectively.

Conclusions: These results clearly demonstrate that an enzyme-based method is required to obtain the classically defined stromal vascular fraction from adipose tissue. Neither of the alternative methods yielded comparable populations in terms of cell number, identity, or viability. Until claims of alternative method efficacy are supported by rigorous scientific data, enzymatic isolation is and will remain the gold standard approach to obtain SVF.

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CHARACTERIZATION OF HUMAN ADIPOSE DERIVED MESENCHYMAL STEM CELLS USING DIFFERENT SEPARATION TECHNIQUES

Presenter: Maryam Niapour, PhD
Authors: Niapour M, Sliwin SJ, Rice S
Adisave

Introduction: Established in 2011, AdiSave has focused on finding optimal methods for harvesting, separation, extraction, and cryopreservation of Adipose Derived Stem Cells (ADSCs) from lipoaspirates. The main focus of this abstract presentation is to demonstrate how enzymatic and mechanical separations affect characteristics of hADSCs.

Methods: Enzymatic separation was performed on lipoaspirate using four different enzymes from three different companies. For mechanical separation we used an ultrasonic instrument manufactured by Sonics. We compared cell number per gram of lipoaspirate, apoptosis, colony forming unit capacity, cell surface marker expression, integrin expression and senescence index between groups and within groups. We analyzed data using ANOVA-PAST statistical program. Data presented are mean+/- SE (N=16 and n=3).

Results: 1- Enzymatic separation performed by the enzyme manufactured by Biospecifics yields statistically significant fewer cell number per gram of fat tissue $p<0.05$, but no statistically significant differences in CFU-f numbers. 2- Enzymatically separated cells expressed very low levels of hematopoietic markers and high levels of mesenchymal stem cell markers. 3- Enzymatically separated cells expressed B1-Integrin and CD54 >60% and >30% respectively. 4- Mean of C12FDG fluorescent, a senescence marker, was 32+/- 6.7 in cells separated enzymatically, compared to the positive control 2456+/- 122. 5- In mechanical separation smaller tips, higher amplitudes and longer impulses induce cell death. 5- Mechanical separation yields statistically significant less cell number per gram of fat tissue compared to enzymatic separations ($p<0.005$), cells are not plastic adherent, do not form CFU-f and lose expression of B1-integrin and CD54 irreversibly.

Conclusion: Adipose derived stem cells hold great promise in cellular therapy. They may surpass any other source of adult mesenchymal stem cells by their particular characteristics such as abundance, accessibility, homing and immuno-privileges. While the ultimate goal is to use these characteristics as a unique opportunity to change the paradigm of medicine, care must be taken to avoid any manipulations that introduce irreversible changes in ADSCs characteristics.

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VIABILITY OF ISOLATED ADIPOSE-DERIVED STEM CELLS VIA ULTRASONIC SEPARATION

Presenter: Joseph A. Broujerdi, MD, DMD
Authors: Broujerdi JA, Schendel SA, Jacobson RL

DID NOT PRESENT AT THE MEETING

40
ADIPOSE-DERIVED STROMAL CELLS FOR THE RECONSTRUCTION OF A HUMAN VESICAL EQUIVALENT

Presenter: Alexandre Rousseau, MSc
Author: Rousseau A, Bernard G, Marceau Fortier G, Bouhout S, Fradette J, Bolduc S
Centre LOEX de l'Université Laval

Tissue engineering offers a variety of models for vesical reconstruction. Currently, most of the complications and limitations regarding functionality are attributed to the lack of a differentiated urothelium. We evaluated the potential benefits of using adipose-derived stromal cells (ASCs) to generate the stromal compartment (without the use of exogenous matrix) of a tissue-engineered vesical equivalent (VE), including a functional urothelium. Using the self-assembly method, we developed and compared three VE models using cell sheets engineered from human fibroblasts (Fbs) and ASCs. These were: the previously reported classical Fb-VE, the ASC-VE, and an Hybrid-VE containing both cell types. The properties of these engineered tissues were also compared to native pig bladders. Cell differentiation and functionality of the various VE models were assessed by histological analyses, immunolabelling, mechanical testing and scanning electron microscopy. The barrier function of the VE was evaluated by permeability studies using 14C-urea. Unlike the ASC-VE, both the Hybrid-VE and the Fb-VE promoted the differentiation of a transitional urothelium. Four weeks after the seeding of urothelial cells (Uc), the differentiated urothelium expressed the uroplakins Ib, II and III as well as ZO-1, a tight junction marker. Microvilli were also observed by scanning electron microscopy on the surface of both VE, which is usually the result of a certain level of urothelium maturation. These two engineered VE displayed a barrier function nearly identical to a native pig bladder. Finally, all VE featured adequate mechanical properties. ASCs can greatly improve the development of an effective engineered human vesical equivalent due to their matrix secretion. However, a direct contact of the Uc with Fbs was found to be essential in order to obtain a differentiated and functional urothelium in vitro.

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PRE-VASCULARIZED CELL SHEETS COMPRISED OF HUMAN FIBROBLASTS, ENDOTHELIAL CELLS AND ADIPOSE-DERIVED STEM CELLS

Presenter: YenChih Lin, PhD
Authors: Lin YC, Grahovac T, Oh SJ, Rubin JP, Marra KG
University of Pittsburgh

Introduction: There is a clinical need for wound healing that will enhance skin regeneration. Adipose-derived stem cell (ASC) sheets have shown promise in this setting but effective vascularization remains a challenge. Co-cultured systems of different cells can result in a suitable extracellular matrix with angiogenic factor production to enhance vascularization in neo-tissue. We hypothesize that human fibroblasts (Fb), endothelial cells (EC), and ASC multi-layered cell sheets can be fabricated to directly enhance angiogenesis.

Methods: Human FB, EC, and ASC were isolated from discarded human abdominal skin or subcutaneous adipose tissue. Fb and EC mixtures were seeded to fabricate the co-cultured sheet. ASC cell sheets were produced on the surface of fibrin-grafted culture dishes. The cell layer was detached with sterile forceps and the membrane was transferred to ASC sheets with the attached cell layer facing downwards. The previous steps were repeated to create a multi-layered ASC-Fb/EC-ASC sheet cultured for 10 days. Control sheets consisted of ASCs only. The induction of angiogenesis in an in vitro model was analyzed.

Results: All cell sheets were easily removed from the dishes using forceps. Morphological analyses of haematoxylin & eosin staining revealed that tubular structures were formed in multi-layered ASC-Fb/EC-ASC sheet constructs. As an indicator of pre-vascularization, ±?SMA and CD31+ immunohistochemical staining was measured at day 10. The multi-layered ASC-Fb/EC-ASC sheet showed significantly higher pre-vascularization as compared to ASC-only sheets. Quantitative RT-PCR results compared gene expression ratios for FABP4, PPAR-?, Collagen? and VEGF from multi-layered ASC-Fb/EC-ASC sheet and ASC only cell sheets. There were significant differences in expression of Collagen? and VEGF gene expression between multi-layered ASC-Fb/EC-ASC sheet and ASC only cell sheets; there was no significant difference FABP4, PPAR-? gene expression in both groups.

Conclusions: Novel multi-layered ASC-Fb/EC-ASC sheets were fabricated and characterized. This multi-layered ASC-Fb/EC-ASC sheet technology provides a substantial advance for vascularization of tissue engineered matrices, and will be examined in a murine full thickness excisional wound model.

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EVALUATION OF HUMAN ADIPOSE TISSUE STROMAL/STEM CELLS FOR BLOOD VESSEL TISSUE ENGINEERING

Presenter: Maxime Tondreau, MS
Authors: Tondreau M, Vallieres K, Laterreur V, Bourget JM, Germain L, Fradette J, Auger FA
Laval University, LOEX

Rationale: There is an important clinical need for small diameter blood vessels, notably for coronary artery bypasses. Indeed, current small-diameter synthetic grafts do not perform satisfactorily whereas autologous tissue, such as the saphenous vein, is limited. We have therefore developed at the LOEX a novel way to tissue engineer entirely biological autologous blood vessels using the self-assembly technique. The current method is based on the isolation of dermal fibroblasts and of smooth muscle cells from a vascular biopsy to form the tunica externa and media respectively. Recently, adipose tissue stromal/stem cells (ASCs) have been shown to have possible anti-inflammatory and pro-angiogenic properties, two attributes that could benefit the integration of vascular prostheses. We hypothesized that we could tissue engineer blood vessels using ASCs as an alternative to fibroblasts and smooth muscle cells.

Methods: Briefly, ASCs, fibroblasts and smooth muscle cells were cultured in the same medium in the presence of ascorbic acid to allow collagen production. Three weeks later, living cellular sheets comprised of the cells and their self-assembled matrix were rolled around a 4.8-mm mandrel. Adjoining cellular sheets spontaneously fused following a maturation phase.

Results: ASCs formed cellular sheets comparable to fibroblasts and smooth muscle cells. The cellular sheets could be handled with forceps and were sufficiently resistant to be rolled around a mandrel. To verify the mechanical properties, traction tests were performed. ASCs rings, cut from the tubular constructs, could sustain a tension slightly less than similar fibroblast constructs. To verify their contractile properties, ASCs rings were placed in a myograph and stimulated with increasing doses of the common vasoactive agonists histamine and U46619. The ASCs rings exhibited a contractile behaviour similar to smooth muscle cells.

Conclusion: Our results suggest that ASCs could be used to replace fibroblasts and smooth muscle cells. The next logical step will be to verify the capacity to endothelialize ASCs tissue engineered blood vessels, a necessary step to obtain optimal anti-thrombotic properties.

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EFFECT OF RECOMBINANT HUMAN BONE MORPHOGENETIC PROTEIN-2 AND ADIPOSE TISSUE-DERIVED STEM CELL ON NEW BONE FORMATION IN HIGH-SPEED DISTRACTION OSTEOGENESIS OF ADULT RABBIT CRANIUM

Presenter: ByeongKyu Kim, PhD
Authors: Kim BK, Lee SJ, Choi TH, Kim SH
Seoul National University College of Medicine

DID NOT PRESENT AT THE MEETING

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A SHORT BMP-2 STIMULUS SUFFICES FOR OSTEOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS SEEDED ON CALCIUM PHOSPHATE SCAFFOLDS

Presenter: Janice Overman, PhD
Authors: Farre-Guasch E, ten Bruggenkate CM, Schulten EA, Klein-Nulend J, Helder MN
VU University Medical Center

A one-step concept for bone regeneration has been postulated in which human adipose tissue mesenchymal stem cells (hASCs) are harvested, triggered to differentiate, seeded on biosynthetic substitute carriers, and implanted in the same operative procedure. Toward this goal it was investigated whether short incubation with a physiological dose of BMP-2 suffices to trigger osteogenic differentiation of hASCs seeded on calcium phosphate carriers.

hASCs were isolated from subcutaneous abdominal wall adipose tissue. hASCs were treated +/- BMP-2 (10 ng/ml) for 15 min, and seeded on fl-tricalcium phosphate (fl-TCP) or biphasic calcium phosphate (BCP) granules. Attachment was determined after 10-30 min. After 4, 14, and 21 days of culture, proliferation (DNA content), and osteogenic differentiation (alkaline phosphatase (ALP) activity; osteogenic gene expression of CBFA1, collagen-1, osteonectin, and osteocalcin), as well as expression of the adipogenic marker PPAR-? were analyzed. Gene expression was determined by real-time PCR.

hASC attachment to fl-TCP and BCP scaffolds was similar, and unaffected by BMP-2. BMP-2 increased the DNA content of hASCs seeded on the scaffolds by 2.1-fold (day 14) and 2.7-fold (day 21) compared to untreated controls. ALP activity increased in time on both scaffold types, unaffected by BMP-2. ALP gene expression was increased (3.0-fold) by BMP-2 on BCP scaffolds only at day 14. In hASCs seeded on BCP and fl-TCP, BMP-2 also stimulated gene expression of the osteogenic markers CBFA1 by 4.9-fold (BCP) and 3.7-fold (fl-TCP), collagen-1 by 6.8-fold (BCP) and 8.9-fold (fl-TCP), osteonectin by 8.6-fold (BCP) and 7.3-fold (fl-TCP), and osteocalcin by 1.9-fold (BCP) and 2.2-fold (fl-TCP) at day 21. In contrast, BMP-2 treatment inhibited expression of the adipogenic marker PPAR-? by 4.5-fold (BCP) and 4.0-fold (fl-TCP) at day 21.

In conclusion, this study revealed that 15 minutes incubation with a physiological dose BMP-2 had a long-lasting stimulating effect on osteogenic differentiation of hASCs after culturing on BCP or fl-TCP scaffolds. Our findings indicate that this short pre-treatment with BMP-2 is a very promising tool for its use in a clinical one-step surgical procedure, and strongly support a one-step clinical concept for bone regeneration.

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IN VITRO RECONSTRUCTION OF A VASCULARISED TENDON-LIKE STRUCTURE WITH ADIPOSE DERIVED STEM CELLS (ADSCS)

Presenter: Franco Bassetto, MD
Authors: Bassetto F, Lancerotto L, Tonello C, Abatangelo G, Cortivo R, Zavan B, Vindigni V
University of Padova

Introduction: Tissue-engineered bioartificial tendons would be useful in a wide range of surgical conditions. For in vitro development of tendon-like structures, it is well known that adipose derived stem cells (ADSCs) have the same differentiating capacity as mesenchymal stem cells (MSCs) yet have the advantage of being easily isolated, and obtainable in abundance from adipose tissue with minor procedures. In the present study, we used the ability of ADSCs to differentiate in tenocyte-like cells under an external mechanical stimulus to successfully create an in vitro reconstructed tendon-like structure with a microvascular network.

Materials and Methods: Human ADSCs isolated from lipoaspirate were seeded onto hyaluronic acid mesh scaffolds, and the cellularized constructs were cultured in a bioreactor under mechanical stress for up to 15 days. Human tenocytes were used in the same conditions as control. Constructs evolution was assessed by histology, immunochemistry, ultrastructural, and biomolecular analysis.

Results: ADSCs adhered and differentiated along the entire surface of the biomaterial, began to infiltrate within its structure and to secrete collagen type I, with cells and matrix lining up along the traction vector. Furthermore, CD31 and anti-vWF staining demonstrated the presence of endothelial cells arranged in capillary-like ring structures with lumen in the matrix of ADSCs but not tenocytes seeded matrix.

Conclusions: Our preliminary data show the suitability of a hyaluronan mesh scaffold to support growth and differentiation of ADSCs into tendon-like structures when subjected to mechanical forces. Seeding with lipoaspirate-derived ADSCs also seems to have the additional potential of microcapillary network formation into such structures, which may improve the performances of bioartificial tendons once grafted in vivo.

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SELECTIVE ISOLATION OF ADIPOSE-DERIVED STEM/STROMAL CELLS FROM LIQUID PORTION OF LIPOSUCTION ASPIRATES USING AN ADHERENT COLUMN

Presenter: Kentaro Doi, MD
Authors: Doi K, Kato H, Kuno S, Mineda K, Kinoshita K, Yang S, Yoshimura K
University of Tokyo

Introduction: Stromal vascular fraction is a heterogeneous cell mixture including adipose-derived stem/progenitor cells (ASC). Although the adipose portion of liposuction aspirates is the major contribute as a source of ASC, the liquid portion (LAF: liposuction aspirate fluid) also contains ASC. For isolation of LAF cells, we evaluated an adherent column which is already available for isolation of mesenchymal stem cells from bone marrow.

Methods: Three different solutions (peripheral blood [PB], peripheral blood mixed with cultured ASC [PB+ASC], and LAF) were applied to the column for cell isolation and sorted into positive (adherent/trapped) and negative (non-adherent) fractions. The column isolation was compared with our hemolysis method (manual hypotonic hemolysis using distilled water: lysis). Using cell-counter and flow cytometry, red blood cells (RBC) number, nucleated cell yield and cell composition was investigated. Isolated LAF cells were cultured for 7 days to measure viable ASC yield and further used for mesenchymal differentiation assay and network formation assay.

Results: RBC removal effect for PB and LAF was similar by both lysis and column (more than 95% of RBCs were removed). Nucleated cell in PB decreased both by the lysis and column. WBC number in positive fraction from PB was one third of that obtained by lysis (though cell composition was similar). After processing PB+ASC solution, 60.5% of ASC (53.2% by lysis) were collected in positive fraction, while no ASCs were present in the negative fraction. As for the processing of LAF, nucleated cell yield, ASC composition and viable ASC yield was not significantly different between column and lysis methods. Negative fraction contained less numbers of nucleated cells (22.7% of positive fraction), ASC (11.3%) and viable ASC after culture (4.4%), suggesting some selectivity of the column. ASC obtained from the column and lysis methods did not show significant functional difference in differentiation assay and network formation assay.

Discussions/Conclusions: Our results suggested that the column is as a reliable method as the lysis method for isolation of ASC from heterogeneous solutions such as LAF. The column can avoid air exposure in cell isolation process and may be useful for standardization.

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DIFFERENTIAL DIGESTION OF ADIPOSE TISSUE. ROLE OF SPECIFIC PROTEASES ON CELL YIELD. IMMUNOMODULATORY AND ANGIOGENIC PROPERTIES OF STROMAL VASCULAR FRACTION OBTAINED

Presenter: Severiano Dos Anjos Vilaboa Sr., PhD
Authors: Dos Anjos Vilaboa S, Mercader J, Llull R, Katz A, Futrell W
Stem Center SL

Lipoaspirate, has been shown to contain an heterogeneous population of cells termed stromal vascular fraction cells, that contains a significant amount of mesenchymal stem cells with multilineage differentiation capacity. The enzymatic digestion of human lipoaspirate is a critical step in the isolation of SVF. The collagenase is the most effective and used enzyme for this purpose. However, we hypothesize that the digestion conditions used could influence both cell yield and viability, and also the cell populations obtained, which could be useful from a therapeutic point of view. In this study we have analyzed different proteases that could affect this process. We have performed immunomodulatory, angiogenic and immunophenotypic assays to analyze the properties of the isolated cells. The results obtained indicate that the digestion conditions determine changes in the cell populations obtained. The therapeutic properties of the stromal vascular fraction cells isolated are affected in some way, what is demonstrated with the potency assays used.

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EXTRACTION OF EQUINE STEM CELL RICH FAT TISSUE IN A MINIMALLY INVASIVE LIPOASPIRATE PROCEDURE AND CLINICAL APPLICATIONS

Presenters: Sharon McQuillan, MD
Authors: McQuillan S, Comella K

Through the use of adult stem cells, regenerative medicine is a revolutionary approach to treating many degenerative conditions and injuries. This field joins nearly all disciplines of science and holds the realistic promise of repairing damaged tissue by harnessing the body's ability to heal itself. Adipose (fat) tissue contains a stromal vascular fraction that can be easily isolated and provides a rich source of adipose tissue-derived mesenchymal stem cells (ADSCs). These ADSCs are a great source of cells for tissue engineering. ADSCs include mesenchymal stem cells, which have adipogenic, myogenic and chondrogenic potential and are very angiogenic in nature. This population also includes hematopoetic stem cells, pericytes and endothelial progenitor cells. ADSCs are multipotent cells that can be utilized in regenerating diseased or damaged tissue in horses.

In order to obtain adipose tissue and large amounts of regenerative stem cells, we have developed a procedure called Equine VetLipo™. The Equine VetLipo™ procedure is an innovative technique to remove stem cell rich adipose (fat) tissue from horses in a minimally invasive, scar-free method. The technique involves using a proprietary cannula and syringe which creates enough of a vacuum (without needing a vacuum pump) to remove, or aspirate, fat tissue from the base of the tail in horses. The incision needed is only about a millimeter and does not even require stitches. Equine VetLipo™ is also designed to remove fat in a way that protects the millions of regenerative stem cells within the tissue. Traditional techniques of removing fat tissue involve a more invasive surgical approach which requires stitches and tends to leave a noticeable scar on the horse. The fat tissue that is extracted by the VetLipo™ procedure can then be processed rapidly on-site to isolate millions of regenerative stem cells. The stem cells are directly injected into a diseased or damaged area in order to accelerate healing and repair in horses suffering from tendon injuries, ligament injuries and/or arthritis. Clinical results and case studies will be presented.

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STROMAL VASCULAR CELL THERAPEUTICS: SORTING OUT FACT FROM FICTION

Presenter: John Fraser, MD
Authors: Zhu M, Shanahan R, Hicok KC, Fraser JK, Arm D
Cytori Therapeutics Inc

Non-culture expanded adipose stromal vascular cells (SVF) are increasingly gaining acceptance as a viable cellular therapy for treatment of ischemic injury and disease. As with any new technology, numerous claims are made regarding what the therapeutic is and what it can do. Some are confirmed by rigorous validation; others are unsubstantiated, causing confusion among users. Here we report our decade of experience in determining how much SVF is actually present in human adipose, and the cellular identity of SVF cells obtained after enzymatic release from tissue.

Methods: A mathematical model was developed based upon mean adipocyte and stromal vascular cell diameter to determine the potential number of nucleated stromal vascular cells obtainable per gram of fat. Model accuracy was validated by histomorphometric assessment of lipoaspirate. Enzymatic processing of >300 individual human donor adipose tissue samples was performed by either manual or automated enzymatic processing for 20-30 min at 37C. Validation of viable SVF cell number counting methods was performed by comparing cell number and viability obtained using a dual fluorescent dye live/dead staining hemocytometer method to use of a semi-automated Nucleo Counter device. Cell surface marker expression for major cell subpopulations was determined by flow cytometry with CD31, CD34, and CD45 antibodies.

Results: The predicted number of SVF cells obtained per gram of fat is in the range of 1.87 x 106 to 3.74 x 106 cells depending on the ratio of mature adipocytes: SVF. SVF density based upon empirical histomorphometric nuclei count assuming a 1:1 ratio was found concordant with our model at 3.68x106 ± 1.55 x 106 SVF cells per cc. Enzymatic processing yielded between ~1.5 x 105 to ~10.0 x 105 SVF cells/g tissue. NucleoCounter was found to be more reproducible than live/dead cell counting. Flow data demonstrated a large variation of major cell subpopulations between individuals.

Conclusion: Characterization of SVF is Not as straight forward as some might think. Contaminating irrelevant cell populations, high interpersonal sample variability, and use of nonvalidated analysis methods contribute to the generation of misinformation about SVF that could ultimately compromise patient safety and therapy.

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SERVA COLLAGENASE NB 6 GMP GRADE FOR ADIPOSE TISSUE DIGESTION

Presenter: Rowena A. Soriano, BS
Authors: Soriano RA, Torfi H
Invitrx Therapeutics Inc

The use of human adipose derived stem cells (ASCs) has become more common in cosmetic and regenerative applications. A reason for this is ASCs, as well as other types of cells such as endothelial progenitors, are higher in concentrations in adipose tissue than in other sources. In addition, adipose tissue is easily accessible through liposuction. Because of the cosmetic and regenerative use of ASCs in the clinical setting, a safe and effective procedure is necessary to assure optimal cell survivability and efficacy. The purpose of this study is to examine the use of Collagenase NB 6 GMP Grade and determine its safety of use, and quantitative yield of cells. Collagenase NB 6 GMP Grade meets the criteria described in the current version of the monograph Products with risk of transmitting agents of animal spongiform encephalopathies no. 1483 of the European Pharmacopoeia and has a certificate of suitability (No. R1-CEP 2002-003-REV 00). It is therefore safe to utilize in isolating stem cells from adipose tissue for re-implantation for cosmetic and regenerative procedures. Collagenase NB 6 GMP Grade was reconstituted in chilled 1x Phosphate Buffered Saline Solution 2.78mg/ml. Solution was used at equal ratio to adipose tissue. Digestion time was 25 minutes at 37°C yielding 5.04x10^6 cells per ml of tissue. Although further investigation is required to maximize optimized Collagenase NB 6 GMP Grade use, Serva's enzyme demonstrated significant digestive properties while taking into account the safety of the enzyme for clinical applications.

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CASE REPORT: OPTIMIZATION OF ROCHE LIBERASE IN THE ENZYMATIC DIGESTION OF HUMAN ADIPOSE TISSUE FOR THE ISOLATION OF STEM & REGENERATIVE CELLS

Presenter: Habib Torfi, MD
Authors: Soriano RA, Lamblet H, Mohammadi SA, Torfi H
Invitrx Therapeutics Inc

DID NOT PRESENT AT THE MEETING

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ADIPOSE STROMAL CELLS PREVENT AND RESCUE ACUTE HEMATOPOIETIC TOXICITY OF CIGARETTE SMOKE THROUGH SECRETION OF THE ANTI-INFLAMMATORY CYTOKINE TSG6

Presenter: Jie Xie, MD
Authors: Xie J, Feng D, Cook TG, Van Demark M, Schweitzer K, Johnstone BH, Petrache I, Broxmeyer HE, March KL
Indiana University School of Medicine

Introduction: Cigarette smoking (CS) increases the risk of numerous hematopoietic progenitor dysfunctions, including myelodysplasia, myeloid leukemia, and bone marrow (BM) transplant failure. No treatment is available due to limited understanding of the etiology. TNF±-induced Glycoprotein 6 (TSG6) has previously been established as an anti-inflammatory cytokine to inhibit TNF± secretion from macrophages. Secretion of TSG6 from adipose stromal cells (ASC) has not been described due to extremely low levels of secretion at baseline. Furthermore, its specific function in hematopoiesis remains unexplored.

Methods: Human ASC or vehicle control were injected (i.v.) into mice (C57BL/6) either before exposing to cigarette smoke (CS) for 1 day to examine marrow toxicity prevention or on day 2 of CS exposure for 3 days to examine marrow rescue. Secretion of TSG6 from ASC was measured by Western blot and enzyme-linked immunosorbent assay (ELISA). Expression of TSG-6 in ASC was knocked down by siRNA. BM hematopoietic progenitors were quantified by colony forming-unit assays.

Results: BM hematopoietic progenitors (e.g., granulocyte & macrophage progenitors, CFU-GM) declined acutely with CS exposure for 1 day (vs. air exposure, 65.7±5.3%, $p<0.05$, n=3). Human ASC prevented this decline (vs. air, 125±9.1%, $p>0.05$, n=3). Prolonged exposure (3 days) further reduced hematopoietic progenitors (vs. air, 47.1±3.3%, $p<0.01$, n=6), which was fully rescued by human ASC (vs. air, 95.8±4.9%, $p>0.05$). Secretion of TSG6 from ASC was low at baseline (6.0±6.5 ng/10ˆ5 cells/day) but can be strongly activated by tumor necrosis factor (TNF) Œ± (354.0±8.9 ng/10ˆ5 cells/day), an inflammatory cytokine known to be elevated in smokers (Kuschner et al, 1996). Knocking-down TSG6 secretion (>95%) resulted in complete loss of rescuing effect of ASC in smoker mice (vs. air exposure, 49.3±3.3%, $p<0.01$, n=6).

Conclusions: Cigarette smoking, even for brief time periods, causes dose-dependent acute suppression of hematopoiesis. Human ASC demonstrate a strong protective effect on hematopoiesis, both as a preventive and rescue regime. TSG6 supplied exogenously by ASC plays a significant role in the modulatory pathway; presumably by modulating the damage caused by smoke-induced inflammatory cytokines.

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ADIPOSE STROMAL CELLS PREVENT AND RESCUE ACUTE HEMATOPOIETIC TOXICITY OF CIGARETTE SMOKE THROUGH SECRETION OF THE ANTI-INFLAMMATORY CYTOKINE TSG6

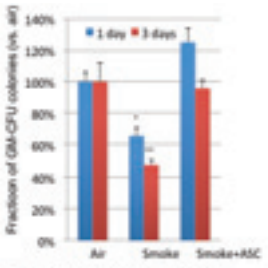

Fig 1. Increased toxicity with prolonged exposure, prevented / rescued by ASC injection. Mean ± SEM, n=3-6/group. *p<0.05, **p<0.01 (vs. air), ANOVA.

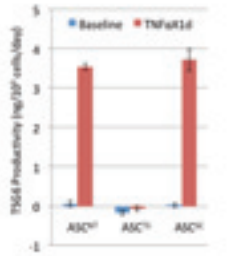

Fig 2. Knock-down of TSG6 production from ASC by siRNA. NT=Non-transfected ASC; TS=TSG6 siRNA; SC=scramble siRNA.

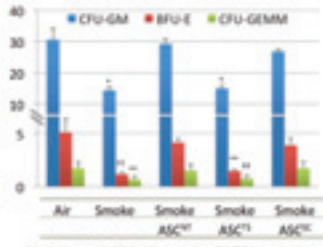

Fig 3. Loss of ASC effects after TSG6 knock-down. Mean ± SEM, n=6/group. *p<0.05, **p<0.01 (vs. air), ANOVA. CFU-GM: Granulocyte & Monocyte progenitors; BFU-E: Erythrocyte progenitors; CFU-GEMM: common progenitors

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HUMAN ADIPOSE DERIVED STROMAL CELLS IN A NOVEL 3D CULTURE SYSTEM FOR OSTEOGENIC DIFFERENTIATION: AN IN-VITRO AND IN-VIVO INVESTIGATION

Presenter: Brian C. Werner, MD
Authors: Werner BC, Shen FH, Liang H, Shang H, Katz AJ
University of Virginia

Introduction: Human adipose-derived stromal cells (hADSCs) are an excellent source of stem cells for bone regeneration. Current conventional techniques that grow cells in a two dimensional (2D) monolayer fail to reproduce the environment that is observed in vivo. Healthy mammalian cells in normal tissues are organized in complex 3D networks that display nutrient and signaling gradients. In recent years, 3D culture systems have been utilized to mimic tumor microenvironments in cancer research, however, there have been no studies exploring the ability hADSCs in a 3D culture system to undergo osteogenic differentiation.

Methods: HADSCs were prepared as either a 2D monolayer or as 3D multicellular aggregates (MAs). MAs were formed using a novel hanging droplet technique. Cells were treated in osteogenic medium (OM) in vitro and differentiation assessed using gene expression, histology, and microCT. In vivo investigation involved creating a muscle pouch in male athymic rats. Specimens were then pretreated with OM and surgically implanted either as (1) matrigel carrier alone, (2) carrier with human ADS cells in monolayer or (3) human ADS cells as MAs. In vivo evidence of osteogenesis was evaluated at 8 weeks with microCT and histology.

Results: HADSCs cultured by the hanging droplet technique successfully formed MAs. (1A) hADSCs cultured in monolayer or as 3D MAs retain the ability to undergo osteogenic differentiation as confirmed by increased ALP and OCN expression. MAs expressed increased differentiation potential and extracellular matrix production over the same cells cultured in monolayer. (1B-C, 2) When implanted in vivo, significantly greater bone volume and ECM was present in the implanted specimens of MAs confirmed by both microCT and histology. (3)

Conclusions: This is the first study to investigate the capability of hADSCs in a 3D culture system to undergo osteogenic differentiation. When compared to analogous cells in monolayer culture, the hADCs as MAs exhibit increased osteogenic differentiation and matrix mineralization both in-vitro and in-vivo. These findings support the concept that 3D culture systems remain not only a viable option for stem cell culture, but possibly a more attractive alternative to traditional culture techniques.

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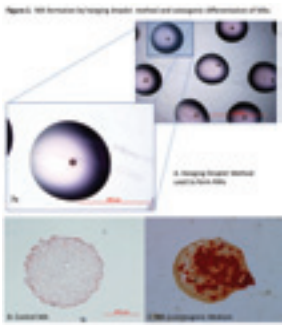
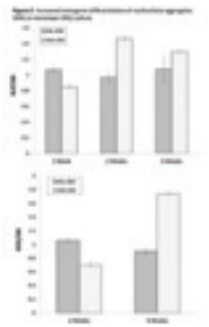
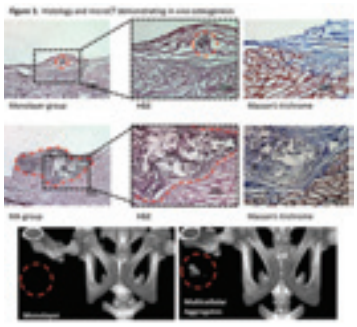
HUMAN ADIPOSE DERIVED STROMAL CELLS IN A NOVEL 3D CULTURE SYSTEM FOR OSTEOGENIC DIFFERENTIATION: AN IN-VITRO AND IN-VIVO INVESTIGATION







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VOLUMETRIC EVALUATION OF FAT GRAFT SURVIVAL IN THE RADIATED BREAST

Presenter: Kevin H. Small, MD
Authors: Small KH, Karp N, Choi M, Lee C, Levovitz C
New York Presbyterian Hospital

Introduction: Radiation therapy for breast cancer alters underlying tissue perfusion, which can result in skin discoloration and tissue fibrosis of the reconstructed breast. Management of these deformities remains a challenge, but fat grafting (FG) has emerged to correct contour in the radiated breast. Advancements have been made in techniques of fat graft harvest and delivery, but our ability to judge the incorporation of FG to radiated tissue remains limited. The following study applies 3D imaging to assess the stability of breast shape following autologous FG to the radiated reconstructed breast.

Methods: All patients receiving FG to the reconstructed breast from 2009-2010 were enrolled in the study. The average time interval between radiation and FG was greater than six months. FG surgery was performed using a modified Coleman technique. Preoperative and post-operative 3D scans were obtained on all patients. 3D imaging was performed using the Canfield VECTRA system with Geomagic software analysis. As previously described, breasts were isolated as closed objects and total breast volume was calculated on every scan.

Results: In the observed period, 59 non-radiated patients (88 breasts) and 26 radiated patients (28 breasts) received FG and associated images. Average fat injected to the breast was 91cc to the non-radiated breast and 110cc to the radiated breast. For the non-radiated breast, one month post-operatively, the breast had 74.09% volume retention and 1.30%/day resorption rate, and two months post-operatively, the breast had 70.03% volume retention and 0.57%/day resorption rate. For the radiated breast, one month post-operatively, the breast had 77.86% volume retention and 1.25%/day resorption rate, and two months post-operatively, the breast had 62.77% volume retention and 0.53%/day resorption rate. Comparison of percent volume retention and resorption rate at both time points were statistically insignificant.

Conclusion: FG is a useful method for breast contouring in the reconstructed radiated patient. Our data suggests that radiation therapy does not affect short term percent volume retention and resorption rate.

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POROUS DECELLULARIZED ADIPOSE TISSUE FOAMS FOR SOFT TISSUE REGENERATION

Presenter: Claire Yu, BASc
Authors: Yu C, Bianco J, Brown C, Watkins JF, Flynn LE
Queens University

Introduction: Porous bioscaffolds derived from the extracellular matrix (ECM) can support cellular infiltration and promote the cell signalling processes required for soft tissue regeneration. In previous work, decellularized adipose tissue (DAT) has been shown to provide an adipo-inductive substrate for human adipose-derived stem cells (ASCs) [3]. To engineer 3-D adipogenic scaffolds with a defined shape and volume, we developed non-crosslinked porous foams from solubilized human DAT for use in reconstructive surgery.

Materials & Methods: Human DAT was enzyme digested and acid solubilized [1,2]. The DAT foams were prepared using a controlled freezing and lyophilisation process. The porosity can be tuned by the DAT concentration and freezing temperature (Fig.1). The swelling, stability and structure of the foams were characterized. Foams were seeded with human ASCs (P2) and adipogenesis was assessed by RT-PCR, GPDH enzyme activity, and Oil Red O staining over 14 days. For all assays, (n=3, N=3). A preliminary in vivo study was performed to assess the early response to the seeded and unseeded DAT foams in a subcutaneous Wistar rat model.

Results: The foams fabricated at 50 mg DAT/mL frozen at -20°C exhibited the greatest stability, with minimal swelling and high water content. The in vitro cell culture studies indicated that the DAT foams were pro-adipogenic, with the strongest expression of adipogenic markers in the foams cultured in adipogenic medium. Similarly, high GPDH levels were observed for the induced foams, while the non-induced foams had comparable levels to the induced tissue culture positive controls. Oil red O staining showed extensive intracellular lipid accumulation on both the induced and non-induced foams. In vivo observations indicated that the DAT foams were stable and stimulated angiogenesis at early timepoints (Fig.2).

Conclusions: Porous human DAT foams are novel adipo-inductive, natural biomaterials that show great potential as 3-D bioscaffolds for volume augmentation. The fabrication process does not require stabilizing crosslinkers, which may be advantageous for clinical translation.

[1] Flynn LE. Biomaterials 2010;31(17):4715.
[2] Stevens FS. Ann. rheum. Dis. 1962;23:300.
[3] Turner AEB et al. Biomaterials 2012;33(18):44

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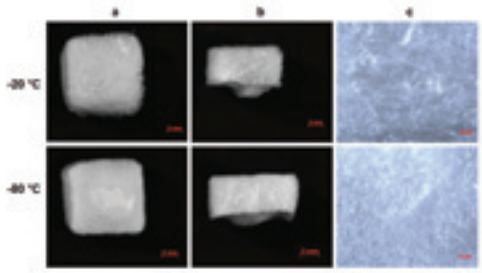
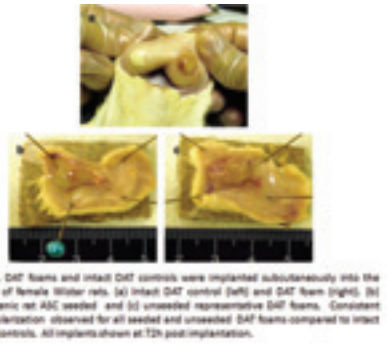
POROUS DECELLULARIZED ADIPOSE TISSUE FOAMS FOR SOFT TISSUE REGENERATION





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SENESCENT CELLS COMPROMISE FAT TISSUE FUNCTION

Presenter: Ming Xu, PhD
Authors: Xu M, Zhu Y, Pirtskhalava T, Giorgadze N, Baker DJ, Jensen MD, van Deursen J, Tchkonia T, Kirkland JL
Mayo Clinic

Introduction: Senescent cells with essentially irreversible loss of replicative potential accumulate in various tissues with aging. Cellular senescence is associated with a pro-inflammatory senescence-associated secretory phenotype. We tested the hypotheses that senescent preadipocytes accumulate with aging, contribute to age-related inhibition of adipogenesis and fat tissue inflammation, and whether eliminating them restores fat tissue function.

Methods: Senescent cell abundance was determined by assaying senescence-associated beta-galactosidase activity and p16Ink4A mRNA. Preadipocytes from lean subjects were X-irradiated or serially passaged to induce senescence. Non-radiated preadipocytes were stained with DiI and co-cultured with non-radiated (control) or irradiated preadipocytes. Preadipocytes were also cultured in conditioned media (CM) from control or irradiated preadipocytes mixed 1:1 with differentiation medium. Fifteen days later, the percent of target (DiI-stained and CM treated) cells with doubly-refractile lipid inclusions was determined. Macrophage migration in response to CM was assayed as well. INK-ATTAC transgenic mice with a p16Ink4A promoter fragment and FKBP-caspase-8 (ATTAC) were bred with BubR1 hypomorphic mice (BubR1H/H), which have accelerated age-related subcutaneous fat loss. Senescent cells are specifically eliminated from INK-ATTAC;BubR1H/H mice by administering AP20187.

Results: Senescent cells increased in fat tissues with aging and obesity. The percent of differentiated DiI-labeled preadipocytes was lower following exposure to senescent than control cells (N=6; 21.3% vs. 51.9%; p<0.0001; T-test). Differentiation was also impaired by CM from senescent preadipocytes. Macrophage migration was greater in response to senescent preadipocytes and primary preadipocytes from elderly lean or obese young subjects. Subcutaneous fat tissue loss was partially reversed by clearing senescent cells from progeroid INK-ATTAC;BubR1H/H mice with AP20187.

Conclusion: We found senescent cells accumulate in fat with aging, inhibit differentiation of adjacent preadipocytes, and induce macrophage infiltration, contributing to age-related fat tissue dysfunction. Eliminating senescent cells partially reverses age-related loss of subcutaneous fat.

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CHARACTERIZATION OF HUMAN ADIPOSE TISSUE-RESIDENT HEMATOPOIETIC CELL POPULATIONS: A NOVEL MACROPHAGE SUBPOPULATION WITH CD34 EXPRESSION AND MESENCHYMAL MULTIPOTENCY

Presenter: Kahori Kinoshita, MD
Authors: Doi K, Eto H, Kato H, Kuno S, Mineda K, Kinoshita K, Yang S, Yoshimura K
University of Tokyo

Introduction: Adipose tissue (AT) contains mature adipocytes and stromal vascular fraction (SVF) cells, including adipose stem/stromal cells (ASCs). We examined the cell composition of hematopoietic cells residing in human non-obese AT and analyzed AT-macrophages (ATM: CD45+/CD14+/CD206+).

Methods: SVF was isolated from human lipoaspirates by collagenase digestion. SVF and peripheral blood (PB) were investigated and sorted by flow cytometer (FACS) according to surface marker expression. Microarray analysis was performed for three monocyte/macrophage populations and ASCs. Sorted ATM subpopulations were further analyzed for mesenchymal differentiation capacity. Whole mount staining and immunohistochemistry of AT were performed.

Results: AT-resident hematopoietic cells were mostly either lymphocytes or ATM. AT-resident lymphocytes were predominantly composed of helper T cells and NK cells. Almost no B cells and few cytotoxic T cells were observed. Both CD206+ (>50%) and CD206- (<50%) macrophages (CD45+/CD14+) in AT were observed in SVF while CD206+ macrophages were not detected in PB, suggesting CD206 is a reliable marker for ATM. Histology showed that most of ATM located periendothelially, though a small number of them were seen in interstitial spaces between adipocytes. CD34+ ATM (11.1% of ATM) were detected in FACS and histologically localized in perivascular region. Then, three subpopulations (CD34+ ATM, CD34- ATM, and CD45-/CD31-/CD34+ ASCs) were sorted from SVF and examined by microarray as well as PB-derived circulating monocytes (CD45+/CD14+). The microarray revealed CD34+ ATM showed both characteristics similar to monocytes and ASCs. CD34+ ATM, but not CD34? ATM, grew on adherent culture and differentiated into multiple mesenchymal lineages. CD34+ ATM grew rapidly and lost expression of CD45, CD14 and CD206 by passage 3, resulting in similar expression profile to ASCs.

Conclusions: We found a novel CD34+ ATM subpopulation with multiple mesenchymal differentiation capacity. CD34+ ATM were localized in perivascular region and possessed both monocytes/macrophage and ASCs characters. It was suspected that a subpopulation of bone-marrow-derived hematopoietic cells may home and localize in AT, working as adipose progenitor cells.

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REGENERATION OF CRITICAL OSTEOCHONDRAL DEFECTS IN MINIPIG BY ADIPOSE-DERIVED STEM CELLS (ASCS) ON HYDROGEL OF OLIGO (POLYETHYLENE GLYCOL) FUMARATE

Presenter: Elena Arrigoni, PhD
Authors: Arrigoni E, de Girolamo L, Niada S, Di Giancamillo A, Domeneghini C, Dadsetan M, Yaszemski M, Vena P, Peretti GM, Brini AT
University of Milan

DID NOT PRESENT AT THE MEETING

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TRANSPLANTATION OF A CHIMERIC STEM CELL GRAFT: THE EX-VIVO ASSEMBLY OF A HYBRID STEM CELL GRAFT USING THE LGR6+ EPITHELIAL STEM CELL AND ADSC TO AUGMENT CELLULAR MASS AND ANGIOGENESIS ON ACELLULAR MATRICES

Presenter: Denver M. Lough, MD, PhD
Authors: Lough DM, Dai H, Yang M, Reichensperger J, Wetter N, Cox L, Harrison C, Neumeister MW
Southern Illinois University School of Medicine

Introduction: Discovery of the adnexal LGR6+ stem cell (LGR6+ SC) of the hair follicular bulge, a cell capable of producing all lineages of the skin, permits researchers to manipulate the fundamental proliferative and differential properties of this cellular entity for broader applications in tissue engineering and wound care. Adding the LGR6+ SC to an acellular matrix allows for ample expansion and the creation of a stem cell-derived epithelial surface. Furthermore, additive substrate and angiogenic support from the mesenchymal ADSc allows for the development of a chimeric ex-vivo hybrid graft system which can be readily transplanted into living organisms for use in wound care and reconstructive transplantation.

Methods: Using C57BL/6(UBC-GFP) and B6.Cg-Tg(CAG-mRFP1) murine tissues, we isolated both the LGR6+ epithelial SC and the mesenchymal ADSc, expressing GFP and RFP respectively using FACS. From these populations, we seeded a spectrum of acellular matrices in order to build an ex-vivo chimeric hybrid graft. Using confocal and scanning electron microscopy, we validated viable population of the grafts by the cells. We further evaluated wound healing and angiogenic properties with RT-PCR, protein immunoblots and within endothelial angiogenesis co-culture systems. Bioluminescent imaging demonstrated stem cell confluence within the graft as well as transplant viability into a Nu/Nu mouse.

Results: LGR6+ stem cells are fully capable of seeding a spectrum of acellular matrices and proliferating freely within the matrix structure as confirmed by imaging. Wnt1 and Wisp1, key adnexal stem cell transcripts, are augmented upon matrix substrate binding. The addition of the ADSc to the LGR6+ SC-seeded matrix also increases VEGF, FGF, TGFB, PDGF, PGF and P63 expression. Placement of the graft into an in-vitro endothelial culture shows enhanced angiogenesis toward the graft. Additionally, the chimeric graft is fully transplantable and viable in a Nu/Nu murine system as depicted by bioluminescent studies and gene array analysis.

Conclusion: Here, we suggest a novel role for a hybrid ADSc-supported LGR6+ epithelial stem cell graft for the development of an ex-vivo chimeric tissue body that is fully transplantable and capable of integration into native tissues.

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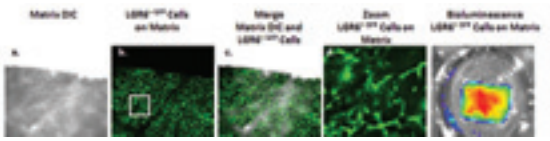

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OPTIMIZING HYPOXIC PRECONDITIONING OF MESENCHYMAL STEM CELLS FOR ANGIOGENIC THERAPIES

Presenter: Julie Beegle
Authors: Fierro FA, Beegle JR, Stewart H, Nolta JA
University of California Davis

In order to repair damaged tissues it is essential to establish blood vessels at the site of injury, which can then provide oxygen (O2), nutrients and different cell types to promote regeneration. Mesenchymal stem cells/multipotent bone marrow stromal cells (MSC) are great candidates for tissue repair. In many cases the cells do not contribute by direct differentiation into the damaged tissue, but rather promote angiogenesis through paracrine mechanisms. It has been suggested that pre-incubation of MSC in low O2 may have positive effects in this regard, by inducing increased secretion of angiogenic factors and improving the migration potential of MSC, among others. Nevertheless, it is unclear what specific O2 concentration and exposure time yields the most beneficial effects. To test this, we cultured MSC at varying concentrations of O2 (20, 10, 5 and 1%) for varying times (1 to 14 days). Using an MTT assay and propidium iodide cell cycle analysis, we found that proliferation of MSC decreases after incubation in hypoxia for 6 or more days, in proportion to the decrease in O2. Similarly, osteogenic and adipogenic differentiation of MSC are with decreasing O2-concentration, as assessed by alkaline phosphatase and Oil Red O measurements, respectively. Exposure to varying O2 levels for 1 to 4 days increases mRNA expression and protein secretion of the angiogenic factor VEGF, where the highest levels of secreted VEGF are obtained after culture for 2 days in 1% O2. Most importantly, pre-incubation for 2 days with reduced O2 concentrations leads to a dramatic reduction in cell apoptosis after a 6 day starvation period (in the absence of fetal bovine serum) as measured by flow cytometry, using Annexin V staining.

Overall, our findings strongly support the strategy of pre-incubation of MSC for 2 days in 1% O2 in order to promote the highest secretion of angiogenic factors and to achieve the highest yields of retention after transplantation.

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MULTI DRUG RESISTANCE PROTEIN BCRP PROTECTS ADIPOSE DERIVED STEM CELLS AGAINST ISCHEMIC DAMAGE

Presenter: Benno A. Naaijkens, MSc
Authors: Naaijkens BA, van Dijk A, Jurgens WF, Oerlemans R, Scheffer G, Visser FC, Schuurmans GJ, Juffermans LJ, van Milligen FJ, Niessen HW
VU Medical Center Amsterdam

Adipose derived stem cells (ASC) are promising candidates for cellular therapy, for instance after myocardial infarction. However, when transplanted in the infarcted heart, ASC are jeopardized by an ischemic environment, leading to cell death of the administered cells. Efflux of harmful substances performed by multi drug resistance (MDR) proteins is a well known characteristic of stem cells. Several MDR proteins have been identified on different types of stem cells, however, not much research to investigate the expression of MDR proteins in ASC has been performed. In this study, we determined the expression and functional activity of the MDR proteins breast cancer resistance protein (BCRP) and P-glycoprotein (P-GP) in ASC, in normal and ischemic conditions.

BCRP and P-GP expression was analyzed over culture (passage 2 to 6) by western blot analysis showing expression of BCRP, but not of P-GP. Interestingly, BCRP expression was most prominent in early passages and decreased during culture. Furthermore, immunohistochemical analysis showed BCRP expression on the nucleus, but not on the cell surface membrane. Using a specific substrate extrusion assay functional activity of BCRP was shown. Moreover, we showed that ischemia induced protein expression of BCRP in the nucleus and cytoplasm. Finally, using flow cytometry we determined that blockage of BCRP results in significantly more cell death during ischemia.

In conclusion, 1) ASC express BCRP, which decreases during culture. 2) BCRP expression increases in ischemic conditions, and 3) BCRP protects ASC from cell death in an ischemic environment. Therefore, cellular therapy during ischemia is optimal using low passage ASC when expression of BCRP is high.

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ENGRAFTMENT OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS AS PERIVASCULAR CELLS OF BIOENGINEERED MICROVESSELS ENHANCES ADIPOSE TISSUE FORMATION

Presenter: RueiZeng Lin, PhD
Authors: Lin RZ, Greene AK, Melero-Martin JM
Childrens Hospital Boston and Harvard Medical School

Purpose: Mesenchymal stem cells (MSCs) isolated from human adipose tissues can generate multiple end-stage mesenchymal cell types and are a promising cell population for regenerative therapies. However, controlling the engraftment of MSCs to maximize their differentiation potential in vivo is challenging. This study investigates whether implanting MSCs as perivascular cells enhances their engraftment and the formation of new adipose tissues.

Methods: Human MSCs were isolated from white adipose tissue. MSCs were subcutaneously injected into SCID-GFP mice in the presence or absence of human endothelial colony-forming cells (ECFCs; 2x106 total cells; 40:60 ECFC/MSC ratio) using 200 ul of Matrigel. MSC engraftment as well as the extent of adipose tissue formation were evaluated.

Results: Co-implanted ECFCs significantly increased MSC survival (2.5 folds) by reducing early apoptosis, a process that was partially mediated by PDGF-BB prior to the onset of blood vessel formation. Additionally, the presence of ECFC-lined microvessels enabled specific perivascular engraftment of PDGFR-beta+ MSCs, which showed higher clonal growth and multilineage potential than non-perivascular PDGFR-beta- MSCs. Thus, implants containing MSCs and ECFCs were able to generate significantly more adipose tissue (up to 20 folds) at 4 weeks than those containing only MSCs. The majority of newly differentiated adipocytes were from human origin (human specific vimentin+ and perilipin-A+), suggesting the implanted human MSCs underwent adipogenic differentiation.

Conclusions: Survival and functionality of implanted human MSCs were significantly improved by the presence of ECFCs, which led to more extensive adipose tissue formation. These results suggest that creating a perivascular niche for MSCs to engraft is critical to maintain MSC stemness in vivo. This two cell based approach has the potential to improve the outcome of future MSC therapies.

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ENGRAFTMENT OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS AS PERIVASCULAR CELLS OF BIOENGINEERED MICROVESSELS ENHANCES ADIPOSE TISSUE FORMATION

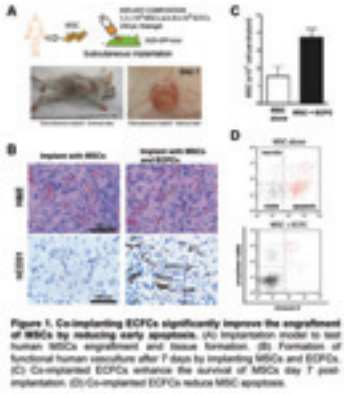


Figure 1. Co-implanting ECFCs significantly improve the engraftment of MSCs by reducing early apoptosis. (A) Implantation model to test human MSCs engraftment and tissue formation. (B) Formation of functional human vasculature after 7 days by implanting MSCs and ECFCs. (C) Co-implanted ECFCs enhance the survival of MSCs day 7 post-implantation. (D) Co-implanted ECFCs reduce MSC apoptosis.

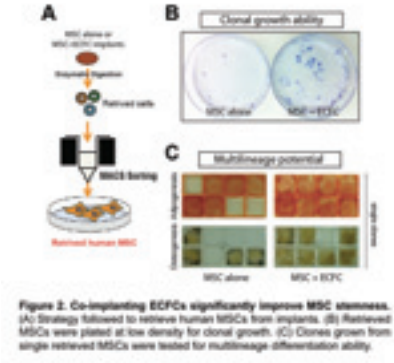
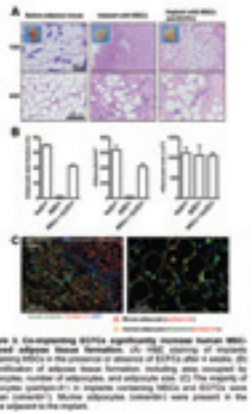


Figure 2. Co-implanting ECFCs significantly improve MSC stemness. (A) Strategy followed to retrieve human MSCs from implants. (B) Retrieved MSCs were plated at low density for clonal growth. (C) Clones grown from single retrieved MSCs were tested for multilineage differentiation ability.



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EFFECT OF HUMAN ADIPOSE-DERIVED STEM CELLS TREATMENT IN A MOUSE MODEL OF NEUROPATHIC PAIN

Presenter: Stefania Niada, Dr
Authors: Niada S, Rossi A, Arrigoni E, Franchi S, Panerai AE, Sacerdote P, Brini AT
University of Milan

DID NOT PRESENT AT THE MEETING

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ACTIVATION OF ADIPOSE-DERIVED STEM/STROMAL CELLS (ASCS) BY ADIPOSE TISSUE-DAMAGE ASSOCIATED FACTORS AND CHEMOKINES

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

Introduction: Chemokines and damage-associated molecular pattern molecules (DAMPs) are known to be involved in initiation of immune-response after tissue injury such as activation and/or attraction of resident or mobilized stem/progenitor cells. However few studies dealt with reaction of ASCs to those tissue damage associated factors.

Methods: Microarray for a whole body of gene expression was performed for freshly isolated ASCs, adipose tissue-resident macrophages (ATM) and circulating monocytes. Adipose tissue damage-associated factors were collected as adipose-tissue soaked buffer (ATSB), which were prepared by incubating a fragmented adipose tissue in buffered saline. Influences of SDF-1± and I-TAC as well as ATSB were examined on migration, proliferation and capillary-like network formation of ASCs.

Results: Microarray assay revealed that, among chemokine receptors, CXCR7 (receptor for SDF-1± and I-TAC) gene expression was specifically upregulated in ASCs compared with ATM and circulating monocytes. ASCs showed a significantly enhanced migration in vivo in response to SDF-1±, while I-TAC and ATSB slightly promoted ASC migration. Proliferation assay (XTT assay) show that SDF-1±, I-TAC and ATSB promoted proliferation of ASCs, but ATSB-treated ASCs presented significantly higher proliferation than SDF-1± or I-TAC. The capillary-like network formation was enhanced in all tested groups; slightly higher level of network formation was observed in ATSB-treated group compared to SDF-1± or I-TAC-treated group.

Discussions/Conclusions: The results suggested that SDF-1±, which is known to appear secondarily in wound healing, substantially promoted migration of ASCs, whereas tissue damage-associated factors, which primarily appear in the initial stage of wound healing, promoted ASC proliferation. It was also suggested that chemokines and damage-associated factors are involved in activation and attraction of ASCs in repairing process after adipose tissue damage.

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WNT PATHWAY ANTAGONIST, SECRETED FRIZZLED-RELATED PROTEIN1 (SFRP1) AS AN INDICATOR OF INNATE ADIPOGENESIS

Presenter: Sudheer K. Ravuri, PhD
Authors: Ravuri SK, Philips BJ, McArdle NL, Opene BA, Meyer EM, Pfeifer ME, Zimmerlin L, Donnenberg VS, Donnenberg AD, Marra KG, Rubin JP
University of Pittsburgh

Introduction: Fat distribution varies among individuals and adipose stem cells (ASCs) from different fat depots are known to display innate differences in levels of lipid accumulation. However, for soft tissue reconstruction, the ability to induce adipogenesis is vital. In our previous studies, we identified higher mRNA/protein levels of sFRP1 (Wnt antagonist) in fully differentiated ASCs, suggesting that overexpression of endogenous sFRP1 results in increased adipogenic potential. This study focused on evaluating variability in adipogenic potential among ASCs and its sub-populations among human subjects.

Methods: ASCs were isolated from 8 human female subjects by standard laboratory procedures and four distinct ASC sub-populations (Endothelial mature, Pericytes, Endothelial progenitor and Pre-adipocytes) were sorted by 8-color multi-parametric flowcytometry. Lentiviruses (sFRP1.GFP and sFRP1.siRNA) were constructed in HEK293T cells by transfection to study gain/loss of function of sFRP1 in ASCs by overexpressing and silencing sFRP1, respectively. Adipogenic differentiation potential and gene expression of ASCs and sub-populations under basal and sFRP1-overexpression conditions were measured by Adipored (lipid accumulation) and qPCR (PPARγ&FABP4). sFRP1 overexpressing ASCs were positively selected by flowcytometry using GFP reporter.

Results: ASCs and associated sorted sub-populations displayed varying levels of baseline sFRP1 mRNA expression in proportion to adipogenic PPARγ & FABP4 gene expression. Specifically, sFRP1 overexpressing cells demonstrated approximately 2-fold increase in adipogenesis and mRNA expression versus control (untransfected) cells. However, sorted (transfected) pre-adipocytes from four subjects showed nearly 5 to 8-fold higher adipogenic potential compared to unsorted (transfected) ASCs. Loss of sFRP1 function was confirmed by siRNA gene silencing.

Conclusions: Variability in adipogenesis among ASCs and its sub-populations was observed and sorted (transfected) pre-adipocytes showed much higher adipogenic potential. This study suggests: 1)Expression levels of sFRP1 could greatly influence expression of adipogenic genes (PPARγ&FABP4) by antagonizing Wnt pathway and 2)sFRP1 may be used as an indicator of adipogenic potential of ASCs.

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SINGLE-CELL TRANSCRIPTION STATE ANALYSIS OF ALDEFLUOR-BRIGHT AND -DIM ADIPOSE STROMAL CELLS AND PERICYTES USING FLUIDIGM MICROFLUIDIC ARRAYS

Presenter: Winters R. Hardy, PhD
Authors: Hardy WR, Datta K, Livak K, Lupov I, Traktuev D, Corselli M, Peault B, Srour E, March K
IUPUI

Adipose stromal cells (ASC) and pericytes are both readily and abundantly procured from adipose tissue, and each show promise for regenerative medicine. In some cases, clonal analysis has revealed subsets of cells within each group that differ in multipotency, paracrine properties, and immunomodulatory potential. The purpose of this study was to identify and profile individual cells within phenotypically similar subpopulations comprising ASC and pericytes. Our strategy is to establish criteria for cells possessing greater pluripotency and enhanced paracrine support, and establish a facile method to identify and select these regenerative cells in complex populations. Experimentally, we isolated from stromal vascular fraction 50 to 100 single cells belonging to each of four subpopulations using Index sorting, a BD Biosciences application that stores intensity values for each label on each cell. The four subpopulations consisted of both ASCs (CD31-/CD45-/CD34+/CD146-) and pericytes (CD31-/CD45-/CD34-/CD146+), isolated as either Aldefluor-dim or -bright cells, according to immunophenotype and ALDH1A activity, respectively. Following sorting, single-cell transcription state analysis was performed on Fluidigm microfluidic arrays, and principle component analysis and hierarchical clustering were employed to assess subpopulation heterogeneity based upon the multiplexed qPCR analysis of ~430 genes. These genes were selected to represent transcripts involved in chromatin remodeling, DNA methylation, protein turnover, metabolism, cell cycle status, and various transcription factors and functional/structural markers associated with self-renewal or differentiation along a adipocyte, myocyte, chrondrocyte, or osteocyte lineage. Transcripts for Pou5FI, Nanog, Sox2, cMyc, and Klf4, associated with pluripotency in embryonic stem cells, were detected in all four subpopulations. Additionally, comparative analysis of digital gene expression (i.e., either detected or undetected) across all ~430 genes revealed 52 and 41 genes that were differentially expressed between the Aldefluor-dim or -bright subpopulations of pericytes and ASCs, respectively. The functional implications of these results, and their potential application for regenerative medicine, will be discussed.

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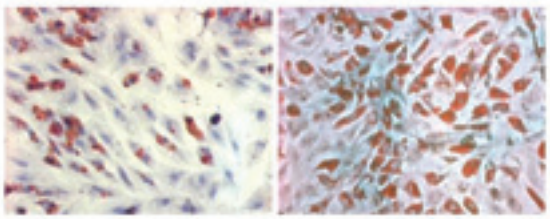
THE USE OF A HYALURONATE-BASED INJECTABLE HYDROGEL AS A DELIVERY VEHICLE OF STROMAL VASCULAR FRACTION FOR ADIPOSE TISSUE REPAIR: PRELIMINARY RESULTS

Presenter: Thomas Zarembinski, PhD
Authors: Zarembinshi T, Atzet SK, Doty N, Tandeski T, Tew WP
BioTime Inc

More than five million reconstructive plastic surgery procedures are performed each year in the United States, many of which were to repair soft tissue contour defects due to trauma, tumor surgeries, cancer therapies, therapeutic drug regimens, and congenital defects. Cell-based autologous adipose tissue engineering has emerged as a powerful approach to regenerate lost adipose tissue. While there has been some success in transplanting lipoaspirated fat for reconstructive and plastic surgical procedures, outcomes can be highly variable with up to 90% resorption rates of the implanted fat [1]. An alternative approach to improving patient outcome is to implant adipose-derived stem cells in a biocompatible, injectable matrix that both localizes the cells and promotes their survival. Here we will describe initial experiments with a hyaluronate-based injectable hydrogel (HyStem®-C) which, when combined with adipose-derived stem cells, improves adipogenesis both in vitro and in vivo. Whole genome expression analysis will also be presented that indicates this improvement is likely due to subtle changes in gene expression in adipogenic signal transduction pathways.

1. Thanik, V.D., et al., A murine model for studying diffusely injected human fat. Plast Reconstr Surg, 2009. 124(1): p. 74-81.



ADSCs on culture plastic (left) or HyStem-C (right)

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TEAR TROUGH DEFORMITY TREATMENT: FAT GRAFTING X HYALURONIC ACID. UNRAVELING BENEFITS AND PITFALLS

Presenter: Katarina Andjelkov, PhD
Authors: Andjelkov, K, Sforza, Zaccheddu R

Goals: The depression of the nasojugal fold may change the lower eyelid's shape, which is rather difficult to be corrected by a simple blepharoplasty. We present a retrospective study of 100 patients who had fat injections or hyaluronic acid in order to correct this deformity ("tear trough depression").

Methods: All 100 patients presented "tear trough" depression. The age range was between 36 and 62 years. Patients were offered fat transfer (FT) or hyaluronic acid injection (HAI). The treatment decision was made following the evaluation of cost, recovery time, risks and complications. Fat transfer was done under local anesthetics. The evaluation of results was made after 3 and 6 months following the procedure. In all FT patients, the fat was harvested and processed using the Puregraft® system. Patients who requested HAI, had Juvederm 3®. Around 0,3-0,5 cc of grafted fat or hyaluronic acid were placed in a deep plane; posterior to the orbicularis muscle.

Results: The results were evaluated by comparing before and after pictures and a patients' satisfaction rate. In patients with HAI (n= 50), the results at 3 months were excellent" in 90% of cases, "good" in 6%, and "fair" in 4%. After 6 months the results were "excellent" in 4% of cases and "good" in 26% and inexistent in 70% as per full reabsorption of the product. In patients with FT (n= 50), the ratings after 3 months were "excellent" in 96% of cases and "good" in 4%. After 6 months the results were "excellent" in 66% of cases and "good" in 22% and fair in 12%. At 6 months, a percentage of the injected fat had been reabsorbed, but the high satisfaction rate was sustained. In all cases, successful correction was achieved within the first 3 months. The complication rate was 2% with FT (overcorrecting) and 6% with HAI (hyper pigmentation, persistent edema and granuloma). (Picture 1)

Conclusion: The HAI procedure is comprehensible, quick, safe, cost effective and practical approach, with impermanent results. Patients with FT had fewer complications and despite an initial higher cost, the long-term result leads to a lower cost of treatments. Moreover, the preadipocytes can improve the tissue quality, especially of the skin. (Picture 2)

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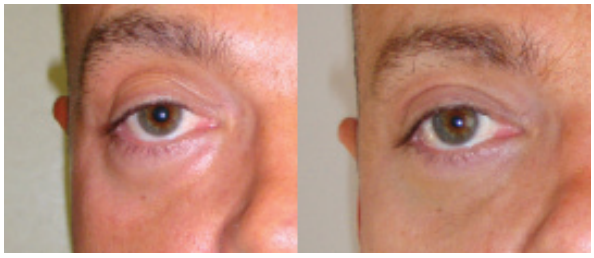
TEAR TROUGH DEFORMITY TREATMENT: FAT GRAFTING X HYALURONIC ACID. UNRAVELING BENEFITS AND PITFALLS



69
HIGH DEFINITION ULTRASOUND MONITORING OF CRYOPRESERVED AND FRESH FAT GRAFTS IN THE BREASTS

Presenter: Jeffrey M. Hartog, MD
Author: Hartog JM
The Adrecyte Regenerative Medicine and Surgery Center

Fat grafting to the breast for reconstruction and augmentation is becoming an increasingly popular technique. Modern techniques of fat grafting have shown increasing reliability and long term survival. Present techniques of fat grafting nevertheless do not result in 100% survival of the grafted fat, and the sequelae related to even small amounts of fat necrosis are the formation of oil droplets, oil cysts and calcifications. In general, small oil cysts or microcysts, less than 5mm in diameter are considered of no consequence and are expected to resorb within approximately one year. Larger oil cysts may be more problematic and are indicative of more extensive necrosis of the grafted fat, poor technique or both. These larger cysts may require drainage and may be more likely to result in significant dystrophic calcification.

Additionally, there is significant interest in utilizing cryopreserved fat to minimize the morbidity of repeated liposuction procedures when larger volume staged fat grafting is indicated.

High definition ultrasound is a relatively simple and economical method of monitoring breast tissues before, during, and after fat grafting, and particularly for cyst formation can be considered of similar or better value to other imaging techniques such as MRI or mammography.

High definition ultrasonography is capable of resolving oil droplets or microcysts of 1mm or less, and in conjunction with 3-D computer imaging has proved useful in monitoring the progress of fat grafting procedures for breast augmentation and reconstruction in our patients.

We have developed a repeatable scanning method for documenting and monitoring oil cyst formation and resolution in cases using either fresh or cryopreserved fat, or combinations of both.

Cases are presented demonstrating the usefulness of high definition ultrasonography. Additionally, the use of ultrasonography to observe intraoperative anatomy and graft placement is presented.

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CORRECTING DEFORMITIES AFTER BREAST AUGMENTATION WITH SILICONE IMPLANTS: DOES FAT GRAFTING HAVE THE X FACTOR?

Presenter: Marcos Sforza, MD
Authors: Sforza M, Andjelkov K, Zaccheddu R
Dola Park Hospital

Goals/Purpose: Breast Augmentation with implants is probably the most frequently performed cosmetic surgery in the world. Unfortunately, due to the fact that breasts have a natural asymmetry and silicone implants come in pre manufactured sizes and shapes, fine symmetry in volume and contour is often difficult to achieve. Moreover, as implants are foreign bodies, natural capsular contraction is increasingly a common complication. We present a retrospective study of 24 patients who had fat injections to correct deformities or asymmetries after previous breast enlargement surgery with silicone implants.

Methods/Technique: All 24 patients presented unsatisfactory results after breast enlargement surgery. We divided the patients in two groups: asymmetries (difference in volume, n=15) and deformities (difference in shape, rippling, capsular contracture, double bubble, n= 9). The age range was between 19 and 32 years. Patients were offered fat transfers to correct their problems as opposed to breast implant replacement. The evaluation of results was made after 6 months. In all patients, the fat was harvested and processed using the Puregraft® system. The fat was usually harvested from the abdominal area and the volume of fat transferred ranged from 160cc to 360cc, with average of 280cc per procedure.

Results/Complications: The results were evaluated by comparing before and after pictures and a satisfaction rate obtained from the patients. Patients with satisfaction rate after 6 months were excellent" in 83,3% of cases, "good" in 12,5%, and "fair" in 4,2%. The medical team evaluation after 6 months rated as "excellent" 75% of cases, "good" 20,3% and fair 4,2%. In all cases, a successful correction of the previous problems was achieved without any complications in this series.

Conclusion: The medical team was highly satisfied with the Puregraft® system as we could process larger amounts of fat, within shorter periods of time. Patients also had the benefit of a liposculpture to harvest the fat, which made major contributions to their satisfaction rate. At 6 months, a percentage of the injected fat had been reabsorbed, but the high satisfaction rate was sustained. This technique has minimal associated risks and complications and has been shown to be very effective.

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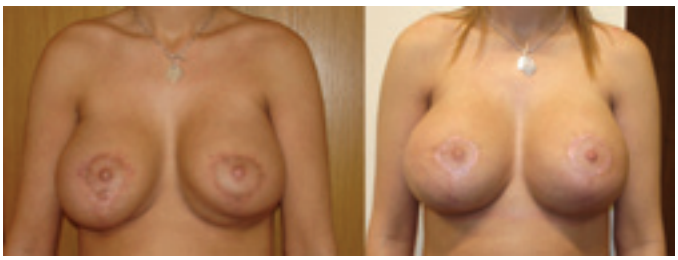
CORRECTING DEFORMITIES AFTER BREAST AUGMENTATION WITH SILICONE IMPLANTS: DOES FAT GRAFTING HAVE THE X FACTOR?



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WRITING AN INVESTIGATIONAL REVIEW BOARD PROPOSAL FOR FAT GRAFTING TO THE BREAST: WHY IT SHOULD BE DONE

Presenter: Brannon R. Claytor, MD
Author: Claytor BR
Atlantic Plastic Surgery

Goals: Development of novel techniques in surgery should be encouraged. It is amazing how rapidly treatment options progress; sometimes however, with little evidence of long term results. Solid tissue organ transplantation revolutionized medical care 40 years ago and autogenous tissue transplantation is similarly transforming treatment algorithms today. One of the most controversial topics of autogenous fat grafting is to the female breast. Currently there are multiple techniques for fat grafting to the breast, with little basic science backing up tissue viability or tissue incorporation. Instituting IRB protocols for fat grafting to the breast would ensure that appropriate steps are taken to ensure patient safety, provide open discussion about effectiveness and a process for evaluation outcomes.

Technique: Become CITI (Collaborative Institute Training Initiative) certified. Establish the questions you want answered and write a protocol and an informed consent. Establish a standard evaluation form. Select local physicians to act as a peer review group. Standardize the surgical technique. Establish imaging follow up. Submit your proposal to the local hospital IRB.

Results: Establishing an IRB protocol for fat grafting to the breast at the local hospital establishes a standard of care as well as an opportunity to critically evaluate results. Increased interest in publications and presentations with higher Levels of Evidence suggest that prospective examination of our results will result in better outcomes.

Conclusion: Fat grafting to the breast is a controversial procedure. However, the process of IRB protocols is not new to plastic surgeons. We became very involved in the process when silicone implants were removed from the market. Establishing an IRB protocol is lengthy, confusing, time consuming and burdensome. There are few guidelines and often little to no institutional support. We do not have a large corporation providing logistical support as we did with the breast implant issue. Despite these challenges, establishing IRB protocols at local hospitals will translate into better results, a more collaborative approach and increased patient safety. The process is a straight-forward algorithm.

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FAT GRAFTING IN AESTHETIC SURGERY OF THE FACE. GOOD FILLER MATERIAL

Presenter: Gennadiy Patlazhan, MD, PhD
Author: Patlazhan G
Institute of Plastic Surgery Virtus

DID NOT PRESENT AT THE MEETING

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COMPOSITE TISSUE-SPECIFIC BIOSCAFFOLDS FOR ADIPOSE TISSUE REGENERATION

Presenter: Lauren E. Flynn, PhD
Authors: Flynn LE, Cheung HK, Watkins JF, Amsden BG
Queens University

Introduction: Bioscaffolds for delivering adipose-derived stem cells (ASCs) via minimally invasive methods, while supporting cell retention, viability and adipogenesis, would have great utility in soft tissue regeneration. Building on previous work with decellularized adipose tissue (DAT) as a bioactive matrix [1], our aim was to investigate the human ASC response in composite bioscaffolds incorporating micronized DAT within injectable, photo-crosslinkable methacrylated glycol chitosan (MGC) or chondroitin sulphate (MCS) [2,3].

Materials & Methods: MCS and MGC pre-polymers were synthesized and characterized by 1H-NMR. Cryomilled DAT (0, 3, 5 w/v%) was added and composite scaffolds were crosslinked with UV light (Fig. 1) [2]. The sol content, equilibrium water content, and mechanical properties were measured. Human ASCs (P2) were encapsulated to assess cell viability and adipogenic differentiation over 14 days [1]. Viability was quantified using LIVE/DEAD staining. Adipogenesis was evaluated in terms of GPDH enzyme activity, end-point RT-PCR, and Oil red O staining. For all assays, (n=3, N=3).

Results: The characterization data indicated that the composites were highly hydrated, with mechanical properties mimicking soft tissues. The DAT improved ASC viability and retention, with the MCS+5 w/v% DAT composites having the highest cell viability and cell number at all time points. Additionally, the DAT enhanced ASC adipogenesis within the hydrogels. The highest GPDH levels were observed in the MCS+5 w/v% DAT scaffolds, as compared to all other groups and controls (Fig. 2). The RT-PCR results confirmed the expression of the adipogenic markers LPL, PPARg, and CEPBa, with the highest levels in the MCS+DAT composites. Oil red O staining demonstrated extensive intracellular lipid accumulation in the bioscaffolds with the DAT.

Conclusions: Photo-crosslinkable composite MCS+DAT bioscaffolds maintained viability while promoting adipogenesis, demonstrating great promise for ASC delivery and 3-D volume augmentation in plastic surgery. Incorporating the hydrogel phase enhanced cell retention and differentiation on the DAT. [1] Flynn L. Biomaterials, 2010, 31, 4715. [2] Amsden BG et al. Biomacromolecules, 2007, 8, 3758. [3] SJ Bryant et al. Macromolecules, 2004, 37, 6726.

74
ADIPOSE TISSUE-DERIVED ECM AND SVF CELLS AS BUILDING BLOCKS FOR TISSUE ENGINEERED CONSTRUCTS

Presenter: Hyun J. Paek, PhD
Authors: Iwami S, Shimoda C, Lee JQ, Kim C, Paek HJ
Tissue Genesis Inc

Autologous extracellular matrix (ECM)-based scaffolds can function in a pivotal role in tissue engineering by supporting cell adhesion and expansion, while eliminating the potential for transmission of xenogeneic pathogens and foreign body reaction. Adipose-derived stromal vascular fraction (SVF) represents a rich source of cells and growth factors critical for regenerative medicine. The SVF has the potential to create a number of tissue constructs to repair and replace afflicted organs.

We isolated SVF from lipoaspirated human adipose tissue using the fully automated Cell Isolation System™ and cultured them for further evaluation. Adipose tissue was subjected to various methods of decellularization to obtain adipose tissue-derived ECM (ATEM). Surface topography of fixed ATEM was observed using SEM and fiber diameter was measured using Image J software. Expression of growth factors by SVF cells were evaluated using a Human Growth Factor real-time PCR Array.

Morphologically, ATEM procured by enzymatic or mechanical digestion followed by chemical treatment appeared uniform, smooth, and loosely associated with other fibers. ATEM by freeze/thaw disruption with no chemical treatment appeared clumpy with globular particulate coating the surface and fiber diameters that measured significantly smaller (433+/-44 nm, $p<0.001$) compared to enzymatic (717+/-180 nm) or mechanical methods (616+/-73 nm). Comparative analyses within groups show no significant differences in fiber diameter, suggesting little to no patient variability. These results demonstrate that chemical treatment is necessary for complete lipid and particulate removal and cellular disruption can be achieved by mechanical disruption in place of enzymatic digestion, while maintaining fibrotic integrity. Quantitative gene analysis of cultured SVF revealed the expression of valuable growth factors, including VEGF, BMPs, and TGF-.

Future studies involve the characterization of self-assembled cellular aggregates and seeding onto ATEM, as they have been shown to display architectural and functional characteristics similar to that of native tissues. Preliminary studies show that SVF cultured in aggregate micro-molds exhibit high levels of CD31, suggesting cells exhibit adhesive and angiogenic properties.

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3D ASC SPHEROIDS AS TOOL FOR BASIC RESEARCH AND BUILDING BLOCKS FOR ADIPOSE TISSUE ENGINEERING

Presenter: Torsten Blunk, PhD
Authors: Blunk T, Muhr C, Dietl S, Goepferich A, Winnefeld M, Bauer-Kreisel P
University of Wuerzburg

3-dimensional (3D) spheroids of human adipose-derived stem cells (ASC) provide cell-cell and cell-ECM interactions in a more in vivo-like context, as compared to conventional 2D cell culture. Such spheroids can be utilized to study adipogenesis in a 3D environment, but they may also have the potential to serve as building blocks in adipose tissue regeneration. Here we present a) adipogenesis of ASC in 3D monoculture spheroids in comparison to conventional 2D culture, b) adipogensis in 3D coculture spheroids made from human ASC and human microvascular endothelial cells (MVEC), and c) the in vitro evaluation of different hydrogels as carriers for the spheroids and their ability to support adipose tissue development.

3D monoculture (ASC) and coculture (ASC:MVEC 1:1) spheroids were produced in 96-well plates using the liquid overlay technique. In ASC monoculture spheroids, adipogenesis proved to be by far less dependent on exogenous hormonal stimulation than in conventional 2D culture. High lipid content was demonstrated even after short-term induction (negligible in 2D), as shown by histology and triglyceride (TG) quantification. This was well reflected on the molecular level with increased transcription factor and adipokine gene expression (TaqMan array and qRT-PCR). In cocultures, MVEC assembled either in clusters or in network-like structures (confocal microscopy, immunohistochemistry). ASC within coculture spheroids also showed substantial adipogenesis (histology, TG quantification, TaqMan array), however, TG synthesis appeared to be locally inhibited in the immediate vicinity of the MVEC structures (histology, immunohistochemistry).

Exhibiting strong adipogenesis, as compared to conventional 2D culture, the ASC spheroids may be utilized to further elucidate the impact of cell-ECM interaction. Furthermore, the coculture spheroids appear suitable to investigate the molecular crosstalk of ASC and MVEC, likely contributing to the elucidation of the interplay between adipogenesis and vascularization which is regarded to be crucial for tissue development. Adipogenesis of ASC spheroids within different hydrogels was assessed and the materials will be discussed with regard to their suitability to serve as carriers for the spheroids in engineering adipose tissue.

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TRANSCRIPTOME ANALYSIS OF RECONSTRUCTED ADIPOSE TISSUES ENGINEERED FROM HUMAN STEM CELLS COMPARED TO NATIVE ADIPOSE TISSUES

Presenter: Marie-Ève Ouellette, MSc
Authors: Ouellette M, Vallée M, Bérubé J, Bossé Y, Fradette J
Genie tissulaire et regeneration LOEX Centre de recherche FRSQ du CHA universitaire de Quebec Universite Laval

The self-assembly method of tissue engineering takes advantage of the endogenous capacity of mesenchymal cells to produce and organize extracellular matrix components upon ascorbic acid stimulation, leading to natural and entirely human substitutes. Adipose-derived stem cells were used to reconstruct functional adipose tissues by this method. We investigated variations in mRNA expression between our in vitro 3D human model and native subcutaneous lipoaspirated fat. Whole-genome gene expression was performed using the Illumina HumanWG-6 v3 BeadChip. The expression values were log2-transformed and quantile normalized using the lumi package in R. The Significance Analysis of Microarrays method was used to identify genes differentially expressed (false discovery rate < 5% and fold change > 2.0). Pathway analysis was performed using Ingenuity pathway analysis 8.5 (IPA) and Fisher's exact test was used to calculate p value determining the probability that the association between the genes in the dataset and the canonical pathway is explained by chance alone. By assessing the mRNAs that were modulated in reconstructed adipose tissues compared to native adipose tissues, interesting pathways were revealed. As expected, basic biological processes associated with adipocytes were similar such as PPARgamma signalling (p value = 8.5E-04), fatty acid metabolism (p value = 0.001) and IGF-1 signalling (p value = 0.001). Interestingly, cell death-related processes were not increased in our in vitro tissues compared to native fat (p value= 0.006). The engineered tissues displayed an increased association with stemness-associated pathways (p value = 4.9E-04) compared to native fat, which will prompt us to further investigate that no propensity towards tumorigenicity is suggested. Globally, our human tissue-engineered adipose substitutes were closely related to native adipose tissue in regard to the major metabolic and biological functions. Such comparative analyses provide helpful information to ensure the safety profile of these tissues before grafting and to further improve their production if deemed necessary. Supported by NSERC.

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DIABETIC ADIPOSE TISSUE WITHIN A THREE-DIMENSIONAL HOLLOW FIBER-BASED BIOREACTOR

Presenter: Danielle M. Minteer, BS
Authors: Minteer DM, Lin YC, Young M, Over P, Gerlach JC, Rubin JP, Marra KG
University of Pittsburgh

Introduction: Our laboratory previously developed a hollow fiber-based bioreactor for 3D perfusion of adipose cells and tissue formation in vitro. Unlike traditional 2D culture, 3D tissue culture creates a stable system in which long-term culture of adipocytes is possible, providing a model useful for drug discovery screening in treatment of diseases such as type 2 diabetes mellitus. Our studies aimed to explore the metabolic activity and differentiation of adipose-derived stem cells from a diabetic human patient into adipocytes within our established 3D hollow fiber-based bioreactor model.

Materials and Methods: Adipose stem cells (ASCs) were isolated from discarded human fat tissue isolated from a Type II diabetic patient and expanded in culture. 80 x 106 cells were inoculated into the bioreactor and cultured at physiological conditions for 6 weeks. Weeks 1-2, cells were treated with DMEM/F12 culture medium, differentiation of ASCs into adipose tissue was implemented during weeks 3-4, and adipocytes were maintained within the bioreactor during final 2 weeks. Physiological parameters within the bioreactor were controlled and metabolic activity measured daily. To evaluate mature adipocytes in the system, immunohistochemical/histological analyses and qPCR were performed.

Results: AdipoRed/AlexaFluor 488/DAPI staining confirmed presence of mature adipose tissue within the bioreactor. Daily metabolic activity of the diabetic adipose stem cells behaved as expected initially; glucose uptake was severely lessened, lactate dehydrogenase levels indicated more cell lysis, and less lactate was produced as when compared to non-diabetic stem cells in the bioreactor system. After differentiation, however, adipocytes from a diabetic patient behaved similarly to previous adipocytes from non-diabetic patients we studied in the bioreactor.

Conclusions: Adipose stem cells from a diabetic patient were inoculated into a 3D, hollow fiber membrane-based bioreactor for 8 weeks. Metabolic behavior of the adipocytes after differentiation followed the same trends as adipocytes from non-diabetic patients. Future repetitive studies will provide further insight to the behavior of diabetic ASCs within a bioreactor and the potential application as a screening tool for diabetic drug testing.

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ADIPOSE-DERIVED STEM CELLS SEEDED ONTO ACELLULAR DERMAL AND PERITONEAL EXTRACELLULAR MATRICES AS INJECTABLE CONSTRUCTS FOR SOFT TISSUE RECONSTRUCTION

Presenter: Jolene E. Valentin, PhD
Authors: Valentin JE, Bechtel J, McLaughlin MM, Hoffman DF, Bowley MR, Goldman S, Marra KG, Rubin JP
University of Pittsburgh

Background: Injectable biomaterials are currently being investigated as a way to deliver biologically active compounds such as growth factors, provide three-dimensional architecture for mechanical support, and act as a carrier for the local administration of mesenchymal stem cells to the site of tissue reconstruction. Scaffolds derived from extracellular matrix (ECM) are currently used clinically for the repair and restoration of soft tissue defects. In combination with adipose-derived stem cells (ASCs) and autologous lipoaspirate, our aim of the study is to investigate acellular dermal (ADM) and acellular peritoneal matrices (APM) as injectable carriers for the local administration of ASCs to the site of soft tissue repair.

Methods: ASCs were isolated from human abdominal fat and cultured. Porcine acellular peritoneum matrix (APM) and acellular dermal matrix (ADM) were prepared in powder form with varying size distributions. ECM powders were characterized by SEM imaging, and mixed with human lipoaspirate to determine the consistence and injectibility through a 16G cannula. ASC viability and proliferation on ECM powders were determined. SEM images were taken of the ECM-ASC constructs to characterize the morphology and distribution of cells on the powders.

Results: The ECM powders mixed well with human lipoaspirate and injected easily through a cannula without clogging. Greater than 85% ASCs seeded onto APM and 95% onto ADM powders were viable after 14 days in culture. By day 3 of culture ASCs proliferated up to 260% when cultured on APM and 115% when cultured on ADM. ASCs cultured on APM and ADM powders showed that ASCs attached uniformly onto the surface of the ECM.

Conclusion: This study showed that ASCs are viable and proliferative on ECM powders and are injectable using a clinically relevant delivery system. Experiments are planned which will investigate secretion of growth factors and ASC differentiation, and a small animal study will inject grafts composed of lipoaspirate mixed with ECM-ASC constructs to determine volume retention, adipogenesis, vascularity, and tissue response.

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MEGA VOLUME FAT GRAFTING FOLLOWING AUTOLOGOUS BREAST RECONSTRUCTION

Presenter: Marwan H. Abboud, MD
Authors: Abboud MH, Dibo SA
CHU Tivoli

The purpose is to report a new conception in breast fat grafting following autologous breast reconstruction with pedicled myocutaneous flaps, achieving large volumes while maintaining a reduced operative time. The concept is to exploit the autologous flap as a matrix in order to optimize fat grafting. Fat is harvested, allowed to decant and is finally filled into large syringes attached to an injection gun. The technique consists of performing multidirectional and multilayered tunneling in the reconstructed breast, using the vibroliposuction machine, focusing on the flap in a way to fashion a matrix for fat grafting. A multiple holes thin cannula is utilized to carry out fat injection and fill the created tunnels. The final step consists of a vibration phase, by means of the vibrating cannula -hand piece unit disconnected from suction, the aim of which is to maximize diffusion of the injected fat in the created matrix.The technique was applied for 70 patients, between 2008 and 2011. The patient population included 31 immediate and 39 delayed reconstructions, subdivided into unilateral and bilateral cases, all performed under general anesthesia. Injections were performed between 3 and 12 months following reconstruction. The injected volumes ranged between 100 and 450 ml (AV 280 ml) per session and the operative time ranged between 30 to 90 min (AV 45 min). One to two injection sessions were required, performed at 6 months intervals. The follow up period ranged between 12 and 36 months. The average resorption rate was 50 % at 6 months follow up. Complications included liponecrotic cysts in 6.1 % of the Breasts. In this described technique,the autologous flap is exploited as a matrix for mega volume fat grafting. Multidirectional, multilayered tunneling of the recipient site followed by the vibrational phase optimize diffusion and survival of the grafted fat. The technique remains a reliable option to achieve breast mega volume fat grafting while maintaining a reduced operative time.

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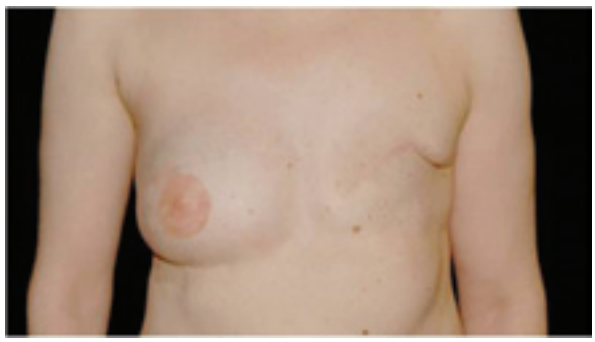
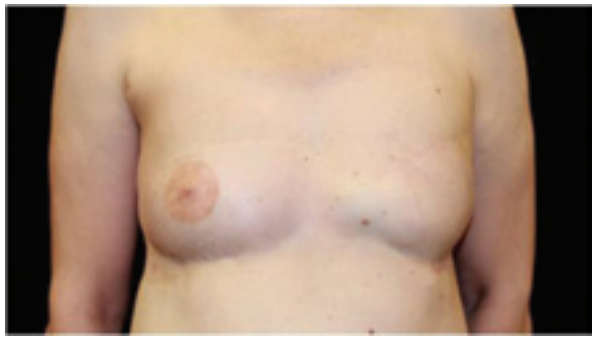
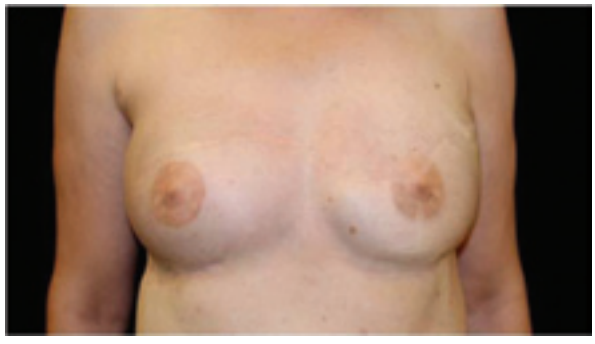
MEGA VOLUME FAT GRAFTING FOLLOWING AUTOLOGOUS BREAST RECONSTRUCTION







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BEAUTY AND THE DIEP: IMPROVED AESTHETIC RESULTS WITH AUTOLOGOUS FAT GRAFTING TO THE RECONSTRUCTED BREAST

Presenter: Sybile Val, MD
Author: Val S, Sadeghi A
Louisiana State University

Background: Breast reconstruction after mastectomy has changed dramatically over the last decade. With improvements in surgical technique and technology, both the expectation of the patients and surgeons have expanded to include not simply an acceptable reconstructed breast mound, but one with aesthetic properties as well. The use of autologous fat has gained popularity since the early 1980s with its success in aesthetic and reconstructive applications to the face and hand. With its known safety in the breast, autologous fat grafting (AFG) is now being used as an adjunct in the contouring of the reconstructed breast.

This study was designed to add to the growing literature of successful autologous fat grafting to the reconstructed breast. Our goal is to demonstrate that autologous fat grafting allows for an improved aesthetic result when used during second stage breast reconstruction after primary free flaps.

Methods: This is a retrospective review of 68 patients with 118 free flaps treated with autologous fat grafting between August 2010 to December 2011 by one senior surgeon. All patients underwent free flap reconstruction with either DIEP/SIEA, PAP, TUG, or GAP after either skin sparing mastectomy or modified radical mastectomy. Autologous fat grafting was performed three to six months after primary reconstruction. Patient follow up was twelve months with clinical and photographic evaluation of symmetry, superior medial fullness, projection, overall aesthetic appearance and patient satisfaction.

Results: All patients demonstrated clinical and photographic evidence of improved aesthetic results based upon symmetry, projection and patient satisfaction. No complications were encountered.

Conclusion: The improved aesthetic result provided by autologous fat grafting cannot be achieved with other surgical adjuncts. AFG allows the reconstructed breast to have a more natural appearance particularly assisting in providing adequate size and projection. The well vascularized bed of a free flap provides the ideal environment for successful placement and retention of AFGs. With the ease of procurement and transfer AFG is the ideal adjunct for ensuring a superior aesthetic result in the free flap reconstructed breast.

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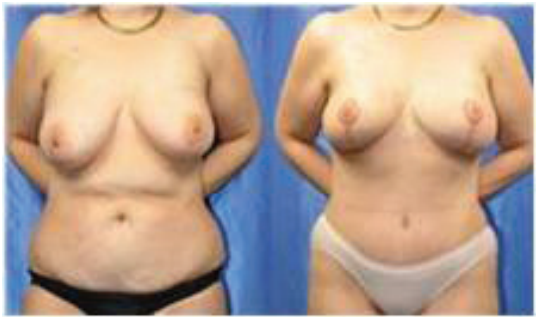
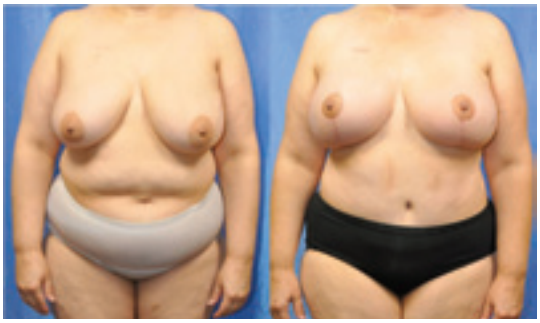
BEAUTY AND THE DIEP: IMPROVED AESTHETIC RESULTS WITH AUTOLOGOUS FAT GRAFTING TO THE RECONSTRUCTED BREAST





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A COMPARISON OF CELL ENRICHED FAT TRANSFER TO CONVENTIONAL FAT GRAFTING AFTER AESTHETIC PROCEDURES USING A PATIENT SATISFACTION SURVEY

Presenter: Brian Mailey, MD
Authors: Cohen SR, Mailey B, Wallace AM
University of California San Diego

Introduction: The role of stem cells in adult human fat is unclear. Pre-clinical studies have shown improvement in fat graft retention when stem cells are used to enrich the fat graft. At present, limited clinical studies have been performed comparing patient satisfaction with cell enriched fat transfer (CEFT) to conventional autogenous fat transfer (AFT) for aesthetic indications. Herein we present our preliminary data obtained from patient satisfaction questionnaires.

Methods: Patients undergoing autologous fat transfer received conventional AFT or CEFT. Study participants were surveyed for overall satisfaction, symmetry, deformity, scarring and pigmentation. Patient responses in the two groups were compared using the Student's t-test.

Results: Eighty-four patients underwent fat grafting procedures between January 2009 and September 2011. Of these, 17 patients (12 CEFT and 5 AFT) returned completed Patient Satisfaction Rating surveys. The most common site of injection was the face (N=14), followed by the breast (N=2), and neck (N=1). The overall mean satisfaction rate was 5.2 out of 6 (5.3 vs. 5.0 for CEFT and AFT, respectively, p=0.24) at a median follow-up time of 10.0 months (11.0 and 5.0 for CEFT and AFT, respectively). There were no significant differences in regard to deformity (5.1 vs. 4.7, p=0.49), symmetry (4.5 vs. 5.0, p=0.47), or scarring (5.3 vs. 4.5, p=0.23). However, pigmentation was dramatically improved in the CEFT vs. the AFT groups ($p<0.001$). Patient satisfaction scores in emotional health were rated 4.2/5 in the CEFT group and 4 in the AFT. 2.9/3 CEFT patients and 2.6/3 AFT patients would do surgery again. Interestingly, none of the patients in the AFT group noted skin pigmentation improvement, while 7/11 having CEFT noted improvement in skin pigmentation. Estimates of fat retention from clinical photos may show greater retention from CEFT.

Conclusion: CEFT to the face and body of aesthetic patients produces high satisfaction rates. Our preliminary data demonstrates similar satisfaction with regard to symmetry, scarring and deformity in patients treated with CEFT vs. AFT. However, a stark improvement in pigmentation was seen for patients treated with CEFT. Larger studies need to be done to better understand this phenomenon.

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ADIPOSE MATRIX-BASED SCAFFOLDS AS AN ALTERNATIVE TO FAT GRAFTING

Presenter: Iwen Wu, MS
Authors: Wu I, Conde-Green A, Graham I, Chae J, Elisseeff J
Johns Hopkins University

Introduction: Current clinical options for the repair of soft tissue defects often require the use of autologous tissue through flap reconstructions or fat grafting. An adipose-derived biomaterial could potentially provide an extracellular matrix-based alternative that can be used allogenically. Additionally, the adipose matrix retains the biological cues to promote regeneration of host adipose tissue.

Methods: The adipose matrix is prepared by removing lipids and cells through mechanical processing and chemical treatments using 3% peracetic acid for 3 hours and 1% Triton X-100 for 16 hours. A Boyden chamber migration assay and in vitro differentiation was carried out with adipose-derived stem cells seeded on the adipose matrix and compared with acellular dermis. A 12 week in vivo study with athymic mice (n=12) was used to compare the adipose matrix and fat grafts from human lipoaspirate with 200 ul subcutaneous injections of each condition.

Results: The migration assay showed an increase in adipose stem cell migration in response to the adipose matrix, showing the chemoattractant properties of the adipose matrix in encouraging stem cell migration. Adipose stem cells cultured on the adipose matrix showed improved adipogenic differentiation in comparison to cells grown on acellular dermis, suggesting that tissue specificity is retained in the respective scaffolds. In vivo, the acellular adipose matrix was compared with fat grafting from human lipoaspirate to evaluate the quality and extent of new tissue formation as well as the volume persistence. Upon completion of the study, we observed that the fat grafts suffered from significant central necrosis, resulting in microcystic changes and secondary calcification. In contrast, the adipose matrix did not face the same challenges and maintained a stable volume as a biological scaffold for host tissue regeneration. Host cells migrated into the scaffold and new adipose tissue formation was observed at the periphery of the implant.

Conclusions: Based on our studies, the adipose extracellular matrix is a promising biomaterial for soft tissue reconstruction that can take advantage of host tissue regenerative capacities for the repair of soft tissue defects.

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ENGRAFTMENT OF HUMAN ADIPOSE DERIVED STEM CELLS DELIVERED IN A HYALURONIC ACID PREPARATION IN MICE

Presenter: Isa Dietrich, MD, PhD
Authors: Dietrich I, Cochet O, Villageois P, Rodrigues CJ
Sao Paulo University Medical School

Purpose: To evaluate the implant of human adipose derived stem cells (ADSC) delivered in hyaluronic acid gel (HA), injected in the subcutaneous of athymic mice.

Methods: Control implants -HA plus culture media was injected in the subcutaneous of the left sub scapular area of 12 athymic mice. ADSC implants: HA plus ADSC suspended in culture media was injected in the subcutaneous, at the contra lateral area, of the same animals. With eight weeks, animals were sacrificed and the recovered implants were processed for extraction of genomic DNA, and histological study by hematoxilin-eosin staining and immunufluorescence using anti human vimentin and anti von Willebrand factor antibodies.

Results: Controls: Not visualized at the injection site. An amorphous substance was observed in hematoxilin-eosin stained sections. Human vimentin and anti von Willebrand factor were not detected. No human DNA was detected. ADSC implants - A plug was visible at the site of injection. Fusiform cells were observed in sections stained by hematoxilin- eosin and both human vimentin and anti von Willebrand factor were detected by immunofluorescence. The presence of human DNA was confirmed.

Conclusion: The delivery of human adipose derived stem cells in preparations of hyaluronic acid assured cells engraftment at the site of injection.

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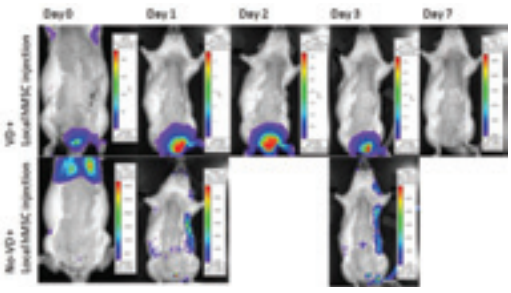
FATE OF STEM CELLS IN INJURY: IN-VIVO REAL TIME TRACKING OF MESENCHYMAL STEM CELLS (MSCS) IN A RAT MODEL OF STRESS URINARY INCONTINENCE (SUI)

Presenter: Kerry O. Grimberg, PhD
Authors: Molter J, Lennon D, Kavran M, Grimberg KO, Daneshgari F, Caplan AI, Lee Z, Flask CA, Hijaz AK
Case Western Reserve University and University Hospitals Case Medical Center

Previous studies have shown that human MSCs (hMSCs) improved incontinence in a rat model of vaginal distension (VD). This study sought to track periurethrally injected hMSCs using in vivo bioluminescence imaging (BLI). Bone marrow-derived hMSCs were transfected with a triple-fusion imaging reporter gene fluc-mrfp-ttk, encoding firefly luciferase, monomeric red fluorescent protein, and truncated herpes simplex virus type 1 sr39 thymidine kinase. One Sprague-Dawley rat underwent 4hr of VD followed by injection of triple-fusion labeled hMSCs (1.5 million cells). Another age-matched control rat received labeled hMSCs without VD. Animals were imaged immediately and daily after injection with BLI until the signal disappeared. Animals were then sacrificed and sections of the midurethral and anterior vaginal wall at the site of injection were processed and stained. Another group of rats underwent VD followed by local injection of Dil-labeled hMSC and were sacrificed 2 hours and 24 hours after hMSC injection (n=4 per group). Sections of the midurethra were prepared and imaged under immunofluorescent microscope. BLI signal increased within 3d in the pelvis of VD rats injected with hMSC. Interestingly, rats that received hMSC in the absence of VD demonstrated immediate localization to the lungs (Figure). The signal disappeared 8 days post-injection in both animals. Staining for Human-specific alu stain at 8 days did not demonstrate positive cells in the urethra or connective tissue around urethra. Dil-labeled cells 2 hours and 24 hours after local injection demonstrated positive preurethral signal that coalesced in the VD group at 24 hours. Transplanted hMSCs home to injured tissues where they exert their effect and proliferate. Cells were not found at the injection site 1 week later. Follow up studies are needed to determine the sequence of events contributing to distribution of MSCs at 1 week.



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THE CHEMOKINE, STROMAL DERIVED FACTOR-1 ALPHA, PROMOTES ENDOTHELIAL PROGENITOR CELL-MEDIATED NEOVASCULARIZATION OF HUMAN TRANSPLANTED FAT TISSUE IN DIABETIC IMMUNOCOMPROMISED MICE

Presenter: Saher Hamed, MD, PhD
Authors: Hamed S, Egozi D, Malyarova N, Keren A, Kruchevsky D, Dawood H, Ben-Nun O, Gilhar A, Brenner B, Ullmann Y
Technion Israel Institute of Technology

DID NOT PRESENT AT THE MEETING

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THE EFFECT FOR BONE REGENERATION WITH COMBINATION OF ADIPOSE-DERIVED STEM CELLS AND PLATELET-RICH PLASMA

Presenter: Morikuni Tobita, DDS, PhD
Authors: Tobita M, Orbay H, Hyakusoku H, Mizuno H
Juntendo University School of Medicine

Introduction: The goal of bone regeneration is to bridge a bone defect over healing procedures stable and durable. Adipose-derived stem cells (ASCs) with/without several scaffolds can differentiate into osteogenic cells. However the functional bone tissue regeneration is a future crucial issue for clinical therapy. Meanwhile, platelet-rich plasma (PRP) therapy represents an interesting biological technique to provide tissue repair by inducing chemotactic, proliferative and anabolic cellular responses. PRP could be an ideal biological autologous product providing a balanced combination of mediators able to improve the healing process. The purpose of this study was to evaluate the ability of bone regeneration with the combination of ASCs and PRP in a rat calvarial defect model.

Materials and Methods: The cultured ASCs, which were isolated from inguinal fat pads (Fischer rat), were mixed with PRP obtained from inbred rats before implantation. Bone defect (a diameter of 5mm) was generated at the left calvarial bone. The animals were randomly assigned to 4 experimental groups with 8 animals in each group as following, Group 1: ASCs with PRP implantation group, Group 2: ASCs with Type I collagen gel implantation group, Group 3: PRP implantation group, Group 4: Type I collagen gel implantation group. The samples were harvested at 4 and 8 weeks after implantation. Micro-CT imaging was used to assess the newly formed bone in the defect. The sections of samples were stained with hematoxylin and eosin.

Results: In the Micro-CT analysis of group 1, both the surface area and three-dimensional volume of newly formed hard tissue were significantly greater than other groups. The mean value of newly formed hard tissue surface area at 4 and 8 weeks after implantation of the Group 1 was 95% and 95%, the Group 2 was 75% and 65%, the Group 3 was 50% and 55%, and the Group4 was 44% and 55% respectively. The mean value of newly formed hard tissue three-dimensional volume in the 4 and 8 weeks after implantation of the Group 1 was 49% and 65%, the Group 2 was 39% and 33%, the Group 3 was 23% and 28%, and the Group 4 was 15% and 24% respectively.

Conclusion: This study demonstrates that the combination of ASCs and PRP could improve bone healing in a rat model effectively.

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EXPERIMENTAL MODEL OF ISCHEMIA-REPERFUSION INJURY OF A MUSCULAR FREE FLAP OF THE MUSCULUS LATISSIMUS DORSI OF DOMESTIC SWINE

Presenter: Patrik Richtr, MD
Authors: Richtr P, Liska V, Racek J, Trefil L, Lavicka P
Medical School and Teaching Hospital Plzen Czech Republic

Introduction: The transfer of free flaps in plastic and reconstructive surgery is currently experiencing considerable expansion. In these tissue transfers there is a change of ischemia and reperfusion: ischemia - reperfusion syndrome (I/R). It is a complex of changes influencing the function and morphology of tissue. All these processes could lead to damage of the transferred flap and in extreme cases the damage is irreversible and therefore noticeably prolongs the morbidity of the patient.

Methods: In this experimental study the authors expect creating the most complex and real model of ischemia reperfusion injury (I/R) of musculus latissimus dorsi (MLD) of domestic swine (15 animals). In each animal MLD is ambilaterally preserved, the muscle is nurtured only through vascular pedicle of thoracodorsal vessels. One MLD serves as revisory flap and the vascular pedicle of the other MLD is temporarily clamped (for 60 min.) which simulates I/R in real conditions. Subsequently, blood samples are taken in given time periods (before clamping and 0, +30, +45, +60 min. of reperfusion) both from a vascular pedicle vein and from artery (a. carotis comm.). In the samples the values of quantities are measured - Na, K, Cl, CK, AST, myoglobin, ICAM-1, VCAM-1, AGE, CB, TBARS, GSHPx, GSH, SOD, AOPP, isoprostans. Their AV difference is also surveyed. In the same time intervals bioptic samples from MLD muscular tissue are taken for homogenates in which the same oxidative stress enzyme activity is determined (see above).

Results: In blood samples in first 7 animals we found significant changes of pyruvate and creatin kinase. Significantly higher levels of lactate in the minute 60 and 90 in ischemic muscle compared to control muscle. No significant changes of oxidative stress markers.

Conclusion: The experimental model is successfully created, its further development will enable research and elimination of unfavourable I/R effects in free lobe tissue.

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ERYTHROPOIETIN IMPROVES FAT GRAFTING IN NUDE MICE MODEL

Presenter: Yehuda Ullmann, MD
Authors: Ullmann Y, Fishelzon O, Hamed S, Kruchevsky D, Sliman L, Gilhar A
Rambam Healthcare Campus

Background: Autologous fat grafting is a safe and common method for the filling of soft tissue defects and is currently used for both aesthetic and reconstructive purposes. The unpredictable and relatively high rate of post-grafting fat-resorption reduces the efficacy of this technique. This phenomenon is attributed to an inadequate vascularization of the grafted fat causing reduced fat cell take. Erythropoietin (EPO) has non-hematopoietic effects, and exhibits proangiogenic and antiapoptotic properties.

Hypothesis: EPO may improve long-term fat graft survival in mice.

Materials and Methods: Human fat tissue was obtained by aspiration from a volunteer. The aspirated fat was injected subcutaneously into the scalp of nude mice that were intra- and post-operatively treated with either low dose EPO (20 IU), high dose EPO (100 IU) or saline. The follow-up continued for 15 weeks before the engrafted fat tissues were dissected out and blindedly evaluated for volume, weight, VEGF content, extent of apoptosis, tissue integrity, fibrosis, inflammation and angiogenesis.

Results: EPO was proved to prevent fat resorption as reflected by the preservation of the transplanted fat weight and volume and the restoration of the fat tissue architecture. EPO increased new blood vessel formation via VEGF-induced angiogenesis, and decreased inflammation and fat tissue apoptosis in a dose dependent manner.

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THE LIPOINJECTION OF DEFECTIVE VOCAL FOLD: THE GROWTH KINETIC OF THE ADIPOSE TISSUE STEM CELLS (HATSC) AND THE VOICE OUTCOME

Presenter: Maria R. Marchese, MD, PhD
Authors: Machese MR, Fetoni AR, Lattanzi W, Almadori A, Salgarello M
Catholic Univeristy of the Sacred Heart

DID NOT PRESENT AT THE MEETING

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INVESTIGATION OF P107 DOWN REGULATION IN DETERMINING THE BROWN ADIPOCYTE LINEAGE

Presenter: Anthony Scimè, PhD
Authors: Isse M, Scimè A
York University

Excess white adipose tissue (WAT) is a major risk factor for metabolic diseases, whereas brown adipocytes have a profound beneficial effect by increasing energy expenditure. Improved metabolic function and decreased body weight is enhanced by the presence of brown adipocytes. In this study, we found that p107, an Rb family member, is at the crux in determining white versus brown adipose lineages in mouse. It was completely absent in brown adipocyte tissue depots and only expressed in the stem cell compartment of mouse WAT depots. Moreover, p107 has a stem cell autonomous role in brown adipocyte formation as demonstrated by the in vivo and in vitro formation of brown-like adipocytes in p107 knockout MEFs and knockdown mesenchymal stem cell lines. Contrary to mouse, there is no data available for the role of p107 in human white and brown adipocyte lineage determination. In human adipose-derived stem/stromal cells (ASCs), we found that the p107 expression pattern increased slightly and then gradually decreased over a time course of adipocyte differentiation, suggesting a role in adipocyte lineage determination. To test the putative role of p107 in human adipocyte lineage determination, we have generated a stem cell line, shp107-ASC, with a knockdown of p107 in ASCs. In summary, these results will provide important insight into the pivotal role for p107 in determining the brown adipose lineage in humans, essential for formulating therapeutic interventions for the treatment of obesity.

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SIRTUIN REGULATION OF HUMAN AGING IN PRIMARY ADIPOSE TISSUE

Presenter: Ivona Percec, MD PhD
Authors: Percec I, Dierov R, Auman D, Chang B
University of Pennsylvania

Understanding the molecular mechanisms responsible for human aging has become critically important as the world's population lives longer and expects a higher quality of life. To meet the medical challenges posed by the aging population, we must understand the pathways of normal human tissue aging and derive therapeutic interventions by modulating these pathways. Epigenetic regulation through chromatin modifications, histone acetylation, and DNA methylation plays a critical role in controlling gene regulation, cellular differentiation and aging. Sirtuins are a family of protein deacetylases that regulate the caloric restriction pathway and are also involved in adipose metabolism and cellular aging. Recent knock-out studies in model organisms have implicated SIRT1, SIRT3, SIRT6 and SIRT7 specifically in the regulation of cellular aging and adipose tissue metabolism. We hypothesized that the sirtuin genes play an important role in normal human tissue aging and that primary human adipose tissue can serve as a robust model with which to study human aging. Here, we validate the use of primary human adipose tissue in a cell-specific manner as an effective system with which to study the epigenetic aspects of aging in primary human cells. Further, we demonstrate that SIRT1, SIRT3, SIRT6, and SIRT7 genes are differentially expressed with advancing age in primary human adipose tissue in a cell-specific manner in adipocytes, stromal vascular fractions and adipose-derived stem cells. We observed predicted changes in the acetylation status of specific SIRT1, SIRT3, SIRT6 and SIRT7 target proteins in adipocytes, stromal vascular fractions and ASCs confirming that sirtuin activity is indeed compromised in aging adipose tissue. Intriguingly, certain protein targets demonstrated the onset of acetylation changes in midlife, while others a decade later, indicating that aging is a progressively orchestrated molecular response that likely begins in the fourth decade of life. Together our data suggest that adipose tissue is an excellent model for the study of human aging, the sirtuin genes are important regulators of normal human tissue aging, and that sirtuin dosage modulation in adipose-derived stem cells may be an effective therapeutic intervention in anti-aging medicine.

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OBESITY-ASSOCIATED DYSREGULATION OF ADIPOSE STEM CELL BIOLOGY INFLUENCES BREAST CANCER TUMORIGENESIS AND PROGRESSION

Presenter: Amy F. Lin, MPH
Authors: Lin AF, Semon JA, Strong TT, Rhodes LV, Shi Z, Santoke TT, Zhang X, Zhang S, McFerrin HE, Burow ME, Gimble JM, Bunnell BA
Tulane University School of Medicine

Adipose stem cells (ASCs) have been shown to influence breast cancer progression through a number of cellular mechanisms. However, with respect to obesity, the role of ASCs in the increased incidence of breast cancer in centrally obese patients remains to be determined. The goal of this study was to determine whether ASCs conditioned by their local microenvironment in obese patients possess properties that distinguishes them from ASCs isolated from lean patients. More specifically, ASCs were categorized based on BMI (BMI 18.5-24.99 or BMI > 30) and depot site (abdominal or non-abdominal). As ASCs secrete factors that aid in their ability to traffic to sites of inflammation, this study focused on the migration and invasion of ASCs towards breast cancer conditioned media. In order to investigate the mechanism by which these cells traffic to breast cancer, detailed mRNA and protein analyses and silencing of identified proteases with RNAi technology confirmed the role of calpains and MMPs in ASC invasion. Furthermore, since ASCs have been shown to influence tumor initiation and early tumor growth, the potential for ASCs isolated from different donors to affect tumorigenesis and tumor progression in an in vivo model was also assessed. ASCs were co-cultured and injected into the mammary fat pad of immunodeficient mice. Tumor latency and volume was influenced by the ASC donors obesity status and depot site of origin. Additional immunohistochemistry studies revealed a hormone dependent mechanism by which ASCs may influence the growth of breast cancer cells. In summary, the data from our work indicate that the donors obesity status and depot site of origin markedly influences the properties of subcutaneous-derived ASCs. More specifically, ASCs isolated from the abdomen of obese patients demonstrated enhanced invasion towards breast cancer and increase the tumor burden of breast cancer.

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COMPARING THE IMMUNOREGULATORY EFFECTS OF BONE MARROW- AND ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

Presenter: Lehao W. Wu, MD
Authors: Wu LW, Yuan N, Rubin JP, Lee WP, Brandacher G, Cooney DS
Johns Hopkins Medical Institution

DID NOT PRESENT AT THE MEETING

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AGE-DEPENDENT ALTERATIONS IN MIRNA PROFILES REVEALS DIFFERENTIAL EXPRESSION OF NF-KB AND MAPK IN ADIPOSE STEM CELLS

Presenter: Bruce A. Bunnell, PhD
Authors: Bunnell BA, Pandey AC, Semon JA, Kaushal D, OSullivan RP, Glowacki J, Gimble JM
Tulane University School of Medicine

Adipose stem cells (ASCs) play a central role in mediating endogenous repair of damaged tissues and are widely investigated as a therapy in the treatment of numerous diseases. Biological aging is a universal process that results in changes at the tissue, cellular and molecular levels. The role of microRNA (miRNA) in age-induced molecular alterations in ASCs derived from young and old human donors were investigated using an unbiased genome-wide approach. Human ASCs were expanded and total cellular RNA was isolated for analysis. The miRNA fraction was enriched and used to determine the expression profile of ASCs from each age group. Based on the miRNA expression, differences in donor ASCs were further investigated utlizing differentiation assays, Western blot, immunocytochemistry and bioinformatics. Biological aging resulted in reduced osteogenic and adipogenic potential in ASCs isolated from older donors, while cell size, complexity and cell surface antigen profiles remained unaltered with aging. Analysis of miRNA profiles revealed that small subsets of active mRNAs changed secondary to aging. Evaluation of miRNA showed significantly decreased levels of gene expression of inhibitory kappa B kinase (IkB), interleukin-1alpha, inducible nitric oxide synthase (iNOS), mitogen activated protein kinase/p38, ERK1/2, c-fos and c-jun in ASCs from older donors by both bioinformatics and Western blot analysis. Nucelar factor kappa B (NF-kB), myc and interleukin-4 receptor mRNA levels were signficantly elevated in aged cells. Immunocytochemistry demonstrated nuclear localization of phosphorylated NF-kB in young donors, however, ASCs from aged donors revealed a cytoplasmic localization. Westerm blot demonstrated significantly elevated levels of NF-kB subunits, p65 and p50 and AKT. These findings suggest that differential expression of miRNA is an integral component of biological aging in ASCS.

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COMPARISON OF HUMAN ADIPOSE-DERIVED STEM CELLS ISOLATED FROM DIFFERENT DEPOTS FOR APPLICATIONS IN CELL-BASED REGENERATION

Presenter: Valerio Russo, MS
Authors: Russo V, Belliveau P, Watkins JF, Hamilton A, Flynn LE
Queens University

Introduction: Adipose-derived stem cells (ASCs) meet the requirements for an ideal regenerative cell population. Given the presence of different fat depots in the body, each having unique characteristics, the focus of this study was on the comparison of human ASCs extracted from (i) subcutaneous adipose tissue, (ii) the omentum, (iii) pericardial fat and (iv) the thymic remnant under both normoxic (21% O2) and hypoxic (5% O2) conditions.

Materials & Methods: Cells were isolated from the different depots using established methods. Flow cytometry was used to characterize the immunophenotype of ASCs, as well as to assess cell proliferation with live/dead staining (Invitrogen) and cell counting. A colony forming unit (CFU) assay was used to estimate the proportion of early progenitors in each of the cultures. Finally, the differentiation potential was assessed for the adipogenic, osteogenic and chondrogenic lineages through enzyme activity quantification, gene expression analysis and staining techniques. For all assays, (n=3, N=3).

Results: Colony formation and proliferation were enhanced under hypoxic conditions for all sources. The CFU assay showed varying populations, with the thymic remnant yielding the lowest number of colonies and subcutaneous fat the highest. ASCs from all of the depots successfully differentiated along the three lineages. Hypoxic conditions enhanced chondrogenesis and decreased osteogenesis, but did not influence adipogenesis. Interestingly, despite the advanced age of the pericardial fat/thymic remnant donors (average 70 yrs), adipogenic differentiation was comparable to those extracted from the younger subcutaneous donors (average 46 yrs). ASCs from the omentum demonstrated higher levels of osteogenesis when compared to ASCs extracted from subcutaneous fat from the same donor.

Conclusions: Overall, our results demonstrate that the stem cell yield, as well as the proliferation and differentiation capacities of ASCs, vary between different adipose tissue depots and that oxygen tension is a key ASC mediator. An in-depth understanding of each ASC source will allow for the development of optimized cell-based therapies.

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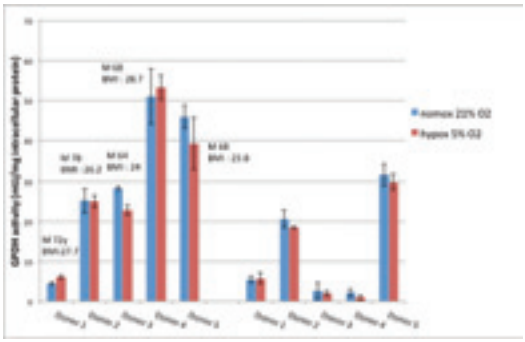
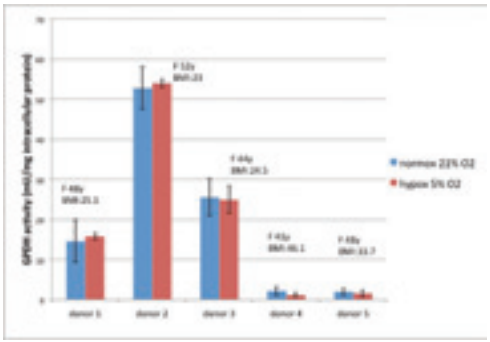
COMPARISON OF HUMAN ADIPOSE-DERIVED STEM CELLS ISOLATED FROM DIFFERENT DEPOTS FOR APPLICATIONS IN CELL-BASED REGENERATION





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CORRELATING BODY MASS INDEX TO ADIPOSE STEM CELL FUNCTIONALITY AND FAT GRAFT RETENTION

Presenter: Jacqueline Bliley, BS, MS
Authors: Bliley J, Grahovac TL, Nayar HS, Philips BJ, Courcoulas AP, Marra KG, Rubin JP
University of Pittsburgh

Background: Adipose-derived stem cells (ASCs) have shown promise in a wide range of clinical applications in the areas of soft tissue reconstruction, wound healing, and treatment of other ischemic conditions. Despite this, little is known about the effects of donor characteristics on harvested ASCs-factors which may influence the selection of patients for fat grafting procedures. We hypothesize that variation in BMI could lead to important functional variations in ASCs with downstream clinical impact.

Methods: Subjects were characterized by BMI into three groups: lean (BMI<30), obese (BMI>40), and massive weight loss (BMI reduction to <35 or ?20% loss of body weight). Harvested cells were exposed to hypoxic (>1% and 10% O2) and normoxic (21% O2) conditions. Supernatant was removed and assessed for growth factor secretion. Baseline differentiation and proliferation were also assessed. Lipoaspirate from each BMI category was processed into fat grafts and injected subcutaneously into the flanks of athymic nude mice. Grafts were explanted at week 8, and volume retention was calculated.

Results: The lean group displayed significantly more lipid accumulation than the obese group ($p < .01$) after a 14-day differentiation study. Lean group ASCs had significantly higher VEGF secretion under hypoxic conditions when compared to obese group ASCs ($p<.05$). Massive weight loss ASCs exhibited significantly lower VEGF secretion than lean ASCs in the lowest O2 concentration ($p<.05$), but not under 10% 02 conditions. Difference in VEGF secretion among BMI categories was not observed under normoxic conditions, suggesting that ability to handle ischemic stress may be a more important indicator of ASC function.

Discussion: Our findings suggest that variations in BMI can alter ASC behavior. We plan to further characterize these contributions by studying a more complete growth factor panel corroborated by RNA expression analysis. Recent studies have shown that endothelial progenitor cells are dysfunctional in obese subjects, a finding which was reversible after weight loss. Similarly, we found that ASCs isolated from massive weight loss patients partially regained their function. Future studies will investigate whether BMI can account for differences in fat graft retention.

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THE ROLE AND THERAPEUTIC POTENTIAL OF THE ADSC ISOLATED FROM HUMAN BURN TISSUE

Presenter: Hui Dai, MD, PhD
Authors: Dai H, Lough DM, Derby B, Wetter N, Reichensperger J, Cox L, Harrison C, Bueno R, Neumeister MW
Southern Illinois University School of Medicine

Background: Recent studies have suggested that adipose-derived stem cells (ADSC) within the hypodermis are preserved during and after severe burn injury. Additionally, it has been shown that these autologous stem cells can be isolated directly from the debrided skin of burn patients in quantities that can be clinically useful for burn wound bed repair and tissue regeneration. Here, we seek to understand the potential roles and possible applications for the ADSC isolated from human burn tissue so as to augment wound healing and skin regeneration.

Methods: We isolated and characterized viable human ADSCs from both burned and non-burn tissue controls using FACS. At first passage, we evaluated stem cell-related gene expression profiles, paracrine effects, angiogenic properties and wound healing capacity using both in vitro and xenograft Nu/Nu murine models.

Results: Our data indicates that after severe burn injuries, stem cells still present within tissue survive and are available for therapeutic application as verified using confocal microscopy and FACS. In addition, we found that ADSCs from burn patients could be expanded in vitro and differentiate into other cellular lineages using induction media. Interestingly, the proliferation of ADSCs from burn patients was found to be faster than control non-burned ADSC isolates and a subset of key embryonic cell lineage markers: ACTC1, PDX1 (IPF1), KRT15, MSX1, MYOD1, T were significantly up-regulated. These data suggest that burn isolated ADSCs may have greater functional potential than those cells isolated from non-traumatized fat. Further studies utilizing both angiogenesis and cytokine protein arrays showed significant up-regulation of VEGF, ICAM-1, HGF, PDGF- x, FGF-1, G-CSF, GM-CSF, IL-6, and TNF-R±, TGF-x3 when compared to controls.

Conclusion: Our results indicate that human ADSCs isolated from burn tissues demonstrate accelerated proliferative and developmental potential, which can be manipulated for clinical use. Here, we highlight the role and therapeutic applications of the burn induced ADSC as a patients own source of autologous progenitor cells for the treatment of skin regeneration and hope to develop a new strategy for cell-based therapy in burn, wound and reconstructive transplant settings.

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UNDERSTANDING THE DEDIFFERENTIATION PROCESS OF HUMAN MATURE ADIPOCYTES FOR OPTIMAL UTILIZATION OF THEIR STEM CELL CAPACITIES

Presenter: Julie Lessard, PhD
Authors: Lessard J, Pelletier M, Biertho L, Biron S, Moustarah F, Marceau P, Tchernof A
Centre de Recherche de l'Institut de Cardiologie et Pneumologie de Québec Laval University

Background: Ceiling culture is a relatively recent technique used to dedifferentiate mature adipocytes into fibroblast-like cells with a multipotent capacity. Little is known about the dedifferentiation process, especially in human cells. Omental and subcutaneous cells originate from different cell precursors and they are known to have distinct metabolic activities, indirectly suggesting possible depot differences in the dedifferentiation process. Our research objectives were to compare the dedifferentiation potential of human cells from omental and subcutaneous fat compartments and study their resulting cell lines to determine if they are both reliable sources of human multipotent cells.

Method: Omental and subcutaneous abdominal adipose tissue samples were obtained from patients undergoing gynaecological (n=4) or bariatric surgery (n=16). They were digested with collagenase, and mature cell suspensions were cultivated in ceiling culture using DMEM/F12 supplemented with 20% serum. Flasks were reversed at day 7 and maintained in DMEM/F12 with 20% serum until day 12. Media was collected at day 7 and day 12 to perform cytokine arrays.

Results: Both subcutaneous and omental mature adipocytes from normal weight or morbidly obese subjects had the capacity to dedifferentiate. However, slight differences were observed according to the compartments: subcutaneous adipocytes dedifferentiated earlier than omental adipose cells. Dedifferentiated cells from both depots had similar replication rates and capacity to redifferentiate into lipid-storing cells thereafter. During the process, both cell types secreted IL-6 and IL-8.

Conclusion: Subcutaneous cells reach the dedifferentiated state earlier than omental cells. Further analyses are necessary to evaluate the capacity of each of the different adipose tissue sources to differentiate into other cell lineages. Supported by a NSERC discovery grant (A.Tchernof).

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COMPARATIVE STUDY OF ADIPOSE DERIVED STROMAL CELLS CHARACTERISTICS FROM PEDIATRIC TO SENIOR HEALTHY DONORS

Presenter: Valérie Planat-Benard, PhD
Authors: Planat-Benard V, Abbo O, de Barros S, Arnaud E, Casteilla L
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Introduction: Adipose tissue mesenchymal stromal cells are very potential and effective to support tissue reconstruction and regeneration as demonstrated in various experimental models, recently leading to clinical applications. However most of the experimental data were obtained with cells from adult donors. In this study we performed a comparative analysis of various adipose derived cell populations, i.e. the crude stromal vascular fraction (SVF) and adipose stromal cells in primary culture (ASC-P0) or after 1 passage (ASC-P1) prepared from adipose samples obtained from pediatric to senior donors.

Methods: 50 pediatric adipose tissue samples were collected from newborns (0 to 12 months) and children (1 to 10 years old) undergoing programmed visceral surgery. Abdominal adipose tissue samples from young (20-35 years old) and older (over 50 years old) healthy donors were also collected to isolate cells from the SVF and processed in culture to obtain ASC. In vitro studies were performed to compare cell properties from the different groups of donors.

Results: Although, no major differences in SVF or ASC number, phenotype and proliferation were observed within the different groups of age, there is a decrease in the adipose differentiation potential with aging and a decrease in the ability of ASC from children and older donors to differentiate into endothelial cells compare to young adults. The microarray analysis of 28,869 genes revealed limited developmental differences in gene expression profile of ASC from adult versus children; around 717 genes (2.5%), 1.5-fold change with p-value<0.05. This signature was unexpected and is under investigation.

Conclusion: Taken together, these results show that in vitro characteristics of the adipose tissue derived stromal cells are maintained in each group of age with no or minor differences. However slight modifications could be depicted at the gene expression level that may provide clues to investigate molecular mechanisms that may be associated with therapeutic potential differences in tissue regeneration.

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DIFFERENTIAL PROPERTIES OF STROMAL VASCULAR CELLS DERIVED FROM ARTERIES, VEINS, SMALL VESSELS AND ADIPOSE TISSUE

Presenter: Santsun Yang, MD, Msc
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Introduction: Adipose-derived stem/stromal cells (ASC) are perivascularly localized, while vascular-wall resident progenitor cells were reported to localize in the adventitia of vessels and possess differentiation capacity into endothelial cells. In this study, we compared biological properties of stromal vascular cells originated from four tissues; 1) arterial wall, 2) venous wall, 3) small vessels in adipose tissue (AT) and 4) lipoaspirates (corresponds to ASCs).

Methods: Stromal vascular cells from the four tissues were collected from 11 healthy human with consent. Tissues were observed with HE staining, immunofluorescent staining for isolectin and acLDL, and imunostaining for CD34, CD31, CD271, CD146, CD140b and SMA. The four types of stromal cell populations were compared with proliferation assay, network formation assay and mesenchymal lineage differentiation, and fluorescence-activated cell sorting (FACS).

Results: In histology, CD34+ or CD271+ stromal cells located in the adventitia layer in all tissues. FACS showed that the cultured stromal cells partly presented CD34, but rarely CD271. EPC markers including Flk1, Tie2, CXCR4 and CD117 distributed differently positive in small number. Muse cell markers, CD105 and SSEA-3, were expressed at small percentages in all cell populations without significant difference. All stromal cell populations differentiated into mesenchymal lineages. Arterial stromal cells presented significantly higher (p=0.02) osteogenic differentiation capacity than other three populations and may take part in arthrosclerosis and related calcification, whereas ASCs showed the most potential in adipogenesis (p=0.016). Stromal cells from small vessels presented significance in capillary-like network formation assay (p=0.03).

Conclusions: Stromal vascular cells with distinct origins shared major biological properties such as surface antigen expression, but showed differential potency in mesenchymal differentiation such as osteogenesis. The understanding of these cells would be helpful in developing cell-based therapies and dissecting mechanistic underlying in pathological phenomena, though further researches are needed to elucidate.

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LYMPHATIC POTENTIAL OF ADIPOSE DERIVED STROMAL VASCULAR FRACTION IN WOUND HEALING

Presenter: Catherine J. Baty, DVM, PhD
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Background: Until recently the significance of the lymphatic vasculature has been underestimated, and thought to be primarily important for return of excess interstitial fluid from the bodys periphery back to the heart. In the last few years, its unique and active roles in inflammation, immunity, infection, and tumor metastasis has begun to be appreciated. Despite evidence of the importance of lymphatic vessels (LVs) in these crucial physiologic processes, their role in wound healing continues to be ignored. We hypothesize that lymphatic vessels play a crucial role in wound healing and are pursuing experiments in vitro and in vivo experiments to test this.

Methods: Human adipose derived stromal vascular fraction (SVF) was obtained from discarded tissue and isolated routinely. Immunofluorescent confocal microscopy and low magnification bright field microscopy were used to determine the lymphatic identity and morphology, respectively, of freshly isolated cells. Cultures were grown in an endothelial optimized media (EGM-2MV; Lonza) with and without VEGFC supplementation and re-evaluated for lymphatic identity and gross morphology and then tested using conventional in vitro functional assays: tube formation and migration. Assay results were compared to primary human adult dermal microvascular endothelial cells (LECs; Lonza).

Results: Untreated SVF showed detectable but low expression of lymphatic markers PROX-1 and VEGFR-3 by immunofluorescent microscopy. Lymphatic optimized SVF showed strong expression of LYVE-1 and moderate VEGFR3. No detectable expression of CD31 or podoplanin was detected by immunofluorescent microscopy. SVF cells cultured in EGM without lymphatic optimization formed a network initially but did not persist over 24 hrs as compared to the primary LEC and LOSVF cells. LOSVF cells showed more sprouting and more cellular structures than the LECs. Similarly LOSVF demonstrated enhanced motility relative to SVF.

Conclusions: Preliminary in vitro studies show that ADSVF has strong lymphatic endothelial potential and supports the potential for in vivo wound healing.

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ADIPOSE STEM CELLS ALLEVIATE BARRIER DYSFUNCTION OF ENDOTHELIAL MONOLAYERS

Presenter: Natalia V. Bogatcheva, PhD
Authors: Bogatcheva NV, Merfeld-Clauss S, March KL
IUPUI

Endothelial barrier dysfunction is known to contribute to the development of pulmonary edema and the impairment of gas exchange, major clinical manifestation of Acute Lung Injury and Adult Respiratory Distress Syndrome. The loss of endothelial barrier can be induced by the exogenous factors (bacterial toxins), several host inflammatory cytokines, and products of oxidative burst released by neutrophils. Here, we used human pulmonary endothelial cell (EC) monolayers to evaluate the potential effect of adipose stem cells (ASC) on regulation of endothelial permeability. To assess endothelial permeability, we used 1) Electric Cell-Substrate Impedance Sensor (ECIS), and 2) Transwell chambers, where EC were grown on polyester inserts and tested for permeability to FITC-dextran. We have shown that, similar to other fibroblasts, ASC increase permeability of endothelial monolayers to water and ions if they are allowed to establish a direct contact with EC; while in contrast, EC monolayers in direct contact with ASC displayed attenuated permeability to macromolecules. To study the effect of ASC on EC monolayer in more detail, we established ASC/EC co-cultures in which ASC were not allowed direct contact with EC. EC monolayers, thus grown in proximity to ASC, displayed markedly reduced FITC-dextran permeability and re-arrangement of junctional organization in response to H2O2. These data suggested that ASC exert significant effects on EC monolayer barrier function via secreted factors. To confirm this hypothesis, we pre-treated EC monolayers with ASC conditioned media (ASC-CM). Our data show that ASC-CM -pretreated monolayers display enhanced basal barrier and attenuated barrier dysfunction in response to H2O2. We conclude that both ASC and ASC-CM alleviate H2O2-induced barrier dysfunction. These data prompt further investigation of the potential application of ASC for the endothelial barrier stabilization in clinical situation where barrier dysfunction can be detrimental.

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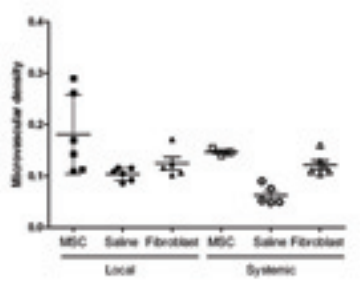
INCREASED ANGIOGENISIS IN URETHRAL TISSUES AFTER TREATMENT WITH MESENCHYMAL STEM CELLS (MSCS) IN A RAT MODEL OF STRESS URINARY INCONTINENCE (SUI)

Presenter: Adonis Hijaz
Authors: Izgi K, Isariyawongse J, Tasdemir S, Tasdemir C, Kavran M, Grimberg KO, Daneshgari F, Caplan AI, Hijaz AK
Case Western Reserve University and University Hospitals Case Medical Center

We recently demonstrated improved functional continence in a rat model of SUI (vaginal distension [VD]) after treatment with MSCs. The current study evaluated angiogenesis in urethral tissue after periurethral and systemic injections of human MSCs in our VD model. Adult female Sprague-Dawley rats (n=20) underwent serial VD for 4hr followed by immediate treatment with either periurethral or systemic injection of either 0.1mL hMSCs (10^7 cells/mL;n=60), 0.1mL rat dermofibroblast (10^7 cells/mL;n=60) or 0.1mL saline (n=60). Leak point pressure was measured 4, 10, or 14 days (n=20 each treatment group at each time point) after VD and treatment. Rats were sacrificed and urethras harvested for morphometric evaluation. Sections were stained for CD31 (an endothelial cell marker) and Masson's Trichrome stained cells were used to analyze vascular density. Slides were scanned with Leica Image Scanner and imaged under immunofluorescent microscope. The images analyzed using Image-Pro Plus. Two midurethral sections were randomly chosen from each rat for quantitative analysis of microvascular structural density measured in pixels.The ratio of vascular structures to the total cross sectional area was determined for each group. Microvascular density in the urethra was increased after both local ($P<0.05$) and systemic-injection of hMSCs after VD compared with saline treated rats (Figure). Injection of dermofibroblasts did not increase microvascular density compared with saline treated rats. Our results demonstrate increased urethral angiogenesis after systemic and periurethral injections of hMSCs, but not dermofibroblasts in a rat model of SUI. These findings suggest that increased vascularity can be one of the mechanisms involved in improvement of urinary continence through MSC therapy. The probable series of events including paracrine and differentiation pathways need to be investigated to fully understand the exact mechanism of MSC actions in increasing angiogenesis in this model of SUI.



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LONG-TERM REMODELING AND STABILIZATION OF ADIPOSE TISSUE AFTER NON-VASCULARIZED GRAFTING

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

Introduction: We previously reported cellular events during dynamic remodeling after fat grafting in mice model. We have shown early death (even on day 1) and subsequent replacement of adipocytes in the early phase of adipose remodeling. In this study, we analyzed long-term changes in fat grafting in an experimental mice models as well as clinical samples.

Methods: Inguinal adipose pad was transplanted under the scalp in mice, and was harvested at 1, 2, 4, 8 and 12 weeks. The tissue was evaluated by immunohistology for perilipin, Ki67 and CD34. Clinical samples of oil cysts resulted from necrosis of grafted fat were also immunohistologically evaluated as well. In addition, sequential changes of CT images and mammography were examined for oil-cysts and calcifications in post-lipoinjection patients.

Results: In mice model, most adipocytes except for those superficially localized in the periphery died during the first week. Ki67+ proliferating cells appeared from the periphery as early as 1 week, its number reached peak at 4weeks, and only macrophages surrounding oil droplets were Ki67+ after 12 weeks. At 1-2 weeks, inflammatory cells infiltrated into the tissue, but unexpectedly some of them surrounding dead adipocytes expressed CD34+. The CD34+ macrophages around oil droplets were observed even at 12 weeks. Clinically, oil drops changed in size until 1 year, cyst wall was formed by 1 year, and the cyst did not change in size thereafter, suggesting that grafted fat tissue is stabilized by 1 year. Oil cyst wall was consist of six components, internal fibrous layer, degenerated fat, healthy fat, vasculature zone, degenerated fat, and outer fibrous layer. Inflammatory cells markedly observed at the outer fibrous layer. Mammography findings suggested that calcification appears as early as 1 year and can further develop until 5 years.

Discussions/Conclusions: The results suggested that not only ASCs but macrophages plays pivotal roles in dynamic remodeling and stabilization processes after fat grafting. CD34+ macrophages were strongly involved in the repairing process, though its functional difference from regular (CD34-) macrophages remains unclear. Low-grade chronic inflammation in the wall of or around oil cysts may induce further calcification even after 1 year.

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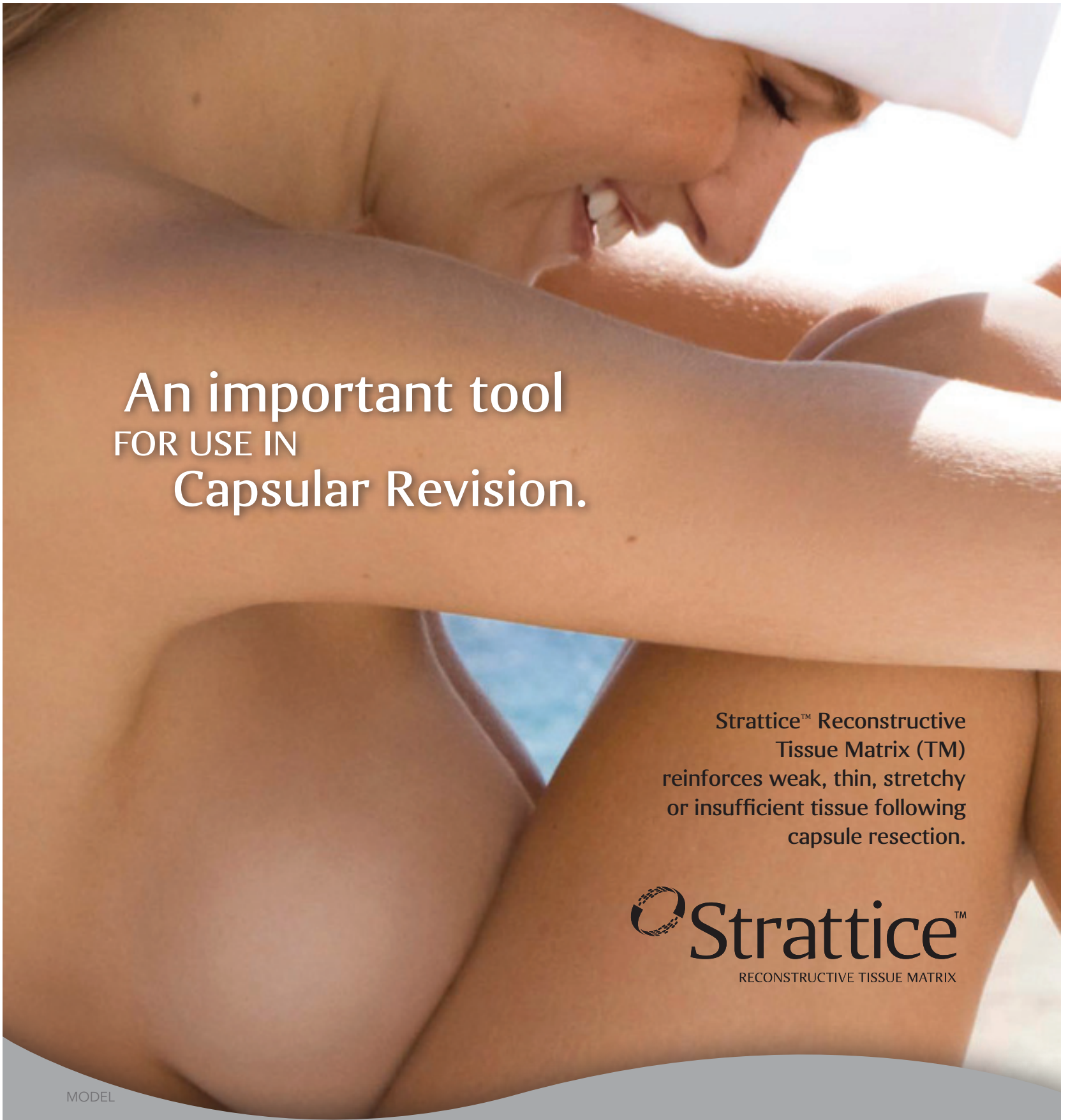

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