IFATS Miami 2017 Conference

15th Annual IFATS Meeting

IFATS

International Federation for Adipose Therapeutics and Science

November 30 - December 3, 2017
Loews Miami Beach Hotel
Miami, Florida
www.ifats.org

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IFATS
International Federation for Adipose Therapeutics and Science

Message from the President:

It is my distinct honor and pleasure to welcome each and every one of you to Miami Beach for the **15th Annual IFATS Meeting**!

The program committee and meeting organizers have been hard at work assembling an exemplary program to be showcased within a gorgeous venue. The meeting will provide an enriching platform to stimulate new ideas and collaborations. The agenda includes invited guest speakers who will provide overviews of current hot topics, presentations of research advances made by meeting participants as well as topical informational seminars presented by representatives from industry.

I encourage all participants to take full advantage of the rich daytime program as well as the many opportunities to connect with others during breaks. There will be additional opportunities to socialize and network at the end of each day, especially Saturday night with the White Nights Party at Nikki Beach.

Hopefully all of you will attend the entire four days of the meeting. You especially don't want to miss the Sunday morning session, which leads off with a special presentation by Aubrey de Grey who will discuss the science of longevity and rejuvenation medicine. Dr. de Grey's presentation is sure to be stimulating and thought provoking.

I want to end by thanking each of you for attending this year's meeting. It is the members who make IFATS a great society. I want to encourage everyone of you to become involved in the organization through serving on the many important committees. This will ensure that IFATS maintains its influential status as an internationally recognized society.

Sincerely,

Brian Johnstone, PhD
IFATS President & Program Chair

SCIENTIFIC PROGRAM COMMITTEE

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Jeffrey Hartog, MD, DMD
Marco Helder, PhD
Joseph Itskovitz-Eldor, MD, DSc
Brian Johnstone, PhD
Adam Katz, MD, FACS
Lauren Kokai, PhD
Malgorzata Kolenda, PhD
Gorana Kuka Epstein, MD
Janice F. Lalikos, MD, FACS
Hebert Lamblet, MD
Keith March, MD, PhD
Kacey G. Marra, PhD
Richard Martin, PhD
Susanna Miettinen, PhD
Ali Modarressi, MD
Ivona Percec, MD, PhD
Stephen Ray, MD, FACS
Ricardo Rodriguez, MD
Brooke Seckel, MD
Shigeki Sugii, PhD
Nir Shani, PhD
Sammy Sliwin, MD, FRCSC
Filip Stillaert, MD, FCCP
Deborah Sullivan, PhD
Dmitry Traktuev, PhD
Ning Yang, PhD
Kevin Zwezdaryk, PhD

INVITED SPEAKERS AND SESSION MODERATORS

Thomas Albini, MD
Katarina Andjelkov, MD, PhD
Torsten Blunk, PhD
Robert Bowen, MD, FCCP
Bruce Bunnell, PhD
Sherman Canapp Jr., DVM, MS, CCRT
Mary Ann Chirba, JD, DSc, MPH
Bryan Choi, MD
Sherry Collawn, MD, PhD
Sydney Coleman, MD
Diego Correa, PhD
Peter Everts, PhD
Philippe Foubert, PhD
Julie Fradette, PhD
Jeffrey M. Gimble, MD, PhD
Aubrey de Grey, PhD
Jeffrey Hartog, MD, DMD
Marco Helder, PhD
Adam Katz, MD, FACS
Lauren Kokai, PhD
Gorana Kuka Epstein, MD
Ramon Llull, MD, PhD
Guy Magalon, MD
Keith March, MD, PhD
Robert Marx, DDS
Susanna Miettinen, PhD
Randy Miller, MD
Ali Modaressi, MD
Camillo Ricordi, MD
Paul Robbins, PhD
Ricardo Rodriguez, MD
J. Peter Rubin, MD, FACS
Bernard Siegal, JD
Filip Stillaert, MD, FCCP
Dmitry Traktuev, PhD
Stuart Williams, PhD
Abdolreza Zarnegar, PhD
Kevin Zwezdaryk, PhD

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MARK YOUR CALENDAR

International Federation for Adipose Therapeutics and Science

16th Annual Meeting

IFATS LAS VEGAS 2018

December 13 - 16, 2018

The Cosmopolitan of Las Vegas
Las Vegas, Nevada

Abstract Deadline:

Midnight EST, Wednesday, June 13, 2018

The Call for Abstracts will be sent this winter. All members of IFATS and all registered attendees of the 2017 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.

IFATS Executive Office
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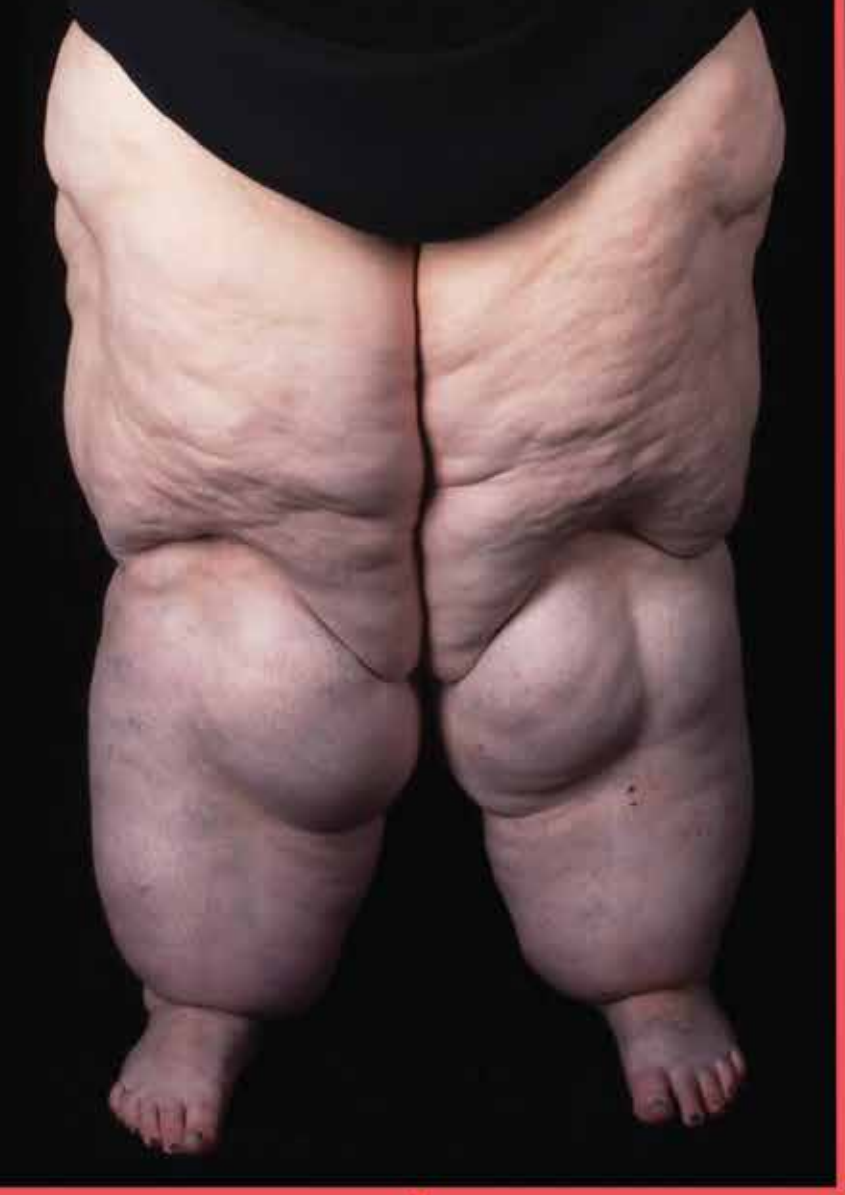

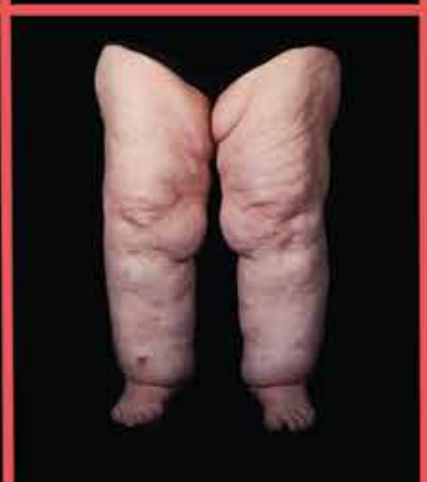

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PROGRAM IN BRIEF

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

Thursday - November 30, 2017

7:30 am	Coffee in the Exhibit Hall
8:00 - 8:30 am	**Welcome Remarks** Brian Johnstone, PhD - IFATS President
8:30 - 9:30 am	**Keynote Speaker**
	Overview of Adipose Tissue Derived Stem Cell Regenerative Medicine Bernard Siegal, JD - *Executive Director, Regenerative Medicine Foundation, Palm Beach, FL*
9:30 - 10:30 am	**Plenary Session 1 - Mechanisms of ASC Function**
	Moderators: Bruce Bunnell, PhD & Jeff Gimble, MD, PhD
10:30 - 11:00 am	Coffee Break in Exhibit Hall
11:00 am - 12:00 pm	**Symposium: Part I - Exosomes**
	Role of Exosomes and Inter-Cellular Messaging in Adipose Derived MSC Behavior Speaker: Camillo Ricordi, MD - *Stacy Joy Goodman Professor of Surgery, Distinguished Professor of Medicine, Professor of Biomedical Engineering, and Microbiology and Immunology at the University of Miami Director of the Diabetes Research Institute and the Cell Transplant Program; Miami, FL* Moderator: Ricardo Rodriguez, MD
12:00 - 1:00 pm	**Symposium: Part II - Exosomes and Cell Messaging**
	Speaker: Paul Robbins, PhD - *Department of Molecular Medicine, Director of the TSRI Center on Aging; The Scripps Research Institute, Jupiter, FL* Moderator: Diego Correa, PhD
1:00 - 2:00 pm	Lunch in Exhibit Hall
2:00 - 3:00 pm	**Industry Showcase I**
	Moderator: Ricardo Rodriguez, MD Biologica Technologies, Millennium Medical Technologies, Inc., Regen Lab S.A., SERVA Electrophosphoresis GmbH/Crescent Medical, Tulip Medical, Worthington Biochemical Company
3:00 - 4:00 pm	**Guest Speaker - PRP**
	Regenerative Potential and Clinical Applications of Combined Adipose Tissue Grafts and Platelet Rich Plasma (PRP) from Bone Marrow Aspirates Robert Marx, DDS - *Professor of Surgery, Division of Oral and Maxillofacial Surgery; University of Miami - Miller School of Medicine, Miami, FL* Moderator: Randy Miller, MD
4:00 - 4:30 pm	Coffee Break in Exhibit Hall
4:30 - 5:30 pm	**Guest Speaker - HGF**
	Role of Hepatocyte Growth Factor (HGF)/HGFR Signaling in Glucose and Fat Metabolism Abdolreza Zarnegar, PhD - *Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA* Moderator: Ricardo Rodriguez, MD
5:30 - 6:40 pm	**Plenary Session 2 - Clinical Studies with SVF**
	Moderators: J. Peter Rubin, MD, FACS & Keith March, MD, PhD
6:40 pm	Adjourn for the day

Friday - December 1, 2017

7:00 am	Coffee in the Exhibit Hall
7:30 - 8:30 am	**Case Report of Blindness After SVF Injections for Treatment of Macular Degeneration**
	Speaker: Thomas Albini, MD - *University of Miami Ophthalmology*
8:30 - 9:45 am	**Plenary Session 3 - Highest Scoring Abstracts** *(Mixed categories)*
	Moderators: Bruce Bunnell, PhD & Julie Fradette, PhD
9:45 - 11:00 am	**Concurrent 1 - ASC Isolation and Culture**
	Moderators: Susanna Miettinen, PhD & Sherry Collawn, MD, PhD

9:45 - 11:00 am	**Concurrent 2 - Fat Grafting I** Moderators: Ali Modaressi, MD & Filip Stillaert, MD, FCCP
11:00 - 11:20 am	Coffee Break in Exhibit Hall
11:20 am - 12:30 pm	**Concurrent 3 - Scaffolds** Moderators: Stuart Williams, PhD & Bryan Choi, MS
11:20 am - 12:30 pm	**Concurrent 4 - Wound Healing** Moderators: Robert Bowen, MD, FCCP & Philippe Foubert, PhD
12:30 - 1:30 pm	Lunch & Learn I
1:30 - 2:30 pm	**Panel - PRP Basic Science and Clinical Applications** Moderator: Randy Miller, MD Panelists: Peter Everts, PhD; Sherman O. Canapp Jr., DVM, MS, CCRT; Guy Magalon, MD
2:30 - 3:30 pm	**Industry Showcase II** Moderator: Stuart Williams, PhD Allergan, Amano Enzyme, Inc., CAREstream America, MTF Biologics, Shippert Medical - an Innovia Medical Company, Stem Europe
3:30 - 4:00 pm	Coffee Break in Exhibit Hall
4:00 - 5:00 pm	**Concurrent 5 - ASC Functional Characterization** Moderators: Torsten Blunk, PhD & Kevin Zwezdaryk, PhD
4:00 - 5:00 pm	**Concurrent 6 - Defining SVF** Moderators: Gorana Kuka Epstein, MD & Dmitry Traktuev, PhD
5:00 - 6:30 pm	**Poster Session**
6:30 pm	Dinner on own

Saturday - December 2, 2017

7:30 am	Coffee in the Exhibit Hall
8:00 - 9:00 am	IFATS Members Meeting
9:00 - 10:30 am	**Skills Course** **Tissue Engraftment and Neogenesis for Reconstructive and Cosmetic Indications** Ramon Llull, MD, PhD & Sydney Coleman, MD
9:00 - 10:30 am	**Concurrent 7 - Effects of Donor Physiology on ASC** Moderators: Lauren Kokai, PhD & Susanna Miettinen, PhD
10:30 - 11:00 am	Coffee Break in Exhibit Hall
11:00 am - 1:00 pm	**Skills Course** **How to Get Published and Gain Impact** Jinnie Kim - Publisher, Wiley; Jeffrey M. Gimble, MD, PhD - Chief Scientific Officer, LaCell LLC; Ann Murphy, PhD - Executive Editor, *Stem Cells*, President, AlphaMed Press
11:00 am - 1:00 pm	**Concurrent 8 - Fat Grafting II** Moderators: Jeffrey Hartog, MD, DMD & Katarina Andjelkov, MD, PhD
1:00 - 2:00 pm	Lunch & Learn II
2:00 - 3:00 pm	**Concurrent 9 - Tissue Engineering** Moderators: Julie Fradette, PhD & Torsten Blunk, PhD
2:00 - 3:00 pm	**Concurrent 10 - Bone and Soft Tissues** Moderators: Marco Helder, PhD & Susanna Miettinen, PhD
3:00 - 3:30 pm	Coffee Break in Exhibit Hall

3:30 - 4:30 pm	**Guest Speaker**
	The Turbulent Healthcare and Regulatory Environment and What it Means for Adipose Derived Regenerative Therapies Mary Ann Chirba, JD, DSc, MPH - *Professor, Boston College Law School, Boston, MA; Joint faculty appointment, Tufts Medical School JD/MPH program; Adjunct Professor of Law, NYU Law School, New York, NY, IFATS Advisory Board* Moderator: Adam Katz, MD, FACS
4:30 - 5:30 pm	**Panel: Buttock Fat Grafting - Catastrophic Events**
	Moderator: J. Peter Rubin, MD, FACS Panelists: Sydney Coleman, MD & Ricardo Rodriquez, MD
7:00 pm	**White Nights Party at Nikki Beach**

Sunday - December 3, 2017

7:30 am	Coffee
8:00 - 8:10 am	Introductory Remarks
8:10 - 10:10 am	**Plenary Session 4 - Hot Topics**
	Moderators: Marco Helder, PhD & Ramon Llull, MD, PhD
10:10 - 10:25 am	Coffee Break
10:25 - 11:45 am	**Guest Speaker**
	The Science of Regenerative Medicine and Longevity Aubrey de Grey, PhD - *Chief Science Officer and Co-founder of SENS Research Foundation* Moderator: Brian Johnstone, PhD
11:45 am	Concluding Remarks

Lunch and Learn table discussions - Friday, December 1, 2017

1. Clinical Fat Grafting - Tips, Techniques & Regulatory Status
2. Designing and Implementing Clinical Studies with Adipose and/or SVF
3. Using Adipose to Improve Reconstructive Outcomes After Radiation Therapy
4. Experience with Acellular Matrices for Soft Tissue Reconstruction
5. Basic Research in Adipose Tissue Immune Cells and Immunomodulation
6. Basic Research in ASC Characterization: How Should we Assess Identity and Functionality?
7. Fat Grafting for Septuagenarians and Beyond: Identifying Risks or Special Considerations
8. Basic Research in Brown and Beige Fat
9. Basic Research in Adipose Cell Messaging and Exosomes
10. Basic and Clinical Research with Adipose and PRP
11. Allergan - Science of REVOLVE Fat Grafting: Introduction to New Fat Processing Technology
12. Biospherix - Overcoming Obstacles: A Journey From Startup to Clinical Manufacturing
13. CAREstream America - Cell Harvesting & Processing Techniques - Dr. Klaus Ueberreiter
14. Shippert/Summit Medical - Adipose Tissue Collection Device Options

Lunch and Learn table discussions - Saturday, December 2, 2017

1. Clinical Fat Grafting - Tips, Techniques & Regulatory Status
2. Designing and Implementing Clinical Studies with Adipose and/or SVF
3. Using Adipose to Improve Reconstructive Outcomes After Radiation Therapy
4. Experience with Acellular Matrices for Soft Tissue Reconstruction
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7. Fat Grafting for Septuagenarians and Beyond: Identifying Risks or Special Considerations
8. Basic Research in Brown and Beige Fat
9. Basic Research in Adipose Cell Messaging and Exosomes
10. Basic and Clinical Research with Adipose and PRP
11. Allergan - Optimizing Fat Grafting Outcomes with Surgical Technique and New Fat Processing Technology
12. CAREstream America - Cell Processing & Counting for Regenerative Medicine - Dr. Todd Malan
13. Stem Cell Centers - How Stem Cell Centers Built a $4 Million a Year Regenerative Medicine Practice
14. Tulip Medical - Fat Injection and Nanofat

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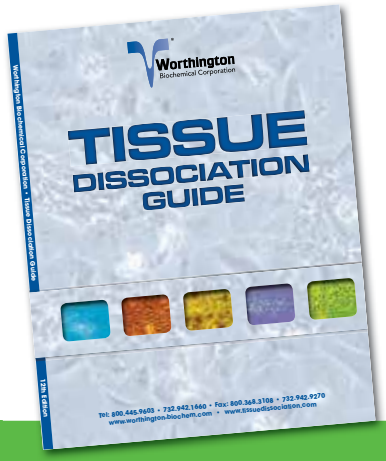

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NOTES

PROGRAM SCHEDULE

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9:30 - 10:30 am	**Plenary Session 1 - Mechanisms of ASC Function** Moderators: Bruce Bunnell, PhD & Jeff Gimble, MD, PhD
9:30 am	1 **INFLAMMATORY FACTORS INHIBIT THE EFFICIENCY OF ASC-MEDIATED VASCULOGENESIS BY INCREASING THE LOCAL ACCUMULATION OF ACTIVIN A** Presenter: Dmitry Traktuev, PhD (USA) Affiliation: Indiana University Authors: Traktuev D, Merfeld-Clauss S, Lu H, March KL
9:40 am	2 **MECHANISTIC EFFECTS OF ADIPOSE STROMAL VASCULAR FRACTION THERAPY DURING INFLAMMATION AND AUTOIMMUNITY** Presenter: Annie Bowles, MS (USA) Affiliation: Tulane University Authors: Bowles A, Wise RM, Thomas RC, Gerstein BY, Ogelman R, Manayan RC, Bunnell BA
9:50 am	3 **SHAKING THE GROUND YOU WALK ON: ARE ADIPOSE-DERIVED STEM CELLS (ASCS) TRUE STEM CELLS?** Presenter: Angelo A. Leto Barone, MD (Italy) Affiliation: University of Palermo Authors: Di Stefano AB, Leto Barone AA, Grisafi F, Montesano L, Russo A, Cordova A, Moschella F
10:00 am	4 **THE SECRETOMES OF ADIPOSE DERIVED STEM CELLS AND COMPLETE STROMAL VASCULAR FRACTION ENHANCE MYOBLAST PROLIFERATION VIA DIVERSE SIGNALLING FACTORS** Presenter: Paul Kingham, PhD (Sweden) Affiliation: Umeå University Authors: Kingham P, El-Habta R, Backman LJ
10:10 am	5 **DECREASED SIRT1 ACTIVITY NEGATIVELY AFFECTS MITOCHONDRIAL FUNCTION AND BIOGENESIS LEADING TO BLUNTED ADIPOGENESIS IN AGING CELLS** Presenter: Ivona Percec, MD, PhD (USA) Affiliation: University of Pennsylvania Authors: Percec I, Raum JC, Dierov R
10:20 am	6 **THE REDUCED REGENERATIVE POTENTIAL OF AGED AND DIABETIC ADIPOSE DERIVED STROMAL CELLS IS CAUSE BY A DISRUPTION OF SUBPOPULATION DYNAMICS** Presenter: Dominik Duscher, MD (Germany) Affiliation: Klinikum Rechts der Isar- Technical University of Munich Authors: Duscher D, Aitzetmueller MM, Hopfner U, Machens HG
10:30 - 11:00 am	Coffee Break in Exhibit Hall

11:00 am - 12:00 pm	**Symposium: Part I - Exosomes**
	Role of Exosomes and Inter-Cellular Messaging in Adipose Derived MSC Behavior Speaker: Camillo Ricordi, MD - *Stacy Joy Goodman Professor of Surgery, Distinguished Professor of Medicine, Professor of Biomedical Engineering, and Microbiology and Immunology at the University of Miami Director of the Diabetes Research Institute and the Cell Transplant Program; Miami, FL* Moderator: Ricardo Rodriguez, MD
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	Biologica Technologies Millennium Medical Technologies, Inc. Regen Lab S.A. SERVA Electrophosphoresis GmbH/Crescent Chemical Tulip Medical Worthington Biochemical Company
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5:30 - 6:40 pm	**Plenary Session 2 - Clinical Studies with SVF** Moderators: J. Peter Rubin, MD, FACS & Keith March, MD, PhD
5:30 pm	7 **EFFECTS OF THE INTRADISCAL IMPLANTATION OF STROMAL VASCULAR FRACTION PLUS PLATELET RICH PLASMA IN PATIENTS WITH DEGENERATIVE DISC DISEASE** Presenter: Kristin Comella, MS (USA) Affiliation: Bioheart Authors: Comella K, Silbert RK, Parlo M
5:40 pm	8 **AN EXPERIMENTAL STUDY AND A CLINICAL COHORT STUDY OF SVF-GEL ASSISTED LIPOTRANSFER** Presenter: Xiuying Shan, MD (China) Affiliation: The First Affiliated Hospital of Fujian Medical University Authors: Shan X, Chen L, Lei C, Liu Z, Wang B
5:50 pm	9 **MESOAMERICAN NEPHROPATHY, CHRONIC KIDNEY FAILURE OF UNKNOWN ETIOLOGY, IN 19 PATIENTS TREATED WITH ADIPOSE-DERIVED STROMVAL VASCULAR FRACTION (SVF) CELLS VIA INTRA-ARTERIAL INFUSION: A PRELIMINARY REPORT QUANTITATIVE REDUCTION OF RESISTANCE INDEX** Presenter: Michael Carstens, MD (USA) Affiliation: Saint Louis University Authors: Carstens M, Jarquín M, García N, Pastora I, Chavarría D, Correa D

6:00 pm

10
THE USE OF ADIPOSE TISSUE IN SCALP REGENERATION
Presenter: Marco Pellon, MD (Brazil)
Affiliation: Clinica Sao Vicente
Author: Pellon M

6:10 pm

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EFFICACY OF LIPOINJECTION COMBINED WITH STROMAL VASCULAR FACTOR IN PATIENTS WITH SEVERE CONTRACTURES DUE TO BURNS
Presenter: Mehmet Bozkurt, MD (Turkey)
Affiliation: Istanbul Lutfi Kirdar Kartal Education and Training Hospital
Authors: Bozkurt M, Ceran F

6:20 pm

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AUTOLOGOUS FREE FAT TRANSFER FOR ANAL FISTULAE ENABLES CLOSURE OF TEMPORARY STOMAS IN ANTI-TNF RESISTANT MB CROHN PATIENTS
Presenter: Susanna Kauhanen, MD, PhD (Finland)
Affiliation: Helsinki University Hospital
Authors: Kauhanen S, Salmenkylä S

6:30 pm

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DECELLULARIZED ADIPOSE TISSUE SCAFFOLD FOR SOFT TISSUE RECONSTRUCTION
Presenter: Francesco M. Egro, MBChB, MSc, MRCS (USA)
Affiliation: University of Pittsburgh Medical Center
Authors: Egro FM, Schusterman M, Sivak WN, Grybowski D, Mahoney C, Minteer DM, Kokai LE, Marra KG, Rubin JP

6:40 pm

Adjourn for the day

Friday - December 1, 2017

7:00 am

Coffee in the Exhibit Hall

7:30 - 8:30 am

Case Report of Blindness After SVF Injections for Treatment of Macular Degeneration
Speaker: Thomas Albini, MD - *University of Miami Ophthalmology*

8:30 - 9:45 am

Plenary Session 3 - Highest Scoring Abstracts *(Mixed categories)*
Moderators: Bruce Bunnell, PhD & Julie Fradette, PhD

8:30 am

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TRANSLATIONAL ADIPOSE-DERIVED STEM CELL-BASED IMMUNOMODULATORY THERAPY IN A PORCINE HIND-LIMB TRANSPLANT MODEL
Presenter: Deokyeol Kim, MD (USA)
Affiliation: University of Pittsburgh
Authors: Kim D, Waldner M, Zhang W, Barone A, Cooney D, Solari M, Washington K, Marra KG, Gorantla V, Brandacher G, Rubin JP

8:40 am

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LIPOTRANSFER IMPROVES MEASURES OF APPEARANCE AND FUNCTION DUE TO FACIAL FIBROSIS IN SYSTEMIC SCLEROSIS
Presenter: Aurora Almadori, MD (Italy)
Affiliation: Second University of Naples
Authors: Almadori A, Ryan C, Griffin M, Hansen E, Denton C, Butlet P

8:50 am

16
ADIPOSE-DERIVED STEM CELL EXTRACELLULAR VESICLES CAN BE SPECIFICALLY TUNED FOR SOFT TISSUE REPAIR AND WOUND HEALING
Presenter: Benjamin Buehrer, PhD (USA)
Affiliation: ZenBio
Authors: Buehrer B, Nicoll JB, Ludlow JW, Oschwald DL

9:00 am

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WITHDRAWN

INTRAVENOUS DELIVERY OF AUTOLOGOUS ADIPOSE DERIVED REGENERATIVE CELLS (ADRCS) IMPROVES HEALING IN A PORCINE THERMAL BURN MODEL INVOLVING 20% BODY SURFACE AREA

Presenter: John K. Fraser, PhD (USA)
Affiliation: Cytori Therapeutics
Authors: Foubert P, Liu M, Anderson S, Zafra D, Rajoria R, Gutierrez D, Tenenhaus M, Fraser JK

9:10 am

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THE BRCA1 MUTATION LEADS TO INCREASED SECRETION OF INTERLEUKIN-6 AND INTERLEUKIN-8 FROM ADIPOSE DERIVED STEM CELLS: IDENTIFYING A POTENTIAL TARGET IN THE TUMOR MICROENVIRONMENT

Presenter: Ruya Zhao, BS (USA)
Affiliation: Duke University School of Medicine
Authors: Zhao R, Fan L, Lee A, Pien I, Liu X, Seewaldt V, Li C, Hollenbeck S

9:20 am

19

DONOR-SPECIFIC ADIPOSE-DERIVED STROMAL CELLS ATTENUATE GRAFT VASCULOPATHY AND REJECTION IN RODENT VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Presenter: Riccardo Schweizer, MD (Switzerland)
Affiliation: UniversityHospital Zurich
Authors: Schweizer R, Klein H, Waldner M, Kollar B, Fuchs N, Lehner F, Taddeo A, Salemi S, Eberli D, Giovanoli P, Plock J

9:45 - 11:00 am

Concurrent 1 - ASC Isolation and Culture

Moderators: Susanna Miettinen, PhD & Sherry Collawn, MD, PhD

9:45 am

20

A NOVEL POINT OF CARE, AUTOMATED, AND CLOSED SYSTEM FOR PROCESSING STROMAL VASCULAR FRACTION EITHER WITH OR WITHOUT COLLAGENASE

Presenter: Todd Malan, MD (USA)
Affiliation: Roxbury Regenerative
Author: Malan T

9:55 am

21

Q-GRAFT (R), A NEW DEVICE FOR ISOLATION OF SVF AND STEM CELLS FROM FATTY TISSUE

Presenter: Klaus Ueberreiter, MD (Germany)
Affiliation: Park Klinik Birkenwerder
Authors: Ueberreiter K, Meyer J

10:05 am

22

OPTIMIZATION OF ENZYMATIC DIGESTION FOR SVF ISOLATION FROM HUMAN LIPOASPIRATES

Presenter: Rintaro Asahi, MD (Japan)
Affiliation: Jichi Medical University
Authors: Asahi R, Shirado T, Saitoh N, Furukawa K, Yoshimura K

10:15 am

23

EFFECT OF THE BOWL STRUCTURE ON ISOLATION YIELD OF STROMAL VASCULAR FRACTION USING AUTOMATED CELL ISOLATION DEVICE

Presenter: Hyung Min Hahn, MD (Korea)
Affiliation: Ajou University Hospital
Authors: Hahn HM, Yoo BY, Park JH, Jung HJ, Lee IJ

10:25 am

24

LONG-TERM IN VITRO CULTURE AND OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS IN CGMP-COMPLIANT MEDIA

Presenter: Napat Tandikul, MS (USA)
Affiliation: Epibone Inc.
Author: Tandikul N

10:35 am

25
GROWTH SUPPLEMENTS FOR LARGE-SCALE CLINICAL EXPANSION OF ADIPOSE-DERIVED STEM CELLS: A TEST OF FOUR TYPES OF PLATELET LYSATES FROM OUTDATED OR FRESH BUFFY COAT-DERIVED PLATELETS
Presenter: Peter Vester-Glowinski, MD (Denmark)
Affiliation: Copenhagen University Hospital
Authors: Vester-Glowinski P, Koelle SK, Svalgaard JD, Herly M, Drzewiecki KT, Fischer-Nielsen A

10:45 am

26
OPTIMIZATION OF CELL ADHESION RATE ON SILK/FIBROIN MICROCARRIERS FOR ONE-STEP ADIPOSE STEM/STROMAL CELL DELIVERY: DESIGN OF EXPERIMENT APPROACH
Presenter: Carlotta Perucca Orfei, PhD (Italy)
Affiliation: IRCCS Galeazzi Orthopaedic Institute
Authors: Perucca Orfei C, Taló G, Chlapanidas T, Viganó M, Perteghella S, Fabro Fontana F, Torre ML, De Girolamo L

9:45 - 11:00 am

Concurrent 2 - Fat Grafting I
Moderators: Ali Modaressi, MD & Filip Stillaert, MD, FCCP

9:45 am

27
VOLUME RETENTION, METABOLISM, AND CELLULAR COMPOSITION OF HUMAN FAT XENOGRAFTS
Presenter: Brittany Merrifield, BS (USA)
Affiliation: Michigan State University
Authors: Merrifield B, Komorowska-Timek E, Chang A, Hostetter G

9:55 am

28
LONG-TERM RETENTION OF EXCISED FAT GRAFTS: A LONGITUDINAL, RETROSPECTIVE COHORT STUDY OF 108 PATIENTS FOLLOWED FOR UP TO 8.4 YEARS
Presenter: Mikkel Herly, MD (Denmark)
Affiliation: University Hospital Copenhagen
Authors: Herly M, Pipper CB, Broholm H, Poulsgaard L, Fugleholm K, Glovinski PV, Orholt M, Thomsen C, Drzewiecki KT

10:05 am

29
MOLECULAR MECHANISMS OF FAT GRAFT FAILURE: EXPLORING PATHWAYS THAT CONFER HYPOXIA-INDUCED APOPTOSIS RESISTANCE IN ADIPOSE TISSUE
Presenter: Lauren Kokai, PhD (USA)
Affiliation: University of Pittsburgh
Authors: Kokai LE, Johngrass MG, Schroth RN, Gusenoff JA, Rubin JP

10:15 am

30
THE HYBRID BREAST RECONSTRUCTION: IMPLANT-BASED BREAST RECONSTRUCTION WITH ADDITIONAL LIPOFILLING
Presenter: Filip Stillaert, MD (Belgium)
Affiliation: University Hospital Gent
Author: Stillaert F

10:25 am

31
22 YEARS OF 3 DIMENSIONAL FACE LIFTING EVOLUTION USING FAT TRANSFER AS THE PRIMARY Z AXIS TOOL
Presenter: Andrew Wolin, MD (USA)
Affiliation: Private Practice
Author: Wolin A

10:35 am

32
TWO-STAGE BREAST RECONSTRUCTION WITH A SUBPECTORAL SILICONE TISSUE EXPANDER AND AUTOLOGOUS FAT GRAFTING
Presenter: Ruomiao Chen, MD (China)
Affiliation: The First Affiliated Hospital of Fujian Medical University
Authors: Chen R, Shan X, Xu L, Wang B

10:45 am	**33** **LIPOLOOP: A STATE-OF-THE-ART, CLOSED, AUTOMATIC, VIBRATORY SYSTEM FOR FAT GRAFTING, MECHANICAL SVF PROCUREMENT AND CELL AND TISSUE SELECTION** Presenter: Steven Cohen, MD, FACS (USA) Affiliation: University of California San Diego Authors: Cohen S, Ramos C, Angeloni D, Miles G
11:00 - 11:20 am	Coffee Break in Exhibit Hall
11:20 am - 12:30 pm	**Concurrent 3 - Scaffolds** Moderators: Stuart Williams, PhD & Bryan Choi, MS
11:20 am	**34** **PRODUCTION OF NATURALLY DERIVED TISSUE-ENGINEERED BIOLOGICAL DRESSINGS UNDER SERUM-FREE CONDITIONS TO STIMULATE WOUND HEALING IN DIABETIC MICE** Presenter: Julie Fradette, PhD (Canada) Affiliation: Universite Laval Quebec Canada Authors: Fradette J, Safoine M, Coté A, Paquette C, Plourde-Campagna MA, Ruel J
11:30 am	**35** **COMBINED USE OF EXTERNAL VOLUME EXPANSION (EVE) AND A DECELLULARIZED ALLOGRAFT ADIPOSE MATRIX (AAM) TO INDUCE ADIPOSE TISSUE REGENERATION: A NOVEL PARADIGM IN SOFT TISSUE RECONSTRUCTION?** Presenter: Giorgio Giatsidis, MD (USA) Affiliation: Brigham and Women's Hospital - Harvard Medical School Authors: Giatsidis G, Succar JS, Haddad AH, Nilsen TN, Chnari EC, Orgill DO
11:40 am	**36** **DECONSTRUCTING ALLOGRAFT ADIPOSE MATRIX** Presenter: Lohrasb Ross Sayadi, MD (USA) Affiliation: UCI Authors: Ziegler M, Sayadi LR, Banyard DA, Tylutki T, Chnari EC, Evans GR, Widgerow AD
11:50 am	**37** **A PRE EMULSIFICATED PRODUCT: THE MILLIMICROFAT** Presenter: Angelo Trivisonno, MD (Italy) Affiliation: Sapienza University Author: Trivisonno A
12:00 pm	**38** **ADIPOSE CONSTRUCTS WITH BIODEGRADABLE SURFACE TOPOGRAPHY INFLUENCE THE NEOVASCULARIZATION ONSET IN SKIN TISSUE REGENERATION** Presenter: Michelle McLuckie, MSc (Switzerland) Affiliation: Zurich University Hospital Authors: McLuckie M, Robotti F, Sanchez-Macedo N, Enderlin D, Egana JT, Poulikakos D, Giovanoli P, Ferrari A, Lindenblatt N
12:10 pm	**39** **POLYMER-MINERAL SCAFFOLD TO AUGMENT IN VIVO EQUINE MULTIPOTENT STROMAL CELL OSTEOGENESIS** Presenter: Mandi Lopez, DVM, MS, PhD (USA) Affiliation: Louisiana State University Authors: Duan W, Chen C, Haque M, Hayes D, Lopez MJ
12:20 pm	**40** **ANTI-AGING EFFECT OF THE STROMAL VASCULAR FRACTIONS/ADIPOSE-DERIVED STEM CELL IN A MOUSE MODEL OF SKIN AGING INDUCED BY UVB IRRADIATION** Presenter: Hongwei Liu, MD, PhD (China) Affiliation: The First Affiliated Hospital of Jinan University Author: Liu H

11:20 am - 12:30 pm	**Concurrent 4 - Wound Healing** Moderators: Robert Bowen, MD, FCCP & Philippe Foubert, PhD
11:20 am	**41** **A FIBRIN SPRAY SYSTEM FOR THE DELIVERY OF ASCS TO DIABETIC WOUNDS** Presenter: Philipp Nessbach, MSc (Germany) Affiliation: Klinikum Rechts der Isar - Technical University of Munich Authors: Aitzetmüller M, Nessbach P, Centeno Cerdas C, Hopfner U, Kirsch M, Machens HG, Duscher D
11:30 am	**42** **WOUNDS REPARATION AFTER HUMAN STROMAL VASCULAR FRACTION EXPOSURE IN A MOUSE MODEL OF PRESSURE ULCERS** Presenter: Joanna Bukowska, PhD (USA) Affiliation: LaCell LLC Authors: Bukowska J, Smith S, Wu X, Frazier T, Brown T, Kosnik P, Katz AJ, Mehrara B, Gawronska-Kozak B, Bunnell BA, Gimble JM
11:40 am	**43** **ADIPOSE-DERIVED STEM CELLS IN AN IN VIVO RAT MODEL OF ACHILLES TENDON EXCISION INJURY** Presenter: Jolanta Norelli, BA (USA) Affiliation: Northwell Health System Authors: Norelli J, Plaza DP, Varghese AM, Stal D, Liang H, Grande DA
11:50 am	**44** **ADIPOSE-DERIVED STROMAL CELLS INHIBITED MAST CELL FUNCTIONS: IMPORTANT ROLE IN POST-BURN HYPERTROPHIC SCAR TREATMENT** Presenter: Benoit Chaput, MD (France) Affiliation: STROMALab Authors: Chaput B, Laloze J, Grolleau JL, Sensebe L, Varin A
12:00 pm	**45** **THE FATE OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS ADMINISTERED SYSTEMICALLY AND LOCALLY FOR WOUND REPAIR** Presenter: Karlien Kallmeyer, MSc (Switzerland) Affiliation: University of Geneva Authors: Kallmeyer K, Andrè-Lévigne D, Pepper MS, Pittet-Cuénod B, Modarressi A
12:10 pm	**46** **ADIPOSE STEM CELLS AND ADIPOSE STEM CELL CONDITIONED MEDIA EXHIBIT SIMILAR ACTIVITY IN A PORCINE MODEL OF EXCISIONAL WOUND HEALING** Presenter: J. Peter Rubin, MD, FACS (USA) Affiliation: University of Pittsburgh Authors: Grybowski D, Schusterman MA, Kim D, March KL, Johnstone B, James IB, Venkatesh K, Kokai LE, Marra KG, Rubin JP
12:20 pm	**47** **EFFECT OF HUMAN ADIPOSE TISSUE DERIVED MSC AND EXOSOMES ON WOUND HEALING** Presenter: Marta Garcia-Contreras, PhDc (USA) Affiliation: University of Miami Authors: Garcia-Contreras M, Messaggio F, Mendez AJ, Robbins PD, Ricordi C
12:30 - 1:30 pm	Lunch & Learn I *(see topics on page 10)*
1:30 - 2:30 pm	**Panel - PRP Basic Science and Clinical Applications** Moderator: Randy Miller, MD Panelists: Peter Everts, PhD; Sherman O. Canapp Jr., DVM, MS, CCRT; Guy Magalon, MD

2:30 - 3:30 pm	**Industry Showcase II** Moderator: Stuart Williams, PhD
	Allergan Amano Enzyme, Inc. CAREstream America MTF Biologics Shippert Medical - an Innovia Medical Company Stem Europe
3:30 - 4:00 pm	Coffee Break in Exhibit Hall
4:00 - 5:00 pm	**Concurrent 5 - ASC Functional Characterization** Moderators: Torsten Blunk, PhD & Kevin Zwezdaryk, PhD
4:00 pm	48 **MAINTENANCE OF STEMNESS AND REJUVENATION POTENTIAL OF ADIPOSE-DERIVE STEM CELLS (ASCS) ISOLATED FROM PREMATURE/PROGEROID MICE MODEL** Presenter: Xiaodong Mu, PhD (USA) Affiliation: University of Texas Authors: Mu X, Chen W, Ravuri S, Huard J
4:10 pm	49 **GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN ADIPOSE-DERIVED STROMAL/ STEM CELLS IS CELL DENSITY-DEPENDENT AND POSITIVELY IMPACTS ADIPOGENIC DIFFERENTIATION** Presenter: Torsten Blunk, PhD (Germany) Affiliation: University of Wuerzburg Authors: Blunk T, Wiesner M, Berberich O, Hoefner C, Bauer-Kreisel P
4:20 pm	50 **DFO PRECONDITIONING RESTORES THE THERAPEUTIC POTENTIAL OF DIABETIC ASCS** Presenter: Matthias Aitzetmüller, MD (Germany) Affiliation: Klinikum rechts der Isar - Technical University of Munich Authors: Aitzetmüller M, Nessbach P, Hopfner U, Kirsch M, Centeno Cerdas C, Machens HG, Duscher D
4:30 pm	51 **FUNCTIONAL CHARACTERISTICS OF FIBROBLASTS DIFFERENTIATED FROM ADIPOSE-DERIVED STEM CELLS** Presenter: Ivona Percec, MD, PhD (USA) Affiliation: University of Pennsylvania Authors: Percec I, Gersch RP
4:40 pm	52 **ADIPOSE STEM CELLS FROM OBESE INDIVIDUALS PROMOTE TRIPLE NEGATIVE BREAST CANCER METASTASIS IN A PATIENT DERIVED XENOGRAFT (PDX)** Presenter: Rachel Sabol, MS (USA) Affiliation: Tulane University School of Medicine Authors: Sabol R, Matossian M, Dutreil MF, Cote A, Bowles AC, Burks HE, Collins-Burow B, Burow ME, Bunnell BA
4:50 pm	53 **AUTOLOGOUS ADIPOSE DERIVED MULTIPOTENT STROMAL CELLS ON XENOGRAFTS REDUCE THE SYSTEMIC LYMPHOCYTE RESPONSE** Presenter: Mandi Lopez, DVM, MS, PhD (USA) Affiliation: Louisiana State University Authors: Lopez MJ, Takawira C, Bova J, Stout R, Dietrich M

4:00 - 5:00 pm	**Concurrent 6 - Defining SVF** Moderators: Gorana Kuka Epstein, MD & Dmitry Traktuev, PhD
4:00 pm	54 **FRACTIONAL LASER + MICRONEEDLING + NANOFAT: EVIDENCE OF RAPID HEALING AND ELASTIN AND COLLAGEN REGENERATION** Presenter: Steven Cohen, MD, FACS (USA) Affiliation: University of California San Diego Authors: Cohen S, Goodache A, Delauney F
4:10 pm	55 **METABOLOMIC CHANGES IN HUMAN ADIPOSE TISSUE DERIVED PRODUCTS FOLLOWING NON-ENZYMATIC MICROFRACTURING** Presenter: Marta Garcia-Contreras, PhDc (USA) Affiliation: University of Miami Authors: Garcia-Contreras M, Messaggio F, Mendez AJ, Ricordi C
4:20 pm	56 **A CRITICAL EVALUATION OF THE ENDOTHELIAL PLASTICITY OF ADIPOSE-DERIVED STEM CELLS** Presenter: Jeremy Antonyshyn, BSc (Canada) Affiliation: University of Toronto Authors: Antonyshyn J, Santerre JP
4:30 pm	57 **EFFECT OF CRYOPRESERVATION ON INTACT ADIPOSE TISSUE AND ISOLATED STROMAL VASCULAR FRACTION CELLS IN VITRO AND IN VIVO ANALYSES** Presenter: Fabiana Zanata, MD (Brazil) Affiliation: Federal University of Sao Paulo Authors: Zanata F, Bowle A, Frazier T, Curley L, Bunnell BA, Wu X, Wade J, Devireddy R, Gimble J, Ferreira LM
4:40 pm	58 **ISOLATION AND EXPANSION OF VASCULAR ENDOTHELIAL CELLS FROM HUMAN LIPOASPIRATES** Presenter: Natsumi Saito, PhD (Japan) Affiliation: Jichi Medical University Authors: Saito N, Asahi R, Shirado T, Odbayar B, Yoshimura K
4:50 pm	59 **ADIPOSE-DERIVED STEM CELL CONDITION MEDIUM ATTENUATED CISPLATIN-TRIGGERED APOPTOSIS IN TONGUE SQUAMOUS CELL CARCINOMA CELLS** Presenter: Yu-Jen Chiu, MD (Taiwan) Affiliation: Taipei Veterans General Hospital Authors: Chiu YJ, Ma H, Hsu H, Yang J
5:00 - 6:30 pm	**Poster Session**
	1P **HUMAN ADIPOSE STEM CELL MEDIATED MATRIX REMODELING AS A MECHANISM FOR CANCER PROGRESSION** Presenter: Ethan Byrne, BSBE (USA) Affiliation: Louisiana State University Authors: Byrne E, King C, Bunnell BA, Martin EC
	2P **ALTERED VASCULATURE, ANGIOGENESIS AND ADIPOCYTE HYPERTROPHY IN SUBCUTANEOUS ADIPOSE TISSUE (SAT) DISORDERS** Presenter: Sara Al Ghadban, PhD (USA) Affiliation: University of Arizona Authors: Al Ghadban S, Herbst K, Ussery C, Harris D, Badowski M, Allen M

3P
SODIUM BICARBONATE BUFFERING IMPROVES ADIPOCYTE STEM CELL VIABILITY AFTER LIPOSUCTION
Presenter: Wei Z. Wang, MD (USA)
Affiliation: University of Nevada Reno School of Medicine
Authors: Francis A, Wang WZ, Goldman JJ, Fang XH, Williams SJ, Baynosa RC

4P
RE-CLASSIFICATION OF THE FAT GRAFT
Presenter: Steven Cohen, MD, FACS (USA)
Affiliation: University of California San Diego
Authors: Cohen S, Hewett S, Ross L, Goodache A

5P
BIOLOGIC AUGMENTATION OF A SURGICALLY REPAIRED MENISCUS IN AN ELITE HIGH SCHOOL ATHLETE. A CASE REPORT
Presenter: Jay Bowen, DO (USA)
Affiliation: New Jersey Regenerative Institute
Authors: Bowen J, Malanga GA, Raja A

6P
~~**A PHASE I SAFETY STUDY USING STROMAL VASCULAR FRACTION FROM LIPOASPIRATE IN THE TREATMENT OF CHRONIC NON-HEALING WOUNDS VIA THE ANTRIA CELL PREPARATION PROCESS (ACPP)**~~
WITHDRAWN
Presenter: Leonard Maliver, MD (USA)
Affiliation: Antria Inc.
Authors: Maliver L, Bizousky DT

7P
~~**TREATMENT AND OUTCOMES OF LIPOFILLING IN TUBEROUS BREASTS**~~
WITHDRAWN
Presenter: Patricia Gutierrez-Ontalvilla, MD (Spain)
Affiliation: Hospital La Fe
Authors: Gutierrez-Ontalvilla P, Lopez E

8P
~~**LIPOFILLING UNDER THE SCARS WITH AND WITHOUT PLATEL RIC PLASMA**~~
WITHDRAWN
Presenter: Yasser Helmy Ali, MD (Egypt)
Affiliation: Faculty of Medicine Al-Azhar University
Author: Yasser Helmy A

9P
EFFECT OF PLATELET-RICH PLASMA ON THE PROLIFERATION OF HUMAN ADIPOSE STEM CELLS
Presenter: Fangyuan Lai, MD (Japan)
Affiliation: Kansai Medical University
Author: Lai F, Kakudo NA, Morimoto NA, Taketani SH, Hara TO, Ogawa TA, Kusumoto KE

10P
LARGE VOLUME LIPOFILLING WITH CLOSE SYSTEM IN AESTHETIC PLASTIC SURGERY
Presenter: Aristides Arellano-Huacuja, MD, FICS (Mexico)
Affiliation: Clinica Dermatologica y Cirugia Estetica de Puebla SA de CV
Authors: Arellano-Huacuja A, Arellano-Montalvo A, Arellano-Montalvo D

11P
THE TROPHIC EFFECT OF VETAP-17® ON HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS IN A 3D HUMAN PLATELET LYSATE GEL MODEL
Presenter: Thitikan Jirakittisonthon, DVM (USA)
Affiliation: Kansas State University
Authors: Jirakittisonthon T, Murnane JM, Weiss ML

WITHDRAWN	**12P** **~~CORRECTION~~ DEPRESSED SCARS WITH PRP ENRICHED FAT GRAFTING** Presenter: Sameh Elshawadfy, MD (Egypt) Affiliation: Faculty of Medicine Tanta University Authors: Elshawadfy S, Shalaby H, Hammad S
	13P **PEROXICAM REDUCES RESIDUAL COLLAGENASE ACTIVITY IN ENZYMATICALLY-DERIVED STROMAL VASCULAR FRACTION** Presenter: Joseph Zakhari, MA (USA) Affiliation: University of Louisville School of Medicine Authors: Zakhari J, Williams SK
	14P **STEM CELLS FROM HUMAN HAIR FOLLICLES: FIRST MECHANICAL ISOLATION FOR IMMEDIATE AUTOLOGOUS CLINICAL USE IN ANDROGENETIC ALOPECIA AND HAIR LOSS** Presenter: Pietro Gentile, MD, PhD (Italy) Affiliation: University of Rome Tor Vergata Author: Gentile P
6:30 pm	Dinner on own

Saturday - December 2, 2017

7:30 am	Coffee in the Exhibit Hall
8:00 - 9:00 am	IFATS Members Meeting
9:00 - 10:30 am	**Skills Course**
	Tissue Engraftment and Neogenesis for Reconstructive and Cosmetic Indications Ramon Llull, MD, PhD & Sydney Coleman, MD
9:00 - 10:30 am	**Concurrent 7 - Effects of Donor Physiology on ASC** Moderators: Lauren Kokai, PhD & Susanna Miettinen, PhD
9:00 am	**60** **CIGARETTE SMOKING-INDUCED RENAL PATHOLOGIES ARE AMELIORATED BY ADIPOSE STEM CELL THERAPY** Presenter: Daria Barwinska, BA (USA) Affiliation: Indiana University Authors: Barwinska D, Cook T, Traktuev DO, Saliba J, Bacallao R, Basile DP, March KL
9:10 am	**61** **MILD HYPOTHERMIA ATTENUATES OXIDATIVE STRESS AND INFLAMMATORY RESPONSE FACILITATING EXPANSION AND DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS** Presenter: Gal Tirza, MD, BSc (Israel) Affiliation: Tel Aviv Sourasky Medical Center Authors: Tirza G, Sela M, Solodeev I, Pasmanik-Chor M, Gur E, Shani N
9:20 am	**62** **THE EFFECT OF DONOR BMI ON ADIPOSE STEM CELL CHARACTERISTICS - OBESITY-DISCORDANT AND WEIGHT-CONCORDANT MONOZYGOTIC TWIN STUDY** Presenter: Miia Juntunen, MS (Finland) Affiliation: University of Tampere Authors: Juntunen M, Patrikoski M, Rissanen A, Pietiläinen K, Miettinen S
9:30 am	**63** **INTERACTION WITH PRO-INFLAMMATORY MACROPHAGES INHIBITS IMMUNOSUPPRESSIVE FUNCTION IN HUMAN ADIPOSE-DERIVED STROMAL CELLS** Presenter: Audrey Varin, PhD (France) Affiliation: Department of Plastic and Reconstructive Surgery Authors: Varin A, Espagnolle N, Balguerie A, Arnaud E, Chaput B, Sensebe L

9:40 am	64 **ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS RESCUE APOPTOTIC RETINAL PIGMENT EPITHELIAL CELLS UNDER OXIDATIVE STRESS, AND DIFFERENTIATE TOWARDS RETINAL PIGMENT EPITHELIAL CELLS IN VITRO AND IN VIVO** Presenter: Aya Barzelay, MD, PhD (Israel) Affiliation: Tel Aviv Medical Center Authors: Barzelay A, Wheisthal S, Nitzan A, Ben Hemo M, Loewenstein A, Barak A
9:50 am	65 **MOLECULAR BASIS OF ADIPOSE-DERIVED STEM CELLS (ASC) THERAPY FOR MANAGEMENT OF RADIATION INDUCED FIBROSIS (RIF)** Presenter: Asim Ejaz, PhD (USA) Affiliation: University of Pittsburgh Authors: Ejaz A, Epperly MW, Fisher R, Zhang X, Johngrass MG, Schusterman MA, Kokai LE, Greenberger JS, Rubin JP
10:00 am	66 **IMPACT OF AGE AND DIABETES ON HUMAN ADIPOSE STEM CELLS FOR A BONE TISSUE-ENGINEERED PRODUCT** Presenter: Sophie Veriter, PhD (Belgium) Affiliation: Novadip Biosciences Authors: Veriter S, Lafosse A, Palacios P, Bidias D, Demir C, Adnet PY, Dufrane D
10:10 am	67 **IMMUNOLOGICAL CHANGES IN ADIPOSE TISSUE DEPOTS INDUCED BY HUMAN CYTOMEGALOVIRUS INFECTION** Presenter: Kevin Zwezdaryk, PhD (USA) Affiliation: Tulane University Authors: Zwezdaryk K, Norton EB, Sullivan DE
10:20 am	68 **MICROENVIRONMENT DIVERSIFICATION OF YOUNG VS. AGED ADIPOSE-DERIVED STEM CELLS** Presenter: Katie Hamel, BS (USA) Affiliation: Louisiana State University Authors: Hamel K, Gimble JM, Jung JP, Martin EC
10:30 - 11:00 am	Coffee Break in Exhibit Hall
11:00 am - 1:00 pm	**Skills Course** **How to Get Published and Gain Impact** Jinnie Kim - Publisher, Wiley Jeffrey M. Gimble, MD, PhD - Chief Scientific Officer, LaCell LLC Ann Murphy, PhD - Executive Editor, *Stem Cells*; President, AlphaMed Press
11:00 am - 1:00 pm	**Concurrent 8 - Fat Grafting II** Moderators: Jeffrey Hartog, MD, DMD & Katarina Andjelkov, MD, PhD
11:00 am	69 **FAT GRAFTING FOR FAT PAD ATROPHY OF THE HEEL: EARLY FINDINGS FROM A RANDOMIZED CONTROLLED CLINICAL TRIAL** Presenter: Isaac James, MD (USA) Affiliation: University of Pittsburgh Authors: James IB, Gusenoff BR, Minteer DM, Wang S, Dibernardo G, Gusenoff JA
11:10 am	70 **BREAST RECONSTRUCTION USING POLY-4-HYDROXYBUTYRATE MESH SCAFFOLD AND AUTOLOGOUS FAT GRAFTING** Presenter: Mark Schusterman, MD (USA) Affiliation: University of Pittsburgh Medical Center Authors: Schusterman M, Rehnke RD, Badylak SF, Rubin JP

11:20 am

71
LONG TERM RESULTS AFTER WATER ASSISTED FAT GRAFT WITH BEAULI PROTOCOL
Presenter: Klaus Ueberreiter, MD (Germany)
Affiliation: Park Klinik Birkenwerder
Authors: Ueberreiter K, Tanzella U

11:30 am

72
THE IMPACT OF DIFFERENT RECIPIENT SITE PRE-CONDITIONING TECHNIQUES IN FAT GRAFTING SURGICAL OUTCOMES
Presenter: Carlo Oranges, MD (Switzerland)
Affiliation: Basel University Hospital
Authors: Oranges C, Striebel J, Tremp M, Kalbermatten DF, Schaefer DJ

11:40 am

73
SAFETY AND EFFICACY OF PERCUTANEOUS INJECTION OF AUTOLOGOUS MICRO-FRACTURED ADIPOSE TISSUE FOR OSTEOARTHRITIC KNEES
Presenter: Jay Bowen, DO (USA)
Affiliation: New Jersey Regenerative Institute
Authors: Panchal J, Malanga GA, Bowen JE

11:50 am

74
FAT GRAFT RETENTION IN PEDAL FAT GRAFTING: ASSOCIATION WITH CD34+ ADIPOSE STEM CELLS AND COLLAGEN
Presenter: Sheri Wang, BS (USA)
Affiliation: University of Pittsburgh
Authors: Wang S, James IB, DiBernardo G, Lannau B, Grybowski D, Zhang W, Gusenoff BR, Marra KG, Gusenoff JA

12:00 pm

75
THE EFFICACY AND VOLUME RETENTION OUTCOME OF MICROFAT TRANSFER FOR FACIAL CONTOURING AND FACIAL REJUVENATION: A RETROSPECTIVE STUDY OF 1279 CASE IN 7 YEARS
Presenter: Pichansak Bunmas, MD (Thailand)
Affiliation: Vplast Institute of Aesthetic and Plastic Surgery
Author: Bunmas P

12:10 pm

76
THE COMBINED USE OF ENHANCED STROMAL VASCULAR FRACTION AND PLATELET-RICH PLASMA IMPROVES FAT GRAFTING MAINTENANCE IN BREAST SOFT TISSUE DEFECTS
Presenter: Pietro Gentile, MD, PhD (Italy)
Affiliation: University of Rome Tor Vergata
Author: Gentile P

12:20 pm

77
EFFECT OF 650-NM GAALAS LASER IRRADIATION ON THE THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE-DERIVED STEM CELLS
Presenter: Hongwei Liu, MD, PhD (China)
Affiliation: The First Affiliated Hospital of Jinan University
Author: Liu H

12:30 pm

WITHDRAWN

78
SETUP, CHARACTERIZATION AND VALIDATION OF AN ADIPOSE TISSUE MODEL INCLUDING HUMAN MATURE ADIPOCYTES
Presenter: Ann-Cathrin Volz, MS (Germany)
Affiliation: Reutlingen University
Authors: Volz AC, Dieckmann S, Huber B, Kluger PJ

12:40 pm

79
ADIPOGENIC FACTOR EXPRESSION IN LIPEDEMA ADIPOSE STEM CELLS DIFFERS FROM CONTROL ADIPOSE STEM CELLS
Presenter: Anna-Theresa Bauer, MD (Germany)
Affiliation: University Clinic for Plastic Surgery and Handsurgery
Authors: Bauer AT, Von Lukowicz D, Lossagk K, Hopfner U, Kirsch BM, Moog P, Schmauss D, Machens HG

12:50 pm

80
OXY133 AS A DETERRENT OF ADIPOGENESIS, IN VIVO STUDIES
Presenter: Akishige Hokugo, DDS, PhD (USA)
Affiliation: UCLA
Authors: Bakshi R, Hokugo A, Zhou S, Rezzadeh K, Jarrahy R

1:00 - 2:00 pm

Lunch & Learn II *(see topics on page 10)*

2:00 - 3:00 pm

Concurrent 9 - Tissue Engineering
Moderators: Julie Fradette, PhD & Torsten Blunk, PhD

2:00 pm

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UTILIZING A SELF-ASSEMBLING, ZOONOTIC FREE 3-D CELLULAR MATRIX CONSTRUCTED FROM HUMAN PLATELET LYSATE AND HUMAN ADIPOSE STROMAL VASCULAR FRACTION FOR THE GENERATION OF FUNCTIONALIZED 3-D FAT PAD
Presenter: Michelle McCarthy, MS (USA)
Affiliation: Tulane University School of Medicine
Authors: McCarthy M, Bender RJ, Brown TA, Smith SE, Xiying W, Gimble JM, Frazier TP

2:10 pm

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BIOACTIVITY OF A TISSUE-ENGINEERED PRODUCT: BRIDGING THE GAP BETWEEN ACADEMIC AND CLINICAL STUDIES
Presenter: Sophie Veriter, PhD (Belgium)
Affiliation: Novadip Biosciences
Authors: Veriter S, Palacios P, Bidias D, Demir C, Luseau A, Plougonven E, Dufrane D

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TISSUE-ENGINEERED 3D EAR CARTILAGE CONSTRUCT
Presenter: Derek Banyard, MD, MBA (USA)
Affiliation: UCI
Authors: Ziegler M, Banyard DA, Jaffurs D, Evans GR, Widgerow AD

2:30 pm

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EFFECT OF MESENCHYMAL STEM CELLS DERIVED FROM BONE MARROW, ADIPOSE TISSUE AND UMBILICAL CORD ON HUMAN OSTEOARTHRITIC CHONDROCYTES
Presenter: Nada Alaaeddine, PhD (Lebanon)
Affiliation: University of St Joseph
Authors: Alaaeddine N, Moussa M, Sayegh S, Hilal G, Haykal G, Khalil C

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NEW APPROACHES TO FACIAL REJUVENATION USING INJECTABLE TISSUE REGENERATION AND INJECTABLE TISSUE ENGINEERING
Presenter: Steven Cohen, MD, FACS (USA)
Affiliation: University of California San Diego
Authors: Cohen S, Hewett S, Delauney F, Goodache A

~~2:50 pm~~

WITHDRAWN

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PERITENDINOUS PLATELET-RICH PLASMA INJECTIONS IN THE TREATMENT OF TENDINOPATHIES: A RETROSPECTIVE EVALUATION
Presenter: Isik Akgun, MD (Turkey)
Affiliation: Istanbul University
Authors: Akgun I, Kivrak A, Kayaalp ME, Ünlü MC

2:00 - 3:00 pm	**Concurrent 10 - Bone and Soft Tissues** Moderators: Marco Helder, PhD & Susanna Miettinen, PhD
2:00 pm	87 **SAFETY CONSIDERATIONS FOR COMPOSITE GLUTEAL AND CALF AUGMENTATION PROTOCOLS** Presenter: Katarina Andjelkov, MD, PhD (Serbia) Affiliation: BelPrime Clinic Belgrade Serbia Authors: Andjelkov K, Llull R
2:10 pm	88 **NON-RESPONSIVE SHOULDER PAIN WITH OSTEOARTHRITIS AND ROTATOR CUFF TEARS TREATED WITH AUTOLOGOUS, MICRO-FRAGMENTED AND MINIMALLY MANIPULATED ADIPOSE TISSUE UNDER CONTINUOUS ULTRASOUND GUIDANCE** Presenter: Jay Bowen, DO (USA) Affiliation: OptimumJoint Integrated Joint Spine Authors: Striano R, Malanga G, Bowen J, Bilbool N, Azatullah K
2:20 pm	89 **CLINICAL EVALUATION OF AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE AS A TREATMENT OPTION FOR MENISCUS TEARS** Presenter: Jay Bowen, DO (USA) Affiliation: New Jersey Regenerative Institute Authors: Malanga G, Raja A, Bowen JE, Wolf B
2:30 pm	90 **MICRO-FRAGMENTED ADIPOSE TISSUE INJECTION ASSOCIATED WITH ARTHROSCOPIC PROCEDURES IN PATIENTS WITH EARLY KNEE OSTEOARTHRITIS** Authors: Laura De Girolamo, PhD (Italy) Affiliation: IRCCS Galeazzi Orthopaedic Institute Authors: Cattaneo G, De Caro A, Napoli F, Chiapale D, De Girolamo L, Perucca Orfei C, Trada P, Camera A
2:40 pm	91 **STRUCTURAL FAT GRAFTING OF THE FOOT** Presenter: Solomon Azouz, MD (USA) Affiliation: Mayo Clinic Authors: Teausant AM, Bryant L, Boyll P, Azouz S, Rebecca A
2:50 pm	92 **IMPACT OF THE COMBINATION OF A PEPTIDE-MODIFIED GELLAN GUM HYDROGEL WITH ADIPOSE TISSUE DERIVED STEM CELLS IN A LUMBAR SPINAL CORD INJURY ANIMAL MODEL** Presenter: Ant Salgado, PhD (Portugal) Affiliation: University of Minho Authors: Salgado A, Gomes E, Mendes S, Leite-Almeida H, Gimble J, Tam R, Schoichet M, Sousa N, Silva N
3:00 - 3:30 pm	Coffee Break in Exhibit Hall
3:30 - 4:30 pm	**Guest Speaker** **The Turbulent Healthcare and Regulatory Environment and What it Means for Adipose Derived Regenerative Therapies** Mary Ann Chirba, JD, DSc, MPH - *Professor, Boston College Law School, Boston, MA; Joint faculty appointment, Tufts Medical School JD/MPH program; Adjunct Professor of Law, NYU Law School, New York, NY, IFATS Advisory Board* Moderator: Adam Katz, MD, FACS
4:30 - 5:30 pm	**Panel: Buttock Fat Grafting - Catastrophic Events** Moderator: J. Peter Rubin, MD, FACS Panelists: Sydney Coleman, MD & Ricardo Rodriquez, MD

7:00 pm	**White Nights Party at Nikki Beach**

Sunday - December 3, 2017

7:30 am	Coffee
8:00 - 8:10 am	Introductory Remarks
8:10 - 10:10 am	**Plenary Session 4 - Hot Topics** Moderators: Marco Helder, PhD & Ramon Llull, MD, PhD
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8:20 am	94 **APPLICATION OF GOOD MANUFACTURING PRACTICE TO STROMAL VASCULAR FRACTION PRODUCTION IN EUROPE : EXPERIENCE IN A PUBLIC HOSPITAL CELL THERAPY UNIT** Presenter: Guy Magalon, MD (France) Affiliation: AMU AIX Marseille University Authors: Magalon G, Magalon J, Veran J, Sabatier F
8:30 am	95 **A MICROPHYSIOLOGIC PLATFORM FOR HUMAN FAT: SANDWICHED WHITE ADIPOSE TISSUE** Presenter: Steven Scahill, BS (USA) Affiliation: Louisiana State University HSC Authors: Scahill S, Vogel K, Luckett JP, Meyer A, Rogers C, Tessler O, Dupin C, St. Hilaire H, Islam KN, Gimble J, Lau FH
8:40 am	96 **STROMAL VASCULAR FRACTION (SVF) FOR THE TREATMENT OF ERECTILE DYSFUNCTION- A PILOT STUDY** Presenter: Elliot Lander, MD (USA) Affiliation: Cell Surgical Network Authors: Lander E, Berman MH
8:50 am	97 **IMPROVEMENT OF BONE ALLOGRAFT RECOLONIZATION BY ADIPOSE STEM CELLS: IMPACT OF BONE GRAFT DEMINERALIZATION** Presenter: Sophie Veriter, PhD (Belgium) Affiliation: Novadip Biosciences Authors: Veriter S, Palacios P, Mauquoy S, Plougonven E, Dufrane D
9:00 am	98 **NON-RECONSTRUCTABLE PERIPHEERAL VAXCULAR DISEASE IN 10 PATIENTS TREATED WITH ADIPOSE-DERIVED STROMAL VASCULAR FRACTION CELLS** Presenter: Michael Carstens, MD (USA) Affiliation: Saint Louis University Authors: Carstens M, Correa D, Gomez A, Cortes R, Turner E, Perez C, Ocon M
9:10 am	99 **ADIPOSE DERIVED STEM CELLS (ASCS) ISOLATED FROM SUPER HEALER MICE HAVE IMPROVED REGENERATIVE POTENTIAL: IMPLICATION FOR WOUND HEALING AND BETTER AGING** Presenter: Xiaodong Mu, PhD (USA) Affiliation: University of Texas Authors: Mu X, Chen W, Ravuri S, Huard J

9:20 am

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MICROCIRCULATORY RESPONSES IN-VIVO ON LOCAL INTRA-ARTERIAL INFUSION OF AUTOGENIC ADIPOSE-DERIVED STEM CELLS OR STROMAL VASCULAR FRACTION
Presenter: Wei Wang, MD (USA)
Affiliation: UNLV School of Medicine
Author: Wang W

9:30 am

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IDENTIFICATION OF THE ADIPOGENIC RESPONSE OF ADIPOSE DERIVE STEM CELL FOLLOWING BREAST CANCER STIMULATION
Presenter: Connor King, BS (USA)
Affiliation: Louisiana State University
Authors: King C, Byrne CE, Bunnell BA, Martin EC

9:40 am

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VASCULAR SMOOTH MUSCLE CELLS AND PERICYTES DERIVED FROM THE ADIPOSE TISSUE EXHIBIT A MYOFIBROBLAST PHENOTYPE IN PATHOLOGIC CONDITIONS
Presenter: Howard Ray, BSE (USA)
Affiliation: University of Virginia
Authors: Ray H, Kesting SG, Bruce AC, Peirce SM, Yates PA

9:50 am

WITHDRAWN

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~~A COMPARISON OF AMPK-TARGETED DRUG RESPONSES IN HUMAN STROMAL VASCULAR FRACTION-DERIVED WHITE AND BROWN FAT IN 2D AND 3D~~
Presenter: Robert Bender, MS (USA)
Affiliation: Tulane University and LaCell
Authors: Bender R, McCarthy M, Wu X, Smith S, Gimble J, Frazier T

10:00 am

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ENRICHMENT OF AUTOLOGOUS FAT GRAFTS WITH EX VIVO-EXPANDED ADIPOSE TISSUE-DERIVED STROMAL CELLS IN COSMETIC BREAST AUGMENTATION: A RANDOMIZED CONTROLLED CLINICAL TRIAL
Presenter: Stig-Frederik Trojahn Kølle, MD, PhD (Denmark)
Affiliation: Aleris Hamlet Hospitals
Authors: Trojahn Kølle SF, Fischer-Nielsen A, Asirvatham Gjorup C, Sonnich Rasmussen B, Taudorf M, Katz AJ, Jønsson B

10:10 - 10:25 am

Coffee Break

10:25 - 11:45 am

Guest Speaker

The Science of Regenerative Medicine and Longevity
Aubrey de Grey, PhD - *Chief Science Officer and Co-founder of SENS Research Foundation*
Moderator: Brian Johnstone, PhD

11:45 am

Concluding Remarks

AUTHOR INDEX

PAPER PRESENTATIONS

1

INFLAMMATORY FACTORS INHIBIT THE EFFICIENCY OF ASC-MEDIATED VASCULOGENESIS BY INCREASING THE LOCAL ACCUMULATION OF ACTIVIN A

Presenter: Dmitry Traktuev, PhD (USA)
Affiliation: Indiana University
Authors: Traktuev D, Merfeld-Clauss S, Lu H, March KL

Adipose stem/stromal cells (ASC) reside in a perivascular location in adipose tissue, share multiple phenotypical and functional properties with pericytes, and are able to support endothelial cell (EC) survival and vasculogenesis. These features prompted ASC to be used for vascular applications. Inflammation, which dominates many ischemic pathologies, is detrimental to vasculature. Using an *in vitro* model of vasculogenesis, which is based on EC cultivation on ASC, the effects of inflammatory factors TNFα and IL-1β on vessel formation were tested. Incubation of EC-ASC co-cultures in control media led to EC reorganization into dense networks of vascular structures. However, addition of inflammatory factors to the incubation media decreased the vessel density by 65% for TNFα and by 37% for IL-1β. To determine the mechanism responsible for these effects, the responses of EC and ASC monocultures to these factors was tested. In ASC, both TNFα and IL-1β decreased expression of the important mediator of vasculogenesis as PDGF-Rβ, but stimulated secretion of VEGF; and, surprisingly, when combined with PDGF-BB produced an additive effect. In addition, the expression of Activin A, an angiostatic and pro-inflammatory factor, was evaluated. At baseline, EC show low level of Activin A expression and both TNFα and IL-1β stimulated Activin A secretion. ASC, which do not secrete activin A at baseline, were nonresponsive to TNFα, but induced Activin A secretion in response to IL-1β. To test whether the decrease in vasculogenesis was attributable to Activin A activity, EC-ASC co-cultures incubated in the presence of TNFα/IL-1β were supplemented with neutralizing Activin A or isotype control IgG. As expected, TNFα/IL-1β decreased vessel density by 50% but silencing Activin A signaling completely eliminated the inflammatory factor's detrimental effect. In conclusion, both EC and ASC respond to TNFα and IL-1β by induction/upregulation of Activin A, which then diminishes the efficiency of vasculogenesis. This suggests that therapies based on local injection of ASC alone or in combination with EC to areas with inflammatory processes designed to promote tissue vascularization may benefit from supplemental anti-Activin A therapy.

2

MECHANISTIC EFFECTS OF ADIPOSE STROMAL VASCULAR FRACTION THERAPY DURING INFLAMMATION AND AUTOIMMUNITY

Presenter: Annie Bowles, MS (USA)
Affiliation: Tulane University
Authors: Bowles A, Wise RM, Thomas RC, Gerstein BY, Ogelman R, Manayan RC, Bunnell BA

The robust anti-inflammatory and immunomodulatory effects of cultured adipose-derived stem cells (ASCs) have been well demonstrated for numerous clinical conditions, however less is known about the stromal vascular fraction (SVF). These freshly isolated heterogeneous SVF cells are widely used in reconstructive procedures to improve fat graft retention, yet the mechanisms which mediate these outcomes are still unknown. Furthermore, the immunomodulatory potential of the SVF support the growing evidence for its use to treat clinical indications such as inflammatory and autoimmune diseases. In this study, we investigated the mechanistic effects following treatment with SVF cells and ASCs in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis. We showed the comprehensive effects of these therapies during active autoimmunity and after the pathogenesis of disease, thus early and late-state treatment, by analyzing the central nervous system (CNS) and peripheral lymphoid tissues and blood. This evidence revealed that SVF cells, and not ASCs, markedly suppressed effector T cell differentiation during autoimmunity which led to attenuation of the disease progression. At late-stage disease, SVF cells rapidly induced anti-inflammatory macrophages which led to repair, and possibly regeneration, of CNS tissues. Together with our previous work, SVF cells are capable of robust immunomodulation by promoting regulatory T cells and high levels of interleukin-10 while diminishing pathogenic cell and associated inflammatory mediators. The translation evidence from these studies suggest the use of SVF cells and ASCs at an optimal time of administration as well as the mechanistic effects following these therapies that mediate improvements in vivo.

3

SHAKING THE GROUND YOU WALK ON: ARE ADIPOSE-DERIVED STEM CELLS (ASCS) TRUE STEM CELLS?

Presenter: Angelo A. Leto Barone, MD (Italy)
Affiliation: Plastic and Reconstructive Surgery
Authors: Di Stefano AB, Leto Barone AA, Grisafi F, Montesano L, Russo A, Cordova A, Moschella F

Introduction: Adhesion-based culture conditions have been the standard technique for in vitro expansion of ASCs. However, stem cells from different organs grow in suspension and display a more primordial phenotype characterized aggregation in clusters as spheroids. We hypothesized that S-ASCs could represent an upstream stage of the traditional adherent ASCs (aASCs) before they enter an early differentiation pathway leading to their adhesion. Molecular profiles of miRNAs and mRNAs were used between aASCs and S-ASCs to investigate our hypothesis.

Methods: Lipoaspirate samples were processed for the extraction of S-ASCs as previously described by our lab. The miRNAs profile was analyzed using Taqman Array Human MiRNA A Cards in S-ASCs and aASCs cells. Statistically significant changes are considered up- or down-regulation of miRNA expression higher than 2 folds compared to control ($p<0.001$).

Results: After a screening analysis of several miRNAs, we compared molecular patterns between S-ASCs with aASCs and the principal miRNAs and mRNAs involved with the stemness and mesenchymal differentiation. S-ASCs displayed significant up-regulation of miR-142-3p and SOX2, OCT4, NANOG, (5-fold increase) typically expressed in the pluripotent stem cells. Consequently, the early (SOX-9, RUNX-2, PPARg, miR-495, miR-221, miR-30c) and late (LPL, ALP, COL10A, miR-140, miR-143 and miR-100) RNAs correlated with mesenchymal differentiation was up-regulated in aASCs. Furthermore, we have assessed the same molecular analysis in S-ASCs and aASCs during different time in vitro culture up to 28 days. The results have demonstrated the maintenance of stemness only in S-ASCs, expressing high level of pluripotent stem cells markers and low level of differentiation markers.

Conclusion: S-ASCs overexpress important pluripotent stem cells markers typical of iPS cells that are not present in aASCs. Furthermore, miRNAs and mRNAs typical of differentiated cells in multiple lineages were significantly under-expressed in S-ASCs while being over-expressed in aASCs. This molecular pattern supports the upstream nature and the stemness maintenance of S-ASCs and the down-stream and more differentiated precursor nature of aASCs. This data represents the first step in the recognition of S-ASCs as the true stem cell population within adipose tissue.

4

THE SECRETOMES OF ADIPOSE DERIVED STEM CELLS AND COMPLETE STROMAL VASCULAR FRACTION ENHANCE MYOBLAST PROLIFERATION VIA DIVERSE SIGNALLING FACTORS

Presenter: Paul Kingham, PhD (Sweden)
Affiliation: Umeå University
Authors: Kingham P, El-Habta R, Backman LJ

Introduction: Functional muscle recovery after peripheral nerve injury is far from optimal, mainly because of atrophy of the muscle due to prolonged denervation. Previously, using an experimental animal model, we showed that injections of Schwann cell-like differentiated adipose derived stem cells (dASCs) into denervated muscle reduced the atrophy and enhanced hind limb functionality. In this current study, we have investigated the in vitro interactions between the stem cells and myoblasts.

Methods: Adipose derived stem cells were stimulated with a mix of factors (bFGF, PDGF-AA, neuregulin-1 and forskolin) to induce the Schwann-cell like phenotype. The dASCs, undifferentiated stem cells (uASCs) or complete stromal vascular fraction (SVF) were indirectly co-cultured with primary myoblasts or the L6 cell line. Proliferation of the myoblasts was assessed by MTS, BrdU and qRT-PCR assays. The signalling mechanisms between the cells were investigated using a range of biochemical, molecular and pharmacological assays.

Results: Both dASCs and SVF enhanced proliferation of myoblasts whereas uASCs had no effect. The dASCs and SVF activated ERK1/2 signalling in the myoblasts and inhibition of this pathway with a MEK inhibitor abolished the proliferative effects on the cells. However, the dASCs and SVF appear to act via diverse upstream mechanisms. The dASCs expressed the protein machinery necessary for acetylcholine production but this was not detected in the SVF or uASCs. Treatment of dASCs/myoblast co-cultures with the muscarinic acetylcholine receptor blocker atropine attenuated the proliferation of the myoblasts. In contrast, atropine had no effect on the SVF-evoked myoblast proliferation. Comparison of dASCs and SVF using PCR growth factor arrays showed a number of genes with greater than 20 fold higher expression in the SVF. These included Hgf, Vegfd and Fgf10 and the relative importance of these molecules in SVF-mediated proliferation of myoblasts is under investigation.

Conclusions: These studies provide new insights into how adipose tissue derived cells mediate effects on myoblasts via their secretomes. The knowledge gained could be used to improve regenerative cell therapy strategies for treatment of the injured neuromuscular system.

5

DECREASED SIRT1 ACTIVITY NEGATIVELY AFFECTS MITOCHONDRIAL FUNCTION AND BIOGENESIS LEADING TO BLUNTED ADIPOGENESIS IN AGING CELLS

Presenter: Ivona Percec, MD, PhD (USA)
Affiliation: University of Pennsylvania
Authors: Percec I, Raum JC, Dierov R

Introduction: With the increase in the number of aging and obese patients with metabolic syndrome, understanding the mechanisms of adipocyte-driven insulin resistance is of prime importance. Defects in the differentiation of resident adipose stem cells (ASC) to adipocytes have been implicated as a causative agent in insulin resistance. It has been recently demonstrated that mitochondrial function is critical for differentiation of stem cells into adipocytes. SIRT1 is a protein deacetylase with various targets including transcription factors and histones. Critically, SIRT1 function has been linked to mitochondrial biogenesis in multiple cell types. Here we examine the role that aging has on mitochondrial function and biogenesis during adipogenesis and specifically how SIRT1 regulates these processes.

Methods: ASCs were isolated from adipose tissue collected from young (<35 years old) and old (>55 years old) patients (BMI<28) underduring abdominoplasty. To generate SIRT1 knockdown cells, young ASCs were infected with virus containing either scrambled or SIRT1 targeting siRNAs. To examine the effect aging and SIRT1 play during adipogenesis, ASCs were differentiated for 14 days and mRNA and gDNA were harvested at day 0, 7 or 14. Furthermore young and old untreated cells were differentiated and mitochondrial function assessed.

Results: Aging negatively affected mitochondrial biogenesis and function during adipogenesis. Mitochondrial biogenesis (mtDNA) was decreased in older (5.15±0.05 fold) vs younger (8.9±0.1 fold) ASCs. Furthermore, mitochondrial function at Day 7 of adipogenesis was significantly blunted at baseline (4.42±0.01 vs 2.51±0.16 pmol/min/ug/20ul) and maximal mitochondrial output (10.01±0.47 vs 7.21±0.29 pmol/min/ug/20ul). Lastly we noted that cells infected with siSIRT1 had decreased accumulation of Oil Red O, indicating incomplete differentiation, a phenotype similar to that of aging cells.

Conclusion: Our results indicate that aging affects adipogenesis by modulating mitochondrial function. This is potentially due to altered SIRT1 function. Future experiments are aimed at elucidating the exact molecular mechanism for SIRT1 function on mitochondria and how this affects adipogenesis in aging humans.

6

THE REDUCED REGENERATIVE POTENTIAL OF AGED AND DIABETIC ADIPOSE DERIVED STROMAL CELLS IS CAUSE BY A DISRUPTION OF SUBPOPULATION DYNAMICS

Presenter: Dominik Duscher, MD (Germany)
Affiliation: Klinikum Rechts der Isar - Technical University of Munich
Authors: Duscher D, Aitzetmueller MM, Hopfner U, Machens HG

Introduction: Mesenchymal stromal cells derived from adipose tissue (ASCs) have been used clinically to promote wound healing. However, the regenerative capacity of ASCs is impaired in diabetic and aged populations, limiting the efficacy of autologous cell based therapies in these patients. Exploring cell enrichment strategies to overcome this deficiency, we employed transcriptional analysis paired with a novel bioinformatics approach to identify and isolate a subpopulation of ASCs with increased regenerative potential.

Methods: Primary ASCs were isolated from human and murine healthy, diabetic and aged adipose tissue, and microfluidic-based single cell transcriptional analysis was employed to characterize the expression of 96 genes. A novel bioinformatic clustering analysis was used to identify ASC subpopulations based on transcriptional profiles, and a putatively pro-vasculogenic subpopulation was prospectively isolated using fluorescence assisted cell sorting (FACS) for assessment of enhanced functional capacity in vitro and in vivo.

Results: Single cell transcriptional analysis revealed a subpopulation of human and murine ASCs characterized by an elevated expression of multiple stemness-associated and pro-angiogenic genes, which was significantly depleted in ASCs isolated from diabetic and aged samples. Prospective subpopulation isolation using correlative surface markers resulted in prolonged retention of progenitor associated surface antigens, increased cell survival, proliferative capacity and clonogenicity, and upregulation of angiogenic cytokines when compared to negatively selected and parent populations. When applied to an in vivo diabetic wound healing model, this newly defined ASC subpopulation significantly improved healing compared to negatively selected and parent populations, and critically restored normal healing kinetics to diabetic wounds.

Conclusion: Functionally distinct ASC subpopulations can be transcriptionally identified and linked to surface marker expression for prospective isolation and verification of predictive functionality. Demonstrating the validity of this approach, enrichment of a putatively pro-angiogenic ASC subpopulation was found to enhance the regenerative potential of ASC-based therapies in diabetic wounds.

7

EFFECTS OF THE INTRADISCAL IMPLANTATION OF STROMAL VASCULAR FRACTION PLUS PLATELET RICH PLASMA IN PATIENTS WITH DEGENERATIVE DISC DISEASE

Presenter: Kristin Comella, MS (USA)
Affiliation: Bioheart
Authors: Comella K, Silbert RK, Parlo M

Background: Stromal vascular fraction (SVF) can easily be obtained from a mini-lipoaspirate procedure of fat tissue and platelet rich plasma (PRP) can be obtained from peripheral blood. The SVF contains a mixture of cells including ADSCs and growth factors and has been depleted of the adipocyte (fat cell) population. We evaluated the safety and efficacy of administering SVF and PRP intra-discally into patients with degenerative disc disease.

Methods: A total of 15 patients underwent a local tumescent liposuction procedure to remove approximately 60 ml of fat tissue. The fat was separated to isolate the SVF and the cells were delivered into the disc nucleus of patients with degenerative disc disease. The subjects were then monitored for adverse events, range of motion, visual analog scale (VAS), present pain intensity (PPI), Oswestry Disability Index (ODI), Beck Depression Inventory (BDI), Dallas Pain Questionnaire and Short Form (SF)-12 scores over a period of 6 months. Safety events were followed for 12 months.

Results: No severe adverse events (SAEs) were reported during a 12 month follow up period with no incidences of infection. Patients demonstrated statistically significant improvements in several parameters including flexion, pain ratings, VAS, PPI, and short form questionnaires. In addition, both ODI and BDI data was trending positive and a majority of patients reported improvements in their Dallas Pain Questionnaire scores.

Conclusions: Overall, patients were pleased with the treatment results. More importantly, the procedure demonstrated a strong safety profile with no severe adverse events or complications linked to the therapy.

8

AN EXPERIMENTAL STUDY AND A CLINICAL COHORT STUDY OF SVF-GEL ASSISTED LIPOTRANSFER

Presenter: Xiuying Shan, MD (China)
Affiliation: The First Affiliated Hospital of Fujian Medical University
Authors: Shan X, Chen L, Lei C, Liu Z, Wang B

Objection: To investigate the validity of SVF-GEL assisted lipotransfer via animal experiments and evaluate its efficacy in facial rejuvenation treatment.

Methods: 1. BALB/c nude mice were separated into four groups. Adipose tissue compounded with NS, SVF-GEL, PRP or PRF was transplanted to back of mice from different groups respectively. Then gross morphology of adipose granule or its compounds, volume of adipose granule and microvascular density were measured and statistically analyzed at postoperative 1 week, 2 week, 4 week, 8 week, 12 week. 2. A cohort study which enrolled facial hollow patients between December 2016 and March 2017 was carried out. Fat tissue from patients' thigh or hypogastrium was compounded with SVF-GEL and then implanted to correct facial concave deformity.

Results: 1. At all postoperative time points, gross morphology of compound, volume of adipose granule and microvascular density in SVF-GEL+AG group was superior to the other two groups with statistical significance. There was no necrosis, cyst, hard junction or calcification in any group. 2. Adipose granule compounded with SVF-GEL was used in facial rejuvenation treatment for 20 patients. AG- SVF-GEL was implanted to fill up areas including fronto-temporal region, peri-orbital region, nasal dorsum, nasolabial groove, chin, etc. After 3 months follow-up, all patients have got satisfactory outcome without revision surgery.

Conclusion: SVF-GEL assisted lipotransfer promotes early vascularization and adipogenic differentiation in grafts, which leads to acceleration in fat tissue remodeling and improvement in the survival rate of the fat grafts. SVF-GEL assisted lipotransfer achieved satisfactory outcomes in facial rejuvenation treatment.

Keywords: ADSCs, SVF-GEL, PRF, PRP, Fat Grafting

9

MESOAMERICAN NEPHROPATHY, CHRONIC KIDNEY FAILURE OF UNKNOWN ETIOLOGY, IN 19 PATIENTS TREATED WITH ADIPOSE-DERIVED STROMVAL VASCULAR FRACTION (SVF) CELLS VIA INTRA-ARTERIAL INFUSION: A PRELIMINARY REPORT QUANTITATIVE REDUCTION OF RESISTANCE INDEX

Presenter: Michael Carstens, MD (USA)
Affiliation: Saint Louis University
Authors: Carstens M, Jarquín M, García N, Pastora I, Chavarría D, Correa D

Mesoamerican nephropathy (MeN) is a form of chronic kidney disease of unknown etiology that is endemic to rural inhabitants of the Pacific coast of Central America, where agricultural manual labor is the predominant form of employment. MeN patients are relatively young, have elevated creatinine levels, normal or sub-clinical levels of albuminuria, and are normotensive. The disease is characterized by chronic tubulointerstial fibrosis and ischemia, leading to small, scarred kidneys. We present a series of nineteen patients with MeN in clinical stages 3-4 treated with non-expanded autologous adipose-derived stromal vascular fraction (SVF) cells for the purposes of reducing inflammation and fibrosis and enhancing neovascularization in the kidneys for Adipose tissue was surgically harvested and processed to yield heterogeneous SVF cells for immediate point-of-care administration via intra-arterial injection. Response to treatment was evaluated clinically, based upon symptoms, measurements of serum/urine parameters, and biomarkers; and by imaging using renal ultrasound with power Doppler to measure kidney dimensions and renal vascular resistance indices (RI). All patients demonstrated clinical improvement in constitutional symptoms, creatinine levels, and (when applicable) improvements in proteinuria. Ultrasound demonstrated significant increases in flow. Whereas arterial perfusion preop was limited to the renal arteries, by 2 months it had extended to the level of the interlobar arteries in 100% and to the arcuate arteries in 40% of patients. Measurements of RI provided another indicator of blood flow, showing at 2 months clinically significant decreases in 35 of 38 kidneys (92%). 18/19 patients had at least one responsive kidney (94%). The parameters will be followed at 4 and 6 months to determine if improvement is progressive or static. This series suggests a potential role for non-expanded adipose-derived heterogeneous SVF cells processed at the point-of-care, to treat patients with MeN in the hopes of augmenting perfusion, reducing ischemia and fibrosis and thereby forestalling further progression of the disease.

10

THE USE OF ADIPOSE TISSUE IN SCALP REGENERATION

Presenter: Marco Pellon, MD (Brazil)
Affiliation: Clinica Sao Vicente
Author: Pellon M

Introduction: The reconstruction of defects of the scalp with exposure of the cap is a challenge that stays throughout the history of plastic surgery. Despite the effectiveness of technics such as those described by Orticochea in 1964 up to modern techniques of microsurgery, there are situations where no technique seems to solve the problem. Cases where there is large exposure of the bone board, with involvement of the surrounding structures, remains difficult to solve. The mechanisms of wound regeneration are regulated by cells form bone marrow via bloodstream and by multiple types of resident cells of skin and fat tissue. Remembering that the bone marrow is located just below the exposed bone surface, we have created a direct line between the fat grafts and the bone marrow, increasing the effectiveness of the regenerative response.

Method: The author conducted a clinical trial in 8 patients, treated at the Burn Care and Trauma Unit, from 2016 to 2017. The lesions size ranged from 3 to 8 cm of diameter and were produced by electrical burns, necrosis of flaps and skin cancer resections. The age ranged between 22 and 65. Perforations were drilled in the external table of the exposed bone, to access the bone marrow. We applied the fat graft 'in natura' on the wound, and then we covered with synthetic gauze and applied a layer of hydrocolloid to reduce cell desiccation. This dressing is reviewed after 3 or 5 days. In two cases the fat graft procedure was repeated after 10 days.

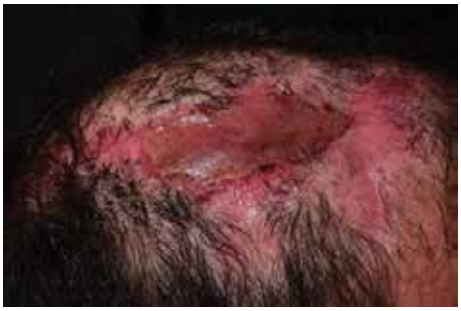
Results: The author observed a rapid migration of vascular structures from the edges of the wound and from the bone marrow, through the perforations produced on exposed bone. This vascular network allowed the skin grafting for definitive wound closure. On average the lesions took two months to reach the expected result.

Conclusion: The application of adipose tissue associated with perforations in the external bone plate to stimulate the bone marrow, was shown to be a very effective method in regeneration of these lesions, opening a new possibility of treatment for these patients.

10

THE USE OF ADIPOSE TISSUE IN SCALP REGENERATION

Presenter: Marco Pellon, MD (Brazil)
Affiliation: Clinica Sao Vicente
Author: Pellon M



11

EFFICACY OF LIPOINJECTION COMBINED WITH STROMAL VASCULAR FACTOR IN PATIENTS WITH SEVERE CONTRACTURES DUE TO BURNS

Presenter: Mehmet Bozkurt, MD (Turkey)
Affiliation: Istanbul Lutfi Kirdar Kartal Education and Training Hospital
Authors: Bozkurt M, Ceran F

Introduction: Burn injuries have high mortality in the acute phase and morbidity in the long term. The mortality risk was minimized in parallel with the developments in the management of the acute period. It can not be said for persistent burns contractures (BC), which is one of the morbidities. In advanced stages, BC are likely to be encountered with extremity contractures may lead to extensive functional impairment. Contractures tend to repeat likely, so reconstructive surgical initiatives should increase the function and reduce the recurrence. We demonstrated the efficacy of lipoinjection enriched with stromal vascular factor (SVF) in addition to conventional skin grafting (SG) in the treatment of BC in this study.

Method: Fifty patients with complaints of the BC were admitted to our clinic. Patients were operated for contracture releasing under general anaesthesia. After contracture releasing, dermal scaffolds and SG were applied. 100 cc of lipoinjection combined with SVF procedures were performed on postoperative 6 month for all patients to create sufficient soft tissue under the grafted skin and contour. Preoperative, early and late postoperative range of motion (ROM) was measured.

Results: Fifty patients between the ages of 2 and 44 (Mean:15) were included in the study. Follow-up time was 18 months. Significant differences were found between preoperative and postoperative ROM. There was improvement in skin quality and flexibility, reduction of hypertrophic scarring.

Conclusions: Inadequate skin quality and flexibility, insufficient ROM and high reccurence incidence are common problems in BC treatment. Inability to use local or regional flaps is another trouble for extremities with BC. The options are limited to SG and free flaps (FF) for these patients. Donor area morbidity, long hospital stay and economical limitations are disadvantages for FF. SG tend to contract in the long term when used alone. Lipoinjection provides sufficient amount of subcutaneous soft tissue, and SVF provides an excellent nutritive environment for the injected fat. This procedure creates shorter hospitalization times for patients, reduces the repetition tendency of the contractures, achieves near perfection in terms of aesthetic and functional, and results in less economic losses.

12

AUTOLOGOUS FREE FAT TRANSFER FOR ANAL FISTULAE ENABLES CLOSURE OF TEMPORARY STOMAS IN ANTI-TNF RESISTANT MB CROHN PATIENTS

Presenter: Susanna Kauhanen, MD, PhD (Finland)
Affiliation: Helsinki University Hospital
Authors: Kauhanen S, Salmenkylä S

Introduction: Perianal Crohn's disease with complex fistulas leads to a permanent stoma in 20% of patients even in the era of biological drugs. Lately adipose-derived stem cells (ASC) have been advocated in treating complex fistulas. In world-wide public health care, however, isolation of SVF with subsequent cell culturing is not available for every patient. Therefore we conducted a pilot study, using simple, ordinary, yet high quality, free fat transfer as a treatment for persistent fistulae in Mb Crohn patients.

Methods: Two patients with severe long-standing fistulizing Crohn's disease refractory to anti-TNF and temporary stoma were treated. In general anesthesia, lipoaspirate was harvested with a water assisted closed system from the abdominal region or the thigh. The aspirate was decanted on the table for a few minutes. Then fat was re-injected around the fistular tracts penetrating the crypts from several different directions in a three-dimensional manner and also performing curettage of the surface of cavities and covering the surfaces with lipoaspirate. The mean volume of fat injected was 30 ml (range 24-40 ml). Closure of the inner opening of fistulae was performed. Patients were followed with MRI scans 3 and 6 months postoperatively and clinically while continuing the anti TNF-therapy.

Results: In both cases, 6 months after two autologous fat transfers 4 months apart full clinical response was achieved. After the first procedure fistulae diminished in size and secretion and after the second spontaneous closure of fistular tracts could be seen. Temporary stomas were reversed. Follow-up comprised 1 year and 3 months after the second session of fat grafting. There has not been a relapse.

Conclusion: Persisting fistulae prevent closure of stoma and thus a need for other treatment options is eminent. The fistulae biologically behave like chronic wounds stuck in an inflammatory phase. Transfer of high quality autologous free fat into the area, containing ADSC;s, macrophages etc, offers a versatile but simple tool to mechanically remove biofilm, soften the scars tissue, cushion and fill tissue defects, improve blood supply and eventually shift the inflammatory phase of the tissue in a regenerative direction moderating immune responses and repair.

13

DECELLULARIZED ADIPOSE TISSUE SCAFFOLD FOR SOFT TISSUE RECONSTRUCTION

Presenter: Francesco M. Egro, MBChB, MSc, MRCS (USA)
Affiliation: University of Pittsburgh Medical Center
Authors: Egro FM, Schusterman M, Sivak WN, Grybowski D, Mahoney C, Minteer DM, Kokai LE, Marra KG, Rubin JP

Introduction: Fat grafting has been shown to be a potent tool for soft tissue augmentation in a variety of settings. Animal studies have shown the efficacy of decellularized adipose tissue to serve as a scaffold for neovascularization and ingrowth of adipose tissue, representing a potential new option for soft tissue reconstruction in addition to traditional fat grafting. This study reports our clinical and histological results after testing this product in human subjects.

Methods: 10 subjects are being enrolled in an IRB approved clinical trial testing a decellularized adipose tissue product in human subjects. All subjects are initially evaluated by a plastic surgeon and must be an appropriate candidate for panniculectomy or abdominoplasty. Subjects must have a thickness of at least 2 cm of subcutaneous fat on the anterior abdominal wall. After passing screening, each subject undergoes injection of 20cc of the product into 6 individual 5cm x 3cm areas in the subcutaneous fat within the predetermined area of pannus excision. Each subject is randomized to panniculectomy either 3 or 6 months after injection. Photos and abdominal wall ultrasound are performed at each clinic visit, and biopsies of one site are performed in the clinic at 1 and 2 months. Product specimens obtained at biopsy and surgery are fixed in formalin and stained with H&E, Masson's Trichrome, and Verhoeff's stain. In addition, specimens were stained for CD31, perilipin, collagen 1, and collagen 3.

Results: Injection has been performed in 6 subjects with no adverse reactions directly related to the product. Three subjects have successfully undergone panniculectomy or abdominoplasty at 3 months post-injection. The only adverse event was a surgical site infection in one patient which occurred after a biopsy and was successfully treated with oral antibiotics. Thus far, tissue specimens have been obtained at 1, 2 and 3 months. Histological evaluation is in progress.

Conclusion: Early results demonstrate the safety of this decellularized adipose tissue for use in human subjects. We hypothesize that completion of the histological evaluation will demonstrate the generation of native adipose tissue, making the decellularized adipose product a unique tool for use in soft tissue reconstruction.

14

TRANSLATIONAL ADIPOSE-DERIVED STEM CELL-BASED IMMUNOMODULATORY THERAPY IN A PORCINE HIND-LIMB TRANSPLANT MODEL

Presenter: Deokyeol Kim, MD (USA)
Affiliation: University of Pittsburgh
Authors: Kim D, Waldner M, Zhang W, Barone A, Cooney D, Solari M, Washington K, Marra KG, Gorantla V, Brandacher G, Rubin JP

Background: Immunomodulatory effects and low immunogenicity of mesenchymal stem cells have been confirmed in experimental and clinical studies. Our team was the first to use bone marrow-derived stem cells (BMSCs) for immunomodulation in clinical vascularized composite allotransplantation (VCA). The significance of adipose-derived stem cells (ASCs) as an immunomodulatory or tolerogenic cell therapy in VCA is just emerging. This study was designed to investigate whether ASC treatment could prolong graft survival in a porcine large animal VCA model.

Methods: Full and partial swine leukocyte antigen mismatched heterotopic hind-limb transplantations were performed in MGH mini-swine. Animals receiving no therapy and animals treated with tacrolimus for 30 days served as negative and positive controls, respectively. Experimental animals were treated with pre-surgical radiation and a standard immunosuppressive protocol including tacrolimus for 30 days, followed by ASC or BMSC therapy (donor-derived [1.0x10^6 cells/kg] cells were administered intravenously in VCA recipients at POD 7). Allograft survival was compared across the different treatment protocols.

Results: A total of eleven allogenic hind limb transplantations in the mini-swine were performed in six groups. Untreated controls reached Banff grade 4 acute rejection by average 7.5 days after transplantation. In a partial mismatch, the mean allograft survival day of BMSCs therapy group was 92.5, which is not significantly different compared to the standard immunosuppressive group. Allotransplants in the ASCs therapy group showed donor-specific unresponsiveness at day 50 and 100 postoperatively. Allografts treated with ASCs across a full mismatch demonstrated grade 4 rejection at 119 days and rejection-free survival over 190 days postoperatively which showed no histological signs of rejection.

Conclusion: Early results of ongoing in vivo experimentation showed promising results of ASC therapy in prolonging rejection-free VCA survival across a full SLA mismatch. Correlation of graft survival with in vitro immune surrogates is currently in progress to further delineate the immunomodulatory effects of ASCs in VCA.

15

LIPOTRANSFER IMPROVES MEASURES OF APPEARANCE AND FUNCTION DUE TO FACIAL FIBROSIS IN SYSTEMIC SCLEROSIS

Presenter: Aurora Almadori, MD (Italy)
Affiliation: Second University of Naples
Authors: Almadori A, Ryan C, Griffin M, Hansen E, Denton C, Butlet P

Background: Systemic sclerosis (SSc) is characterised by fibrosis of the skin, underlying connective tissue structures, and localised loss of subcutaneous fat. Oro-facial fibrosis has major impact on oro-facial function, facial appearance, and quality of life. Based upon theoretical rationale for antifibrotic potential and improvement in connective tissue bulk we have evaluated the benefit of autologous lipotransfer in a large cohort of SSc patients including validated outcome tools.

Methods: 62 SSc patients with oro-facial fibrosis were included. 61 were female and 1 was male. The mean age was 54. Of the 62 patients, 36 were affected by limited cutaneous systemic sclerosis (lcSSc) and 26 by diffuse cutaneous systemic sclerosis (dcSSc); 31 patients were on immunosuppressant medication and 31 patients were not. Efficacy was assessed by pre- and post-operative mouth function (Mouth Handicap in Systemic Sclerosis Scale, MHISS), validated psychological measurements (Darriford Appearance Scale, DAS24; Hospital Anxiety and Depression Scale-anxiety, HADS; Brief Fear of Negative Evaluation Scale, BFNE; Visual Analogic Scale for mood, emotion, and distress, VAS), and volumetric assessment (3dMD imaging system).

Results: We found a significant improvement of mouth function (MHISS) (6.85 ± 5.07) ($p<0.0001$) and all the psychological measures: DAS 24 (12.1 ± 9.5) ($p<0.0001$); HADS-anxiety (2.8 ± 3.2) ($p<0.0001$), HADS-depression (2.0 ± 3.1) ($p<0.0001$); BFNE (2.9 ± 4.3) ($p<0.0001$); VAS (3.56 ± 4.1) ($p<0.0001$). Multiple procedures further improved MHISS ($p<0.05$), DAS ($p<0.0001$) and VAS ($p=0.01$). Disease subset or concomitant immunosuppression did not appear to affect outcome measures. Injected volume was retained variably in all facial areas: cheeks (93.7%), nasolabial folds (81.9%), nose (67.4%), chin (68.2%), upper lips (35.5%) and lower lips (27.3%).

Conclusion: Autologous lipotransfer is a feasible treatment that reversed the effects of oro-facial fibrosis in SSc in this open cohort study. It has been shown to be both feasible and beneficial. Our findings warrant further testing in a randomised controlled trial.

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ADIPOSE-DERIVED STEM CELL EXTRACELLULAR VESICLES CAN BE SPECIFICALLY TUNED FOR SOFT TISSUE REPAIR AND WOUND HEALING

Presenter: Benjamin Buehrer, PhD (USA)
Affiliation: ZenBio
Authors: Buehrer B, Nicoll JB, Ludlow JW, Oschwald DL

Introduction: Stem cell therapies represent a compelling means of tissue repair and have repeatedly demonstrated wound healing and soft tissue regeneration in animal models. However, the intuitive concept that therapeutic stem cells engraft and differentiate at sites of tissue damage has not always been well supported in these models. This suggests that their mechanisms of action occur through paracrine modalities such as secretion of bioactive vesicles. Exploiting stem cell-derived extracellular vesicles (EVs) as a biologic-derived therapy, rather than delivering transient stem cells, is an enticing regenerative and wound healing approach.

Methods: Secreted EVs are readily isolated from our established ASC bioreactor by ultracentrifugation. To tune the EVs, the cellular environment was modulated to induce pro-healing protein and miRNA EV packaging by the cells. The isolated EVs were carefully analyzed for biophysical character (size, number, protein content, miRNA content) and in vitro repair and healing functionality (proliferation, ECM production and remodeling, inflammation reduction, neovascularization). Two common in vivo models (excisional wounds in rats; rodent model of tendon injury) were used to assess repair and healing in vivo through digital analysis and histopathology.

Results: Trillions of EVs can be produced per batch of ASCs using the bioreactor system. ASC-derived EVs are capable of inducing pro-healing responses in vitro and changes to the cellular environment are able to improve these activities. We observed significant tuned EV-induced increases in cellular proliferation, increased collagen I production, reduced inflammation, and increased neovascularization. The in vivo models recapitulated the observed in vitro responses with increased wound closure rates, increased collagen deposition and organization, increased proliferation, and reduced collagen degradation.

Conclusions: ZenBio has shown that EVs can be reliably and cost-effectively manufactured in a bioreactor system. By manipulating the bioreactor, we can amplify the pro-healing activities associated with stem cell-derived EVs. This potential therapeutic has the unique advantage of harnessing the power of stem cells without the need for utilizing complex cell therapies in vivo.

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INTRAVENOUS DELIVERY OF AUTOLOGOUS ADIPOSE DERIVED REGENERATIVE CELLS (ADRCS) IMPROVES HEALING IN A PORCINE THERMAL BURN MODEL INVOLVING 20% BODY SURFACE AREA

Presenter: John K. Fraser, PhD (USA)
Affiliation: Cytori Therapeutics
Authors: Foubert P, Liu M, Anderson S, Zafra D, Rajoria R, Gutierrez D, Tenenhaus M, Fraser JK

WITHDRAWN

18

THE BRCA1 MUTATION LEADS TO INCREASED SECRETION OF INTERLEUKIN-6 AND INTERLEUKIN-8 FROM ADIPOSE DERIVED STEM CELLS: IDENTIFYING A POTENTIAL TARGET IN THE TUMOR MICROENVIRONMENT

Presenter: Ruya Zhao, BS (USA)
Affiliation: Duke University School of Medicine
Authors: Zhao R, Fan L, Lee A, Pien I, Liu X, Seewaldt V, Li C, Hollenbeck S

Introduction: The tumor microenvironment plays an important role in breast cancer development and progression. We have previously shown that BRCA1 mutation in human adipose-derived stem cells (ASCs) leads to an altered secretome that promotes breast cancer aggression. In this study, we further investigate the mechanism responsible for this finding. We hypothesize that alterations in the DNA damage response and its downstream cytokine production are the major players in this pathway.

Methods: BRCA1-knockdown ASCs were generated using the CRISPR/Cas9 system in commercially available human ASC lines. After 48 hr incubation, ELISA assays were performed on cell supernatants of BRCA1-knockdown ASCs and control ASCs. Transwell assays were used to assess cell migration using human recombinant cytokines as attractants for human ER-positive breast cancer cells (MCF7). Gamma-H2AX immunofluorescence assays were performed to assess DNA damage in BRCA1-knockout ASCs and controls.

Results: We were able to generate effective BRCA1 knockdown in human ASCs using CRISPR/Cas9 system, confirmed by western blot (Figure 1A). BRCA1-knockdown ASCs demonstrated significantly elevated levels of interleukin-6 and interleukin-8 (Figure 1B, P=0.03, P=0.01, respectively). Transwell assays demonstrated significantly increased cell migration of MCF7 cells in media with IL-6 and/or IL-8 (Figure 2, P=0.03 for IL-6, P=0.01 for IL-8, P=0.002 for IL-6&IL-8). Gamma-H2AX assays demonstrated co-localization of BRCA1 and γ-H2AX protein, confirming the role of BRCA1 in DNA damage repair. BRCA1 knockout cells were confirmed by the lack of BRCA1 immunofluorescence within the nucleus. Interestingly, BRCA1-knockout ASCs showed decreased level of Gamma-H2AX foci, suggesting that the increase of the cytokine production in BRCA1-knockdown ASCs could be mediated by a pathway independent of DNA damage repair (Figure 3).

Conclusion: We confirmed our hypothesis that the BRCA1 mutation in adipose-derived stem cells leads to a secretory phenotype through IL-6 and IL-8 mediated pathways, which appears to be independent of DNA damage response.

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THE BRCA1 MUTATION LEADS TO INCREASED SECRETION OF INTERLEUKIN-6 AND INTERLEUKIN-8 FROM ADIPOSE DERIVED STEM CELLS: IDENTIFYING A POTENTIAL TARGET IN THE TUMOR MICROENVIRONMENT

Presenter: Ruya Zhao, BS (USA)
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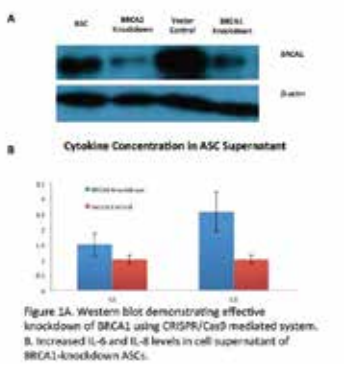

Figure 1A. Western blot demonstrating effective knockdown of BRCA1 using CRISPR/Cas9 mediated system. B. Increased IL-6 and IL-8 levels in cell supernatant of BRCA1-knockdown ASCs.

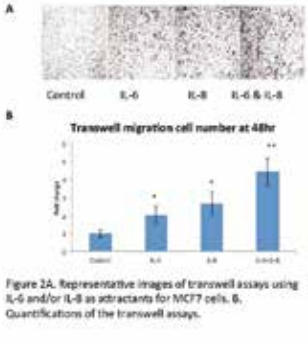

Figure 2A. Representative images of transwell assays using IL-6 and/or IL-8 as attractants for MCF7 cells. B. Quantifications of the transwell assays.

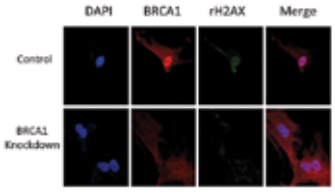

Figure C. Immunofluorescence images of γ-H2AX assays.

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DONOR-SPECIFIC ADIPOSE-DERIVED STROMAL CELLS ATTENUATE GRAFT VASCULOPATHY AND REJECTION IN RODENT VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Presenter: Riccardo Schweizer, MD (Switzerland)
Affiliation: University Hospital Zurich
Authors: Schweizer R, Klein H, Waldner M, Kollar B, Fuchs N, Lehner F, Taddeo A, Salemi S, Eberli D, Giovanoli P, Plock J

Introduction: Vascularized composite allotransplantation (VCA) is increasingly and successfully used for reconstruction of major defects of the upper extremity and face. Graft vasculopathy (GV) seriously endangers long-term outcomes leading to graft failure. GV remains widely unexplored in VCA, and so does the role of Adipose-derived Stromal Cells (ASCs) in reversal or attenuation of rejection.

Methods: ASCs were isolated from donors, characterized and their immunomodulatory capacity investigated. Systemic (SASC) versus local intragraft (LASC) ASC administration was evaluated for therapy of rejection and GV in both acute and chronic models of full-mismatched rat hind-limb transplantation after discontinuation of Tacrolimus immunosuppression. Tissues (skin/muscle/vessels) and blood samples were taken prior and after therapy for histopathology (H&E; Elastin van Gieson) and cytokine analysis (Multiplex; ELISA).

Results: ASCs (CD45-CD29+CD73+CD90+) suppressed alloresponse *in vitro* and reduced pro-inflammatory cytokine levels in mixed lymphocyte reactions (IL-α, IL-β, IL-2, IFN-γ, GM-CSF). *In vivo*, ASC administration at acute rejection grade I-II significantly delayed progression to Grade III (7.57±1.13 days SASC, 7.29±1.11 days LASC vs 2.75±0.7 days Controls; n=23 animals). Blood levels of pro-/anti-inflammatory cytokines showed no significant difference after ASC therapy, except for IL-6, significantly lower in the SASC group. Blood TNFα-stimulated gene 6 (TSG-6) levels correlated with grade of rejection in all groups, whereas skin TSG-6 levels were slightly higher after SASC. ASCs reduced intima/media ratio (IMR) in skin and muscle vessels of the allograft in both the acute and chronic setting. However, GV affected the greater (femoral) vessels only in long-term animals and was significantly attenuated after ASC administration.

Conclusion: Systemic or local ASC therapy significantly reduces progression of onset acute rejection in VCA. Their immunomodulatory function attenuates alloresponse and pro-inflammatory cytokine levels. GV was observed during acute and chronic rejection and was significantly reduced after cytotherapy. Further experiments will explore the mechanisms, immunological pathways and maximal potential of ACSs in therapy of rejection and GV.

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A NOVEL POINT OF CARE, AUTOMATED, AND CLOSED SYSTEM FOR PROCESSING STROMAL VASCULAR FRACTION EITHER WITH OR WITHOUT COLLAGENASE

Presenter: Todd Malan, MD (USA)
Affiliation: Roxbury Regenerative
Author: Malan T

Stromal Vascular Fraction (SVF) is a component of lipoaspirate that can serve as a rich source of multipotent elements with phenotypic and gene expression profiles similar to human Mesenchymal stem cells (hMSCs) and Pericytes. Currently a reliable, automated, and entirely closed point of care system for SVF processing technique does not exist. Here, we present the Q-Graft device from HumanMed AG. The Q-Graft is an entirely closed and sterile point of care automated SVF processing device that can be used either with or without collagenase digestion. Adipose tissue is harvested directly into the device which is placed within the sterile OR field. The device then automates the incubation, filtration, washing, and suspension of the SVF pellet. We will present pre-clinical data gathered in our testing of the Q-Graft device to identify the cellular characterization of SVF product, growth kinetics and self-renewal assay, differentiation potential, ability to maintain sterility, and measures of residual collagenase. Comparisons will also be made in using the device either with or without collagenase. An evaluation as to the feasibility of direct point of care use of the Q-Graft device as an alternative to currently available manual and automated processing techniques will also be discussed.

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Q-GRAFT (R), A NEW DEVICE FOR ISOLATION OF SVF AND STEM CELLS FROM FATTY TISSUE

Presenter: Klaus Ueberreiter, MD (Germany)
Affiliation: Park Klinik Birkenwerder
Authors: Ueberreiter K, Meyer J

Goals/Purpose: The interest in research and clinical treatment using adipose derived progenitor cells has grown very widespread in recent years. Nevertheless the limits consist in the expensive equipment necessary and the time-consuming separation procedure.

From 2013 to 2016 the Company Human Med (Germany) developed in close cooperation with the department of Cell Biology of the University Medecine, Rostock, Germany a new device for the isolation of stem cells in the operating room. The Q-Graft® is a single use device. It consists of 3 chambers to allow a stepwise filtration and separation of the stromal vascular fraction (SVF). In the first chamber the fat will be collected directly by lipo aspiration. To achieve an amount of about 8 million SVF cells 75 ml of fat are to be harvested. In the next processing step collagenase will be added, but only in a very low dosage (1/25th) of the standard otherwise necessary.

An integrated special mixing device is moved by intermittend vacuum of the body-jet®; a heating system for incubation is also included. This process takes about 40 minutes. Fat and SVF cell suspension are separated in the next steps. Due to the very low amount of Collagenase used a centrifugation step is not paramount but will increase the content of SVF/stem cells by 3 to 4 fold. The amount of SVF differs greatly according to the patient and the selected cell counting device; therefore an important issue is the use of evaluated high class devices.

The great advantage of the Q-Graft lies in the high yield of viable cells and the easy handling in the operation room - thus avoiding legal problems in many countries - and the low costs compared to in-house laboratories or other devices.

The device is CE marked and has been tested in our clinic. A video of process will be shown and clinical results be discussed.

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OPTIMIZATION OF ENZYMATIC DIGESTION FOR SVF ISOLATION FROM HUMAN LIPOASPIRATES

Presenter: Rintaro Asahi, MD (Japan)
Affiliation: Jichi Medical University
Authors: Asahi R, Shirado T, Saitoh N, Furukawa K, Yoshimura K

Introduction: Stromal vascular fraction (SVF) was conventionally isolated from lipoaspirates with 0.075% collagenase type 1 in Hank`s Balanced Salt Solution for 1 hour at 37° with gentle agitation [1]. Since then, a number of modified isolation methods have been reported and a recent method employed collagenase digestion using buffer constituents, some additives, and an orbital shaker [2]. Based on our previous studies, 1 gram lipoaspirate is estimated to contain several millions nucleate cells other than adipocytes. However, the SVF yield remains only 0.3-0.8 million, which means more than 80% of nucleate cells are not successfully extracted from lipoaspirates. We sought to optimize the isolation method for better yield of SVF cells.

Methods: We tested crude or purified collagenase products with or without other supplemental enzymes including DNAse, trypsin, dispase, and thermolysin under several different conditions. In addition, non-enzyme additives such as poloxamer and electrolytes are examined to determine an appropriate concentration.

Results/Discussions: After preliminary experiments, the following method consistently enabled isolation of more than one million SVF cells per 1 gram lipoaspirates with high cell viability. Pre-warmed lipoaspirates were digested with prewarmed Hank`s Balanced Salt Solution containing crude collagenase or purified one with thermolysin for 30 min at 37°. The digestive solution also contains 1000 U/ml DNAse, 3 mM calcium chloride, and 0.5% poroxamer-188.

Conclusions: It is possible to improve SVF cell yield by modifying the digestion methods. As a majority of nucleate cells are still not successfully extracted from lipoaspirates, further efforts are needed to maximize the SVF yield.

References:

1) Zuk PA, Zhu M, Ashjian P, et al: Human adipose tissue is a source of multipotent stem cells, 2002: 13: 4279-4295.
2) Tevlin R, McArdle A, Brett E, et al: A novel method of human adipose-derived stem cell isolation with resultant increased cell yield, 2016: 138: 983e-996e.

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EFFECT OF THE BOWL STRUCTURE ON ISOLATION YIELD OF STROMAL VASCULAR FRACTION USING AUTOMATED CELL ISOLATION DEVICE

Presenter: Hyung Min Hahn, MD (Korea)
Affiliation: Ajou University Hospital
Authors: Hahn HM, Yoo BY, Park JH, Jung HJ, Lee IJ

Background: The heterogeneous stromal vascular fraction, containing adipose-derived stromal cells, can be easily isolated through enzymatic digestion of lipoaspirates. To control technical procedures as standard operating procedures and to avoid human errors, automated system for stromal vascular fraction isolation was introduced. However, cell counts and residual collagenase activity acquired with conventional automated system are not satisfactory, compared to manual isolation method. We evaluated the efficiency and reliability of a new automated system of which the bowl has a design of radial protrusion at each angle in order to increase isolation yield.

Methods: Fifteen patients were included in the study as fat tissue donors. The stromal vascular fraction isolated by newly designed automated system was analyzed and compared with conventional automated system. Using the automatic cell counter, the cell viability of the isolated stromal vascular fraction, the total number of cells per 1 mL of solution, and the percentage of viable cells were all measured. Then, these values were compared between conventional bowl and newly-designed top-type bowl.

Results: The average number of cells isolated using the conventional bowl system was 2.5 X 100000 cells/mL of lipoaspirate and 18.0 X 100000 cells/mL of lipoaspirate for the top-type bowl, showing a significant difference ($p < 0.0001$). There were no differences between the two groups in terms of the survival rates of the isolated cells. While the amount of collagenase remaining in the cell layer after centrifugation was shown to be lower in the top-type bowl than in the conventional bowl; therefore, it is reasonable to assume that the top-type bowl is safer to use ($p < 0.0001$).

Conclusions: Total nucleated cell count isolated by newly designed system was higher than conventional system. Decreased residual collagenase activity was achieved with top-type bowl. Automated isolation with improved bowl structure is expected to enable cell-based clinical trials with higher yield of nucleated cell count and decreased residual collagenase activity.

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EFFECT OF THE BOWL STRUCTURE ON ISOLATION YIELD OF STROMAL VASCULAR FRACTION USING AUTOMATED CELL ISOLATION DEVICE

Presenter: Hyung Min Hahn, MD (Korea)
Affiliation: Ajou University Hospital
Authors: Hahn HM, Yoo BY, Park JH, Jung HJ, Lee IJ

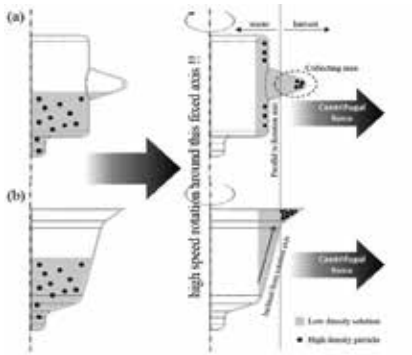

Figure 1. Variation of centrifugal efficacy by bowl structure (a) conventional type bowl (cylinder + lobe) (b) the top-type bowl (the type of no wasting in same area)

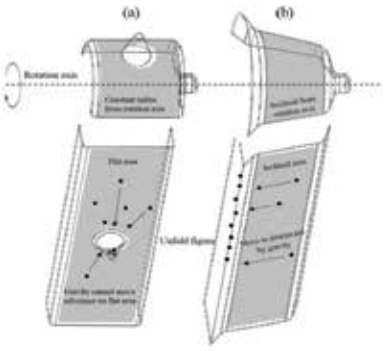

Figure 2. A side view of the structure of the bowl (a) conventional type bowl (b) the top-type bowl

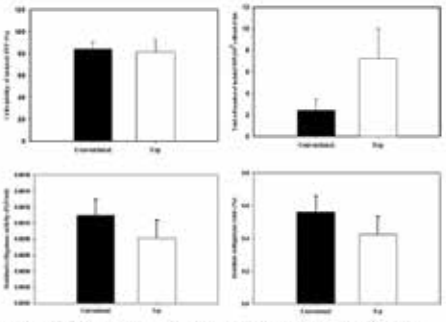

Figure 3. (*left, upper*) The cell viability, (*right, upper*) total cell number, (*left, lower*) residual collagenase activity, and (*right, lower*) residual collagenase ratio of isolated SVF by conventional bowl and the top-type bowl

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LONG-TERM IN VITRO CULTURE AND OSTEOGENIC DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS IN CGMP-COMPLIANT MEDIA

Presenter: Napat Tandikul, MS (USA)
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Author: Tandikul N

Adipose-derived stem cells (ADSCs) are a promising cell source for tissue engineered bone products due to their multilineage differentiation potential, easy accessibility, and abundance. Classical medium with fetal bovine serum (FBS) is commonly used to expand and differentiate human ADSCs. However, FBS can lead to cross-contamination, possible autoimmune rejection and unpredictable outcomes due to lot variation. Our goal is to develop a consistent cell process for ADSC-based tissue-engineered bone products; hence, we investigated the long-term stem cell maintenance of multiple donor ADSC populations in cGMP-compliant, well-defined media. In this study, ADSCs were isolated from 6 donor liposuction samples and expanded in cGMP-compliant XenoFree, Serum-free medium from passage 1 to passage 9. At every passage, the consistency of ADSCs morphology, cell-surface marker expression, population doubling time (PDT), and osteogenic differentiation were examined. The results showed that, across all donors and all passages, ADSCs have spindle-shaped and fibroblast-like morphology with no significant differences in shape, but size increased for late passage cells. Flow cytometry revealed that ADSCs were positive for markers CD73, CD90, and CD105, but negative for CD34, and CD45; however, some minor variations in expression profile were detectable at passage 7, 8 and 9. The results from PDT studies showed that ADSCs can be expanded in this media condition, although late passage cells (passage 8 and 9) grew significantly slower than early passage cells. Osteogenic differentiation capacity was confirmed by quantitation of calcium deposition and alkaline phosphatase (ALP) activity after 21 days of differentiation. For all donors, ADSCs produced abundant calcium and ALP in all passages. Our findings demonstrated that the cGMP-compliant media can be effectively used for ADSC isolation, long-term expansion, and osteogenic differentiation with minimal donor-to-donor variability. Therefore, this process can potentially be used for clinical manufacturing of ADSC-based tissue-engineered bone products.

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GROWTH SUPPLEMENTS FOR LARGE-SCALE CLINICAL EXPANSION OF ADIPOSE-DERIVED STEM CELLS: A TEST OF FOUR TYPES OF PLATELET LYSATES FROM OUTDATED OR FRESH BUFFY COAT-DERIVED PLATELETS

Presenter: Peter Vester-Glowinski, MD (Denmark)
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Authors: Vester-Glowinski P, Koelle SK, Svalgaard JD, Herly MH, Drzewiecki KD, Fischer-Nielsen AF

Background: Platelet lysates (PL) represents a promising replacement for xenogenic growth supplement for adipose-derived stem cell (ASC) expansions. However, fresh platelets from human blood donors are not clinically feasible for large-scale cell expansion based on their limited supply. Therefore, we tested PLs prepared via three methods from outdated buffy coat-derived platelet concentrates (PCs) to establish an efficient and feasible expansion of ASCs for clinical use.

Methods: PLs were prepared by the freeze-thaw method from freshly drawn platelets or from outdated PCs stored in the platelet additive solution, Intersol, modified with different concentrations of plasma and/or Intersol. Using these PLs, we compared ASC population doubling time, cell yield, differentiation potential and cell surface markers. Gene expression profiles were analyzed by microarray assays, and growth factor concentrations in the cell culture medium were measured using ELISA.

Results: Of the three PL compositions produced from outdated PCs, removal of Intersol and resuspension in plasma prior to the first freezing process was overall the best. This specific outdated PL induced media suspension appearance as well as ASC growth kinetics, surface markers, plastic adherence and differentiation potentials comparable to PL from fresh platelets. ASCs expanded in PL from fresh versus outdated PCs exhibited different expressions of 17 overlapping genes, of which 10 were involved in cellular proliferation, although not significantly reflected by cell growth. Also, growth factor turnover revealed only minor differences.

Conclusion: PLs from outdated platelets may be an efficient and reliable source of human growth supplement allowing large-scale ASC expansion for clinical use (Cytotherapy. 2017 Feb;19(2):222-234).

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OPTIMIZATION OF CELL ADHESION RATE ON SILK/FIBROIN MICROCARRIERS FOR ONE-STEP ADIPOSE STEM/STROMAL CELL DELIVERY: DESIGN OF EXPERIMENT APPROACH

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Authors: Perucca Orfei C, Taló G, Chlapanidas T, Viganó M, Perteghella S, Fabro Fontana F, Torre ML, De Girolamo L

Introduction: Recently, the increasing interest in adipose tissue as a source of mesenchymal stem/stromal cells (MSCs) has been accompanied with the mandatory need of developing intra-operative approaches for local therapy, complying with a composite system of regulatory requirements.

In this context, the use of minimally manipulated autologous cells in one-step procedure represents the preferred strategy. However, to improve the efficiency of these approaches, cells could be better delivered to the lesion site using specific carriers and as one-step procedures imply short time of preparation, the time needed to achieve a satisfactory cell adhesion to carriers is crucial. In this view, we used the Design of Experiment (DoE) approach to provide a seeding protocol for one-step administration of Adipose Stem Cells (ASCs), optimizing and speeding up the adhesion efficiency of cells on lyophilized fibroin coated alginate microcarriers (L-FAMs).

Methods: The DoE approach resulted in thirteen different combinations of specific parameters, selected by previous pilot experiments. Each condition has been tested on three donors of ASCs and the final seeding efficiency was evaluated in term of percentage of adhesion, viability and metabolic activity of adhered cells.

Results: The better seeding efficiency has been reached by the combination of the intermittent stirring of the cell-L-FAMs suspension at a speed of 10 rpm and using a seeding volume of 400 μl. These factors resulted to be crucial to obtain a satisfactory and homogeneous adhesion in less than two hours. In specific, the DoE outcomes allowed to observe that the intermittent stirring is the more incisive parameter whereas the time is less influencing one. On the base of the obtained results, it has been possible to obtain a final protocol able to furnish a maximized output.

Conclusions: L-FAMs are adequate delivery systems able to guarantee a fast cell adhesion, preserving MSCs features. Even though further optimizations are essential, the present protocol may represents a starting point for the introduction in the clinic of L-FAMs as a carrier in autologous one-step applications.

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VOLUME RETENTION, METABOLISM, AND CELLULAR COMPOSITION OF HUMAN FAT XENOGRAFTS

Presenter: Brittany Merrifield, BS (USA)
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Authors: Merrifield B, Komorowska-Timek E, Chang A, Hostetter G

Background: To optimize the take of transferred fat, better understanding of fat graft morphology and growth properties in vivo is critical.

Objective: To evaluate survival, volume retention, metabolism, and cellular composition of various aliquots of human fat xenografts.

Methods: Twenty athymic nude mice were injected subcutaneously in opposing flanks with 0.1 ml (small) and 1.0 ml (large) aliquots of human fat graft. Volume (ultrasound) of fat aliquots was measured at baseline, 1, 3, and 12 weeks after implantation. Tissue metabolism (18F-FDG), H&E, special stains, and immunohistochemical analysis were performed at 3 and 12 weeks to determine graft viability, cell origin, and proliferative activity.

Results: Only 1 out of 10 small grafts were detected after 12 weeks by ultrasound and 5 out of 10 were found at necropsy. Volume of large grafts decreased significantly from baseline at 3 (827±195 mm3 vs 953±122 mm3 p=0.004) and 12 weeks (515±163 mm3 vs 953±122 mm3, p=0.0001). Metabolism increased with time in small (0.6±0.4%ID/g vs 2.0±1.1%ID/g, p=0.01) and large grafts (0.4±0.3%ID/g vs 1.4±0.9%ID/g, p=0.005). Large grafts viability decreased between 3 and 12 weeks (72±20% vs 31±30%, p=0.012) while small graft viability remained unchanged. Viable and proliferating human and mouse adipocytes as well as chimeric blood vessels were seen within grafts at both time points.

Conclusions: Larger graft aliquot was associated with better volume retention by ultrasound but lower viability by histology. Graft metabolism increased with time irrespective of aliquot size potentially due to regenerative processes of both donor and recipient origin.

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LONG-TERM RETENTION OF EXCISED FAT GRAFTS: A LONGITUDINAL, RETROSPECTIVE COHORT STUDY OF 108 PATIENTS FOLLOWED FOR UP TO 8.4 YEARS

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Introduction: Most surgeons and patients experience volume loss over time after fat grafting. Final fat graft retention is still poorly defined and the time to reach steady state is unknown.

Methods: We compiled a retrospective, longitudinal cohort of patients with vestibular schwannoma who had undergone ablative surgery and reconstruction with excised fat between the years 2006 and 2015. Fat volume retention was quantified by computed tomography and magnetic resonance imaging and used to model a graft retention trajectory and determine the volumetric steady state. In addition, the authors evaluated the association between graft retention and secondary characteristics, such as sex and transplant volume.

Results: A total of 108 patients were included. The average baseline graft volume was 18.1 ± 4.8 ml. The average time to reach steady state was 806 days (2.2 years) after transplantation. By this time, the average fat graft retention was 50.6 percent (95 percent CI, 46.4 to 54.7 percent). No statistically significant association was found between start volume and graft retention ratio. Fat graft retention over time was significantly higher in men than in women (57.7 percent versus 44.5 percent; $p < 0.001$). The average distance from the recipient periphery to the graft center was 0.96 cm. Histologic and radiologic analysis of the fat grafts years after transplantation showed normal fat tissue.

Conclusions: The authors' data provide evidence that the time to reach fat graft volumetric steady state is considerably longer than previously expected. Fat grafts continue to shrink long after the initial hypoxia-induced tissue necrosis has been cleared, thus indicating that factors other than blood supply may be more influential for fat graft retention. Furthermore the very long distance from graft center to recipient edge of 0.96 cm combined with a histologic and radiologic findings of a high degree of viable fat tissue, contradicts previous studies stating that grafted fat farther than 0.2 cm from the periphery will undergo necrosis. (Plast. Reconstr. Surg. 139: 1223, 2017.)

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MOLECULAR MECHANISMS OF FAT GRAFT FAILURE: EXPLORING PATHWAYS THAT CONFER HYPOXIA-INDUCED APOPTOSIS RESISTANCE IN ADIPOSE TISSUE

Presenter: Lauren Kokai, PhD (USA)
Affiliation: University of Pittsburgh
Authors: Kokai L, Johngrass MG, Schroth RN, Gusenoff JA, Rubin JP

Introduction: Poor and variable retention outcomes remain a significant barrier to autologous fat grafting procedures. Our clinical data strongly suggests that the inherent make-up of donor adipose tissue dictates graft performance, as we have shown that outcomes from primary and secondary procedures in the same patient are highly correlated. We hypothesize that resident adipose tissue macrophages (MΦ) have a central role in modulating graft status during ischemia towards either inflammation or regeneration. To test this hypothesis, we measured the expression of inflammatory and apoptotic genes in five unique tissue donors and compared outcomes to increases in either pro-inflammatory (M1) macrophages or regenerative (M2) macrophages.

Method: Adipose particles were cultured in either 1% O_2 (hypoxic) or 8% O_2 (normoxic) gas blends and gene expression and secreted protein levels were measured at 2 and 7 days with quantitative reverse transcription- PCR and ELISA. Investigated genes included macrophage specific markers including CD68 (pan-MΦ), CD5L (activated MΦ), MERTK (phagocytic MΦ), iNOS (inflammatory MΦ), ARG1 (fibrotic MΦ) and IL10 (regenerative MΦ) as well as apoptosis and hypoxia markers.

Results: Our results support clinical observations that adipose tissue has significantly variable molecular responses to hypoxic stress even amongst patients of similar age and BMI. Unexpectedly, the healthiest subject studied (BMI of 27.81, group mean was 32.05) had the highest overall increase in MΦ number ($p=0.0176$) after 7 days in culture. Further, this subject had significantly more M2 macrophages in hypoxic conditions, but not in normoxic conditions. Expression analysis of hypoxia related genes revealed that adipose tissues with resistance to hypoxia-induced apoptosis are also sensitive to tumor necrosis factor alpha (TNFa), and increase X-linked inhibitor of apoptosis (XIAP) expression through the NF kappa-B signaling pathway.

Conclusions: Our long term goal is to determine intrinsic qualities of human adipose tissue that confer resistance to ischemic stress in order to abrogate graft apoptosis and tissue/volume loss in free fat grafts. Future studies with more subjects will also investigate novel pharmaceuticals that increase XIAP expression.

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THE HYBRID BREAST RECONSTRUCTION: IMPLANT-BASED BREAST RECONSTRUCTION WITH ADDITIONAL LIPOFILLING

Presenter: Filip Stillaert, MD (Belgium)
Affiliation: University Hospital Gent
Author: Stillaert F

Introduction: Options for breast reconstructions enclose autologous tissue transfers or implants. Fat grafting is gaining more interest in this specific field of breast surgery. This study concentrates on the technique and aesthetic results of breast reconstruction with fat grafts combined with implants, in women who have undergone total mastectomy.

Methods: A retrospective data analysis of twenty breast reconstructions in thirteen total mastectomy patients was performed. Surgery was done between October 2011 and April 2015. In all these patients a tissue expander was placed at the time of mastectomy or after removal of a previous breast reconstruction. A protocol of intratissular expansion with serial deflation - lipofilling sessions was executed. In order to achieve the best aesthetic outcome, an additional small implant was placed.

Results: All patients underwent a total mastectomy, uni- or bilateral, because of breast cancer or an elevated risk due to a positive BRCA test. Six patients had a bilateral reconstruction. In four patients the described technique was done as a tertiary reconstruction. The mean age of the study population was 46 years (range 33 - 64). The mean of lipoaspirate material for the reconstruction was 333mL (range 120 – 715mL); to achieve symmetry with the contralateral breast a mean of three lipofilling sessions with a three-month interval was needed. To create an adequate volume of the reconstructed breast, a supplementary small implant was placed, with a mean volume of 222mL (range 125 – 375mL). The mean follow-up was 33 months (range 19 – 50 months).

A MRI analysis was performed in eight patients at least 9 months after the last lipofilling procedure, demonstrating a mean of 183mL (range 65 – 538mL) of fat and a volume ratio of fat graft/implant of 1,036 (range 0,312 – 3,845).

Conclusion: This composite technique of using autologous fat tissue and implants shows aesthetic pleasant results and must be considered as a valid alternative in a subset of patients. Further investigations to optimize the fat graft take must be encouraged.

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22 YEARS OF 3 DIMENSIONAL FACE LIFTING EVOLUTION USING FAT TRANSFER AS THE PRIMARY Z AXIS TOOL

Presenter: Andrew Wolin, MD (USA)
Affiliation: Private Practice
Author: Wolin A

The author retrospectively evaluated the last 22 years of fat transfer to the face carried out during his facelift procedures. A total of 600 patients were involved in the study. Single cases were randomly selected from each year using their one year post op photos. General facial form and ability of the fat to provide not just volume correction but also subjective skin quality improvement were graded on a scale of 1 thru 5.

The general trends showed that as the volumetric aliquots increased from the early cases (average volume 10 cc each hemiface) to the most recent (40 to 50 cc in each hemiface) so did the ability of the fat to produce a more stable 3 dimensional form as well as a produce a definite improvement in the texture and general quality of the skin.

The trending not only exhibited increased volume retention with time, but more importantly an evolution to a full facial global fat approach. This process allows a more artistic blending of facial features and defines the facelift procedure as more than an x and y tissue pull phenomenon and more of a z axis 3 dimensional endeavor.

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TWO-STAGE BREAST RECONSTRUCTION WITH A SUBPECTORAL SILICONE TISSUE EXPANDER AND AUTOLOGOUS FAT GRAFTING

Presenter: Ruomiao Chen, MD (China)
Affiliation: The First Affiliated Hospital of Fujian Medical University
Authors: Chen R, Shan X, Xu L, Wang B

Objective: To explore the application of post- expansion prosthesis implantation with simultaneous autologous fat grafting in post-mastectomy breast reconstructions.

Methods: In the 1st stage of surgery insert a 250ml–350ml round-shaped tissue expander posterior to the pectoralis major. Augment the tissue expander by regularly injecting the saline until the volume of expander is exceeding 30%–50% the volume of the contralateral breast and maintain the volume for over 3 months. During the 2nd stage of surgery, replace the tissue expander with an appropriate prosthesis and perform a simultaneous autologous fat grafting by injecting the fat tissues in different layers of the tissue so that the symmetry of breasts can be achieved.

Results: From 2013 to 2016, our department has accomplished 15 cases with 15 sides of implant-based breast reconstruction post tissue expansion with simultaneous autologous fat grafting. The follow-up periods range from 1 to 3 years, and the 15 reconstructed breasts have all achieved satisfactory appearances and symmetry with the contralateral breast, in terms of size and position.

Conclusions: Implant-based breast reconstruction post tissue expansion with simultaneous autologous fat grafting is a simple technique which lowers the risk of implant rupture involved in 2nd-stage fat grafting whereas ameliorates the tissue thickness over the implant. The post- expansion prosthesis implantation with simultaneous autologous fat grafting is an ideal option for breast reconstruction.

Keywords: Tissue expansion; Breast reconstruction; Breast implant; Skin soft tissue expander; Breast cancer; fat grafting

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LIPOLOOP: A STATE-OF-THE-ART, CLOSED, AUTOMATIC, VIBRATORY SYSTEM FOR FAT GRAFTING, MECHANICAL SVF PROCUREMENT AND CELL AND TISSUE SELECTION

Presenter: Steven Cohen, MD, FACS (USA)
Affiliation: University of California San Diego
Authors: Cohen S, Ramos C, Angeloni D, Miles G

Introduction: LipoLoop is a closed, automatic, vibratory system that is used for large to small volume fat grafting, mechanical SVF procurement and cell and tissue in situ selection.

Materials and Methods: LipoLoop was developed to provide a simple and predictable solution for a number of applications in regenerative medicine, ranging from larger volume fat grafting to smaller volume fat grafting and a number of regenerative applications in sports medicine, podiatry, orthopedics and sports medicine. The device introduces tumescent solution by a vibrating cannula inserted through a 14G needle puncture. Once tumescent solution is infiltrated, the harvest cannula is introduced and vibration for 1 min/100 ml of tumescent solution is performed for loosen the fat and mechanically dissociate the SVF. Then, suction is turned on and the fat is aspirated under low vacuum. Once in the canister, the fat may be rinsed with normal saline if desired or if there is no free fatty acid layer or blood in the lipoaspirate, directly transferred into the patient or removed and further processed. The infranatant from the fat may be used for SVF and nanofat may be obtained using a specially designed harvest cannula.

Results: Over a 2 year period, LipoLoop was used for large volume fat transfer, small volume fat grafting, nonfat grafting and mechanical dissociation of stromal vascular fraction (SVF). Indications were breast surgery, body contouring and small volume applications to the face and hands. To date no serious complications have occurred. There have been no emboli or infections. Two patients of 100 undergoing facial fat grafting required minor surgery for removal of fat around the lower eyelid. There have been no other complications related to either the fat transfer or the use of SVF or Nanofat.

Conclusions: LipoLoop has been developed to provide a safe and effective means for closed system delivery of large volume and small volume fat grafting. In addition, SVF may be easily procured by mechanical means and appears to contain 30% of the SVF cells that can be obtained with enzymatic digestion.

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PRODUCTION OF NATURALLY DERIVED TISSUE-ENGINEERED BIOLOGICAL DRESSINGS UNDER SERUM-FREE CONDITIONS TO STIMULATE WOUND HEALING IN DIABETIC MICE

Presenter: Julie Fradette, PhD (Canada)
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Introduction: Healing of diabetic wounds refractory to usual treatments is a major challenge for the health care system. Lately, stem cells have been investigated for their therapeutic secretome favoring wound healing. Previous work from our team showed that adipose-derived stromal/stem cells (ASC) based tissue-engineered biological dressings enhanced global healing of cutaneous wounds in healthy mice. These dressings were produced under serum-containing culture conditions and featured differentiated adipocytes. In this study, we hypothesize that undifferentiated ASCs dressings produced in serum-free conditions will stimulate skin healing in a diabetic murine model.

Methods: The dressings were produced according to the self-assembly approach using a commercially available serum-free medium for the entire production and compared to the standard medium containing 10% fetal bovine serum (FBS). Mechanical properties were assessed using uniaxial tensile test and tissue functionality by ELISA assays to determine the secreted levels of proangiogenic factors. Two complementary diabetic mice models were used: streptozotocin-induced diabetes in K14H2BGFP mice exhibiting a fluorescent epidermis and polygenic diabetic NONcNZO10/LtJ mice. Full-thickness 8-mm splinted skin wounds were created. Global wound closure was evaluated with macroscopic imaging while reepithelialization kinetics were followed with a non-invasive LuminaIVIS imagery system.

Results: Dressings were thicker (2.9 fold) and exhibited increased mechanical resistance (1.9 fold) when produced using the serum-free system compared to FBS-containing medium. They also secreted more PAI-1, HGF and VEGF (5.9, 6.9 and 9.1 fold respectively). In vivo, these biological dressings accelerated wound closure by 83% at day 8, 57% at day 12 and 35% at day 16, at which moment treated wounds were almost closed while the untreated ones exhibited a 70% closure (NONcNZO10/LtJ). Reepithelialization kinetics were similar between the two groups (K14H2BGFP).

Conclusion: These new tissue-engineered dressings exhibited therapeutic properties promoting in vivo diabetic cutaneous healing. The mechanisms involved are being investigated. Supported by CIHR.

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COMBINED USE OF EXTERNAL VOLUME EXPANSION (EVE) AND A DECELLULARIZED ALLOGRAFT ADIPOSE MATRIX (AAM) TO INDUCE ADIPOSE TISSUE REGENERATION: A NOVEL PARADIGM IN SOFT TISSUE RECONSTRUCTION?

Presenter: Giorgio Giatsidis, MD (USA)
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Introduction: Clinical efficacy of structural fat grafting is hindered by an insufficient metabolic support to the transferred tissue which induces partial graft necrosis and volume loss. Strategies proposed to reduce such phenomenon have shown suboptimal outcomes. Differently from grafts, acellular scaffolds do not need metabolic support; instead, they can provide a biochemical and structural framework that fosters tissue regeneration from endogenous cells. We hereby test in a murine model the hypothesis that the use of an allograft adipose matrix (AAM) combined with External Volume Expansion (EVE) for recipient site preparation could promote adipose tissue regeneration and limit graft volume loss at follow up.

Method: 10 week-old Nu/Nu mice were assigned to six experimental groups undergoing different combinations of EVE treatment, fat grafting, and AAM grafting. Grafts (0.3 cc) were injected subcutaneously in the dorsum of animals; fat grafts were obtained from human lipoaspirate, whereas the AAM was derived from processed cadaveric human adipose tissue. On post-operative day (POD) 28 (n = 4 per group) and 84 (n = 16 per group) grafts were collected for analysis (graft structure and volume, using H&E staining; angiogenesis and inflammation, using immuno-histochemistry for the CD 31 and CD 45 markers; adipogenesis, using immuno-fluorescence for the Perilipin marker).

Results: At a long-term follow up (POD 84) AAM grafts combined with EVE showed significantly higher volume retention compared to control fat grafts (1.8- fold increase, $p<0.05$); treatments mixing AAM and fat grafts (with/without EVE) led to intermediate outcomes. Angiogenesis was significantly increased (1.6-1.9-fold, $p<0.05$) in grafts that included the AAM. At histology the AAM showed complete recellularization with adipocytes and a lack of cystic-like areas as compared to fat grafts. Proliferating adipocytes were observed along areas of inflammation in the AAM grafts.

Conclusions: AAM grafts provide a framework that promotes endogenous adipose tissue regeneration, avoid graft necrosis and support graft volume retention at long-term follow up. Combined use of EVE further enhances outcomes possibly by stimulating inflammation-induced adipogenesis. Addition of fat grafts mitigates outcomes.

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DECONSTRUCTING ALLOGRAFT ADIPOSE MATRIX

Presenter: Lohrasb Ross Sayadi, MD (USA)
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Introduction: Plastic and reconstructive surgeons routinely treat soft-tissue defects and contour abnormalities using autologous fat grafting. However, there is large degree of variability in the fat graft retention rates, which means that most patients have to undergo multiple staged procedures to obtain the desired result. Thus, there is a need to develop a better vehicle for treating these conditions. Recently, human allograft adipose matrix (AAM), as a scaffold for tissue engineering, has shown great promise as a vehicle for the promotion of soft tissue regeneration. The generation of AAM, derived from human cadaveric adipose tissue, involves separating the adipose fraction from the superficial fascia fraction, which is typically discarded. We hypothesize that the matrix derived from the fascia fraction might contain important components required to promote adipogenesis that are not present in the AAM.

Methods: To characterize and identify the proteins in the AAM and the fascia fraction of the adipose complex, we used a mass spectrometry (MS) analysis. Among the proteins identified, we selected the matrisome proteins. The matrisome proteins were annotated using Gene Ontology (GO). To validate the presence or absence of the proteins in each of the sample types, a Western blot was performed on a subset of the candidate proteins.

Results: We identified approximately 100 matrisome proteins in each sample. 30% of these were unique to the AAM while 19% were unique to the fascia. The GO annotation analysis revealed that the fascia matrix contained proteins that are enriched for pathways related to angiogenesis, which are not enriched in the AAM. The presence of these proteins in the fascia and their absence in the AAM were validated by the Western blot analysis.

Conclusions: AAM holds great potential as a scaffold for inducing adipogenesis in vivo. However, in order for the newly established tissue to be retained, angiogenesis must occur concurrently. For the AAM, we revealed that protein components were missing certain key angiogenic inducers, but some of these were identified in the fascia matrix. We propose that a mixture of AAM and the fascia matrix would better support adipogenesis due to the identification of its unique angiogenic components.

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A PRE EMULSIFICATED PRODUCT: THE MILLIMICROFAT

Presenter: Angelo Trivisonno, MD (Italy)
Affiliation: Sapienza University
Author: Trivisonno A

Many procedures have been developed in order to reduce or eliminate the contamination of mature adipocytes and to collect SVF cells by mechanical isolation. It is possible to harvest smaller volumes of adipose tissue and therefore a lower number of mature adipocytes using a microcannula, without affecting the possibility to collect a relevant number of SVF cells. With the purpose to reduce the number of adipocytes and the size of the fragments harvested, without destroying all adipocytes to preserve the niche, we developed a 'millimicrofat' procedure consisting in: 1) collection of dermal adipose tissue using a microcannula; 2) mechanical processing by manually forcing the graft back and forth for 30 times through a 1.2 mm transfer connected between two syringes to obtain a tissue processed in smaller fragments we called 'millimicrofat'. We harvested in the same area 2 types of lipoaspirates from 7 patients: 1) 5 cc of macrofat, collected using a 3 mm cannula with larger 2 mm holes; and 2) 10 cc of microfat, collected using a 2 mm microcannula with 1 mm holes, arranged in a single row. To obtain the millimicrofat sample, 5 cc of microfat were processed by 30 passages between 2 syringes through 1.2 mm transfer. The samples were cultured in complete DMEM medium and the culture was extended for 12 days, before the plastic adherent cells were counted.

The numbers of mesenchymal stromal cells obtained after isolation from cultures of samples of microfat and millimicrofat were similar, indicating that the millimicrofat procedure was not detrimental on the number of viable mesenchymal cells isolated. Most importantly, the number of cells obtained using the millimicrofat procedure was approximately 30% higher than cells isolated from the macrofat sample. The millimicrofat was composed of smaller fragments that we could infiltrate through 25-27 G needles, in a more superficial layer of the tissues.

The millimicrofat can be considered as a pre emulsificated product, maintaining the entire niche and a decreased number of mature adipocytes in a reduced volume. Therefore using the millimicrofat procedure it is possible to obtain a sample with greater regenerative capacity in a small volume suitable for regenerative purposes.

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ADIPOSE CONSTRUCTS WITH BIODEGRADABLE SURFACE TOPOGRAPHY INFLUENCE THE NEOVASCULARIZATION ONSET IN SKIN TISSUE REGENERATION

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Authors: McLuckie M, Robotti F, Sanchez-Macedo N, Enderlin D, Egana JT, Poulikakos D, Giovanoli P, Ferrari A, Lindenblatt N

Introduction: After skin tissue injury or loss, the timing of angiogenesis is critical in ensuring successful subsequent regeneration through nutrient perfusion. Hence any sort of enhancement to stimulate this process could provide a distinct advantage, in particular in chronic wounds and large defects. It has been demonstrated that structured surfaces have an effect on cell mobility and wound healing, both in vitro and in vivo. Therefore, it is proposed that a construct with topographical features and adipose, rich in stem cells, has the potential to be pro-angiogenic and influence the onset of neovascularization.

Methods: Murine lipofragment constructs were formed with a polydimethylsiloxane (PDMS) mold. Transplants (6mm radius and 0.4mm thickness) were implanted into the dorsal skinfold chamber (DSC) of four groups of B6 mice (n=5) as follows: (I) unstructured control, (II) unstructured construct, (III) 5μm construct, and (IV) 50μm construct. Intravital microscopy (IVM) observed the onset of neovascularization over 21 days, and histological analysis assessed regeneration over various time points. Multiphoton microscopy (MPM) evaluated collagen density and molecular investigations detected cytokine presence.

Results: Surface structure influenced the onset of capillary ingrowth based on the topography size. Group (III) presented early neovascularisation on day 3 whereas group (II) started at this time point for only 40% of the mice. Group (IV) was delayed to day 5 in 20% of the mice and day 7 for 80%. Group (III) reached angiogenic stabilization by day 21, whereas groups (II) and (IV) where still normalizing. MPM and immunohistochemistry confirmed these results with Group (III) having the highest collagen density and lowest inflammatory score.

Conclusion: The use of structured constructs may provide control over vascularization, which may be of advantage in wounds requiring regulated healing. Fibrin glue was able to maintain its surface topography and exhibit an effect prior to undergoing fibrinolysis. Not only may such constructs serve as a simple vascularization strategy, in a clinical setting it could be applied immediately without the need for lengthy cell culture and additionally provide soft tissue in cases of full thickness skin defects.

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POLYMER-MINERAL SCAFFOLD TO AUGMENT IN VIVO EQUINE MULTIPOTENT STROMAL CELL OSTEOGENESIS

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Affiliation: Louisiana State University
Authors: Duan W, Chen C, Haque M, Hayes D, Lopez MJ

Bioscaffold direction of adult multipotent stromal cells (MSCs) is a contemporary approach to bone regeneration. This study was designed to quantify in vivo equine bone marrow- (BMSC) and adipose tissue (ASC) derived MSC osteogenesis on synthetic polymer scaffolds with and without minerals in athymic mice. Cryopreserved, culture-expanded MSCs were loaded onto scaffolds of tricalcium phosphate (TCP)/ hydroxyapatite (HA) (40:60, HT), polyethylene glycol (PEG)/ poly-L-lactic acid (PLLA) (60:40, GA), or PEG/PLLA/TCP/ HA (36:24:24:16, GT). Scaffolds with and without cells were implanted subcutaneously in mice that were radiographed every 3 weeks. Nine weeks after surgery, explant composition (dsDNA, collagen, sulfated glycosaminoglycan, protein), equine and murine osteogenic target gene expression (alkaline phosphatase, bone sialoprotein, osteocalcin, osteoprotegerin), micro-computed tomography (μCT) mineralization and microstructure were assessed. Radiographic opacity increased with time in GT-BMSC constructs. Extracellular matrix components increased significantly in GT compared to HT constructs. Equine and murine osteogenic gene expression was highest in BMSC constructs with mineral containing scaffolds. The HT constructs with either cell type had the highest mineral deposition. Scaffolds with cells had more ECM than those without. Osteoid was apparent in all BMSC constructs. Results are limited to ectopic bone formation in a murine model and should be confirmed in an equine orthotopic model prior to clinical translation. Addition of mineral to polymer scaffolds enhances MSC osteogenesis over polymer alone by both exogenous and host MSCs, but pure mineral promotes superior osteogenesis. These results emphasize the need for bioscaffolds that provide osteogenic direction of both exo- and endogenous MSCs for the best regenerative potential.

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ANTI-AGING EFFECT OF THE STROMAL VASCULAR FRACTIONS/ADIPOSE-DERIVED STEM CELL IN A MOUSE MODEL OF SKIN AGING INDUCED BY UVB IRRADIATION

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

Objective: The present study was conducted to investigate the anti-aging effect of the SVF or and ADSCs injection in photo-aging skin.

Methods: Six-week-old nude rats were divided to control group and experimental group. And the experimental group was continuously irradiated with ultraviolet B (UVB) for 8 weeks to develop photo-aging skin model. The animal were treated with the different doses of SVF or ADSCs. The SVF were obtained by collagenase I digestion from the adipose tissue in healthy female, and then injected into the back skin of photo aging nude rats. The ADSCs of the third generation were used. The changes of the epidermal and dermal thickness was examined by HE staining.

Results: Treatment of animals with SVF at 105 or 106cells/100μl and ADSCs at 2 – 104 or 2 – 105 cells/100μl decreased the thickness of epidermis, reduced the percentage of stratum corneum and the abnormal proliferation of epidermal basal cell layer, and increased the dermis thickness. Real-time PCR showed that treatment of animals with SVF or ADSCs at the same doses upregulated the expression of mRNA of Collagen I, and deregulated the expression of mRNA of Collagen III.

Conclusion: Our results indicates that SVF might have anti-aging potential in photo-aging skin and ADSCs situated in SVF play an important role.

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A FIBRIN SPRAY SYSTEM FOR THE DELIVERY OF ASCS TO DIABETIC WOUNDS

Presenter: Philipp Nessbach, MSc (Germany)
Affiliation: Klinikum Rechts der Isar - Technical University of Munich
Authors: Aitzetmüller M, Nessbach P, Centeno Cerdas C, Hopfner U, Kirsch M, Machens HG, Duscher D

Introduction: Adipose derived stem cells (ASCs) are a promising regenerative therapy for chronic wounds. A problem of their therapeutic application is how to apply them to and immobilize them in the wound area. Various scaffolds have been developed but the optimal cell delivery system is not yet determined. Here we evaluated the effectiveness of a fibrin spray system for the delivery of ASCs to a immunocompromised murine diabetic wound model.

Methods: ASCs were harvested from five human adult patients undergoing elective liposuction. After collagenase digestion ASCs were isolated by flow cytometry before culture. ASCs were applied to splinted excisional wounds in NSG mice by means of the Tisseel fibrin spray system or via drip on technique. ASC engraftment and survival was evaluated via IVIS imaging. Wound healing kinetics were analyzed and histology was carried out upon wound closure.

Results: In the fibrin sealent group ASCs remained viable significantly longer after application resulting in significantly accelerated wound closure. Potentially explaining the improved healing we observed a significantly increased blood vessel count on CD31 staining in the wounds of this group.

Conclusions: Human adipose tissue is a rich source of autologous stem cells, which are readily available for regenerative applications such as wound healing. Our results indicate that ASCs can be used as an effective cell therapeutic when applied and immobilized via a clinically approved fibrin sealant spray system.

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WOUNDS REPARATION AFTER HUMAN STROMAL VASCULAR FRACTION EXPOSURE IN A MOUSE MODEL OF PRESSURE ULCERS

Presenter: Joanna Bukowska, PhD (USA)
Affiliation: LaCell LLC
Authors: Bukowska J, Smith S, Wu X, Frazier T, Brown T, Kosnik P, Katz A, Mehrara B, Gawronska-Kozak B, Bunnell BA, Gimble JM

Pressure ulcers (PU) remains a serious unmet medical problem that afflict more than 2.5 million people in the United States with the total treatment cost exceeding $11 billion annually. PU are injures localized to the skin and underlying tissues and suggested to be caused by repeated cycles of ischemia reperfusion, impairing blood and lymph flow locally, that lead to cell necrosis in the epidermis, dermis, subcutaneous adipose and skeletal muscle tissues. The standard of care for PU relies on surgical interventions that are insufficient for effective PU treatment in all cases. In the present study we investigate the therapeutic effect of human adipose tissue-derived stromal vascular fraction (SVF) in the mouse model of PU.

To create PU model 2 month old (mo) female and male mice were subjected to 12 hr cycles of ischemia reperfusion injury via the application of two circular magnets (12 mm diameter) to their dorsal skin over two successive days. Wound were injected with PBS or human SVF (doses of 0.5 x 106, 1.0 x 106 or 2.0 106) isolated with Tissue Genesis, LLC., Icellator device that enables a closed system production of clinical grade SVF. Daily wound assessment over 20 days, histological examination and qPCR were conducted to determine the efficacy of SVF in PU wounds.

Delivery of SVF to the wound site revealed substantial differences in the healing pattern of PU between females and males. Faster removal of scabs was observed in females and started at day 8 after SVF injections when compare to males which displayed scabs removal starting at day 10. Moreover, females demonstrated accelerated wound healing after exposure to SVF relative to PBS. Complete wound closure in female mice occurred at days 12, 14, 13 for SVF delivered to the wounds respectively at the concentrations 0.5 x 106, 1.0 x 106 or 2.0 106 106 relative to control (PBS) at day 17. Comparison of male mice wound assessment is underway.

The current results demonstrated for the first time that human SVF can be applied in the mouse model of PU to accelerate wound recovery. Moreover, therapeutic effects of SVF may be more pronounced in females than in males suggesting a sex - dependent indication for the usage of SVF cell-based PU therapies.

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ADIPOSE-DERIVED STEM CELLS IN AN IN VIVO RAT MODEL OF ACHILLES TENDON EXCISION INJURY

Presenter: Jolanta Norelli, BA (USA)
Affiliation: Northwell Health System
Authors: Norelli J, Plaza DP, Varghese AM, Stal D, Liang H, Grande DA

Introduction: Musculoskeletal repair with adipose-derived stem cells (ADSCs) has shown improvement in tendon histology and biomechanics. No research to date has explored ADSC effects on Achilles tendon excision defect repair in vivo. Our purpose was to characterize rat ADSCs, induce ADSC tenogenesis, and analyze ADSC influence on tendon repair in vivo. We hypothesized that teno-differentiated ADSCs would yield superior tendon repair.

Methods: ADSCs were harvested from Sprague-Dawley rats, isolated, grown in vitro, and analyzed for stemness and in vitro paracrine ability. ADSC tenogenesis was tested in vitro with 24 combinations of GDF-5, 6, 7 and PDGF for 1, 7, 14, and 21 days. 14-day induction GDF-6+PDGF was used based on goal of increasing scleraxis (SCX) and collagen type-I (COL1) expression in vivo. Using rat Achilles tendon excision injury model, midsubstance tendon defects were made and either untreated or repaired using a COL1/alginate hydrogel with or without ADSCs (undifferentiated or teno-differentiated). Tendon tissue was harvested at 1.5, 3, and 4.5 weeks from 180 rats total (n=15) and characterized by histology, biomechanics (load to failure tests), and quantitative real-time PCR. Healing was compared to uninjured tendon. Collagen fiber organization was assessed quantitatively by Picro-sirius Red staining.

Results: Rat ADSCs were characterized as stem cells unique from endothelial, hematopoetic, or BMSC lineages. ADSCs culture supernatant showed high concentration of VEGF-A, IGF-1, FGF-1, 2, and IL-10, 8. Tendon defects treated with ADSCs showed increased expression of COL1, COL3, SCX, and TNMD (Fig. 1), significantly improved tissue architecture on histology (Fig. 2), and biomechanical properties (ultimate load, elastic toughness) over time more than hydrogel alone (Fig. 3), while teno-differentiated ADSCs produced collagen fiber dispersion range closest to normal tendon (Fig. 2).

Conclusions: Rat ADSCs were successfully differentiated into tendon-like cells and showed pro-angiogenic, immunomodulatory, and pro-proliferatory profile in vitro, suggesting benefits for tissue repair. Tendons repaired with teno-differentiated ADSCs exhibited increased COL1 expression and most improved tissue architecture and biomechanical properties.

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ADIPOSE-DERIVED STEM CELLS IN AN IN VIVO RAT MODEL OF ACHILLES TENDON EXCISION INJURY

Presenter: Jolanta Norelli, BA (USA)
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Authors: Norelli J, Plaza DP, Varghese AM, Stal D, Liang H, Grande DA

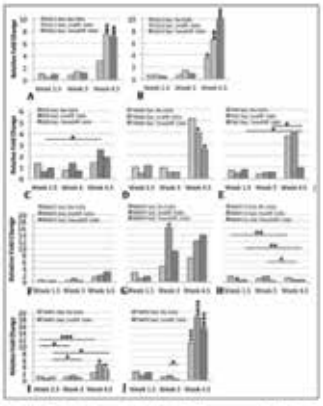
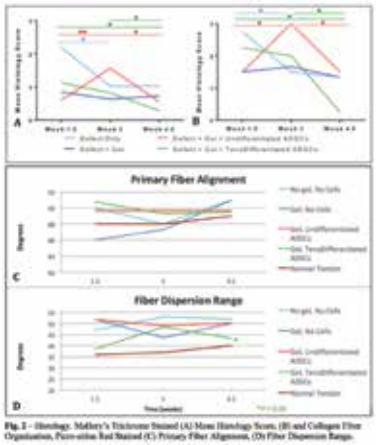


Fig. 1|– Gene Expression (A) Collagen type-I, (B) Collagen type-III, (C) Scleraxis, (D) Tenomodulin, (E) Tenascin C, (F) MMP3, (G) MMP9, (H) MMP13, (I) TIMP1, (J) TIMP2;



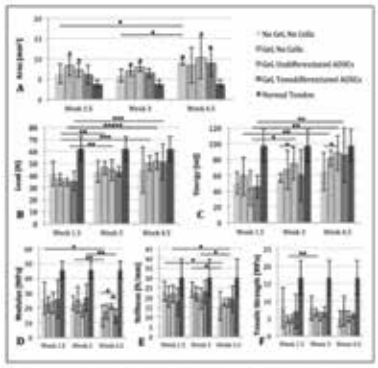


Fig. 3 - Biomechanical Properties (A) Cross-sectional Area, (B) Ultimate Load, (C) Toughness, (D) Elastic Modulus, (E) Stiffness, (F) Strength;

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ADIPOSE-DERIVED STROMAL CELLS INHIBITED MAST CELL FUNCTIONS: IMPORTANT ROLE IN POST-BURN HYPERTROPHIC SCAR TREATMENT

Presenter: Benoit Chaput, MD (France)
Affiliation: Department of Plastic and Reconstructive Surgery
Authors: Chaput B, Laloze J, Grolleau JL, Sensebe L, Varin A

Introduction: Twelve to 40% patients developed hypertrophic scars after skin burns. Post-burn hypertrophic scars may be associated with pain, erythema, pruritus and dysesthesia, that decrease quality of life of the patients, and are characterized by chronic inflammation and skin retraction due to an excess production of connective tissue by fibroblasts. Hypertrophic scars contain significantly more mast cells than mature scars and this infiltration seems to be an important contributor to the pathological evolution of the scar. Indeed, mast cells secreted high amounts of cytokines and growth factors, responsible of chronic inflammation of the scar and they secreted molecules such as histamine and tryptase that stimulate the production of connective tissue by the fibroblasts. Previous study demonstrated that bone marrow-derived mesenchymal stromal cells (BM-MSCs) inhibited the activation and the degranulation of mast cells. Therefore, we studied the effect of adipose-derived stromal cells (ASCs), that have similar immunosuppressive capacities than BM-MSCs, but are easily obtained by liposuction, on mast cells.

Materials and Methods: The human mast cell line HMC-1 was cultivated alone or with different concentration of cultivated ASCs. After 24h of co-culture, mast cells were magnetically separated from ASCs, based on CD73 expression. Effect of ASCs on proliferation and migration of HMC-1 was determined and we analyzed the effect of ASCs on mast cell degranulation as well as cytokine production.

Results: We demonstrated that ASCs decreased in a dose-dependent manner the proliferation of HMC-1 and that ASCs prevented in a dose dependent manner the SCF-induced migration of HMC-1. Moreover, ASCs efficiently inhibited the release of β-hexoaminidase as well as histamine whereas they did not modify tryptase secretion. Interestingly, ASCs modified the cytokine profile of HMC-1. They decreased the secretion of anti-inflammatory cytokines such as IL-10 but increase slightly secretion of TNF-α and IL-12, two the pro-inflammatory cytokines.

Conclusion: Our results demonstrated that ASCs inhibited mast cell degranulation and efficiently prevent mast cell migration, therefore, ASCs could be used as a new efficient cell therapy to treat hypertrophic scar.

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THE FATE OF ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS ADMINISTERED SYSTEMICALLY AND LOCALLY FOR WOUND REPAIR

Presenter: Karlien Kallmeyer, MSc, PhD (Switzerland)
Affiliation: University of Geneva
Authors: Kallmeyer K, Andrè-Lévigne D, Pepper MS, Pittet-Cuénod B, Modarressi A

Introduction: There is increasing interest in the use of adipose-derived mesenchymal stromal cells (ASCs) for wound repair. However, the fate of administered cells as well as the most efficient mode of administration (within a dressing, local injection or systemic treatment) is still poorly defined. Therefore, prior to assessing the benefit of ASCs in wound repair, this study set out to establish the location and survival of ASCs in vivo when administered either systemically or locally using both bioluminescence imaging (BLI) and histological analysis.

Methods: ASCs were transduced with a dual lentivector which expresses both firefly luciferase (Fluc) and green fluorescent protein (GFP). To determine the behaviour of ASCs, a model of physiological wounds in rats was used. Wounds were created bilaterally on the dorsal aspect of the hind paws. Two modes of application were assessed: 2x10^6 ASCs injected systemically into the tail vein, and 2X10^5 ASCs injected locally into the corners of the wound bed. ASC distribution and survival was followed in animals by BLI and histological analysis at 3h, 24h, 48h, 72h, 7 and 15 days post ASC injection.

Results: In animals treated systemically, ASCs were detected in the lungs with a decrease in signal from 3h to 48h, but no luminescent signal or GFP staining was detected in the wound. However, locally administered ASCs remained strongly detectable for at least 7 days at the injection site. At the histological level, locally administered ASCs were detectable by GFP staining at the injection site. Interestingly a few locally administered ASCs seemed to migrate into the wound area as early as 48h post injection.

Conclusion: Using this physiological wound model we have observed that GFP/Fluc labelling allowed ASCs to be tracked in vivo. When administered systemically, the majority of ASCs were filtered out in the lungs. Locally administered ASCs on the other hand remained and survived at the wound site for at least 7 days. Therefore systemic administration of ASCs for local wound repair in the clinical setting, is questionable. To fully understand the role of ASCs in the context of wound repair, further studies using different administration methods in pathological wound models of increased severity should be performed.

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ADIPOSE STEM CELLS AND ADIPOSE STEM CELL CONDITIONED MEDIA EXHIBIT SIMILAR ACTIVITY IN A PORCINE MODEL OF EXCISIONAL WOUND HEALING

Presenter: J. Peter Rubin, MD, FACS (USA)
Affiliation: University of Pittsburgh
Authors: Grybowski D, Schusterman MA, Kim D, March KL, Johnstone B, James IB, Venkatesh K, Kokai LE, Marra KG, Rubin JP

Background: Multiple therapies aim to improve the wound healing process. Cell therapy has many limitations, including immunogenicity of allogenic cells and arduous storage and handling requirements. ASC Conditioned Medium is more versatile in administration and handling, and potentially possesses healing properties of ASC.

Methods: Eight excisional wounds with diameters of 4 cm were created on the back of a female Yorkshire pig. Wounds were treated with injections as follows: 3 with 3ml of ASC Conditioned Medium, 3 with allogenic ASC and 2 with Saline. Injections were performed by delivering 2ml of each treatment into the wound bed and 1ml into the wound edge. CM was prepared by culturing a standard, non-enriched, serum free medium over a course of 60 hours with ASC's from passage 6-9. Wound tracing and standardized photography was performed 3 times per week. At post-operative day 15, the pig was sacrificed and wounds were excised for histology. Tissue was stained for CD31, Elastin and Collagen 1.

Results: ASC Conditioned Medium increased wound epithelialization at day 4 and day 7 ($p<0.05$). Adipose Stem Cells showed increased wound epithelialization at day 7 and decreased scar thickness compared to the Controls ($p<0.05$). CD31, Elastin and Collagen 1 staining were similar across all groups.

Conclusions: ASC Conditioned Medium represents an exciting potential substitute for current treatments in wound healing, and increases wound epithelialization compared to control wounds at post-operative day 4 and 7.

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ADIPOSE STEM CELLS AND ADIPOSE STEM CELL CONDITIONED MEDIA EXHIBIT SIMILAR ACTIVITY IN A PORCINE MODEL OF EXCISIONAL WOUND HEALING

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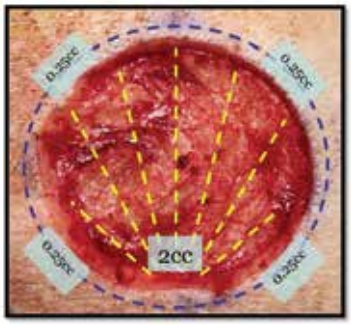

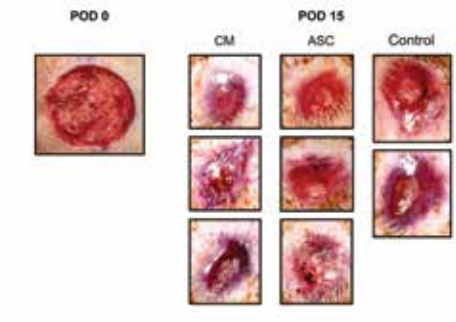

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EFFECT OF HUMAN ADIPOSE TISSUE DERIVED MSC AND EXOSOMES ON WOUND HEALING

Presenter: Marta Garcia-Contreras, PhDc (USA)
Affiliation: University of Miami
Authors: Garcia-Contreras M, Messaggio F, Mendez AJ, Robbins PD, Ricordi C

Wound healing is a complex process delayed in patients with underlying chronic conditions, such as diabetes. Micro Fractured and Purified Adipose Tissue Graft (Lipogems®) has been recently introduced in clinical trials for treatment of selected chronic and acute disease conditions, including wound healing. It has been hypothesized that certain cellular pathways are activated during Lipogems® processing, enabling stem cells and/or exosomes to increase their ability for tissue repair and regeneration. The aim of this study is to examine the therapeutic potential of Lipogems® products in an in vitro wound healing model system.

Lipogems products isolated from lipoaspirates from 5 different donors were cultured for 24 hours in alpha-MEM medium supplemented with 1% BSA. Tissue fractions and supernatants were collected. Briefly, exosomes were isolated from culture supernatants using differential ultracentrifugation and further characterized by Transmission Electron Microscopy and analyzed by Nanosight NS300 system (Malvern Instruments Company). Protein analysis from Lipogems® products and Lipogems®-derived exosomes was performed using The RayBiotech Kit Human Growth Factor, Angiogenesis and Cytokine Antibody Arrays (RayBiotech) and revealed a higher expression of proteins involved in tissue repair and/or regeneration (Angiogenic proteins such as bFGF or CSF, Growth Factors such as TGF or VEGF D and cytokines such as MCP-1 or IL-7), in Lipogems® products. The effect of Lipogems® derived MSC and exosomes was evaluated by scratch wound healing assay using the standard human fibroblasts and keratinocytes migration assay. Both MSC derived condition media and purified exosomes demonstrated a significantly higher in vitro wound healing potential, compared to untreated cells ($p < 0.05$). Our findings suggest that Lipogems® products may facilitate cutaneous wound healing and that the exosome component of these adipose derives tissue products could be responsible for the observed improved effect. These results support the recent clinical observations on the use of Lipogems® for treatment of diabetic wounds and the possible use of MSC and their exosomes for wound healing in diabetes.

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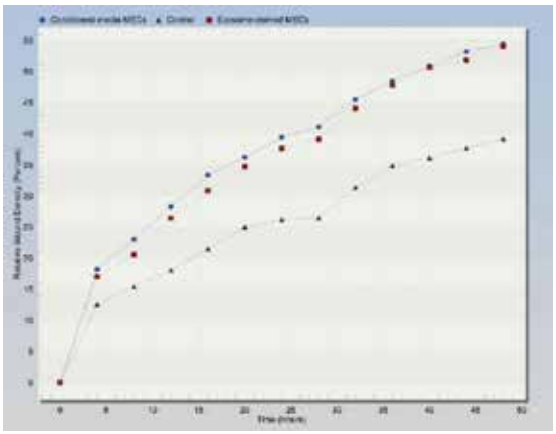

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MAINTENANCE OF STEMNESS AND REJUVENATION POTENTIAL OF ADIPOSE-DERIVE STEM CELLS (ASCS) ISOLATED FROM PREMATURE/PROGEROID MICE MODEL

Presenter: Xiaodong Mu, PhD (USA)
Affiliation: University of Texas
Authors: Mu X, Chen W, Ravuri S, Huard J

Introduction: Exhaustion of stem cell function could lead to decreased rejuvenation and increased age progression. Transplantation of stem cells could potentially slow down or reverse some age related pathologies. Although it was shown that adipose tissues/mature adipocytes could become dysfunctional and acquires pro-inflammatory, senescent-like phenotype with aging, the influence of aging process on adipose stem cells is not well understood. Mice deficient in metalloproteinase, Zmpste24 (Z24-/-), demonstrated premature onset of aging-related pathologies and studied as a model for human Hutchinson-Gilford Progeria Syndrome (HGPS). In current study, we investigated the characteristics and rejuvenation potential of the ASCs from Z24-/- mice *in vitro*.

Methods: ASCs were isolated from subcutaneous fat tissue of Z24-/- mice and WT mice by standard laboratory method (mincing, enzymatic digestion and centrifugation). Culture characteristics of Z24-/- and WT ASCs were determined in stem cell senescence assay in addition to gene expression assay. ASCS + MPCs cell co-culture experiment was performed to find out the potential influence of Z24-/- ASCs on Z24-/- muscle progenitor cells (MPCs) and to determine rescuing potential of ASCs.

Results: Z24-/- ASCs grew much slower than WT ASCs (Fig. 1 A, B), but cell senescence-associated β-galactosidase (SA-β-Gal) staining showed only small fraction (<5%) of Z24-/- ASCs (Fig. 1C). qPCR results showed mTORC1 expression (cell senescence promoting factor) was down-regulated in Z24-/- ASCs, while the expression of p21 (negative cell cycle regulator) was upregulated (Figure 2). TGF-β1 is upregulated in Z24-/- ASCs, but the expression of pro-inflammatory factors (IL-1β, IL-6) was down-regulated. Upregulated VEGF suggests increased regenerative potential of Z24-/- ASCs. WT ASCs or Z24-/- ASCs co-cultured with Z24-/- MPCs showed Z24-/- ASCs positive effect in rescuing defective phenotypes of Z24-/- MPCs, similar to WT ASCs (Fig. 3).

Conclusion: Preliminary results suggest that Z24-/- ASCs are generally not senescent, but could be more quiescent compared to WT ASC. WT-ASCs and Z24-/- ASCs were able to repress pro-inflammatory and pro-fibrogenic factors, but increase anti-inflammatory and energy metabolism factors.

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MAINTENANCE OF STEMNESS AND REJUVENATION POTENTIAL OF ADIPOSE-DERIVE STEM CELLS (ASCS) ISOLATED FROM PREMATURE/PROGEROID MICE MODEL

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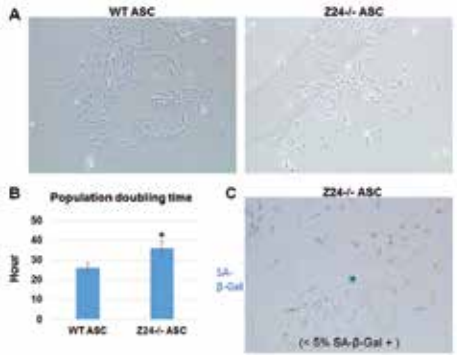

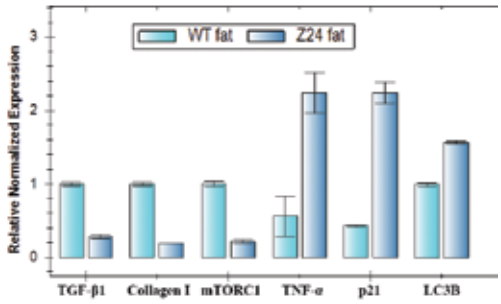

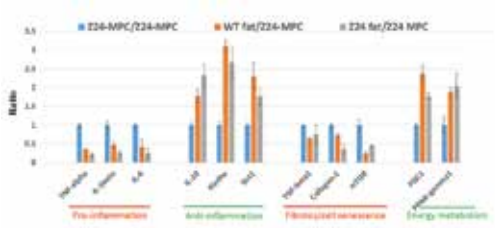

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GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN ADIPOSE-DERIVED STROMAL/STEM CELLS IS CELL DENSITY-DEPENDENT AND POSITIVELY IMPACTS ADIPOGENIC DIFFERENTIATION

Presenter: Torsten Blunk, PhD (Germany)
Affiliation: University of Wuerzburg
Authors: Blunk T, Wiesner M, Berberich O, Hoefner C, Bauer-Kreisel P

Introduction: Direct intercellular communication between cells via connexin 43 (Cx43)-generated gap junctions enables the functional coupling of cells, which has been shown to modulate differentiation processes, determination of stem cell fate and tissue homeostasis. The knowledge of these tissue-inherent processes is of particular importance in tissue engineering in order to understand and drive tissue development. Adipose-derived stromal cells (ASCs) play a pivotal role in adipose tissue engineering approaches, however, little is known about the impact of gap junctional intercellular communication (GJIC) in these cells. Thus, here we focused on the expression of Cx43 and GJIC in human ASCs, and its relevance for adipogenic differentiation.

Methods: hASCs were seeded with 5000, 25,000 and 100,000 cells/cm2 and cultured either in growth medium or adipogenic medium. Expression of Cx43 was characterized by qRT-PCR analysis, Western blot and immunohistochemical staining. Functionality of gap junctions was evaluated by flow cytometry using a dye transfer assay. Inhibition of GJIC was performed by addition of 18α-glycyrrhetinic acid (AGA). Adipogenesis was assessed by histology, analysis of triglyceride content and gene expression analysis of adipogenic marker genes.

Results: Cx43 expression in ASCs was demonstrated histologically and on mRNA and protein level, and was shown to be greatly positively influenced by initial cell seeding density. Functionality of gap junctions was proven by successful dye transfer between cells. Adipogenic differentiation of ASCs was shown to be also distinctly elevated at higher seeding densities. Inhibition of gap-junctional functionality by AGA markedly compromised adipogenesis resulting in decreased lipid accumulation and reduced expression of adipogenic marker genes. Flow cytometry analysis showed a lower proportion of cells undergoing adipogenesis when GJIC was inhibited, further indicating the importance of GJIC in the differentiation process.

Conclusion: Altogether this study demonstrates the relevance of cell-cell communication via gap junctions in the adipogenic differentiation of ASCs and may contribute to the further integration of intercellular crosstalk in rationales for tissue engineering and regenerative medicine.

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DFO PRECONDITIONING RESTORES THE THERAPEUTIC POTENTIAL OF DIABETIC ASCS

Presenter: Matthias Aitzetmüller, MD (Germany)
Affiliation: Klinikum Rechts der Isar - Technical University of Munich
Authors: Aitzetmüller M, Nessbach P, Hopfner U, Kirsch M, Centeno Cerdas C, Machens HG, Duscher D

Introduction: A hallmark of diabetes mellitus is the breakdown of almost every reparative process in the human body, leading to critical impairments of wound healing. Currently, adipose-derived stem cells (ASCs) are considered to be a promising source for cell therapeutics targeting diabetic wounds. However, functionality of these cells is impaired by diabetes which can result from a defect in hypoxiainducible factor-1 (HIF-1), a key mediator involved in neovascularization. In the current study, we sought to explore effectiveness of pharmacological priming with deferoxamine (DFO) as a hypoxia mimetic agent, to restore this compromised angiogenic pathway for attenuating diabetes-associated deficits in cutaneous wound healing.

Materials and Methods: Diabetic ASCs from db/db mice were treated in vitro with DFO and assessed for the expression of key mediators of neovascularization and tissue regeneration at mRNA and protein levels, using qRT-PCR and ELISA. Additionally a matrix tubulization assay was performed. In vivo experiments were conducted using a humanized excisional wound model and histology was taken upon complete healing.

Results: DFO remarkably enhanced expression of regenerative genes and protein levels compared to untreated samples. Compromised angiogenic potential of diabetic ASCs was restored in the tubulization assay. DFO treatment further significantly enhanced the therapeutic effect of db/db ASCs which could be confirmed by CD31 staining demonstrating improved neovascularization.

Conclusion: DFO preconditioning restored the therapeutic potential of diabetic ASCs resulting in enhanced regeneration.

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FUNCTIONAL CHARACTERISTICS OF FIBROBLASTS DIFFERENTIATED FROM ADIPOSE-DERIVED STEM CELLS

Presenter: Ivona Percec, MD, PhD (USA)
Affiliation: University of Pennsylvania
Authors: Percec I, Gersch RP

Introduction: Several dermal wound healing applications have been used to test the therapeutic potential of Adipose-derived stem cells (ASCs); however, we have yet to see dramatic improvement in healing time or scar preventing in a large scale clinical trial. Here, we characterize the fibroblastic differentiation capacity of these cells and extracellular matrix (ECM) production compared to primary fibroblasts as recent literature suggests ECM from ASC differentiated Fibroblasts (dFib cells) may be superior.

Method: ASCs and primary fibroblasts were isolated from healthy female patients undergoing abdominoplasty (n=7, 45.14±14.16 years old). ASCs underwent fibroblastic differentiation for 3 weeks and were then allowed to produce ECM in parallel with fibroblasts for an additional 3 weeks. At this time, mRNA was harvested from ASCs, fibroblasts, and dFib cells for biomarker expression quantification via qPCR and cells were stained using Masson's Trichrome stain to characterize matrix production. Cells further underwent in vitro scratch test migration analysis.

Results: Differentiated cells showed increased expression of fibroblast marker Ephb3 (6.94±1.98-Fold increase over ASC expression) which compares to primary fibroblast expression (4.61±1.8-Fold increase). dFib cells also show increased expression compared to primary fibroblasts for the healthy ECM marker genes Fibronectin (1.25±0.36-Fold vs. 0.80±0.28-Fold respectively, $p<0.05$) and Collagen 1 (1.34±0.41-Fold vs. 0.47±0.09-Fold respectively, $p<0.001$), and Elastin (1.21±1.04-Fold vs. 0.48±0.16-Fold respectively). Masson's Trichrome staining demonstrates a dramatic increase in matrix production by dFib Cells compared to primary fibroblasts. Upon scratch testing, dFib cells demonstrated smoother and more consistent wound margins compared to primary fibroblasts and dFib cells migrated into the wound more quickly resulting in a lower time to wound closure (37.6±12.25 hours) compared to fibroblasts (64±12.39 hours, $p<0.01$).

Conclusions: ASCs can be differentiated toward a fibroblastic lineage. These cells produce a robust ECM more similar to healthy skin as opposed to scar tissue produced by primary fibroblasts and should be investigated further for optimization and wound healing applications.

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ADIPOSE STEM CELLS FROM OBESE INDIVIDUALS PROMOTE TRIPLE NEGATIVE BREAST CANCER METASTASIS IN A PATIENT DERIVED XENOGRAFT (PDX)

Presenter: Rachel Sabol, MS (USA)
Affiliation: Tulane University School of Medicine
Authors: Sabol R, Matossian M, Dutreil MF, Cote A, Bowles AC, Burks HE, Collins-Burow B, Burow ME, Bunnell BA

Obesity is an established risk factor for breast cancer. Obese women have an increased incidence and mortality of breast cancer, however the mechanisms(s) through which obesity enhances tumorigenesis are not well understood. Current models used to study breast cancer in vivo, specifically immortalized cell lines or orthotopic xenografts, are limited by the lack of tissue architecture and human stromal components. Passaging immortalized cell lines results in irreversible alterations of genetic information and behavioral characteristics. Patient-derived xenografts (PDX) have emerged as a novel translational tool for cancer research with the potential to more accurately recapitulate the molecular and behavioral aspects of cancer. Further, PDX represent primary patient tumors providing evidence in the context of humans in vivo. Our team has developed a system to investigate the obesity-breast cancer axis. The protocol involves implanting PDX tumor line 2K1 coated in matrigel alone or co-transplanted with adipose-derived stem cells from lean (lnASCs) and obese (obASCs) donors. PDX tumors were implanted bilaterally into the mammary fat pad of female SCID/Beige mice and passaged when the tumor volume grew to 750-1000 mm3. Tumors with obASCs show decreased expression of CD326 (epithelial cell adhesion molecule) and increased circulating HLA1+ 'human cells' as well as increased CD44+CD24- cancer stem cells in the blood. Additionally, obASCs increased circulating classically associated macrophages as well as alternatively activated macrophages, which have been shown to promote breast cancer tumorigenesis and invasion. Evaluation of lungs and liver revealed increased lung metastases in the obASC group. These data suggest that obASCs promote increased circulating tumor cells leading to increased metastasis of triple negative breast cancer in a PDX model.

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AUTOLOGOUS ADIPOSE DERIVED MULTIPOTENT STROMAL CELLS ON XENOGRAFTS REDUCE THE SYSTEMIC LYMPHOCYTE RESPONSE

Presenter: Mandi Lopez, DVM, MS, PhD (USA)
Affiliation: Louisiana State University
Authors: Lopez M, Takawira C, Bova J, Stout R, Dietrich M

Xenogeneic bone significantly augments options for bone grafts, and addition of adult adipose tissue derived multipotent stromal cells (ASCs) to bone xenografts may enhance integration and direction of native cell tissue generation. This study hypothesis was that autologous ASCs on facial bone xenografts decrease systemic lymphocytes and increase osteogenic biomarkers compared to xenografts alone in a porcine model. Twenty eight Yucatan barrows received one of three treatments: no implant (NI, n= 4), bovine xenograft (S, n= 15) and bovine xenograft with autologous ASCs (ASC, n= 9). Distinct systemic lymphocyte immunophenotype levels were quantified before surgery and regularly up to 6 months post-surgery based on combinations of CD4, CD8, CD3 and CD21 expression using flourochrome labeled, porcine specific antibodies and multi-channel flow cytometry. Serum levels of carboxyl-terminal cross-linked telopeptide of type 1 collagen (ICTP), osteocalcin, and bone specific alkaline phosphatase (BAP) were quantified via enzyme linked immunosorbant assays at the same times. At all time points, the S group had higher levels of CD4+/CD8+ and CD4+/CD8- cells, and CD3+/CD4+ cells were significantly lower in the ASC versus the S group. Serum levels of ICTP and osteocalcin tended to be highest and lowest in the ASC group, respectively, across time points. The BAP levels tended to be highest in the NI group across time points. These results suggest that addition of ASCs to xenografts may effectively reduce the CD8+ and CD4+ T cell response to the foreign material.

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FRACTIONAL LASER + MICRONEEDLING + NANOFAT: EVIDENCE OF RAPID HEALING AND ELASTIN AND COLLAGEN REGENERATION

Presenter: Steven Cohen, MD, FACS (USA)
Affiliation: University of California San Diego
Authors: Cohen S, Goodache A, Delauney F

Introduction: Fractional lasers offer the opportunity of creating a micropuncture into the skin that solicits the host's stem cells to the site of injury as a component of repair. The injury also facilitates transdermal delivery of biologic and synthetic products. Nanofat has an increased number of stromal vascular fraction cells (up to 50,000 cells/ml depending on preparation and patient). These cells can be delivered through micropunctures and using transdermal delivery devices that increase penetration into the tissue. In addition, the cells in Nanofat can be delivered using mesotherapy and microneedling and also as a topical agent in combination with a unique particulate that permits enhanced transdermal delivery.

Materials and Methods: Over the past year we have treated patients with a combination approach of fractional laser and topical application of a specially formulated, patient personalized cream of their stromal vascular fraction cells and maxtrix from fat. As the regenerative cells have been shown to last for 72 hours under refrigeration, patients were instructed to apply the cream for 3 days 4 times a day.

Results: Patients reported faster healing with most indicating healing occurred 2-3 times faster than their prior laser procedure without nanofat biocream. All patients noted much improved skin thickness and texture which was improving at greater than 6 months after treatment. One patient underwent biopsy of a retroauricular region that was lasered on both sides, but only one application of nanofat biocream was made. Her biopsies at 2 months after treatment showed substantially more new elastin fibers and healing was more rapid. There were no complications from the laser or from the fat harvest with the exception of minor bruising at the fat harvest site.

Conclusions: Transdermal delivery of stromal vascular cells via a nanofat gel compounded with a sterile lipoderm that serves as a particulate delivery system that in conjunction with fractional laser appears to improved sun damage skin as well as for the first time regenerate elastin and the skin's blood supply.

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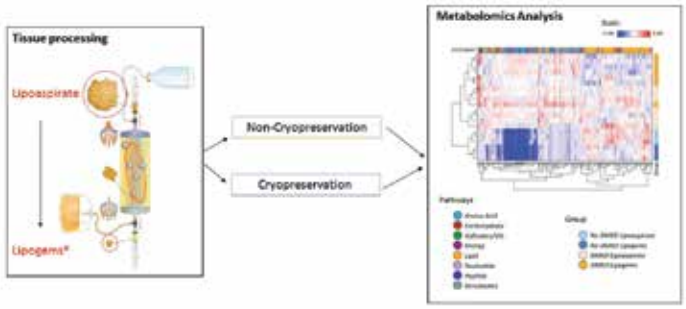
METABOLOMIC CHANGES IN HUMAN ADIPOSE TISSUE DERIVED PRODUCTS FOLLOWING NON-ENZYMATIC MICROFRACTURING

Presenter: Marta Garcia-Contreras, PhDc (USA)
Affiliation: University of Miami
Authors: Garcia-Contreras M, Messaggio F, Mendez AJ, Ricordi C

Human adipose tissue-derived mesenchymal stromal cells (hADSCs) have been widely used in regenerative medicine applications. Processing and cryopreservation methods have been shown to affect the characteristics of the final tissue/cell products that could be clinically used. Microfractured human adipose tissue (Lipogems®) has been shown to provide a more effective source of adult stromal cells compared to the initial lipoaspirated tissue material. Since the potency of hADSCs decreases with age and because of the potential usefulness to have source material available for future use, avoiding repeated liposuctions, cryopreservation could improve the availability of adipose tissue products for subsequent clinical applications.

Metabolomic profiling of cryopreserved Lipogems® tissue products was compared to that of the initial lipoaspirates, before microfracturing, to determine altered metabolites that could result from the non-enzymatic processing and cryopreservation method. Differences were observed in carbohydrate and nucleotide metabolism. These alterations translated in long chain and polyunsaturated fatty acid levels and amino acid metabolites showed divergent trends. When Lipogems® and Lipoaspirate tissue products were cryopreserved with DMSO, aminoacids tended to increase in Lipogems® product. However, in the absence of DMSO aminoacids and their catabolites tended to decreased in Lipogems® fat tissue product. Furthermore, our studies assessing the "stemness" of cells exposed to DMSO could be of assistance to identify possible effects of the cryopreservation and bank adipose tissue methods on hADSCs differentiation potential and paracrine effects in target tissues for further clinical applications.



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A CRITICAL EVALUATION OF THE ENDOTHELIAL PLASTICITY OF ADIPOSE-DERIVED STEM CELLS

Presenter: Jeremy Antonyshyn, BSc (Canada)
Affiliation: University of Toronto
Authors: Antonyshyn J, Santerre JP

Introduction: Considerable effort has been directed at deriving endothelial cells (ECs) from adipose-derived stem cells (ASCs) since Planat-Benard et al. first suggested that adipocytes and ECs share a common progenitor in 2004. The expression of endothelial markers by conditioned ASCs is commonly cited as evidence of their successful differentiation, but these studies most often omit quantitative comparisons to proper EC benchmarks and this has precluded an accurate assessment of their endothelial plasticity. It was the purpose of this investigation to evaluate the extent of endothelial differentiation being achieved with ASCs relative to appropriate controls.

Method: CD45-CD31- ASCs were isolated from subcutaneous abdominal adipose tissue via magnetic-activated cell sorting (N = 3). Their differentiation was induced with EC growth medium (EGM)-2, as commonly performed. After 14 days of conditioning, their morphology was assessed by phase-contrast microscopy and their expression of endothelial genes was evaluated by quantitative polymerase chain reaction. Human umbilical vein ECs (HUVECs), coronary artery ECs (HCAECs) and dermal microvascular ECs (HDMVECs) were used as positive controls to encompass the phenotypic heterogeneity between ECs derived from different vascular beds.

Results: ASCs conditioned with EGM-2 significantly upregulated their expression of PECAM1, NOS3 and arterial marker EFNB2, and significantly downregulated their expression of venous marker EPHB4 (7.1 ± 1.4, 1.4 ± 0.7, 118.3 ± 101.9 and 0.4 ± 0.2 –fold, respectively; $p<0.05$; Fig. 1). However, expression of PECAM1, CDH5, VWF and MCAM were significantly greater in control ECs (1,927.3 ± 980.2, 48,660.4 ± 26,492.0, 10,233.5 ± 6,949.5 and 154.2 ± 78.9 – fold, respectively; $p<0.05$). Furthermore, conditioned ASCs failed to assume a characteristic endothelial cobblestone-like morphology, rather retaining a spindle-shaped morphology.

Conclusion: Despite the phenotypic heterogeneity among ECs, ASCs did not assume an endothelial morphology nor transcriptome after 14 days of inductive culture. These findings underscore the need to include appropriate positive controls in studies of differentiation, and demonstrate the limited extent of endothelial differentiation that can be achieved with ASCs.

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A CRITICAL EVALUATION OF THE ENDOTHELIAL PLASTICITY OF ADIPOSE-DERIVED STEM CELLS
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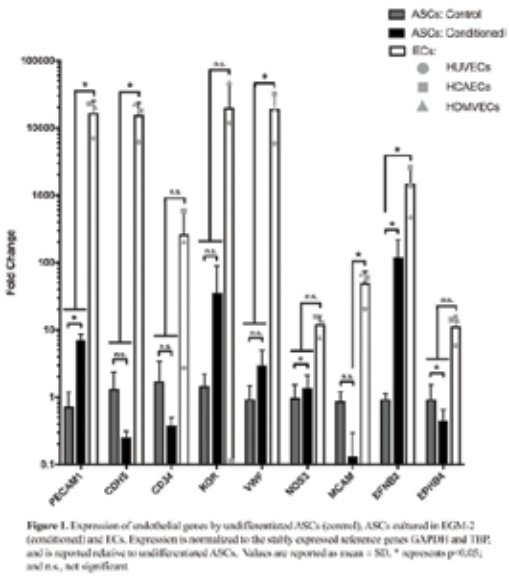

Figure 1. Expression of endothelial genes by undifferentiated ASCs (control), ASCs cultured in EGM-2 (conditioned) and ECs. Expression is normalized to the stably expressed reference genes GAPDH and TBP, and is reported relative to undifferentiated ASCs. Values are reported as mean ± SD, * represents $p<0.05$; and n.s., not significant.

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EFFECT OF CRYOPRESERVATION ON INTACT ADIPOSE TISSUE AND ISOLATED STROMAL VASCULAR FRACTION CELLS IN VITRO AND IN VIVO ANALYSES

Presenter: Fabiana Zanata, MD (Brazil)
Affiliation: Federal University of Sao Paulo
Authors: Zanata F, Bowle A, Frazier T, Curley L, Bunnell B, Wu X, Wade J, Devireddy R, Gimble J, Ferreira LM

Background: Adipose tissue is a source of Adipose-Derived Stromal/Stem Cells for tissue engineering and reconstruction as well as a tissue source for fat grafts. Although liposuction is a simple procedure for the harvest of adipose tissue, the repetition of this surgical intervention can cause adverse effects to the patient and can be a limiting factor for immediate use. Cryopreservation can avoid the morbidity associated with repetitive liposuction, allowing the use of stored tissue after the initial harvest procedure. This paper focuses on the characterization of fresh and cryopreserved human adipose tissue.

Methods: Lipoaspirates from 8 donors were processed as fresh adipose tissue or cryopreserved for 4 to 6 weeks. Fresh and cryopreserved tissue were collagenase digested and the SVF cells were characterized immediately or cryopreserved. Characterization was based on SVF cell proliferation and immunophenotype. In vivo fat grafting was performed in C57BL/6 GFP mice to analyze morphology of the tissue and its adiposity using confocal microscopy, histochemical staining (H&E, Masson's Trichrome) and Immunohistochemistry (GFP, Perilipin, CD31).

Results: Although tissue and SVF cell cryopreservation reduced the total cell yield, the remaining viable cells retained their adhesive and proliferative properties. The SVF cell immunophenotype showed a significant reduction in the hematopoietic surface markers and increased expression of Stromal and Adipogenic markers following cryopreservation. In vivo cryopreserved fat grafts showed a similar morphology to freshly implanted fat grafts.

Conclusion: In this study, we demonstrated that cryopreserved adipose tissue is a potential source of SVF cells and a suitable source for fat grafts.

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ISOLATION AND EXPANSION OF VASCULAR ENDOTHELIAL CELLS FROM HUMAN LIPOASPIRATES

Presenter: Natsumi Saito, PhD (Japan)
Affiliation: Jichi Medical University
Authors: Saito N, Asahi R, Shirado T, Odbayar B, Yoshimura K

Introduction: Vascular endothelial cells (VECs) are one of important cell populations to play pivotal roles in any types of wound healing and tissue remodeling. Thus, VECs are expected to be a potent therapeutic tool in regenerative medicine. Our previous studies indicated that VECs are highly contained in adipose tissue as a component of capillary network and the number of adipose-resident VECs appears to be similar to that of adipose-derived stem/stromal cells (ASCs). However, an effective method for isolation and enrichment of VECs from adipose tissue has not been reported until now.

Method: We have used three steps for isolation and enrichment of VECs from human lipoaspirates. First, stromal vascular fraction (SVF) was extracted from lipoaspirates through enzymatic digestion as already established and standardized, followed by hemolysis. Second, we collected CD45-/CD31+ fraction by conducting magnetic-activated cell sorting (MACS) using CD45 and CD31 antibodies. Finally, the CD45-/CD31+ cell fraction was cultured and expanded in EGM-2 medium, and the second MACS using CD31 antibody alone was performed for further purification of VECs.

Results: SVF cells were extracted from lipoaspirates at 7-10x10^5 nucleated cells per gram of lipoaspirates. The number of CD45-/CD31+ cells obtained from SVF by MACS sorting was less than 2% of that of original SVF cells. In case that VEC purity was <90%, a small number of ASCs grew more rapidly after passage and VECs were totally lost in the end. However, by performing the second MACS to further purify VECs (up to >90%) at the early stage of passage 1 culture, VECs were successfully cultured and expanded.

Conclusions: We found that >90% purification of VECs is a key factor to isolate and expand VECs from human lipoaspirates. MACS seems to be the best way to process a large number of SVF cells within a short time and enables the efficient extraction and purification of VECs.

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ADIPOSE-DERIVED STEM CELL CONDITION MEDIUM ATTENUATED CISPLATIN-TRIGGERED APOPTOSIS IN TONGUE SQUAMOUS CELL CARCINOMA CELLS

Presenter: Yu-Jen Chiu, MD (Taiwan)
Affiliation: Taipei Veterans General Hospital
Authors: Chiu YJ, Ma H, Hsu H, Yang J

Procedures of autologous fat grafting increased dramatically not only for cosmetic purpose but also for deformities after head and neck cancer and breast cancer surgery. Carcinogenesis is always a major concern in cell therapy related issues. However, there is no literature discussing the issue in head and neck squamous cell carcinoma patients. To evaluate the interaction of tongue cancer cells and adipose-derived stem cells, we performed a series in vitro experiment. Our results demonstrated that cisplatin significantly reduced cell viabilities of SCC-25 and CAL-27 cells in a concentration-dependent manner, but it exhibited low cytotoxicity in CAR cells. For the supplement of ASC-CM, there was no significant difference of viability of SCC-25, CAL-27 and CAR cells in ASC-CM and control groups. There was also no significant difference of cell migration by wound scratch assay and transwell invasion assay of SCC-25, CAL-27 and CAR cells between ASC-CM treatment and control treatment. Importantly, the ASC-CM attenuated cisplatin-triggered cell death in SCC-25, CAL-27 cells. Moreover, ASC-CM markedly inhibited cisplatin-induced apoptotic cell death (sub-G1 phase) in CAL-27 cells. Western blotting analyses indicated that cisplatin-induced reductions of pro-caspase-3, pro-caspase-9, phospho-BAD, phospho-IGF-1R, phospho-AKT and phosphor-ERK in CAL-27 cells were rescued by ASC-CM supplement. Taken together, we provide evidence that ASC-CM protects CAL-27 cells from cisplatin-induced cell death, possibly through up-regulation of IGF-1R/AKT/ERK signaling pathway.

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ADIPOSE-DERIVED STEM CELL CONDITION MEDIUM ATTENUATED CISPLATIN-TRIGGERED APOPTOSIS IN TONGUE SQUAMOUS CELL CARCINOMA CELLS

Presenter: Yu-Jen Chiu, MD (Taiwan)
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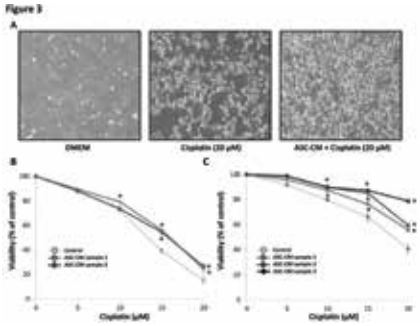
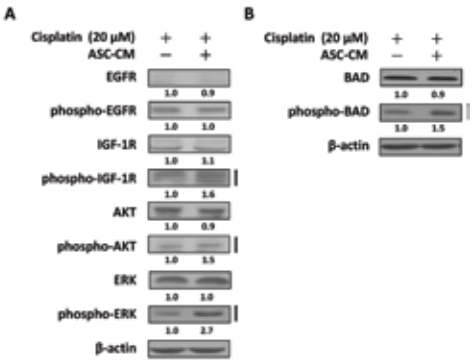
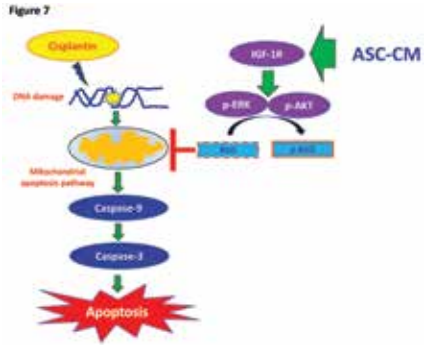


Figure 6





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CIGARETTE SMOKING-INDUCED RENAL PATHOLOGIES ARE AMELIORATED BY ADIPOSE STEM CELL THERAPY

Presenter: Daria Barwinska, BA (USA)
Affiliation: Indiana University
Authors: Barwinska D, Cook T, Traktuev DO, Saliba J, Bacallao R, Basile DP, March KL

Objective: Approximately 10% of the worldwide population is diagnosed with chronic kidney disease. While correlation between cigarette smoking (CS) and decline in kidney function has been observed, little has been done to elucidate the effect of CS on kidney homeostasis. Subsequently, studies are needed to determine the effective treatments of CS-induced kidney pathologies. We previously demonstrated that administration of adipose stem cells (ASC) to rats with kidney ischemia/reperfusion injury protects and regenerates renal morphology and function. Therefore, we hypothesized that ASC-based therapy will ameliorate CS-induced kidney damage.

Methods: C57Bl/6 mice (10-week old) were exposed to CS for five months (five hours a day, five days a week). In parallel, a subset of mice was exposed to ambient air to serve as Control. Following CS-exposure regiment, mice received an i.p. injection of either 3x105 human ASC, or ASC-derived conditioned medium (ASC-CM), or Vehicle once a week for four consecutive weeks. Seven days after last injection, blood flow in kidneys was evaluated by Laser Doppler Perfusion Imager (LDI). Then, kidneys were harvested, weighed, and analyzed for capillary density (Tomato Lectin), collagen (PicroSirius Red) and iron (Perl's Iron) deposition using histology.

Results: When compared to the kidneys of the Control cohort of mice, kidneys of CS-exposed mice manifested decrease in weight by 20%, capillary density by 86% ($p<0.001$), and cortical blood flow by 37% ($p<0.01$), but 9-fold increase in iron and collagen deposition by 53% ($p<0.05$). The latter one is a sign of fibrotic changes. However, post CS-exposure treatment of mice with in vitro expanded human ASC reversed damages caused by CS and restored kidney perfusion to the level of Control cohort.

Conclusion: Our data shows that CS significantly compromises both renal structure and function by decreasing capillary density and inducing collagen and iron deposition, which leads to decrease in kidney perfusion, function and nephrotoxicity. However, systemic administration of human ASC reversed the damage caused by CS, indicating their potential to mitigate CS-induced renal pathology, thus providing additional evidence that ASC-based therapy will benefit patients with compromised renal function.

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MILD HYPOTHERMIA ATTENUATES OXIDATIVE STRESS AND INFLAMMATORY RESPONSE FACILITATING EXPANSION AND DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS

Presenter: Gal Tirza, MD, BSc (Israel)
Affiliation: Tel Aviv Sourasky Medical Center
Authors: Tirza G, Sela M, Solodeev I, Pasmanik-Chor M, Gur E, Shani N

Introduction: Mesenchymal stromal cells (MSCs) are multipotent and can be derived from most adult tissues. Despite their many common characteristics, MSCs also display tissue specific traits. Low oxygen culture conditions reduce the accumulation of reactive oxygen species (ROS) in MSCs derived from intra-abdominal rat adipose tissue (aASCs) preventing oxidative stress leading to apoptosis. Although lowering culture temperature was shown to benefit the survival of bone marrow derived cultures, this was not determined for other MSC types, and the mechanism for this effect remains obscure. The current study was therefore aimed at determining the effect of reduced culture temperature on ASC growth, and to elucidate the mechanism by which temperature changes affect cell cultures.

Methods: Rat ASCs were derived from the adipose intra-abdominal or subcutaneous fat tissues of Lewis rats. Cells were expanded either under 37°C or at hypothermic conditions. ASCs cultured under both conditions were then compared for their proliferation and differentiation potential, cell surface marker expression, apoptosis, reactive oxygen species (ROS) accumulation, gene expression by RT-PCR and a gene array analysis.

Results: We found that culturing of aASCs under mild hypothermia inhibited their NOX1 dependent ROS overproduction and prevented the consequent apoptosis and expansion arrest facilitating long term expansion, as previously seen in low oxygen cultures. Furthermore aASCs cultured under a reduced temperature demonstrated superior adipogenic differentiation potential compared to aASCs cultured at 37°C. Comparison of gene expression profiles of ASCs cultured at 37°C and hypothermic conditions revealed a significant reduction in the expression of multiple immune modulators in hypothermic aASCs, most probably due to their reduced ROS overproduction.

Conclusions: This is first evidence for the effect of culture temperature on ASC growth and differentiation properties. We suggest that reduced temperatures may provide superior ASC cultures, with enhanced expansion capacities in vitro and effectiveness in vivo.

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THE EFFECT OF DONOR BMI ON ADIPOSE STEM CELL CHARACTERISTICS - OBESITY-DISCORDANT AND WEIGHT-CONCORDANT MONOZYGOTIC TWIN STUDY

Presenter: Miia Juntunen, MS (Finland)
Affiliation: University of Tampere
Authors: Juntunen M, Patrikoski M, Rissanen A, Pietiläinen K, Miettinen S

Adipose tissue (AT) derived multipotent cells, known as adipose stem cells (ASCs) are promising candidates for clinical applications because of their regenerative capacity, low immunogenicity and their ability for immunomodulation. The success of the future allogeneic cell-based therapies depends on the appropriate selection of AT donors. Several factors including donor age, sex and harvest location affect the ASC characteristics. These also depend on body mass index (BMI). In this study, ASCs were isolated from AT of obesity-discordant monozygotic twin pairs (n=5) and weight-concordant control twin pairs (n=2) selected from population-based twin cohorts. ASCs proliferation, multipotency and immunophenotype (CD11b, CD14, CD19, CD34, CD36, CD45, CD54, CD73, CD80, CD86, CD90, CD105 CD106, HLA-DR and -ABC) were studied with CCK8 cell proliferation assay, adipogenic and osteogenic differentiation and flow cytometry, respectively. Adipogenic differentiation was confirmed with Oil Red O staining and analysis of adipogenic marker genes PPAR? and AP2. Osteogenic differentiation was confirmed with quantitative alkaline phosphatase activity analysis, Alizarin Red -staining and analysis of osteogenic marker genes RUNX2, OSX, DLX5 and ALP. The effect of BMI on ASC immunogenicity and immunosuppression potential was studied using one-way mixed lymphocyte reaction (MLR), and direct and indirect two-way MLR, respectively. ASC proliferation, immunogenicity or immunosuppression capacity were not related to BMI. ASCs derived from both low and high BMI donors showed low immunogenicity and were equally effective in immunosuppression. Additionally, ASCs derived from both low and high BMI donors showed similar immunophenotype. Differentiation efficacy toward osteogenic and adipogenic lineages was not related to BMI. Our data suggest that under the same genetic background, BMI does not seem to have an effect on ASC proliferation, immunophenotype, immunogenicity, immunomodulation capacity and differentiation.

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INTERACTION WITH PRO-INFLAMMATORY MACROPHAGES INHIBITS IMMUNOSUPPRESSIVE FUNCTION IN HUMAN ADIPOSE-DERIVED STROMAL CELLS

Presenter: Audrey Varin, PhD (France)
Affiliation: Department of Plastic and Reconstructive Surgery
Authors: Varin A, Espagnolle N, Balguerie A, Arnaud E, Chaput B, Sensebe L

Introduction: Adipose-derived stromal cells (ASCs) inhibited adaptive and innate immune responses and represent a potential clinical treatment for immune disorders. ASCs are modulated by soluble factors present in their microenvironment but few data are available on the direct effect of immune cells on ASC functions. Therefore, we study the effect of direct contact with macrophages (M?) on ASC immunomodulatory capacity.

Materials and Methods: Human ASCs were cultivated alone or with pro-inflammatory (M1M?) or anti-inflammatory (M2M?) macrophages. After 24 h of co-culture, ASCs and macrophages were magnetically separated. Then effects of macrophages priming on gene expression profile, and on immunomodulation capacities of ASCs were determined.

Results and Conclusion: Our microarray assay findings revealed that interaction of human ASCs with M1M? modulated a high number of genes involved in the regulation of the immune response and more specifically in the proliferation of T lymphocytes. Indeed, M1M? priming inhibited, in a contact-dependent manner, the immunosuppressive function of ASC and enhanced T cell proliferation. Moreover, the M1M?-primed ASCs generate a similar Th1-Th2 T cells population than ASCs but they were not able to generate CD25+Foxp3+CD4+ T regulatory cells. Using flow microscopy imaging approaches, we demonstrated that the CD54 expression was specifically increased at the M1M?-primed ASC surface and polarized at the contact area of the M1M??ASC interaction. In a lesser extent, similar results were observed after interaction of ASCs with M2M?. Only the physical interaction between ASCs and M1M? was functional and triggered a strong calcium mobilization in ASCs independently of CD54. Interestingly this physical interaction between M? and ASCs was highlighted in human adipose tissue by confocal microscopy.

Therefore, we demonstrated a functional interaction between inflammatory M? and ASCs that inhibited the immunosuppressive activity of ASCs opening new questions of the use of these cells in treatment of inflammatory diseases.

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ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS RESCUE APOPTOTIC RETINAL PIGMENT EPITHELIAL CELLS UNDER OXIDATIVE STRESS, AND DIFFERENTIATE TOWARDS RETINAL PIGMENT EPITHELIAL CELLS IN VITRO AND IN VIVO

Presenter: Aya Barzelay, MD, PhD (Israel)
Affiliation: Tel Aviv Medical Center
Authors: Barzelay A, Wheisthal S, Nitzan A, Ben Hemo M, Loewenstein A, Barak A

Purpose: Oxidative stress leads to degeneration and apoptosis of Retinal pigment epithelial cells (RPE) in age related macular degeneration (AMD). Subcutaneous adipose derived mesenchymal stem cells (ASCs) may serve a therapeutic tool for regenerating RPE. We evaluated ASCs protective effect on apoptosis of RPE, the differentiation potential of ASCs towards RPE, and the efficacy of sub-retinal transplantations of ASCs in a mouse model of AMD.

Methods: Human ASCs were harvested from subcutaneous fat of patients undergoing abdominoplasty. Co-culture of ASCs and human RPE, or human RPE alone, was subjected to oxidative stress by exposure to 1.5mM hydrogen peroxide (H2O2). H2O2 induced RPE apoptosis was measured by Annexin V/ propidium iodide staining and flow cytometry. The differentiation potential of ASCs towards RPE was evaluated using td-tomato marked RPE and GFP marked ASCs seeded in a co-culture. GFP marked ASCs were then isolated by FACS sorter and analyzed for RPE and eyefield markers (BF1, Rx, MITF, PAX6, RPE65) by qRT-PCR and immunostaining. Finally, GFP marked ASCs were transplanted in the subretinal space of sodium iodate (NAIO3) treated mice compared to controls of subretinal saline injection. Evaluation of transplanted ASCs and endogenous RPE was studied by immunostaining for GFP and RPE65 of frozen sections at 0, 7, 14, and 21 days post injection.

Results: Treatment of RPE with ASCs prevented H2O2 induced apoptosis (70% decrease, $p < 0.05$). After 7 days in co-culture, ASCs upregulated RPE and eyefeild markers (BF1 1.9+ 0.2 Rx 4.5+0.8 MITF 1.9+0.17 PAX6 5.5+0.2 RPE65 7.7+2.6, folds of control). In vivo, transplanted ASCs were located in the subretinal space of NAIO3 mice at days 0, 7, 14, and 21 post injections. Level of RPE65 was higher in ASCs treated mice at day 14 (4 folds increase in mean florescence compared to controls).

Conclusions: Treatment of apoptotic RPE with ASCs reduced RPE apoptosis in vitro. ASCs demonstrate a differentiation potential into RPE, evident by upregulation of RPE and eye field markers. Transplantation of ASCs in the subretinal space of NAIO3 mice resulted in an increase in RPE cell count compared to controls. ASCs may have therapeutic potential in regenerating RPE.

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MOLECULAR BASIS OF ADIPOSE-DERIVED STEM CELLS (ASC) THERAPY FOR MANAGEMENT OF RADIATION INDUCED FIBROSIS (RIF)

Presenter: Asim Ejaz, PhD (USA)
Affiliation: University of Pittsburgh
Authors: Ejaz A, Epperly MW, Fisher R, Zhang X, Johngrass M, Schusterman MA, Kokai LE, Greenberger JS, Rubin JP

Introduction: Radiation therapy is one of the most important tools in cancer treatment, however iatrogenic comorbidities including RIF can significantly impair patient healing and life quality. Several published case studies suggest that application of autologous adipose tissue aspirates and adipose stem cells at the irradiation sites can ameliorate RIF, though the mechanism is not clear. In this study, we evaluated the efficacy of adipose tissue aspirates and/or Adipose stem cells (ASCs) in management of radiation fibrosis in a mouse model and investigated the underlying molecular mechanism involved.

Methods: In vitro studies: Transwell co-cultures were performed using irradiated human foreskin fibroblasts (HFFs), ASCs, and human whole fat tissue to determine genes that are down or upregulated in HFFs. In vivo studies: Female C57BL/6 mice were irradiated with 30 Gray (Gy) at the flank region and monitored for expression of fibrosis related genes by quantitative RT PCR at days 1, 3, 14, 21 and 35 post irradiation. Fibrosis was further confirmed by histological analyses and flexibility measure. To assess adipose intervention efficacy, irradiated mice were injected with adipose tissue aspirates from luciferase+ GFP+ mice at day 1, 14 or 21 post irradiation. Luciferase fluorescence emission was quantified weekly for 35 days post injection. Resolution of fibrosis was estimated by flexibility measure.

Results: We successfully developed a C57BL/6 RIF mouse model and confirmed the induction of fibrosis by molecular and histological analyses. Luciferase fluorescence emission analyses confirmed the retention of injected fat at the irradiated site. Intriguingly, we observed an enhanced signal retention in mice injected at day 21 post irradiation. We observed a delayed fibrosis development in mice injected with adipose tissue aspirates. Transwell cultures with whole fat and ASCs demonstrated down regulation of pro-fibrotic genes in irradiated fibroblasts.

Conclusion: Our preliminary data supports clinical observations that grafted adipose tissue has benefit for treating RIF. Further work will help to improve our understanding regarding the molecular mechanism involved and the use of adipose tissue based cell therapy approach in treatment of already established fibrosis.

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IMPACT OF AGE AND DIABETES ON HUMAN ADIPOSE STEM CELLS FOR A BONE TISSUE-ENGINEERED PRODUCT

Presenter: Sophie Veriter, PhD (Belgium)
Affiliation: Novadip Biosciences
Authors: Veriter S, Lafosse A, Palacios P, Bidias D, Demir C, Adnet PY, Dufrane D

Aging and diabetes mellitus are considered as co-morbidity factors associated to increase the incidence of bone non-union. Hence, this work aims to assess the impact of the age and hyperglycemia on the proliferative capacity and the bioactivity of adipose stem cells (ASCs) to produce a bone tissue-engineered product.

Human ASCs were isolated from 5 healthy donors (22-66 yrs) and expanded up to passage 4th in view to assess the cell population doubling levels (PDL), population doubling times (PDT) and the total proliferation time. ASCs (from healthy donors: 19-62 yrs, n=8 and from type 2 diabetic patients: n=4, 53-71 yrs) were also tested in vitro in hypoxic chambers (during 24-72 hours) to study the secretion of angiogenic/osteogenic growth factors (VEGF, SDF1a, IGF-1, BMP2/7 bFGF, HGF) in the following conditions: (i) below 1% O2 (found in a bone non-union) vs. atmospheric conditions (21% O2) in (ii) normo-(1g/L)/hyperglycemic (4.5g/L) conditions. Both undifferentiated and osteogenic ASCs were tested in these conditions (n=3). The capacity of ASCs to produce a scaffold-free osteogenic graft (as demonstrated by osteocalcin expression and mineralization) was also assessed.

No impact of the donor's age on the proliferative properties of ASCs was observed in terms of Cumulative PDL; PDT and total proliferation time. No impact of diabetes and age was found on SDF1a, HGF, IGF-1 and bFGF secretion for undifferentiated ASCs which remained at a low level. A significant elevation of VEGF secretion was found at low oxygen tension in comparison to ASCs exposed at 21% O2 ($p<0.05$) at both normo-/hyperglycemic conditions. The glucose concentration alone and the un-differentiated/osteogenic status of ASCs did not impact the VEGF secretion. Osteogenic differentiation significantly reduced the secretion of SDF1a in comparison to non-differentiated ASCs (50±79 vs 96±73 pg SDF1a/105cells, $p<0.05$, respectively). In each condition, the BMP2/7 secretion remained undetectable. ASCs from Type 2 diabetic patients demonstrated a similar pattern of VEGF secretion. 3D-osteogenic scaffold-free grafts were obtained from all donors.

In conclusion, diabetes and aging are not the limiting factors to use ASCs in the treatment of large bone non-unions with a 3D-osteogenic scaffold-free graft.

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IMMUNOLOGICAL CHANGES IN ADIPOSE TISSUE DEPOTS INDUCED BY HUMAN CYTOMEGALOVIRUS INFECTION

Presenter: Kevin Zwezdaryk, PhD (USA)
Affiliation: Tulane University
Authors: Zwezdaryk K, Norton EB, Sullivan DE

Chronic inflammation in adipose tissue (AT) is associated with the development of diabetes and cardiovascular disease. This results from a dysfunctional relationship between AT and the immune system. Immune populations in AT influence the release of AT-derived secreted factors. How AT drives chronic inflammation and changes to immunometabolism are targets for preventing the development of age-related inflammation. Cytomegalovirus (CMV), a ubiquitous β-herpesvirus found in a majority of the population, significantly alters the metabolic state of infected cells. Although CMV alters immune function and increases inflammatory cytokines, the association between AT, immune cells and CMV infection has not been reported. We hypothesize that acute CMV infection occurs in cells of the SVF, which alters AT immune cell populations resulting in upregulation of inflammatory cytokine expression, release of adipokines and dysregulation of local and systemic metabolism.

Balb/c mice were mock-infected or infected intraperitonelally with sub-lethal doses of CMV for 6 days. Brown AT and white AT were collected and compared to blood and lung immune cell populations. Samples were stained for macrophage, monocyte and T cell markers and analyzed by flow cytometry or snap frozen for downstream qRT-PCR and western blot analysis.

In a murine model of CMV infection, viral DNA and RNA was detected in all AT tested, suggesting viral replication occurs in multiple AT depots. Patrolling (LyC6C-CX3CR1highCD11c-) and inflammatory monocyte (LyC6C+CX3CR1intCD11c-) levels decreased in infected white and brown AT. A monocyte population expressing LyC6C+CX3CR1highCD11c- increased in both AT. Foxp3+ T regulatory cell levels increased in white AT but decreased in brown AT. Macrophage populations increased in infected brown and white AT. CMV infected murine AT exhibited increased levels of inflammatory cytokines such as IL-6.

Our findings suggest that CMV infection involves AT depots with altered transcriptional and phenotypic profiles of AT-associated immune cells and upregulation of inflammatory cytokines. The role of CMV infection to the long-term metabolic status of the AT and immune associated populations may promote low-grade chronic inflammation associated with obesity and aging.

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MICROENVIRONMENT DIVERSIFICATION OF YOUNG VS. AGED ADIPOSE-DERIVED STEM CELLS

Presenter: Katie Hamel, BS (USA)
Affiliation: Louisiana State University
Authors: Hamel K, Gimble JM, Jung JP, Martin EC

Introduction: With aging, composition and architecture of extracellular matrix (ECM) are altered. Such changes will attenuate regeneration capability of tissues, leading to delayed recovery in older adult populations and risks associated with post-surgical complications. Stem cell therapy aims to improve outcomes of older adult patients by rejuvenating aged stem cells. Since stem cell behavior can be directed by modulating biophysical and biochemical properties of the ECM, we aim 1) to identify the impact of age on the ECM of adult stem cell populations and 2) to quantify the expression of age specific ECM genes in older adult patients by rejuvenating stem cell populations.

Materials and Methods: Human adipose-derived stem cells (hASCs) from three young (22-35 years old, YO) and three aged (68-74 YO) donors were grouped by a specific age range and matched according to corresponding body mass indices. Cultures of hASCs (15000 cells/cm2) were maintained with basal medium and followed by lineage-specific chemical induction. hASCs cultured were stained with Crystal Violet at days 0 and 7 to determine the rate of hASC proliferation. qRT-PCR was performed to evaluate differences in ECM associated gene (collagens, fibronectin, glycoprotein and matrix associated transcription factors) expression between young and aged donors.

Results and Discussion: hASCs from the aged population appeared flattened whereas those from the young population showed rectangular morphologies. Crystal Violet staining showed increased proliferation in aged donor hASCs cultured with stromal media, while this effect was lost in osteogenic media. hASCs from the aged donor yielded increased mRNA expression of matrix genes including COL6A1, COL3A1, COL5A1, and COL4A1. The gene expression of FRA-1, a transcription factor of the AP-1 family known to regulate matrix gene expression, was enhanced in hASC cultures of the aged donor. Age appears to affect ECM gene expression and hASC morphology, but further research is needed to determine whether age influences the MAPK signaling pathway. Although MAPK is known to modulate stem cell differentiation and proliferation, the impact of age on the activation and regulation of MAPK is underexplored (Tsang EJ et al., Connective Tissue Res, 2017).

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FAT GRAFTING FOR FAT PAD ATROPHY OF THE HEEL: EARLY FINDINGS FROM A RANDOMIZED CONTROLLED CLINICAL TRIAL

Presenter: Isaac James, MD (USA)
Affiliation: University of Pittsburgh
Authors: James IB, Gusenoff BR, Minteer DM, Wang S, DiBernardo G, Gusenoff JA

Background: The heel contains specialized fat pads which protect the foot from harsh repetitive stress generated during the gait cycle. Heel fat pad atrophy causes pain with ambulation and can cause substantial disability. Current management is limited to offloading orthotics and padding. The present study describes use of autologous fat grafting to treat heel fat pad atrophy.

Methods: 5 patients (3 female, 2 male) underwent fat grafting to the macrochamber (MAC) of the heel as part of an ongoing randomized, controlled crossover trial. Fat was harvested from the abdomen by manual liposuction, processed and injected by Coleman technique, and introduced into the macrochamber compartment of 6 heels (average volume of 6.5cc per heel). Ultrasound-measured tissue thickness (Figure 1), pedobarograph-measured foot pressures, and Manchester Foot Pain and Disability Index (MFPDI) were obtained at preoperative, 1mo, 2mo, and 6mo post-operative visits. Patients were offloaded in a customized Darco shoe for 4wks post-operatively.

Results: Average age was 61yrs. Average BMI was 25.23. No patients had diabetes or were active smokers. The macrochamber was significantly thicker at 1, 2, and 6mo postoperatively ($p<0.05$) (Figure 2). This appears to persist at 12 months based on data from the 3 feet for which we have 12mo follow up data so far. Increased fat pad thickness also translated to significantly reduced heel force and pressure during the gait cycle at 1 and 2mo post-op ($p<0.05$) and trended toward reduced pressure and force at 6mo. Patient-reported foot pain was significantly improved at 6 months ($p<0.05$) with several patients resuming previously untolerated activities.

Conclusions: Although our preliminary findings will need to be corroborated by a larger sample size and longer follow up, analysis of our current data suggests that restoring the heel fat pad with autologous fat grafting can improve foot function, enhance shock absorption, and return patients to activities of daily living.

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FAT GRAFTING FOR FAT PAD ATROPHY OF THE HEEL: EARLY FINDINGS FROM A RANDOMIZED CONTROLLED CLINICAL TRIAL

Presenter: Isaac James, MD (USA)
Affiliation: University of Pittsburgh
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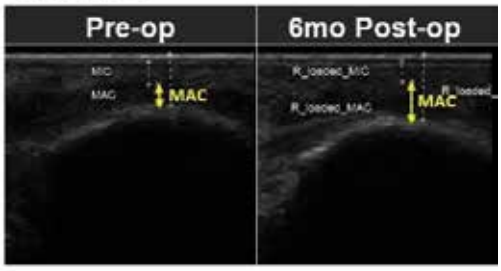
Figure 1:



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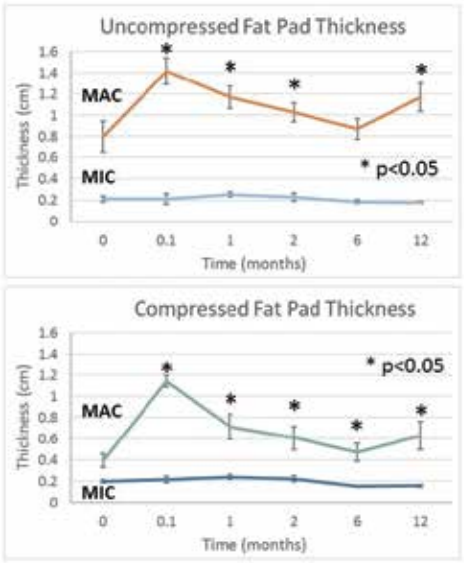

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BREAST RECONSTRUCTION USING POLY-4-HYDROXYBUTYRATE MESH SCAFFOLD AND AUTOLOGOUS FAT GRAFTING

Presenter: Mark Schusterman, MD (USA)
Affiliation: University of Pittsburgh Medical Center
Authors: Schusterman M, Rehnke RD, Badylak SF, Rubin JP

Introduction: Breast reconstruction remains a very important aspect of plastic surgery, with most procedures utilizing implants and/or autologous tissue flaps. The safety of autologous fat grafting (AFG) as an adjunctive treatment to standard breast reconstruction has been widely reported. Few series, however, report on experience with fat grafting as the sole, primary form of breast reconstruction. This study aims to report results and complications a new method of breast reconstruction using a 3-dimensional bio-absorbable mesh with subsequent AFG.

Methods: A retrospective review was performed on a prospectively-maintained database that includes all patients who underwent reconstruction utilizing a bio-absorbable poly-4-hydroxybutyrate (P4HB) mesh scaffold placed in a pre-pectoral plane immediately following mastectomy with subsequent rounds of AFG. Data collected included patient demographics, breast cancer diagnosis, surgical details and postoperative results and complications. Post-operative imaging of the breast was also analyzed and tissue specimens collected at subsequent procedures were stained with H&E for histological evaluation.

Results: 10 patients underwent reconstruction of 14 breasts using P4BH scaffold and AFG between February 2015 and February 2017. All patients were satisfied with final breast shape and size. Mean patient age was 60.5 years and average BMI was 29.1. 10 patients underwent mastectomy with a diagnosis of breast cancer while 4 underwent prophylactic mastectomy. Average follow-up was 14 months. There were no major complications. Patients required on average 2 fat grafting sessions to achieve a successful result (range 1-4). Average amount of fat grafted per breast was 428.7 mL. Post-operative mammogram and MRI show morphology similar to that of a native adipose tissue, and histology revealed no capsule formation with ingrowth of fat tissue into the scaffold.

Conclusions: We present a new option for breast reconstruction, resulting in acceptable outcomes and minimal post-operative complications and morbidity. Post-operative imaging and histology suggests organization of fat cells along the scaffold by approximately one year. Further studies are needed to understand the true potential and mechanism of this procedure.

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LONG TERM RESULTS AFTER WATER ASSISTED FAT GRAFT WITH BEAULI PROTOCOL

Presenter: Klaus Ueberreiter, MD (Germany)
Affiliation: Park Klinik Birkenwerder
Authors: Ueberreiter K, Tanzella U

Goals/Purpose: In the last 9 years we performed far more than 1000 mega fat grafts (100 – 400 ml) mainly to breast and buttocks. The applied BEAULI™ protocol had been evaluated in a prospective clinical study from 2007 to 2010 and a follow up from 2012-2015. The clinical and MRI evaluation showed an high volume persistence and almost complete absence of side effects like cysts etc.

Methods/Technique: The operation was performed according to a standardized (BEAULI™) protocol. The fat was harvested by Water Assisted Liposuction (WAL®), separated in the "Lipocollector" and reinfiltrated by a specially designed cannula (2.5 mm/ 150mm) without any further processing. For quantification of the results, MRI`s of the breasts were taken preoperatively and 6 months. Clinical examinations were done preoperatively, and on day 1, after 1 week, 3 months and 6 months and 5 years postoperatively.

Results/Complications: In all patients, a significant increase of subcutaneous fat tissue was achieved. The volume gain after comparative MRI volumetry was 76 ± 11% of the transplanted fat after 6 months. The five-year results show a complete persistence of the fat grafted and a typical change in breast form. We performed aesthetic breast augmentations, corrections of asymmetries, tuberous breast correction, reconstruction after cancer, buttock augmentation and other defect fillings. Pat. 5 years after two fat grafts of 250 and 280ml each breast (uncentrifuged)

Conclusion: The method is fast and easy to perform with a high reliability in all forms of mega fat grafts. In patients with sufficient fat deposits it can successfully replace silicone implants. The BEAULI™ protocol based on Waterjet® harvested fat has proven to by an excellent method of fat harvesting and transfer.

Literature: (1) Ueberreiter K., von Finckenstein JG., Cromme F., Herold C., Tanzella U., Vogt P.M.: BEAULI™ - eine neue Methode zur einfachen und zuverlässigen Fettzell-Transplantation [BEAULI™ - a new and easy method for large-volume fat grafts]. Handchir Mikrochir Plast Chir. 2010 Dec; 42(6):379-85.

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THE IMPACT OF DIFFERENT RECIPIENT SITE PRE-CONDITIONING TECHNIQUES IN FAT GRAFTING SURGICAL OUTCOMES

Presenter: Carlo Oranges, MD (Switzerland)
Affiliation: Basel University Hospital
Authors: Oranges C, Striebel J, Tremp M, Kalbermatten DF, Schaefer DJ

Introduction: Among the four phases of fat grafting process, which includes harvesting, tissue processing, recipient site preparation and injection, the preparation of the recipient site is the less investigated. The aim of this work is to provide a comprehensive overview of the different recipient site pre-conditioning techniques with the resulting outcomes.

Methods: A search on PubMed/Medline was performed for studies involving the preconditioning of the recipient site in fat grafting using the following key words: "fat grafting" and "recipient site". Resulting articles were reviewed using a priori criteria.

Results: 117 articles were initially identified, 33 of which met inclusion criteria: 18 clinical studies on 2361 patients, 14 animal studies, and one in vitro study. Eight techniques were applied: external expansion, internal expansion, implantation of alloplastic material (silicone sheets), injections of cell-proliferation factors (autologous plasma, vascular growth factor, interleukin-8, adipose tissue derived stromal vascular fraction), ischemia, percutaneous fasciotomies, tunnelization, and microneedling. Pre-clinical studies demonstrated a positive effect on cellular activity (cell proliferation and angiogenesis) achieved with all techniques. Improvement in fat graft survival was demonstrated by the majority of the clinical studies, and was consistently higher than 50% at 3 months to 1 year follow-up.

Conclusions: The pre-conditioning of recipient site in fat grafting provides positive outcomes with different techniques. This can be especially relevant in case of recipient site affected by contracted scars or radiation therapy, where improvement of vascular supply and expansion of soft tissue can be decisive for the success of the procedure.

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SAFETY AND EFFICACY OF PERCUTANEOUS INJECTION OF AUTOLOGOUS MICRO-FRACTURED ADIPOSE TISSUE FOR OSTEOARTHRITIC KNEES

Presenter: Jay Bowen, DO (USA)
Affiliation: New Jersey Regenerative Institute
Authors: Panchal J, Malanga G, Bowen JE

Aims, Objectives, Goal and Purpose: What does the research intend to demonstrate? * To evaluate the safety and efficacy of using autologous, micro-fractured, and minimally manipulated adipose tissue in patients with refractory knee osteoarthritis.

Materials and Methods: 17 subjects (26 knees) with a median age of 72 years (Range: 54-78 years) with a history of knee osteoarthritis (Kellgren-Lawrence grade of 3 to 4) underwent treatment with ultrasound-guided injection of micro-fractured adipose tissue. Micro-fractured fat was obtained by using a minimal manipulation technique in a closed system without the addition of enzymes or any other additives. Study subjects were clinically evaluated using Numerical Pain Scale (NPS), 100 point Knee Society Score (KSS) with its functional component (FXN), and Lower extremity activity scale (LEAS) at six weeks, six months, and twelve months following this procedure.

Results: When compared to baseline, significant improvements were noted in the mean values of NPS, FXN, and LEAS at six weeks, six months, and twelve months. The mean KSS significantly improved at six weeks and twelve months. In particular, average KSS improved from 74 to 82, FXN from 65 to 76, and LEAS from 36 to 47. No serious adverse events were reported.

Conclusions: The injection of autologous, micro-fractured, and minimally manipulated adipose tissue appears to be a safe and effective treatment option in patients with refractory knee osteoarthritis. This intervention may represent a nonsurgical treatment option to avoid knee joint replacement in this population; however, further investigation is needed.

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FAT GRAFT RETENTION IN PEDAL FAT GRAFTING: ASSOCIATION WITH CD34+ ADIPOSE STEM CELLS AND COLLAGEN

Presenter: Sheri Wang, BS (USA)
Affiliation: University of Pittsburgh
Authors: Wang S, James IB, DiBernardo G, Lannau B, Grybowski D, Zhang W, Gusenoff BR, Marra K, Gusenoff JA

Introduction: Fat grafting is an important and versatile procedure, but is limited by unpredictable volume retention. Hazen et al. has demonstrated in mice that high-density fat is associated with increased graft retention, higher progenitor concentration, and fewer collagen bands. Similarly, previous work by our group demonstrated that CD34+ adipose stem cells (ASCs) are predictive of fat graft retention in mice. We hypothesize that CD34+ ASCs are correlated with increased submetatarsal fat graft retention and lower collagen content in humans.

Methods: Fat was harvested by manual liposuction and processed by standard Coleman technique from 24 patients undergoing fat grafting to the forefoot as part of a randomized cross-over clinical trial. Ultrasound-assessed submetatarsal tissue thickness was obtained at baseline, 6mo, & 12mo visits. Processed lipoaspirate was returned to the lab for SVF isolation, flow cytometry, and collagen assessment using Western blot.

Results: Average age was 63.6+/-6.7, and average BMI was 26.1+/-4.6. No patients were diabetic. The proportion of CD34+ ASCs in the fat graft and viability of the SVF isolate were correlated with significantly improved retention of tissue thickness at 6mo ($p=0.044$, $p=0.033$ respectively) but not at 12mo. Fat grafts with lower collagen1 concentration were associated with significantly greater SVF viability and CD34+ ASC content ($p=0.046$, $p=0.005$ respectively).

Conclusions: Volume loss after fat grafting remains a perplexing problem. The inverse association between collagen vs ASC content and SVF viability merits further study, particularly given the fact that ASC content and SVF viability predicted retention of tissue thickness at 6mo in grafted feet.

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THE EFFICACY AND VOLUME RETENTION OUTCOME OF MICROFAT TRANSFER FOR FACIAL CONTOURING AND FACIAL REJUVENATION: A RETROSPECTIVE STUDY OF 1279 CASE IN 7 YEARS

Presenter: Pichansak Bunmas, MD (Thailand)
Affiliation: Vplast Institute of Aesthetic and Plastic Surgerey
Author: Bunmas P

Background: Facial soft tissue atrophy and asymmetry is the common facial aesthetic problems of Thailand and ASEAN woman. This problem is challenge to correction. Fat transfer is the once of most safe and effective to correction than others traditional fillers. The purpose of this study want to showed result of new concept of full facial contouring and rejuvenation with microfat transfer.

Materials and Methods: A retrospective study form July 2009-February 2016, We includes 1279 patients in this study. All patients underwent to full facial contouring and rejuvenation with microfat transfer under local anesthesia and intravenous sedation. All step of procedure and technique was done by same surgeon. We used the concept 'A No Man Land' technique for this procedure. 'A No Man Land' technique mean:
1A = adequate volume injection
2N = 2.1 Normothermia intraoperative and post operative care 2.2 Nutrition status and supplements
3M = 3.1 Manaual system technique, 3.2 Meticulous small amount fat cell injection, 3.3 Multiple tunnel and plan of injection
4L = 4.1 Layer of injection (subdermal to deep), 4.2 Low speed centrifugation, 4.3 Low pressure of fat extraction and reinjection, 4.4 Law of paracrine function and metabolism of fat.

All patients was evaluated of fat survival and volume retention outcome and patients satisfaction at 2 weeks, 1 month, 3 month, 6 month and very 1 year.

Result: All patients were improved of facial appearance by photography when compare with preoperative view. The degree of patients satisfaction for volume retention and rejuvenation effect at one year showed excellent (64.12%), good (21.89%), fair (12.98%) and poor (1.01%).

Conclusion: The concept of 'A No Man Land' technique in this study showed effectively for full facial contouring and facial rejuvenation and long term volume retention outcome.

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THE COMBINED USE OF ENHANCED STROMAL VASCULAR FRACTION AND PLATELET-RICH PLASMA IMPROVES FAT GRAFTING MAINTENANCE IN BREAST SOFT TISSUE DEFECTS

Presenter: Pietro Gentile, MD, PhD (Italy)
Affiliation: University of Rome Tor Vergata
Author: Gentile P

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

Twenty-three patients aged 19-60 years affected by breast soft tissue defects were analyzed at the Plastic and Reconstructive Department of the University of Rome Tor Vergata. Ten patients were treated with SVF-enhanced autologous fat grafts, and 13 patients were treated with fat grafting + platelet-rich plasma. The patients in the control group (n = 10) were treated with centrifuged fat grafting injection according to Coleman's procedure. The patients treated with SVF-enhanced autologous fat grafts showed a 63% maintenance of the contour restoring and of three-dimensional volume after 1 year compared with the patients of the control group treated with centrifuged fat graft, who showed a 39% maintenance. In those patients who were treated with fat grafting and PRP, we observed a 69% maintenance of contour restoring and of three-dimensional volume after 1 year. As reported, the use of either e-SVF or PRP mixed with fat grafting produced an improvement in maintenance of breast volume in patients affected by breast soft tissue defect.

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EFFECT OF 650-NM GAALAS LASER IRRADIATION ON THE THERAPEUTIC POTENTIAL OF HUMAN ADIPOSE-DERIVED STEM CELLS

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

Objective: The present study was conducted to investigate the effects of GaAlAs laser irradiation on the proliferation, cytokine secretion, adipogenic differentiation cultured adipose-derived stem cells (ADSCs).

Background data: Low-level laser (LLL) therapy has been shown to influence the biological behavior of a number of cell types. Human ADSCs are an attractive cell source for repairing injured tissue. Intensive attention has therefore focused on how to enhance their regenerative capacity during cell therapy. Irradiation with a 650-nm GaAlAs laser is reported to have photobiological effects and is widely used for accelerating tissue repair and regeneration. However, less is known about its effect on ADSCs.

Methods: Cultured ADSCs from human adipose tissue were treated with 650-nm GaAlAs laser irradiation at 2 J/m2, 4 J/m2, and 8 J/m2 respectively. Cell proliferation was quantified by MTT assay, cytokine secretion was determined by ELISA assay, and adipogenic differentiation was examined by using oil red O staining. Additionally, the expression profiles of putative ADSC surface markers were analyzed by quantitative Real-time PCR and the anti-aging effect of ADSCs was observed by photoaging skin model.

Results: GaAlAs laser treatment of cells at a radiant exposure of 4 J/cm2 enhanced ADSC proliferation and adipogenic differentiation, and increased the secretion for vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β). Furthermore, GaAlAs laser irradiation at the dosage upregulated the expression profiles of putative ADSC surface markers. In a mice model of photoaging skin, GaAlAs laser irradiation enhanced the anti-aging effects of ADSCs.

Conclusion: Our data indicate that LLL irradiation is an effective biostimulator of ADSCs, and might enhance the therapeutic potential of ADSCs for clinical use.

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SETUP, CHARACTERIZATION AND VALIDATION OF AN ADIPOSE TISSUE MODEL INCLUDING HUMAN MATURE ADIPOCYTES

Presenter: Ann-Cathrin Volz, MS (Germany)
Affiliation: Reutlingen University
Authors: Volz AC, Dieckmann S, Huber B, Kluger PJ

WITHDRAWN

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ADIPOGENIC FACTOR EXPRESSION IN LIPEDEMA ADIPOSE STEM CELLS DIFFERS FROM CONTROL ADIPOSE STEM CELLS

Presenter: Anna-Theresa Bauer, MD (Germany)
Affiliation: University Clinic for Plastic Surgery and Handsurgery
Authors: Bauer AT, Von Lukowicz D, Lossagk K, Hopfner U, Kirsch BM, Moog P, Schmauss D, Machens HG

Introduction: Lipedema is characterized by localized adiposity of the lower extremities, which is typically unresponsive to dietary regimes or physical activity. Additionally to aesthetic deformity women suffer from pressure pain as well as easy bruising and progredient lymphedema. Although the disease is well described and a large number of adult women worldwide is affected hardly any research has been done on the topic. The aim of this study was to investigate the pathophysiology of lipedema cells regarding their expression of adipogenic factors.

M&M: We included 10 lipedema patients diagnosed with stage II lipedema in our study. The control group consisted of 10 comparable healthy female patients with aesthetic liposuction performed in the same areas of the lateral thighs. Fat was collected through aspiration of tumescent liposuction and adipose derived stem cells (ADSCS) were isolated following protocol. Cell culture was carried out under hypoxia and normoxia. After 7 days of culture ELISAS (Adiponectin, Insulin like Growth factor 1, Vascular endothelial growth factor C and Aromatase) were performed using the supernatants of the cell cultures. Cell differentiation was carried out and cells were stained after 7 and 14 days of differentiation with Oil-Red and ELISAS were performed on cell supernatants of differentiated cells.

Results: Our results from the ongoing study showed that cell proliferation in lipedema cell cultures was significantly increased compared to controls. Hypoxia had a significant impact on IGF-1 levels suppressing its expression to a minimum in both groups compared to normoxic cultures. Differences in VEGF C expression showed increased levels after 7 days in lipedema cell supernatants. Aromatase activity in lipedema fat cells was increased indicating significantly higher levels of expression than controls. IGF-1 was decreased in lipedema cell supernatants after 7 days of differentiation. Adiponectin was not detectable in the supernatants of undifferentiated cells, but showed significantly higher levels in normal fat cells compared to lipedema cells after 7 days of differentiation.

Conclusions: Our findings indicate that lipedema adipose stem cells express different patterns of adipogenic factors that normal ADSCs.

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ADIPOGENIC FACTOR EXPRESSION IN LIPEDEMA ADIPOSE STEM CELLS DIFFERS FROM CONTROL ADIPOSE STEM CELLS

Presenter: Anna-Theresa Bauer, MD (Germany)
Affiliation: University Clinic for Plastic Surgery and Handsurgery
Authors: Bauer AT, Von Lukowicz D, Lossagk K, Hopfner U, Kirsch BM, Moog P, Schmauss D, Machens HG

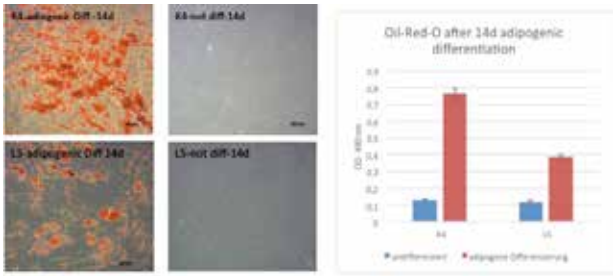

Abb .: significant differences in differentiation after 14 days in lipedema and normal cells

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OXY133 AS A DETERRENT OF ADIPOGENESIS, IN VIVO STUDIES

Presenter: Akishige Hokugo, DDS, PhD (USA)
Affiliation: UCLA
Authors: Bakshi R, Hokugo A, Zhou S, Rezzadeh K, Jarrahy R

Purpose: Therapeutic options for obesity and related diseases are limited and often carry significant consequences. New therapies targeting key stages in adipocyte commitment and maturation have shown clinical promise. Our previous work has demonstrated the potential of oxysterols "naturally occurring bioactive molecules" to attenuate adipose differentiation of committed premature adipose cells in vitro. Here we examine the anti-adipogenic capability of Oxy133 in mouse model.

Methods: Human adipose-derived stem cells (hASC) were cultured and maintained until 90% confluence. Cells were then cultured in adipogenic medium (control) or treated with adipogenic medium and Oxy133. Subsequently, the cells with collected and injected into the subcutaneous tissue on the scalp of nude mice. Specimens were harvested for histologic analysis after 12 weeks. To determine the systemic effects of Oxy133 therapy, mice were fed a high fat diet and given twice weekly subcutaneous injections of either vehicle or with a high or low dose of Oxy133. Weight was tracked weekly over 12 weeks.

Results: Mice injected with hASCs treated with Oxy133 demonstrated significantly decreased adipose tissue as compared to control animals after 12 weeks. Mice fed on a high fat diet who received Oxy133 therapy showed a significant dose dependent decrease in weight gain over the 12 weeks.

Conclusions: Our work shows the ability of Oxy133 to serve as an attenuator of adipose differentiation in vivo. This data, in combination with our previously reported in vitro studies, shows the potential of oxysterols as a viable therapeutic agent to combat obesity.

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UTILIZING A SELF-ASSEMBLING, ZOONOTIC FREE 3-D CELLULAR MATRIX CONSTRUCTED FROM HUMAN PLATELET LYSATE AND HUMAN ADIPOSE STROMAL VASCULAR FRACTION FOR THE GENERATION OF FUNCTIONALIZED 3-D FAT PAD

Presenter: Michelle McCarthy, MS (USA)
Affiliation: Tulane University School of Medicine
Authors: McCarthy M, Bender RJ, Brown TA, Smith SE, Xiying W, Gimble JM, Frazier TP

Background: The use of human adipose tissue in regenerative medicine applications has garnered much interest, particularly due to its ease of collection from the subcutaneous adipose tissue that is commonly discarded after liposuction surgery. Additionally, media supplemented with human platelet lysate (hPL) has been shown to result in a 3D scaffold after addition of human stromal vascular fraction (hSVF). This scaffold is entirely cell-derived, is of human origin, offers the benefit of having no immunogenic properties, and allows for a diverse range of clinical applications.

Yet the success of current clinical applications implementing adipose cellular therapies has been limited to small defects with injected autologous fat, and even these limited applications require repeated treatments to maintain the desired volume. Such major limitations are derived from significant donor site morbidity, rejection, or resorption. Thus, the generation of a functional 3-D adipose matrix using hSVF would serve as a strong candidate to fulfill this gap in clinical care.

Design/Methods: SVF cells were isolated, sub-cultured, and either seeded in traditional 2D culture or in hPL scaffolds. In-vitro hSVF cell/hPL matrix constructs were cultured over extended periods, and assayed for biological properties including: cell surface antigen profiles via flow cytometry, adipogenic differentiation potential via adipogenesis and quantification of intracytoplasmic lipid deposition, cell growth as measured by proliferation assays, and adipose tissue functionality via glucose uptake. hSVF cell/hPL matrices were then injected into dorsal subcutaneous pouches of congenitally athymic (nu/nu) mice and allowed to grow. After eight weeks, the adipose depots were harvested and analyzed via immunohistochemistry (IHC).

Results: Initial in-vitro hPL-SVF 3D cultures demonstrate enhanced proliferation, robust adipogenesis, cellular network formation, and potential for the formation of humanized, functional, adipose 3D matrices. In vivo studies demonstrate highly vascularized tissue formation that resembles native adipose tissue. More work will be conducted to investigate the outlined parameters.

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BIOACTIVITY OF A TISSUE-ENGINEERED PRODUCT: BRIDGING THE GAP BETWEEN ACADEMIC AND CLINICAL STUDIES

Presenter: Sophie Veriter, PhD (Belgium)
Affiliation: Novadip Biosciences
Authors: Veriter S, Palacios P, Bidias B, Demir C, Luseau A, Plougonven E, Dufrane D

The translation of cell-based therapeutics from academic and fundamental sciences to clinical trial settings follows a preclinical pathway with rigorous regulatory oversights to ensure the cell-mediated therapeutic effect. Although international guidelines thoroughly describe single-dose toxicity and biodistribution studies, the quantitative evaluation of the biological activity remains a major challenge for biotechnology companies and authorities. Here we discuss a strategy to demonstrate the bioactivity of an osteogenic tissue-engineered product intended to promote angiogenesis and osteogenesis in a bone defect.

Osteogenic 3-dimensional (3D) grafts were manufactured as a pharmaceutical batch. A sequence of chemical treatment was applied on the native graft in view to obtained a decellularized 3D-matrix as confirmed by a reduction of >90% and 40-80% of the cellular and growth factors (VEGF, IGF1) contents, respectively. Decellularized and native grafts were implanted intra-muscularly (after cauterization of the lumbar region, n=10 nude rats) to assess the mineralization (X-ray micro-CT and histomorphometry on Von Kossa staining) and angiogenesis (histomorphometry on Masson's Trichrome and Von Willebrand Factor immunostaining) at day 29 post-implantation. Human cells detection (in the explanted tissue) were also quantified after KU80/HLA type I immunostaining at the implantation site.

A significant higher mineralization was found for explanted samples from the native osteogenic 3D grafts in comparison to the decellularized tissues (Bone volume/tissue volume of 2.92 ± 1.12% vs. 0.38 ± 0.59%, respectively [p<0.001]) as well as a significant higher number of vessels/area (9,8 +/- 2,7 vs. 4,6 +/- 1,9 vessels/mm^2, respectively [p<0.001]). However, no difference was observed for the percentage of area occupied by vessels while the distribution of vessels size was shifted left in favor of "small-size (<30μm)" vessels for the native fresh grafts in comparison to decellularized tissues (p<0.003). The presence of human cells was associated with the better mineralization found for the native 3D-graft.

Our strategy permits demonstration that adipose stem cells condition activity underlies the success of the osteogenic scaffold-free graft for osteogenesis and angiogenesis.

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TISSUE-ENGINEERED 3D EAR CARTILAGE CONSTRUCT

Presenter: Derek Banyard, MD, MBA (USA)
Affiliation: UCI
Authors: Ziegler M, Banyard DA, Jaffurs D, Evans GR, Widgerow AD

Introduction: Microtia is a congenital condition that results in external ear deformities of varying degrees, of which, the most extreme form is anotia. Although effective surgical reconstruction techniques have been developed over the years, they all require a multi-stage approach and have other inherent disadvantages. These include significant donor site morbidity and unpredictable outcomes, and the long-term results are plagued by implant exposure and/or infection. Tissue engineering technologies offer new approaches in the field of external ear reconstruction. In this treatment setting, chondrocytes isolated from the remnants of auricular cartilage from the patient are cultured in the laboratory with the aim of creating bioengineered cartilage matrices. However, cartilage engineering has many challenges, including the difficulty in culturing sufficient chondrocytes. To overcome these difficulties, we propose a novel model of cartilage engineering that involves the co-culture of chondrogenic cells with adipose-derived stem cells (ADSCs) to expand the number cells available to make cartilage, and this co-culture is seeded into a three-dimensional (3D) acellularized adipose-derived extracellular matrix scaffold (AAM) that resembles the native environment of chondrocytes inside the body.

Methods: Auricular chondrocytes (ACs) were isolated from porcine ear and expanded and characterized by staining. ADSCs were isolated and expanded from human lipoaspirate. ACs and ADSCs were co-cultured at different ratios in 2D and were characterized by Western blotting. The ACs were cultured in the AAM either alone or with the ADSCs and were examined histologically.

Results: ACs were successfully isolated from porcine and showed positive staining for type II collagen and glycosaminoglycans. In the co-culture, the protein expression of chondrogenic-related genes was confirmed. In the AAM, ACs and the co-culture showed cartilage formation.

Conclusions: The comparison of the ACs alone to the co-culture in the 3D AAM revealed that the ADSCs provided sufficient support to induce the formation of cartilage in this setting when the number of ACs available is limited. This novel model of cartilage engineering provides a setting for utilizing the patient's own chondrocytes and adipose

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EFFECT OF MESENCHYMAL STEM CELLS DERIVED FROM BONE MARROW, ADIPOSE TISSUE AND UMBILICAL CORD ON HUMAN OSTEOARTHRITIC CHONDROCYTES

Presenter: Nada Alaaeddine, PhD (Lebanon)
Affiliation: University of St Joseph
Authors: Alaaeddine N, Moussa M, Sayegh S, Hilal G, Haykal G, Khalil C

Osteoarthritis is a chronic debilitating disease characterized by degeneration of cartilage, synovitis and osteophytes formation. This disease does not only affect aged people but it afflicts also young athletes where Until today no medications has proven efficacy in stopping the progression of the disease and/or regenerate the loss of cartilage. Recently cell therapy has attracted attention in many medical fields and especially in rheumatology and orthopedic specialty. Stem cells due to their differentiation capacity, trophic and paracrine effects have been shown to serve as promising new modality in treating osteoarthritis.

Our objective in this study is to investigate the effect and mechanism of action of the three sources of stem cells; bone marrow derived stem cells (B-MSC), adipose derived stem cells (A-MSC) and umbilical cord stem cells (U-MSC) on osteoarthritic chondrocytes.

Methods: Mesenchymal stem cells were isolated from bone marrow, adipose tissue and umbilical cord from 5 different donors respectively and cocultured with human osteoarthritic chondrocytes obtained from 5 patients undergoing total knee arthroplasty. The effect autophagy were determined using flow cytometry analysis. Quantitative polymerase chain reaction (qPCR) and ELISA were used to measure the changes in the major factors playing a role in OA such as (a disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMDTS-5), Metalloproteinases (MMP-3, MMP13), tissue inhibitor of metalloproteinases (TIMP-1-2-3), Collagen, Cox-2, Il-6, the regulators of autophahy FOXO1, FOXO3, and LC3II and Beclin I in the tissues and cocultured media.

Results: In our study we found that the three stem cells sources increased significantly the proliferation of chondrocytes, and increased autophagy via increasing Beclin 1 and LC3II, along with its regulators FOXO1, FOXO3 in human osteoarthritic chondrocytes with $P<0.05$. Furthermore, the three sources of stem cells caused a dose dependent significant decrease in MMP-3, MMP-13, ADAMTS-5, IL-6, CCL20 and COX-2 with $P<0.05$. Aggregan, collagen, TIMPs were also significantly increased by the co-culture of A-MSC, B-MSC, U-MSC.

Conclusion: These results suggest that stem cells could be a promising therapeutic target for the treatment of osteoarthritis.

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NEW APPROACHES TO FACIAL REJUVENATION USING INJECTABLE TISSUE REGENERATION AND INJECTABLE TISSUE ENGINEERING

Presenter: Steven Cohen, MD, FACS (USA)
Affiliation: University of California San Diego
Authors: Cohen S, Hewett S, Delauney F, Goodache A

Introduction: Trophism begins at birth and tapers in the early 20's to a period of gradual replacement of deteriorating tissues until the phenotypic changes of facial aging begin. A new approach in facial rejuvenation was developed to address soft tissue loss and decreases in capillary density to the dermis and subcutaneous fat layers.

Materials and Methods: Patients requesting 2 or more fillers for 2-3 different sites are given the option of full facial fat grafting with injectable tissue regeneration using millifat (fat parcel size = 2.4 mm or <), microfat (1.2 mm or <), nanofat (400-600 microns or <) with or without stromal vascular fraction enrichment. Fat modified into these 3 tissue iterations is used to restore the lost tissue with no attempt to overcorrect. Superficial fat is restored with micro or nanofat, whereas, deep compartment fat loss is restored with milli or microfat. Nanofat was is also used with microneedling as well as compounded with a unique transdermal transport agent and used in conjunction with fractional lasers for dermal and epithelial regeneration.

Results: Using our approach in patients having facelifts, progressive facial volumization was noted up to 24 months and beyond. In addition, single treatment of post-auricular laser injuries with nanofat cream healed more rapidly and had evidence of 3-4 times the amount of new elastin fibers at 2 months after treatment.

Conclusions: Our results point to trophism in the face following treatment with full facial fat regeneration using these types of fat grafts. Instead of using fillers in patients requiring multi-site filling, modified fat grafting to restore losses in subcutaneous fat tissue as well as in capillary density appears to regenerate elastin and may impact cellular aging.

Conclusions: Early treatment using these injectable tissue regeneration approaches with fat for biofilling, biocontouring and injectable tissue engineering eventually in combination with other synthetic and/or autogenous/allogeneic products will become important anti-aging techniques. In patients requiring 2 fillers or fillers in 2 locations, consideration should be given.

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PERITENDINOUS PLATELET-RICH PLASMA INJECTIONS IN THE TREATMENT OF TENDINOPATHIES: A RETROSPECTIVE EVALUATION
Presenter: Isik Akgun, MD (Turkey)
Affiliation: Istanbul University
Authors: Akgun I, Kivrak A, Kayaalp ME, Ünlü MC

WITHDRAWN

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SAFETY CONSIDERATIONS FOR COMPOSITE GLUTEAL AND CALF AUGMENTATION PROTOCOLS
Presenter: Katarina Andjelkov, MD, PhD (Serbia)
Affiliation: BelPrime Clinic
Authors: Andjelkov K, Llull R

Introduction: Fat grafting is associated to daunting complications in gluteal and calf augmentation such as fat macro and micro embolism. We hypothesize that endovascular embolization of adipose tissue fragments are generated by transient increment of muscle compartment interstitial pressures as the non compliant muscle fascia is subject to incremental fat grafted volume. As muscle is a low resistance vascular bed, increased intramuscular pressures could potentially promote migration of mobile dispersed fat graft particles into the abundant muscle venous system with lower pressure gradient as the patient remain in prone decubitus position thus further increasing venous runoff.

Materials and Methods: With this hypothesis in mind, we have reviewed 197 cases on gluteal (63) and calf (134) augmentation for both reconstructive and cosmetic indications and reviewed outcome, complication rates and patient satisfaction. Surgical methods applied were: implant-based augmentation, autologous fat grafting and composite augmentation (combination of implants and fat). In order to establish best practices, we performed fresh cadaveric dissection and determined the zones of high risk for all described methods.

Results: In cases of gluteal implant augmentation, we used intramuscular position of the implant, while in calves the implant was inserted in subfascial pocket. When fat grafting was done, fat was placed in all cases in superficial subcutaneous layer. We analyzed the average size of implant and amount of injected fat both in cases where solely one method was applied as well as in cases were composite augmentation was performed. Comparison between all available methods is given and discussed.

Conclusions: Some of the safety issues that we apply in our practice when doing fat grafting in gluteal and/or calf region are:
1. Avoid muscle injections
2. Limiting fat grafting to superficial subcutaneous placement
3. Limiting amount of grafted fat per session
4. Staged procedure (at least 6 weeks)
5. Hydration, which would increase venous pressure would prevent fat particle migration
6. Changing patient positioning during fat grafting stage

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NON-RESPONSIVE SHOULDER PAIN WITH OSTEOARTHRITIS AND ROTATOR CUFF TEARS TREATED WITH AUTOLOGOUS, MICRO-FRAGMENTED AND MINIMALLY MANIPULATED ADIPOSE TISSUE UNDER CONTINUOUS ULTRASOUND GUIDANCE

Presenter: Jay Bowen, DO (USA)
Affiliation: OptimumJoint Integrated Joint Spine
Authors: Striano R, Malanga G, Bowen J, Bilbool N, Azatullah K

Background: Orthopedic pain from osteoarthritis (OA) and rotator cuff disease affects a large portion of the adult population. Currently, there are limited treatments if non-operative care fails. In this context, autologous fat graft is gaining interest. Fat is readily accessible and simple to harvest, to provide volume, cushion, structural support, repair and replacement of damaged tissues. In addition, fat graft is a heterogeneous mix of cellular and non-cellular elements that produce trophic cascades.

Objective: To study the safety and benefits of using an autologous, minimally manipulated adipose tissue graft for treating degenerative shoulder pathology with pain having failed conventional care.

Study Description: To remove variables no other biologics or pharmacologics were used. 18 subjects (19 shoulders) reached one-year follow-up, which was part of an ongoing IRB study approved by IRCM. The patients were ages: 39 – 89 with moderate to advanced degeneration. Tears (all confirmed on MRI): Glenohumeral Osteoarthritis: Severe n=10, moderate n=8. Acromio-clavicular arthrosis: n=8. Rotator Cuff: Supraspinatus: Full thickness tears n=8, complete tear n=2, partial thickness tears n=7, tendinosis n=8, fatty atrophy n=5. Infraspinatus: Tendinosis n=6, full thickness tear n=1. Subscapularis: Tendinosis n=4, intra-substance tear n=1. Labrum: Tear n=7. Bicep tendon: Tendinosis n=1, partial thickness tear n=4.

Material and Methods: An autologous, fat graft was obtained using a minimal manipulation technology in a closed system, without the addition of enzymes or other additives. The graft was injected in 1cc aliquots under ultrasound guidance, into each joint, tendon or soft tissue defect. Clinical outcomes are shown in Fig. 1-7.

Results: No adverse events were reported. The improvement occurred within a few days after treatment and all measured scores (symptoms, signs, and function) showed significant improvement to one-year follow-up (NPS p<0.0001, ASES p<0.0002, Constant p< 0.0006. Figures 1-7).

Conclusion: Although more investigation is needed, these results show promise. The injection of autologous, micro-fragmented, and minimally manipulated adipose appears safe and effective in patients with shoulder disease that failed conventional care.

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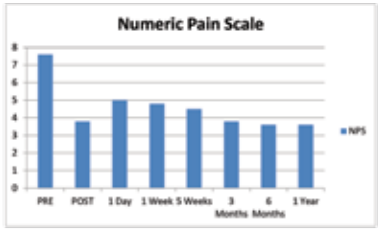

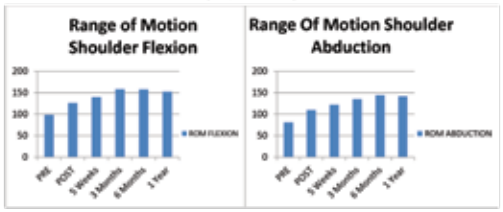

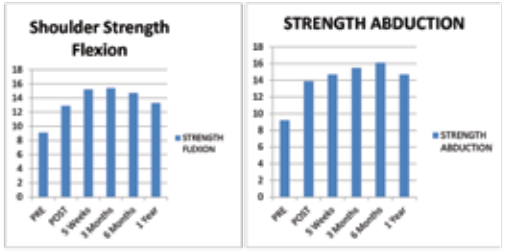

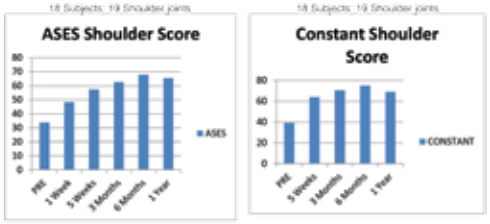

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CLINICAL EVALUATION OF AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE AS A TREATMENT OPTION FOR MENISCUS TEARS

Presenter: Jay Bowen, DO (USA)
Affiliation: New Jersey Regenerative Institute
Authors: Malanga G, Raja A, Bowen JE, Wolf B

Objective: To investigate the potential benefit of using autologous adipose tissue to relieve pain and increase function in patients with meniscus tears refractory to conservative management.

Materials and Methods: In this single-arm, prospective study, 20 patients (ages >35) presenting to a private outpatient sports medicine practice with knee pain, meniscus tear on exam, and MRI evidence of a tear(s) were eligible for this study. Eligible patients were treated with a minimally manipulated, non-digested, micro-fragmented adipose tissue concentrate, under ultrasound guidance. We collected data for factors potentially influencing the outcome, including age, sex, and body mass index (BMI). Clinical outcomes were measured with Numeric Pain Scale (NPS), Knee Injury and Osteoarthritis Outcome Score (KOOS), and physical examination. Pain score and functional questionnaires were evaluated at baseline and then 1, 3, and 6 months follow-up. Range of motion was assessed at every visit.

Results: Patients with previous knee surgery, pathology other than meniscus tears, recent injections, or any contraindications to lipoaspirate were excluded. To date, 19 subjects (10 males, 9 females) have reported outcomes. The average age and BMI were 59.9 ± 7 years and 28.73 ± 5.03 kg/m^2 respectively. Mean baseline pain scores were 5.48 ± 2.2, KOOS=56.05 ± 16.49. At 1 month (n=13) post-procedure NPS scores decreased to 1.04 ± 1.13. KOOS scores improved by 3 months (n=13) to 77.36 ± 18.09 and then again at 6 months (n=5) to 80.05 ± 15.64, while pain scores at 6 months were 2 ± 2.07. Harvest site infection (n=1) was the only significant adverse outcome.

Conclusions: Preliminary results of this case series indicate that autologous adipose-derived stem cells may be a promising treatment for degenerative meniscus tears. These results warrant further investigation with randomized controlled trials involving a comparison group.

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MICRO-FRAGMENTED ADIPOSE TISSUE INJECTION ASSOCIATED WITH ARTHROSCOPIC PROCEDURES IN PATIENTS WITH EARLY KNEE OSTEOARTHRITIS

Presenter: Laura De Girolamo, PhD (Italy)
Affiliation: IRCCS Galeazzi Orthopaedic Institute
Authors: Cattaneo G, De Caro A, Napoli F, Chiapale D, De Girolamo L, Perucca Orfei C, Trada P, Camera A

Introduction: The social impact of degenerative diseases is steadily increasing, because of the continued rise in the mean age of the active population. Articular cartilage lesions are generally associated with disability and symptoms such as joint pain and reduced function, and remain a challenge for the orthopaedic surgeon. Several non-invasive solution have been proposed, but the results achieved to date are far from being completely satisfactory. Recently, new therapeutic approaches, such as the use of mesenchymal stem cells, have been developed. Among the many sources, the adipose tissue is nowadays considered one of the smartest, due to its abundance and easy access. The aim of this retrospective study is to explore whether micro-fragmented adipose tissue associated with a chondral shaving procedure could improve symptoms and function in patients affected by early knee osteoarthritis.

Methods: Thirty-eight patients affected by early knee osteoarthritis were treated in 2015 with an arthroscopic procedure associated with an injection of autologous and micro-fragmented adipose tissue. Micro-fragmented adipose tissue was obtained using a minimal manipulation technique in a closed system. Clinical outcomes were determined at 1, 3, 6, and 12 months follow-up using Knee Injury and Osteoarthritis Outcome Score questionnaire and direct physical examination. Safety of the procedure, recording type and incidence of any adverse event, was also assessed.

Results: A steady and statistically significant improvement of all the clinical scores from pre-operative evaluation to 1, 3, 6, and 12 months follow-up was observed, with KOOS sport and quality of life being the most improved scores. On average, 92% of the patients clinically improved and 100% of them were satisfied with the treatment. No adverse events nor relevant complications were recorded.

Conclusion: The result of the study pointed to micro-fragmented adipose tissue as a safe and effective adjuvant in the surgical treatment of degenerative knee chondropathy. The procedure is simple, sustainable, quick, minimally invasive, one-step, and safe. After one year, the results are very satisfactory and promising. A longer follow-up is needed to draw definitive conclusions and enlarge the indications.

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STRUCTURAL FAT GRAFTING OF THE FOOT

Presenter: Solomon Azouz, MD (USA)
Affiliation: Mayo Clinic
Authors: Teausant AM, Bryant L, Boyll P, Azouz S, Rebecca A

Purpose: Structural fat grafting can be used for soft tissue augmentation in patients suffering from tumor resection, scars, burns, trauma, atrophy associated with aging, and congenital deformities. The purpose of this retrospective study is to evaluate the use of fat grafting to correct anomalies of the foot.

Method: Nine of the patients evaluated were found to have pain, functional or structural deformity and consented to fat grafting to the foot for treatment of their condition. Fat was harvested from the abdomen and injected into the foot deformity using the Coleman technique.

Result: Preoperative and postoperative assessments were used to analyze the morphological changes. The majority of patients received only one operation. The mean volume of fat injected was 21 cubic centimeters. There were no complications. After a minimum follow-up period of 3 months, all patients had improved contour, most had decreased pain and were satisfied with the results. Patients reported less irritation with shoes, and less focal pedal pressure when standing leading to improved overall function.

Conclusion: Fat grafting is a safe and effective option for correcting contour irregularities of the foot. Improvements can be seen with a single operation.

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IMPACT OF THE COMBINATION OF A PEPTIDE-MODIFIED GELLAN GUM HYDROGEL WITH ADIPOSE TISSUE DERIVED STEM CELLS IN A LUMBAR SPINAL CORD INJURY ANIMAL MODEL

Presenter: Ant Salgado, PhD (Portugal)
Affiliation: University of Minho
Authors: Salgado A, Gomes E, Mendes S, Leite-Almeida H, Gimble J, Tam R, Schoichet M, Sousa N, Silva N

Spinal Cord Injury (SCI) is a highly incapacitating condition for which there is still no cure. Current clinical approaches are mainly based on palliative care, so there is a need to find possible treatments to SCI. Cellular transplantation is regarded with great expectation due to the therapeutic potential of cells such as Adipose tissue-derived Stromal/Stem Cells (ASCs) or Olfactory Ensheathing Cells (OECs). Both are accessible sources and present positive paracrine and cell-to-cell interactions, previously reported by our group. Additionally, biomaterials such as hydrogels have been applied in SCI repair with promising results. We propose to combine a GRGDS-modified gellan gum hydrogel with ASCs and OECs in order to promote SCI regeneration. In vitro, ASCs and OECs could be co-cultured within GG-GRGDS hydrogels inducing a more robust neurite outgrowth when compared to controls. In vivo experiments in a hemisection SCI rat model revealed that the administration of ASCs and OECs encapsulated in a GG-GRGDS hydrogel led to significant motor improvements when compared to both control (SCI) and hydrogel alone (GG-GRGDS) groups. This was accompanied by a decreased infiltration of inflammatory cells and astrocytes, and by an increased intensity of neurofilament. These results suggest evident gains induced by the encapsulation of ASCs and OECs in GG-GRGDS based hydrogels.

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IMPACT OF THE COMBINATION OF A PEPTIDE-MODIFIED GELLAN GUM HYDROGEL WITH ADIPOSE TISSUE DERIVED STEM CELLS IN A LUMBAR SPINAL CORD INJURY ANIMAL MODEL

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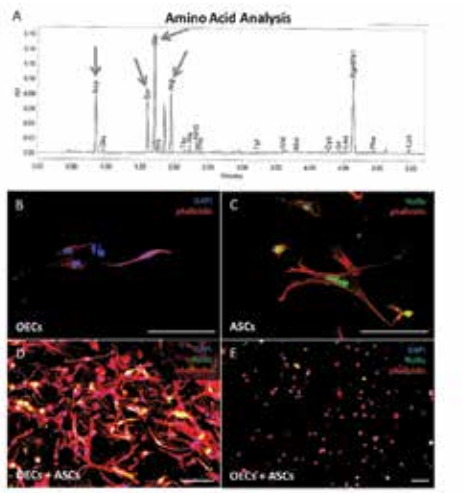
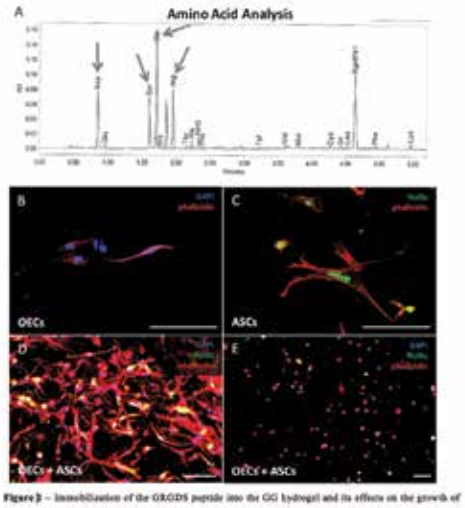


Figure 3 – Immobilization of the GRGDS peptide into the GG hydrogel and its effects on the growth of ASCs and OECs. (A) Amino acid analysis was used to quantify the amount of peptides immobilized to GG hydrogels (approximately 189 nmol GRGDS/mg of GG, grey arrows point to each amino acid). (B-E) Confocal images of ASCs and OECs cultures encapsulated in (B-D) GG-GRGDS hydrogels and (E) in unmodified GG. Cells grown in GG-GRGDS presented their typical morphology either in mono- or co-cultures. In contrast, cells grown in regular GG do not show cellular extensions into the hydrogels. Scale bar: 100 µm. Images are representative of n=3 independent experiments.



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IDENTIFICATION OF OSTEOGENIC MARKERS IN HUMAN ADIPOSE DERIVED STEM CELLS USING NEXT GENERATION SEQUENCING

Presenter: Mullashahensha Shaik, MS (USA)
Affiliation: LSU
Authors: Shaik M, Martin E, Hayes D, Devireddy R

Introduction: Adipose tissue-derived stem cells (ASCs), when grown on collagen coated methylcellulose hydrogels, form cell sheets that are self-detachable at room temperature. The novelty of the format allows to stack the cell sheets into 3D environment and co-differentiate into different pathways within close proximity of each other. Sequencing of mRNA from the isolated single cells from specific locations in cell sheets undergoing osteogenesis will provide insights on the expression of known and unknown genes and further lead to elucidation of intricate and elusive pathways/mechanisms involved and establish baseline data of mRNA sequencing for osteogenically differentiated ASCs. This baseline data will be used in subsequent studies to compare and contrast the spatio-temporal effects on ASC differentiation.

Methods: ASCs at P1, treated with osteogenic media to induce osteogenesis. After 21 days, the RNA was isolated and the samples with A260/280 ratio equivalent to 2 are considered for further processing. The cDNA was synthesized using the Smart seq v4 ultralow input RNA sequencing kit; purified using AMPure XP magnetic beads. The purified cDNA was fragmented using AfaI enzyme to generate library and the quality of library was assessed using fragment analyzer. The libraries were sequenced on ion proton sequencer and the obtained data was analyzed using various bioinformatics tools such as STAR, RSEM, and David bioinformatics on high computing clusters.

Results: Table 1 lists the osteogenic markers that were found to be upregulated. Other genes such as Jun, FOS, and MAP kinases that are known to modulate the osteogenic genes and markers were also found to be upregulated. Further analysis is currently being performed on the sequencing data to elucidate the pathways/mechanisms that are involved with ASC osteogenesis.

Conclusion: Sequencing of mRNA from ASCs undergoing osteogenic differentiation in comparison to undifferentiated ASCs indicated upregulation of several osteogenic markers (shown in Table 1). Further studies, including pathway analysis by david bioinformatics tools and validation of data using QPCR has to be performed to establish osteogenic control which can be used as a reference for the spatio-temporal modulation of osteogenesis.

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IDENTIFICATION OF OSTEOGENIC MARKERS IN HUMAN ADIPOSE DERIVED STEM CELLS USING NEXT GENERATION SEQUENCING

Presenter: Mullashahensha Shaik, MS (USA)
Affiliation: LSU
Authors: Shaik M, Martin E, Hayes D, Devireddy R

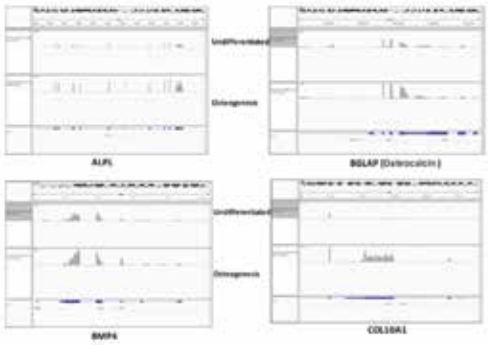

Fig. 1: Representative osteogenic genes visualized in *igv* viewer

Table 1: Upregulated osteogenic markers

Gene Name	Control Count	Control TPM	Control FPKM	Osteo Count	Osteo TPM	Osteol FPKM	Fold Change
ALPL	6860.62	119.98	71.52	20461.99	309.95	207.68	2.983
BGLAP	1.02	0.05	0.03	3.15	0.14	0.1	3.088
BMP4	91	2.84	1.69	144	4.29	2.88	1.582
BMPR1B	214	2.09	1.24	386	4.04	2.71	1.803
COL10A1	5	0.08	0.05	281	5.44	3.65	56.2
CSF1	5126.15	67.05	39.96	17666.67	246.9	165.43	3.446
CTSK	3045.95	84.94	50.63	71657.01	1738.42	1164.82	23.525
EGFR	5323	37.16	22.15	9910.34	61.08	40.93	1.862
FN1	1222356	8119.97	4839.91	3391541	18631.62	12484.05	2.775
ICAM1	1376	22.72	13.54	6718.76	92.86	62.22	4.883
IGF1	50.92	1.21	0.72	137.92	4.92	3.3	2.7086
ITGA3	1370.39	14.54	8.67	2189.73	26.09	17.48	1.598
MMP2	84805.54	1950.48	1162.58	146515.2	2871.53	1924.06	1.728
NOG	2	0.05	0.03	91.04	1.87	1.25	45.52
TGFBR2	14775.1	134.17	79.97	41096.61	329.49	220.77	2.782
TNF	2	0.05	0.03	5	0.12	0.08	2.5
TWIST1	347.99	17.37	10.36	753.89	33.57	22.5	2.166
VCAM1	75	1.03	0.62	801.89	9.83	6.58	10.692
VEGFB	1231	66.86	39.85	3432.78	153.42	102.8	2.789

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APPLICATION OF GOOD MANUFACTURING PRACTICE TO STROMAL VASCULAR FRACTION PRODUCTION IN EUROPE: EXPERIENCE IN A PUBLIC HOSPITAL CELL THERAPY UNIT

Presenter: Guy Magalon, MD (France)
Affiliation: AMU AIX Marseille University
Authors: Magalon G, Magalon J, Veran J, Sabatier F

Introduction: Increasing knowledge in stem cell biology and technological developments in cell engineering now offer a range of very promising innovative cell-based treatment for many clinical indications. Most of these new treatments have recently been regulated in Europe under the specific status of "Advanced Therapy Medicinal Products" (ATMP), thereby creating specific challenges for translation and commercialization. Among stakeholders involved in the development of ATMP, academic cell therapy units are one of the main actors. This communication summarizes the efforts furnished by a European public cell therapy unit to reach the requirement of the European Good Manufacturing Practice level.

Method & Results: Through strong collaboration between Cell Therapy Unit, Plastic Surgery Department, Medical specialists, Quality and Risk management Department, and valorization department, our public university hospital has dramatically improved the level of production of adipose tissue derived stromal vascular fraction as an ATMP during the five past years. All improvements were driven by recommendations from the European Medical Agency through a protocol assistance procedure with the members of the EMA committee for medicinal products for human use in October 2015. It concerns the establishment of validated quality control procedure, the use of appropriate quality rooms for SVF production, an accurate comprehension of SVF mechanism of action or environmental control during production process. These recommendations are currently implemented in three open clinical trials in Crohn's disease, fibrosis vocal cords, Systemic Scleroderma of which the latter is randomized and controlled.

Conclusion: This communication highlights the regulatory framework necessary to develop SVF as ATMP in Europe according to the current guidance. This experience could be shared between European and US units to build recommendations concerning production conditions of ADSVF as ATMP, minimum characterization needed or standardized potency assay to predict SVF efficacy.

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A MICROPHYSIOLOGIC PLATFORM FOR HUMAN FAT: SANDWICHED WHITE ADIPOSE TISSUE

Presenter: Steven Scahill, BS (USA)
Affiliation: LSUHSC
Authors: Scahill S, Vogel K, Luckett JP, Meyer A, Rogers C, Tessler O, Dupin C, St. Hilaire H, Islam KN, Gimble J, Lau FH

Introduction: White adipose tissue (WAT) cannot be reliably cultured. We have overcome this critical barrier for obesity research via tissue engineering methods. This microphysiologic platform is termed "sandwiched WAT" (SWAT).

Methods: SWAT was created by sandwiching minced subcutaneous WAT between sheets of adipose-derived stromal cells. WAT viability was assessed by serial imaging, Oil Red O (ORO), Nile Red, and propidium iodide (PI) staining. Adipocyte protein expression was confirmed with ICC. Transcription of key adipocyte genes was quantified at days 1, 14, and 28 via RT-qPCR and normalized to subject-matched primary WAT. Leptin secretion was quantified by ELISA. Lipolytic function was demonstrated via adrenergic stimulation and glycerol quantification. Whole tissue function was confirmed by SWAT injection into immunodeficient (NOD-SCID) mice with recovery after 10 days.

Results: Tissue from 62 human subjects was cultured via SWAT. Serial imaging demonstrated no visible apoptosis or dedifferentiation during 8 weeks in culture. ORO staining showed intact adipocytes; PI staining revealed no adipocyte death. ICC showed stable adipocyte expression of PPARγ, perilipin, FABP4, and β3-AR. Transcription was evaluated for PPARγ, FABP4, HSL, CEBPα, LPL, and ADIPOQ at days 1, 14, and 28 (fig 1). ELISA of SWAT supernatant showed basal secretion of leptin (d1: 454.17pg/mg protein±82.89, d14: 737.34pg/mg±174.95, d28: 330.13pg/mg±152.33). Adrenergic stimulation induced glycerol release (4.6ng glycerol/mg protein±1.73). NOD-SCID mice engraftment assay had a 100% success rate.

Conclusion: SWAT is a novel, simple, and reliable method for microphysiologically modelling WAT in culture for 8 weeks. SWATs are stable in long-term culture, recapitulating WAT gene expression, protein production, endocrine function, lypolytic function, and engraftment in vivo. This platform allows for characterization of human WAT physiology, the pathophysiology of obesity, and drug screening.

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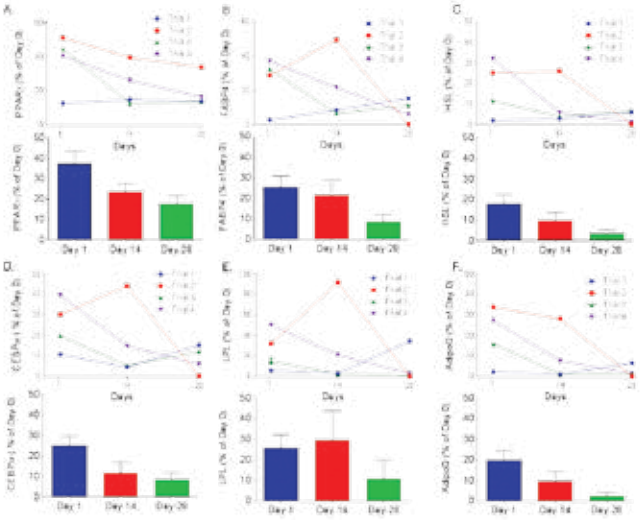

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STROMAL VASCULAR FRACTION (SVF) FOR THE TREATMENT OF ERECTILE DYSFUNCTION - A PILOT STUDY

Presenter: Elliot Lander, MD (USA)
Affiliation: Cell Surgical Network
Authors: Lander E, Berman MH

Introduction: There have been numerous studies in the literature since 2004 demonstrating the effects of Mesenchymal and other progenitor cells deployed in various ways for erectile dysfunction (ED). Nearly all are on animal models (usually murine) with most studies showing some positive efficacy on erectile function. Only seven published papers to date contain human data and the largest included data on 19 patients. This number of publications is extraordinarily low considering that ED is expected in more than 30 million men in the United States alone. We evaluated a series of patients to determine if SVF rich in mesenchymal stem cells may potentially mitigate ED.

Methods: Seventy Six patients with ED of various etiologies but mostly vascular origin received intracavernosal injection of SVF derived from lipo-aspirate using the Cell Surgical Network TimeMachine method. Cell counts in SVF varied as expected with an autologous biologic. A tourniquet was placed at the base of the penis for 20 minutes and SVF was injected intra-cavernosal. 36 patients also received a short series of low intensity acoustic shock waves (1-6) in addition to the SVF therapy. IIEF, EHGS were administered to the patients every 3 months for 2 years. Also patients were asked if they "improved" after their procedures and 35 out of 76 responded affirmative. 23 of these 35 positive responder patients had received shock waves.

Results: Mean IIEF scores showed steady improvement and went from 9.38 baseline to 17.2 at 3 months and then 22.0 at 2 years. Mean EHGS improved from 1.19 baseline to 2.1 at 3 months and then 3.0 at 2 years. There were no significant outcomes differences between patients who received shock waves and those who did not. None of the patients had any adverse reactions related to deployment of SVF or shock wave therapy.

Conclusion: SVF with or without shock waves may offer some benefits to a cross section of patients who suffer from erectile dysfunction.

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IMPROVEMENT OF BONE ALLOGRAFT RECOLONIZATION BY ADIPOSE STEM CELLS: IMPACT OF BONE GRAFT DEMINERALIZATION

Presenter: Sophie Veriter, PhD (Belgium)
Affiliation: Novadip Biosciences
Authors: Veriter S, Palacios P, Mauquoy S, Plougonven E, Dufrane D

Bone allografts combined with mesenchymal stem cells are proposed for bone tissue engineering but remains limited by a low degree of stem cells spreading and cellular recolonization. We postulate to demineralize bone allografts in view to improve adipose stem cells (ASCs) colonization and the bioactivity of the graft.

Bone allografts (n=16) were treated for decellularization (4 groups of demineralization time: 0, 4, 8, 12 hours). The implants were compared in terms of residual calcium, mineral density and bioactivity (for BMP-2/VEGF contents). Each implant was scanned by microtomography to analyze macroporosity and open porosity. Helium pycnometry and Hg porosimetry were performed to assess the absolute density and microporosity. Bone surface analysis was assessed by X-Ray photoelectron spectroscopy and SEM. The bone graft recolonization by ASCs was studied in vitro by SEM, histology and DNA extraction at 24 hours/day 15 post-cellular seeding. Finally, ASCs combined with non-/demineralized bone matrix were implanted into the lumbar muscles of 10 nude rats (in comparison to bone grafts w/o cells) to study the osteoinductivity/angiogenicity by imagery/histology 29 days after implantation.

A significant reduction of the calcium concentration (>-90%) was found in demineralized bone in comparison to native grafts as revealed by ionometry (0.27 vs. 4.1 g/L) and pQCT (0 vs. 0.39 g/cm?). Demineralization significantly increase the macroporosity (>100μm by +13%) and the open porosity (>4 cm?/g vs. 2.1±1.0 cm?/g in comparison to the native graft,p<0.05). A significant increase of microporosity (>10 μm by +158% and <100 nm by 558%) was also found after demineralization. Helium pycnometry confirmed the correlation between the decrease of absolute density and demineralization of the bone graft (R?=0.81). A positive linear correlation between the decrease of calcium/increase of nitrogen atoms (at the bone surface) and the time of demineralization was found (R?=0.99,p<0.001). At day 15 post-incubation, a significant higher ASCs colonization of the bone graft was found for tissue demineralized during 12 hours (p<0.05).

In conclusion, the demineralization of cancellous bones significantly improves the colonization by ASCs in view to return the bioactivity for bone regeneration.

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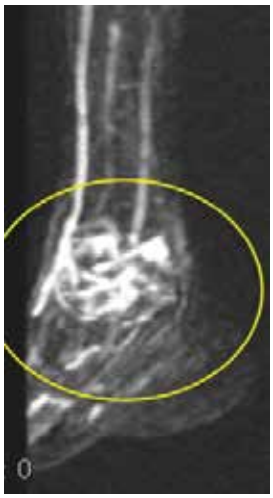
NON-RECONSTRUCTABLE PERIPHEERAL VAXCULAR DISEASE IN 10 PATIENTS TREATED WITH ADIPOSE-DERIVED STROMAL VASCULAR FRACTION CELLS

Presenter: Michael Carstens, MD (USA)
Affiliation: Saint Louis University
Authors: Carstens M, Correa D, Gomez A, Cortes R, Turner E, Perez C, Ocon M

We present a series of ten patients with non-reconstructable peripheral vascular disease (PVD), secondary to arteriosclerosis (AS) and/or diabetes mellitus (DM), treated with local injection of non-expanded autologous, adipose-derived stromal vascular fraction (SVF) cells for the purposes of enhancing neovascularization and chronic wound healing. Adipose tissue was surgically harvested and processed to yield the heterogeneous SVF cells for immediate point-of-care injection. The gastrocnemius muscles and ulcers or wounds where present were locally injected with the resulting SVF. Response to treatment was evaluated both clinically based on pain-free ambula- tion, wound healing capacity over time and ankle/brachial index (ABI) measurements, and by imaging using MRI-based angiography. All patients exhibited clinical improvement (reduction in rest pain and claudication and improvements in ABI), with imaging signs of neovascularization in the majority (5 of 6) of patients in whom the evaluation was feasible. Similarly, 5 of 6 chronic wounds healed without further surgical intervention. This series highlights the utility of non-expanded adipose-derived heterogeneous SVF cell population processed at the point-of-care, to treat patients with end-stage PVD as an alternative to palliation or amputation.



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ADIPOSE DERIVED STEM CELLS (ASCS) ISOLATED FROM SUPER HEALER MICE HAVE IMPROVED REGENERATIVE POTENTIAL: IMPLICATION FOR WOUND HEALING AND BETTER AGING

Presenter: Xiaodong Mu, PhD (USA)
Affiliation: University of Texas
Authors: Mu X, Chen W, Ravuri S, Huard J

Introduction: Aging associated pathologies and muscular dystrophy is the most challenging health problems, and there is still wide area of research that needs to be focused and unfolded. However, Murphy Roths Large (MRL/MpJ) super healer mice, can heal wounds without producing fibrotic scar. Although wound healing and repair mechanism in super healer mice is still unclear, the increased stem cell quantity and/or quality was observed to be the key factors for improved regeneration and repair ability. Adipose-derive stem cells (ASCs) have drawn great attention for potential clinical application and translation in regenerative medicine and anti-aging studies. In this study, we characterized ASCs from super healer mice (SH-ASCs) to determine improved regenerative potential to that of wild type ASCs (WT-ASCs).

Methods: ASCs were isolated from subcutaneous (SC) fat of super healer mice (SH-ASCs) by standard laboratory method. Briefly, fat tissue was minced and enzymatically digested yielded cells were then separated by centrifugation. Isolated ASCs were cultured in adipocyte growth medium. Gene expression analysis was done by real-time PCR and co-culture experiments were conducted to determine effect of SH-ASCs on muscle progenitor cells (MPCs) and cellular responses.

Results: Real time PCR experiments showed that, SH-ASCs have up-regulated expression of anti-inflammatory factors (IL10, Klotho and Sirt1), autophagy marker gene (LC3B), telomerase gene (TERT), and mitochondria activity gene (PGC1). On the other side, expression of pro-fibrogenic factor (TGF-β1) was down-regulated (Figure 1). Compared to WT-ASCs, co-cultured SH-ASCs were able to further repress the pro-inflammatory (IL-1, IL-6) and pro-fibrogenic factor (TGF-β1) in MPCs from aged WT mice (2-year) (Figure 2A), and showed improved myogenic potential (Figure 2B).

Conclusions: Preliminary results showed increased regeneration potential of SH-ASCs compared to WT-ASCs by exerting increased anti-inflammatory and anti-fibrogenic effects. We hypothesize that MPCs function was more positively affected by SH-ASCs in co-culture, compared to WT-ASCs. These findings could be helpful in further application of ASCs in rescuing stem cell dysfunction/exhaustion in muscular dystrophy and systematic aging pathologies.

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ADIPOSE DERIVED STEM CELLS (ASCS) ISOLATED FROM SUPER HEALER MICE HAVE IMPROVED REGENERATIVE POTENTIAL: IMPLICATION FOR WOUND HEALING AND BETTER AGING

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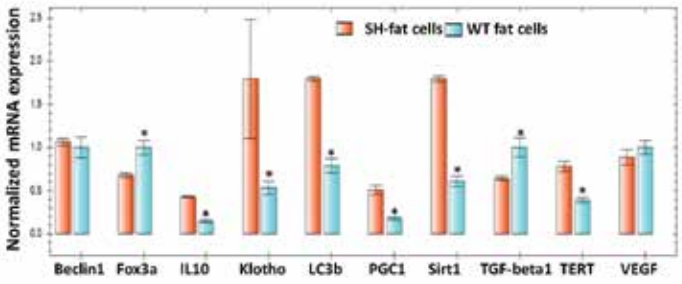

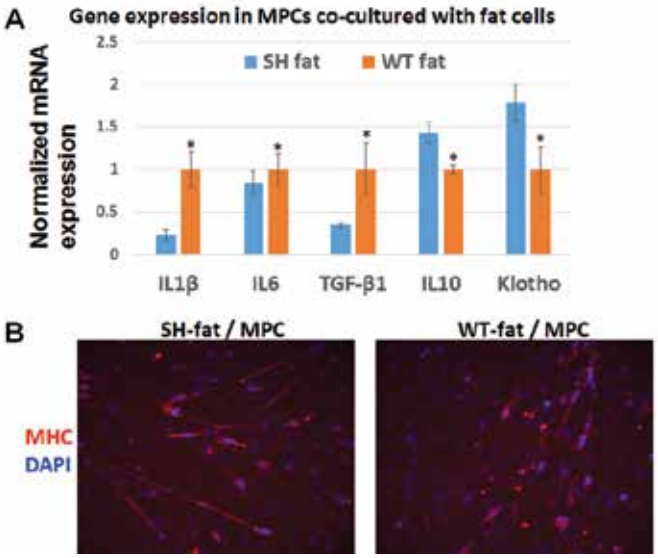

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MICROCIRCULATORY RESPONSES IN-VIVO ON LOCAL INTRA-ARTERIAL INFUSION OF AUTOGENIC ADIPOSE-DERIVED STEM CELLS OR STROMAL VASCULAR FRACTION

Presenter: Wei Wang, MD (USA)
Affiliation: UNLV School of Medicine
Author: Wang W

Background: Both adipose-derived stem cells (ASCs) and stromal vascular fraction (SVF) have been demonstrated the regenerative properties with therapeutic potential for numerous diseases through local or topical applications. However, it is unclear whether ASC or SVF can be delivered systemically through an intra-arterial infusion. The purpose for the present study was to examine the microcirculatory response in-vivo on local intra-arterial infusion of autogenic ASCs or SVF in a vascular pedicle isolated rat cremaster microcirculation model.

Materials and Methods: Fat tissue was surgically harvested from the flanks of male SD rats (n=12) and processed for SVF isolation. Some SVF samples were cultured for 24 hours for ASC purification. The autogenic SVF (1x105) or purified ASC (1x105) cells were infused into the microcirculation of cremaster muscle at speed 0.05ml/min through the cannulation of femoral artery. Since this is a vascular pedicle isolated preparation, the infused SVF or ASC cells went nowhere except the cremaster muscle. The video image of the microcirculation was monitored in real time during infusion.

Results: Arteriole diameter was measured in A1 (100-160μm), A2 (40-80μm), and A3/A4 (10–30μm). Capillary perfusion was quantified in 18 capillary fields of each muscle. There was a significant increase on the diameter of terminal arterioles (P=0.049) and the capillary density (P=0.02) after ASC intra-arterial infusion. However, a significant cell aggregation, embolisms and arterial obstruction were observed in the microcirculation in every case during SVF infusion.

Conclusions: Intra-arterial infusion is an appropriate route for the delivery of autogenic ASCs, but not for SVF. SVF-induced micro-embolism, but not the size of SVF cells, is a significant reason that blocking terminal arterioles resulting in no-flow in the corresponding capillaries.

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IDENTIFICATION OF THE ADIPOGENIC RESPONSE OF ADIPOSE DERIVE STEM CELL FOLLOWING BREAST CANCER STIMULATION

Presenter: Connor King, BS (USA)
Affiliation: Louisiana State University
Authors: King C, Byrne CE, Bunnell BA, Martin EC

There is a known correlation between tumor adipose tissue and breast cancer. Studies demonstrate adipocytes surrounding breast tumors are involved in cancer cell invasion and ASC promoted tumorigenesis in breast cancer. However, a few fundamental gaps exist within this paradigm: specifically, what lasting effects, if any, do cancer cells inflict on adipose tissue? Therefore, evaluation of the effects of the tumor secretome on adipose function and adipogenic differentiation will be invaluable. RNA sequencing of clinical tumor data obtained from The Cancer Genome Atlas (TGCA) was evaluated for infiltration of adipose cells. Results showed a correlation between adipose tissue and estrogen receptor positive (ER+) breast cancer; specifically, that ER+ tumors had a significantly higher fold increase in adipocytes compared to that of ER- tumors. Additionally, initial studies to identify the effects of breast cancer secreted factors on ASCs demonstrated that pre-stimulation of ASCs with media collected from an ER+ breast cancer cell line (cancer conditioned media) increased adipogenesis following one week of adipogenic differentiation. Furthermore, ASCs stimulated for 3 days with cancer conditioned media induced changes in genes associated with adipogenesis (PPARG) and brown fat (UCP1). These studies provide initial data identifying the effects of breast cancer on adipose tissue function. Identifying changes brought on by the tumor to the surrounding stroma could elucidate the lasting effects of cancer on surrounding tissue. Tumor resection and mastectomy are common procedures in breast cancer treatment; therefore, it is of use to identify changes that would occur to the tissue left behind.

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VASCULAR SMOOTH MUSCLE CELLS AND PERICYTES DERIVED FROM THE ADIPOSE TISSUE EXHIBIT A MYOFIBROBLAST PHENOTYPE IN PATHOLOGIC CONDITIONS

Presenter: Howard Ray, BSE (USA)
Affiliation: University of Virginia
Authors: Ray H, Kesting SG, Bruce AC, Peirce SM, Yates PA

Introduction: We have previously shown that myosin heavy chain 11 (Myh11) vascular smooth muscle cells and pericytes (vSMCs-PCs) in the adipose tissue are mesenchymal stem cells. It is reported that mural cells are able to differentiate to a myofibroblast state under pathological conditions, particularly fibrosis. We sought to investigate the role of the cytokine, TGFβ, on myofibroblast phenotype switching of cultured lineage-traced Myh11 vSMCs-PCs derived from adipose tissue. Furthermore, we also investigated the vSMC-PC myofibroblast phenotype switching once locally delivered within an in vivo model for neovascularization and vasculopathy.

Methods: Fluorescently sorted Myh11 lineage-traced vSMCs-PCs from murine epididymal adipose tissue were cultured in standard media and TGFβ-supplemented media for 72 h. Smooth muscle- and fibroblast-related gene expression was measured by confocal imaging and qPCR. The paracrine secretion profiles of cells were also measured by Luminex MAGPIX bead-based multiplex. Lineage-traced vSMCs-PCs were locally delivered in eyes of mice that experienced oxygen-induced retinopathy (OIR), a model for neovascularization and retinal vasculopathy. Two days post-delivery, the retinas were analyzed to determine retinal vasculature remodeling and lineage-traced vSMCs-PCs cell phenotype.

Results: vSMCs-PCs experienced a change in paracrine secretion profiles, and an increased expression of αSMA and a loss of Myh11 under TGFβ-treatment. Locally-delivered vSMCs-PCs were able to integrate into the retinal tissue (1.54%±0.34% of injected cells) and readopt a perivascular position (38.14%±16.06%) in eyes that underwent OIR. When compared to the control eyes, eyes with locally-delivered vSMCs-PCs experienced an 18.4% reduction (n=7, p=0.016) in capillary dropout area. Nearly all locally-delivered vSMCs-PCs expressed cross-linked αSMA among collagen IV deposits. However, locally-delivered vSMCs-PCs did not express the differentiated, smooth muscle contractile protein, Myh11.

Conclusions: We demonstrate that Myh11 vSMCs-PCs derived from the adipose tissue are able to transition from a smooth muscle cell phenotype to a myofibroblast phenotype in pathologic conditions, ultimately aiding in remodeling the local tissue environment.

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A COMPARISON OF AMPK-TARGETED DRUG RESPONSES IN HUMAN STROMAL VASCULAR FRACTION-DERIVED WHITE AND BROWN FAT IN 2D AND 3D

Presenter: Robert Bender, MS (USA)
Affiliation: Tulane University and LaCell
Authors: Bender R, McCarthy M, Wu X, Smith S, Gimble J, Frazier T

WITHDRAWN

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ENRICHMENT OF AUTOLOGOUS FAT GRAFTS WITH EX VIVO-EXPANDED ADIPOSE TISSUE-DERIVED STROMAL CELLS IN COSMETIC BREAST AUGMENTATION: A RANDOMIZED CONTROLLED CLINICAL TRIAL

Presenter: Stig-Frederik Trojahn Kølle, MD, PhD (Denmark)
Affiliation: Aleris Hamlet Hospitals
Authors: Trojahn Kølle SF, Fischer-Nielsen A, Asirvatham Gjorup C, Sonnich Rasmussen B, Taudorf M, Katz AJ, Jønsson B

Background: Autologous fat grafting is increasingly used for cosmetic breast augmentation. However, resorption rates ranging from 25 to 80% have been reported. Therefore, methods to increase graft retention are required for the procedure to be a reliable and attractive alternative to implants, and especially in slim patients where limited fat resources are a challenge. Here, we report the results of a randomized, controlled, blinded, clinical trial comparing the volume retention of fat grafts enriched with ex vivo expanded autologous adipose-derived stem cells (ASCs) with the volume retention of non-enriched fat grafts used in cosmetic breast augmentation.

Methods: Twelve healthy participants were enrolled in the study and divided into two groups by randomization. All participants received a cosmetic breast augmentation using autologous fat grafting. One group received ASC enriched fat grafts and the other served as control and received conventional non-enriched fat grafts. The enriched fat grafts where enriched with at least 20x106 viable ex vivo expanded ASCs/mL fat, depending on the number of cells available after expansion and the amount of fat needed for the augmentation.

Volume retention of the fat grafts was measured by magnetic resonance imaging (MRI). Total breast volume was determined preoperatively and again after four months. Additional MRIs will be conducted after one year and five years as follow up. The primary endpoint was to compare the graft retention of the ASC-enriched fat grafts with that of the control grafts.

Clinical photos were taken simultaneously with the MRIs.

The study is registered at www.clinicaltrials.gov under number H-16046960 and approved by: The local Danish Committee on Biomedical Research Ethics for Copenhagen (EC): H-16046960, The Danish Data Protection Agency: 2016-41-4800 and The Danish National Health and Patient Safety Authority: 5-7410-16/1.

Results: Pending, the results at four months follow up will be presented at IFATS 2017.

POSTER PRESENTATIONS

1P

HUMAN ADIPOSE STEM CELL MEDIATED MATRIX REMODELING AS A MECHANISM FOR CANCER PROGRESSION

Presenter: Ethan Byrne, BSBE (USA)
Affiliation: Louisiana State University
Authors: Byrne E, King C, Bunnell BA, Martin EC

Tumor structure plays an important role in breast cancer progression. The stiffness of breast tissue has been identified as a risk factor for breast cancer as there is a positive correlation with tumor stiffness and cancer progression. Enhanced breast stiffness is associated with increased fibril collagen in the stroma. Aligned fibers from the tumor to the surrounding stroma are believed to facilitate cellular invasion by providing a platform for cell movement. Additionally, within the tumor there is an observed remodeling of the ECM, collagen deposition, and fiber crosslinking, all of which will alter the tumor architecture. The origin for altered matrix is to date unknown, but studies suggest it arises from a stromal component. Here we aim to identify induced changes in matrix genes of human adipose derived stem cells (ASCs) following stimulation with cancer secretome. To investigate induced changes to matrix genes following stimulation with breast cancer secretome, conditioned media from the MCF-7 breast cancer cell line was used to stimulate ASCs for 3 days. ASCs were collected for total RNA and qRT-PCR. Our results demonstrate that stimulation of ASCs with breast cancer secreted factors increased matrix gene expression in ASCs, specifically enhancing collagen IV and matrix regulatory transcription factor FRA-1. The effect of changes in matrix composition on breast cancer cells was evaluated through culturing MCF-7 breast cancer cells on plates coated with different matrix components (laminin, fibronectin, and collagen) and 2D for control. After 24 hours, cells were collected for RNA and qRT-PCR was performed for changes in gene expression that correlate with cancer progression (ER, PGR, CDH1). MCF-7 breast cancer cell line demonstrated decreased expression of epithelial marker CDH1, ER, and PGR when compared to both 2D culture and laminin coated plates. This effect could not be reversed following treatment with primary therapy. These preliminary studies identify novel mechanism for adipose promoted tumorigenesis and cancer resistance to primary therapy.

2P

ALTERED VASCULATURE, ANGIOGENESIS AND ADIPOCYTE HYPERTROPHY IN SUBCUTANEOUS ADIPOSE TISSUE (SAT) DISORDERS

Presenter: Sara Al Ghadban, PhD (USA)
Affiliation: University of Arizona
Authors: Al Ghadban S, Herbst K, Ussery C, Harris D, Badowski M, Allen M

Background: Lipedema and Dercum's disease (DD) are painful SAT disorders affecting millions of women worldwide, characterized by enlargement of SAT on the legs that is resistant to weight loss by usual measures. We propose that blood vessel structure is altered resulting in fluid collection in the matrix, followed by inflammation involving macrophages, mast cells, lymphocytes, and adipocyte hyperplasia and hypertrophy. The aim of the project is to investigate the etiology of SAT disorders with a focus on the blood vasculature and SAT structure.

Methods: Histological sections from skin and SAT of the leg were stained with H&E. Adipocyte cell size and area were measured in two groups with body mass index (BMI) 20-30 kg/m2 (Group I, n=15) and 30-40 kg/m2 (Group II, n=15). Markers for macrophages (CD68), mast cells (CD117), T cells (CD3), endothelial cells (CD31, CD34), and blood vessels (SMA) were investigated by immunohistochemistry (IHC).

Results: Heterogeneity in adipocyte morphology was observed in all SAT tissue. Leg adipocytes were significantly larger in size and area in lipedema ($p<0.0001$; $p<0.005$) and DD ($p<0.0001$) in Group I compared to controls, but not in Group II. Adipocyte size and area were significantly larger in controls in Group II versus Group I ($p<0.0001$). Macrophage numbers trended higher in SAT disorders in Group II leg tissue compared to controls; no differences in mast cells or lymphocytes were detected between groups. SMA stain revealed thickening and dilation of blood vessels in SAT disorder subjects. Unusual papillary dermal vessels were enclosed by the epidermis, and areas of angiolipoma were found in the hypodermis in SAT disorder subjects. Singlets of large ectopic adipocytes were found in a few subjects in all groups. Large circular structures in the hypodermis were consistent with dilated vessels that had lost the endothelium.

Conclusions: Hypertrophic adipocytes in the legs of women with SAT disorders occur independent of BMI. Larger adipocytes are associated with inflammation, in agreement with larger numbers of macrophages in SAT disorders. Our data amplify the role played by altered vasculature in the pathogenesis of SAT disorders and appear consistent with a higher level of angiogenesis.

3P

SODIUM BICARBONATE BUFFERING IMPROVES ADIPOCYTE STEM CELL VIABILITY AFTER LIPOSUCTION

Presenter: Wei Z. Wang, MD (USA)
Affiliation: University of Nevada Reno School of Medicine
Authors: Francis A, Wang WZ, Goldman JJ, Fang XH, Williams SJ, Baynosa RC

Background: Fat grafting is a growing field within plastic surgery and regenerative medicine with many applications. Adipose-derived stem cells (ASCs) and stromal vascular fraction (SVF) are minor components of lipoaspirate and may have roles in fat graft survival. Tumescent anesthesia is the standard technique for liposuction; most standard tumescent solutions contain lidocaine. Previous work by our lab demonstrated that lidocaine-containing tumescent solution negatively affects ASC culture viability and increases SVF apoptosis. Sodium bicarbonate ($NaHCO_3$) improves the pain with lidocaine use as a local anesthetic by buffering the acidity. Standard tumescent solution acidity has been previously demonstrated, but the impact on ASC survival is unknown. The purpose of this study was to determine if $NaHCO_3$ buffering is a practical method to reverse the detrimental effect of lidocaine-containing tumescent on ASC and SFV viability after liposuction.

Methods: Adults undergoing bilateral liposuction were included in the study (n=7). Under general anesthesia, tumescent liposuction on one side of the body was conducted with standard tumescent with lidocaine (one liter lactated Ringer's, 30 mL 1% lidocaine, and 1 mcg epinephrine). On the opposite side, liposuction with $NaHCO_3$ buffering was conducted by adding 7 milliliters of 8.4% $NaHCO_3$ to the standard tumescent (target pH >7.00). Tumescent solution pH was measured. Lipoaspirate was processed for SVF isolation for ASC culture (ASC viability) and flow cytometry (percentage of apoptotic cells).

Results: The pH of the buffered tumescent solution was significantly higher than that of the standard solution (mean±SEM; 7.06±0.05 vs. 6.27±0.11, p=0.001). The average number of viable cultured ASCs in the lipoaspirate treated with $NaHCO_3$ was approximately 56% higher (377,214±116,195 vs. 245,643±75,890, p=0.028) than in the lipoaspirate obtained with standard tumescent. The percentage of apoptotic cells was similar between the groups.

Conclusions: Buffering tumescent solution with $NaHCO_3$ is recommended and can significantly enhance ASC viability. However, $NaHCO_3$ is unable to reverse the apoptosis-inducing effects of lidocaine on SVF. Further investigation is needed to ascertain the mechanisms underlying these processes.

4P

RE-CLASSIFICATION OF THE FAT GRAFT

Presenter: Steven Cohen, MD, FACS (USA)
Affiliation: University of California San Diego
Authors: Cohen S, Hewett S, Ross L, Goodache A

Background: Fat grafting has primarily been performed by Coleman structural fat grafting and some modifications in how the fat is prepared and harvested. Herein, we present a concept of fat graft modification by cleaning and emulsifying into three types of grafts. The rationale is similar for fillers, where they are modified to fit the anatomic region of use.

Materials and Methods: Fat is harvested either using a vibratory device with a vibratory 2.0 mm smooth rectangular hole cannula or a Carraway Harvest (X dimensions) cannula. The fat is first decanted for mechanically released stromal vascular fraction cells and then, the remaining fat is removed and prepared using a specially designed "fat press" which permits simple rinsing of the micronized product. Next, the fat is removed and passed through a 2.4 mm emulsifier, a 1.2 mm emulsifier and a 400 and 600 micron screen to produce Millifat, Microfat and Nanofat, respectively.

Results: Using these techiques most patients undergoing facelift surgeries and/or fractional laser treatments were managed with these injectable tissue regeneration techniques using Millifat for structure (deep fat, temples, pyriform, nose, brow and glabella); Microfat for superficial fat regeneration (forehead, peri-oral region, inferior orbital rim, superior eyelid sulcus) and Nanofat for intradermal rhytids around the lips, deep wrinkles of the neck and as both a mesotherapeutic agent delivered by microneedling or as a gel compounded with an agent that promotes transdermal delivery of cells to the deeper tissues.

Conclusions: Fat is being used for both volume and regeneration. New concepts of fat grafting relies on predictable and rational application of certain key concepts. For one, it makes sense to modify the graft into structural to virtually cellular products to be used safely in the correct anatomic location. Two, the idea of early full facial replacement of superficial and deep fat to maintain trophism in facial aging has been demonstrated by earlier work which demonstrated progressive improvement in facial volume up to 24 months after facelift and fat grafting.

5P

BIOLOGIC AUGMENTATION OF A SURGICALLY REPAIRED MENISCUS IN AN ELITE HIGH SCHOOL ATHLETE. A CASE REPORT.

Presenter: Jay Bowen, DO (USA)
Affiliation: New Jersey Regenerative Institute
Authors: Bowen J, Malanga GA, Raja A

Case Description: A 17-year-old high school football player suffered a contact injury during a game. He immediately felt lateral sided knee pain and weakness. Initial evaluation was consistent with a lateral meniscus tear. MRI confirmed the diagnosis. He underwent a lateral meniscus repair for a full-thickness, high-grade radial split lateral meniscal tear of the posterior horn and body junction. He presented to us 4 days later for adjunctive treatment to enhance recovery. We implemented a treatment course of Platelet-Rich Plasma (PRP) followed by autologous, micro-fractured, and minimally manipulated adipose tissue one week later.

Setting: Outpatient Medical Office.

Results: At 5 weeks post treatment, pain significantly improved and range of motion increased. By week 11, the patient reported no pain or swelling. He was full weight-bearing, physical exam was benign. Week 13 post treatment, he reported no mechanical symptoms or swelling. However, new complaint of mild soreness in the posterior knee, around the lateral head of gastrocnemius. Per the surgeon, this was likely related to suture attachment. Second look arthroscopy revealed complete healing of the peripheral rim and body of the meniscus with the exception of the central free edge. The free edge was trimmed and the meniscal suture removed to decrease irritation. One week later, the patient reported complete resolution of soreness.

Discussion: Meniscal preservation may be a route to prevent early arthritic changes associated with meniscal resection. In this competitive athlete, PRP and adipose-derived tissue injections were used to enhance an arthroscopically repaired meniscus. Within 15 weeks, a complex radial tear in a hypovascular region healed almost in its entirety and allowed for return to sport.

Conclusion: Presently, meniscal repair surgeries are infrequently performed due to the poor intrinsic healing capabilities of these structures. However, autologous cells may help increase this healing potential. This case illustrates the potential for biologic augmentation of current surgical procedures.

6P

A PHASE I SAFETY STUDY USING STROMAL VASCULAR FRACTION FROM LIPOASPIRATE IN THE TREATMENT OF CHRONIC NON-HEALING WOUNDS VIA THE ANTRIA CELL PREPARATION PROCESS (ACPP)

Presenter: Leonard Maliver, MD (USA)
Affiliation: Antria Inc.
Authors: Maliver L, Bizousky DT

WITHDRAWN

7P
TREATMENT AND OUTCOMES OF LIPOFILLING IN TUBEROUS BREASTS
Presenter: Patricia Gutierrez-Ontalvilla, MD (Spain)
Affiliation: Hospital La Fe
Authors: Gutierrez-Ontalvilla P, Lopez E

WITHDRAWN

8P
LIPOFILLING UNDER THE SCARS WITH AND WITHOUT PLATEL RIC PLASMA
Presenter: Yasser Helmy Ali, MD (Egypt)
Affiliation: Faculty of Medicine Al-Azhar University
Author: Ali YH

WITHDRAWN

9P
EFFECT OF PLATELET-RICH PLASMA ON THE PROLIFERATION OF HUMAN ADIPOSE STEM CELLS

Presenter: Fangyuan Lai, MD (Japan)
Affiliation: Kansai Medical University
Authors: Lai F, Kakudo NA, Morimoto NA, Taketani SH, Hara TO, Ogawa TA, Kusumoto KE

Background: Platelet-rich plasma (PRP) is a kind ofn autologous blood product that, which contains a high concentration of growth factors. One of the growth factor is platelet-derived growth factor (PDGF)-BB, which is the potential mitogen of for human adipose adipose-derived stem cells (hASCs). However, the signaling pathway activated by of PRP that stimulates the proliferation of hASCs remains unclear.

Materials and Methods: Cell cultural assay was taken to assess the proliferation degree of hASCs were cultured, treated with 0%, 0.2%, or 1% PRP plus, and 2ng/ml, or 10ng/ml PDGF-BB., and proliferation was assessed. The hASCs were also treated by with PRP and PDGF-BB in the presence or absence of, with or without the PDGF inhibitors, iImatinib and Sorafenib, followed by and cells were counted with the Cell Counting Kit assay. In other experiments, we used 0.5 or 2 ?ganti-PDGF antibody with concentration of 0.5μg/mL and 2μg/mL to suppress the effect of PDGF-BB in PRP and observed the proliferation of hASCs, compared with control. We used inhibitors of various potentially important protein kinases inhibitors such as ERK1/2, JNK, p38, and AKT inhibitors and assessed, to see the proliferation of hASCs.

Results: Proliferation was most remarkably We found that the promoted effect was most remarkable in cells treated with 1% PRP in PRP groups and 10ng/ml PDGF-BB in PDGF-BB groups. Both iImatinib and Sorafenib can hinder inhibited the proliferation of hASCs treated with 1% PRP or10ng/ml PDGF-BB. Also, the 0.5μg/mL and 2μg/mL ml anti-PDGF antibody significantly groups show decreased of the proliferation of hASCs compared with control significantly. PRP PRP-mediated hASCs proliferation was inhibited blocked by inhibitors of ERK1/2, AKT, and JNK inhibitor treatment with certain concentrations, but not by an inhibitor of p38 inhibitor.

Conclusion: These results show that PRP can promote hASCs proliferation. The PDGF-BB in PRP plays an important role in inducing the proliferation of hASCs. PRP promotes hASCss proliferation maybe by via ERK1/2, PI3K/AKT, and JNK signaling pathways.

10P
LARGE VOLUME LIPOFILLING WITH CLOSE SYSTEM IN AESTHETIC PLASTIC SURGERY

Presenter: Aristides Arellano-Huacuja, MD, FICS (Mexico)
Affiliation: Clinica Dermatologica y Cirugia Estetica de Puebla SA de CV
Authors: Arellano-Huacuja A, Arellano-Montalvo A; Arellano-Montalvo D

Introduction: Fat transfer is also called lipofilling injection or fat transplantation. Injecting fat is a natural, safe and non-allergenic procedure. Human adipose tissue contains a cell population of adult stem cells, which proliferate and differentiate into multiple cell types: new adipocytes, myocytes, osteoblasts, etc. This tissue also holds a large number of grow factors (GF's). Notably, Adipose Stem Cells (ASCs) characterize in maintaining it's undifferentiated original form and function. During the natural aging process, the fatty tissue that once had a rounded and youthful appearance can begin to break down, resulting in wrinkles and sagging skin. Therefore, fat injections in these areas help improve not only the appearance of the skin, but also the consistence of it. ASCs can be used to correct large volumes or small imperfections, everything depends on the amount of fatty tissue in good condition that can be obtained from the patient.

Large volume lipofilling Adipose tissue can be obtained by surgical resection, by tumescent liposuction or ultrasonic liposuction. In the case of the tumescent technique infiltration of adipose tissue is performed with saline solution (100ml) plus epinephrine (1ml) and 20ml of lidocaine. To obtain the tissue, aspiration cannulas are used to perform the lipofilling procedure. They vary in diameter; therefore, cannulas with a diameter of maximum 3 mm are used to perform large volume lipofilling. After aspiration, the fatty tissue must be processed to separate the fat cells (adipocytes) of the least useful components (blood, plasma, remains, broken adipocytes, free oil...), allowing the injection of a pure tissue. Gravity is used as a technique to cause spontaneous separation of the oil, fluid and blood components from the fatty tissue. Places of "donation" are mainly the abdomen, abdominal and lumbar flanks, and thighs. Fatty tissue can be transferred to buttocks, face, calves and thighs. It is important to place the "spaghetti-like" threads of fat in different layers at different levels (structural) with the close-system. This technique allows larger volumes of fat to survive more sufficiently with stable results. The volume injected is typically higher than expected.

11P

THE TROPHIC EFFECT OF VETAP-17® ON HUMAN UMBILICAL CORD MESENCHYMAL STROMAL CELLS IN A 3D HUMAN PLATELET LYSATE GEL MODEL

Presenter: Thitikan Jirakittisonthon, DVM (USA)
Affiliation: Kansas State University
Authors: Jirakittisonthon T, Murnane JM, Weiss ML

VETAP-17® is a nutritive product that we test here for its ability to enhance cell growth in vitro. Umbilical Cord derived or adipose-derived Mesenchymal Stromal Cells (MSCs), when grown in vitro, may respond to biological stimuli by changing their migration. When they migrate towards a stimulus, we call that a positive trophic effect. The objective of this study is to observe and document the trophic effects of VETAP-17® on MSCs. We created an in-house method, called 3D invasion model, which mimics in vivo wounds 3D, compared to standard 2D wound model of a scratch assay. In our 3D invasion model, a mold is used to cast human platelet lysate gel. The mold creates three chambers: a central trough and two columns on opposite sides of a trough for the addition of test compounds. After hardening of the gel, we remove the mold and add culture cells suspended in HPL. Usually, MSCs are added at the central trough. HPL media serving as a control and is added to one well. VETAP-17® is added to the opposite well. Preliminary result shows that VETAP-17® has a positive trophic effect on MSCs, compared to the HPL only control well. We are currently characterizing the trophic effects in vitro of VETAP-17® using a dose response assay.

12P

CORRECTION OF DEPRESSED SCARS WITH PRP ENRICHED FAT GRAFTING

Presenter: Sameh Elshawadfy, MD (Egypt)
Affiliation: Faculty of Medicine Tanta University
Authors: Elshawadfy S, Shalaby H, Hammad S

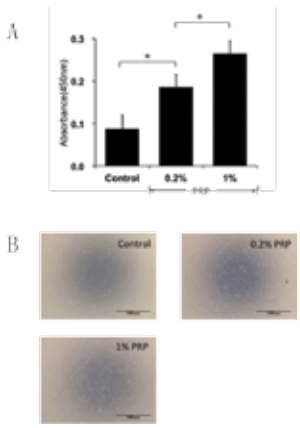
WITHDRAWN

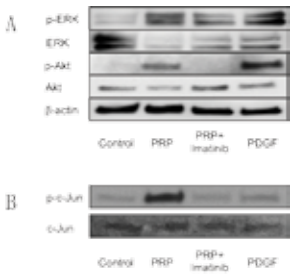
13P

PEROXICAM REDUCES RESIDUAL COLLAGENASE ACTIVITY IN ENZYMATICALLY-DERIVED STROMAL VASCULAR FRACTION

Presenter: Joseph Zakhari, MA (USA)
Affiliation: University of Louisville School of Medicine
Authors: Zakhari J, Williams SK

Both enzymatic and non-enzymatic methods have been used for the isolation of adipose stromal vascular fraction cell populations. It has been established that the tissue dissociation enzymes used for SVF preparation are not completely removed from the isolated cells during their preparation. The current study was performed to evaluate the effect of residual dissociation enzyme on the stability of adipose SVF/collagen gel preparations. Adipose SVF cell populations were prepared using both crude mixtures and purified collagenase enzyme solutions and the isolated SVF cells mixed with soluble collagen type I. The SVF/collagen samples were subsequently placed in multiwell tissue culture plates under conditions that supported collagen gel formation. The stability of the collagen gels was then evaluated over a period of 7 days in vitro in a 37C CO2 incubator. To evaluate the direct effect of collagenase on gel stability, peroxicam was added to the SVF/collagen gel solutions. Peroxicam, a non-selective cox 1 and cox 2 inhibitor is a widely prescribed NSAID pharmacotherapy. Peroxicam has also been reported to selectively inhibit bacterial collagenases. SVF/collagen constructs receiving the IC50 dose of peroxicam retained mechanical stability and shape over a period of 7 days whereas SVF/collagen gels without peroxicam exhibited rapid degradation over a period of 2-4 days. Peroxicam may play an important role in SVF clinical utilization as a means to inhibit residual bacterial collagenase activity especially under conditions where enzymatically derived SVF cell populations are directly injected into soft tissue.





14P

STEM CELLS FROM HUMAN HAIR FOLLICLES: FIRST MECHANICAL ISOLATION FOR IMMEDIATE AUTOLOGOUS CLINICAL USE IN ANDROGENETIC ALOPECIA AND HAIR LOSS

Presenter: Pietro Gentile, MD, PhD (Italy)
Affiliation: University of Rome Tor Vergata
Author: Gentile P

Hair follicles are known to contain a well-characterized niche for adult stem cells: the bulge, which contains epithelial and melanocytic stem cells. Stem cells in the hair bulge, a clearly demarcated structure within the lower permanent portion of hair follicles, can generate the interfollicular epidermis, hair follicle structures, and sebaceous glands. The bulge epithelial stem cells can also reconstitute in an artificial in vivo system to a new hair follicle. In this study, we have developed a new method to isolate human adult stem cells by mechanical centrifugation of punch biopsy from human hair follicles without culture condition. We have shown that the isolated cells are capable to improve the hair density in patients affected by androgenetic alopecia. These cells appear to be located in the bulge area of human hair follicles.

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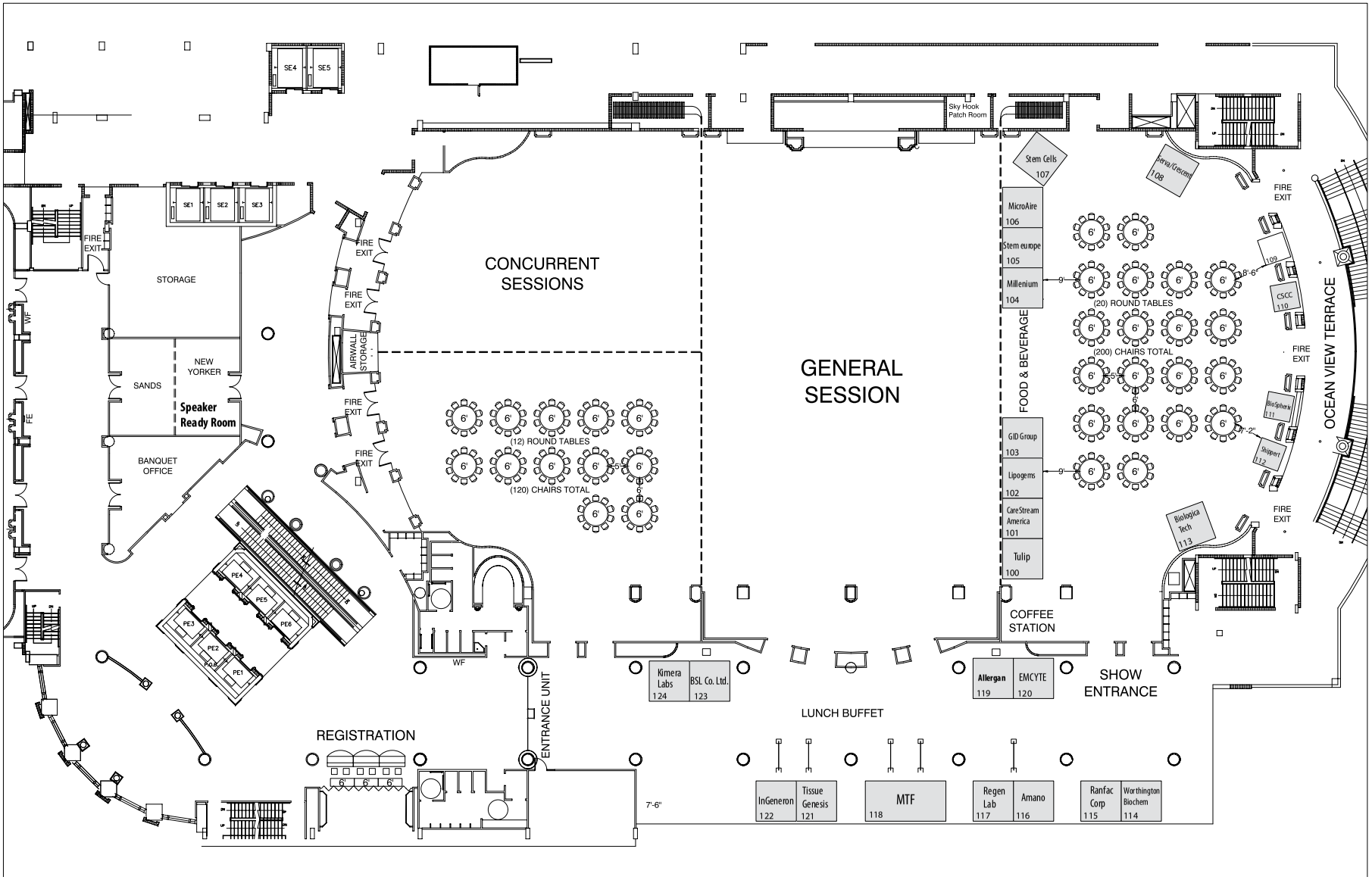
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SE3
Sky Hook Patch Room
Stem Cells 107
Aeria/Crescent 108
MicroAire 106
Stem europe 105
Millenium 104
FIRE EXIT
STORAGE
CONCURRENT SESSIONS
GENERAL SESSION
FOOD & BEVERAGE
(20) ROUND TABLES
(200) CHAIRS TOTAL
109
CSCC 110
BioSphere 111
Shippert 112
Biologica Tech 113
OCEAN VIEW TERRACE
NEW YORKER
Speaker Ready Room
SANDS
BANQUET OFFICE
AIRWALL STORAGE
(12) ROUND TABLES
(120) CHAIRS TOTAL
GID Group 103
Lipogems 102
CareStream America 101
Tulip 100
COFFEE STATION
WF
FE
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PE2
PE3
PE4
PE5
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Kimera Labs 124
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MTF 118
Regen Lab 117
Amano 116
Ranfac Corp 115
Worthington Biochem 114
7'-6"

NOTES

REVOLVE ENVI™ 600
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Artist's rendering, not actual size.

Before use, physicians should review all risk information, which can be found in the *Instructions for Use* for REVOLVE ENVI™ 600.

Allergan

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