

IFATS AMSTERDAM 2014 CONFERENCE



IFATS

International Federation for
Adipose Therapeutics and Science

November 13-16, 2014

NH Grand Hotel Krasnapolsky • Amsterdam, The Netherlands

A commitment to you and your industry that's more than skin deep.

5 things every plastic surgeon should know about MTF.

- 1** As a non-profit organization, we're dedicated to advancing plastic surgery not our bottom line.
- 2** We were founded and governed by surgeons just like you.
- 3** Our high-quality human acellular dermis exceeds the toughest industry standards.
- 4** We proudly help create awareness for what's important to you in the industry.
- 5** MTF believes in contributing to your professional development.

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to learn more about MTF**





International Federation for
Adipose Therapeutics and Science

IFATS AMSTERDAM 2014

November 13-16, 2014

NH Grand Hotel Krasnapolsky • Amsterdam, The Netherlands

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MARK YOUR CALENDAR

International Federation for
Adipose Therapeutics and Science

13th Annual Meeting

IFATS NEW ORLEANS 2015

November 5-8, 2015

JW Marriott New Orleans

New Orleans, Louisiana



ABSTRACT DEADLINE:

Midnight EST, Wednesday, June 4, 2015

The Call for Abstracts will be sent this winter. All members of IFATS and all registered attendees of the 2014 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

45 Lyme Road - Suite 304 Hanover, NH 03755 USA

Tel: 1-603-643-2325 • Fax: 1-603-643-1444

Email: ifats@conmx.net • Web: www.ifats.org

Catherine Foss - Executive Director • IFATS@conmx.net

Jodie Ambrose - Abstract Coordinator and Marketing Manager • Jodie@conmx.net

Jordan Carney - Membership Services Manager • IFATSmembership@conmx.net

Michele Nilsson, CMP - Education Specialist • Michele@conmx.net

Carol Gouin - Accounting Manager • Carol@conmx.net



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Dear Colleagues,

It is my honor to invite you to join us for our 12th Annual Conference on November 13- 16 at the Grand Hotel Krasnapolsky in Amsterdam, The Netherlands. This year's meeting will again provide an excellent opportunity to exchange ideas with leading scientists and clinicians in the exciting field of adult adipose stem cell research and adipose grafting, and to learn about the latest scientific, medical, and technological advances. Our slogan this year is:

THE global bench-to-bedside adipose stem cell and fat grafting congress.

Having organized the meeting for several years in North America, we are very proud to host the IFATS meeting this year in historic Amsterdam. We will expand our scope to novel application fields like cardiology, craniofacial surgeries and orthopedics, as well as other fields recognizing the high potential of adipose stem cell technology. We aim to encourage interaction between industry and the clinical and scientific communities, not only from Europe but from all continents.

To promote these interactions, we have established new aspects of the meeting, such as lunch time seminars and a pre-meeting symposium addressing the regulatory path for translation of research to commercial medicinal products in a global perspective.

The meeting's goal is to promote scientific exchange among basic researchers making key discoveries, technology developers who are creating new cost-effective devices and procedures, and clinicians enabling the use of adipose tissue and stem cells. The meeting will particularly emphasize cutting edge approaches and original data. Scientists and clinicians will have plenty of opportunities to share ideas in both formal and informal settings, thereby gaining insight into the future use of adult adipose stem cells around the world.

On behalf of the IFATS Board of Directors, I look forward to welcoming you to Amsterdam in November.

With best wishes,



Marco Helder, PhD
VU University Medical Center
Amsterdam, The Netherlands
IFATS President 2013-2014



SCIENTIFIC PROGRAM COMMITTEE

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J. Peter Rubin, MD, FACS
Dmitry O. Traktuev, PhD
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INVITED SPEAKERS AND SESSION MODERATORS

Petra Bauer-Kreisel, PhD
Bruce Bunnell, PhD
Prof. Valerio Cervelli
Hans Clevers, MD, PhD
Sydney Coleman, MD
Richard D'Amico, MD
Daniel Del Vecchio, MD
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Kotaro Yoshimura, MD

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Papers are reprinted as they were submitted.

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All papers in this Program Book are listed in numerical order.

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

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





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.



PROGRAM IN BRIEF - Expanded program begins on page 17

Thursday, November 13, 2014

11:00 am - 6:00 pm	Registration
1:00 - 1:15 pm	Welcome Remarks <i>Marco Helder, PhD - IFATS President</i>
1:15 - 1:45 pm	Keynote Speaker 1 Developments in Clinical Fat Grafting <i>Sydney Coleman, MD</i>
1:45 - 2:15 pm	Keynote Speaker 2 Healing Parenchyma with Mesenchyma; A New Paradigm in Cell Surgery <i>Ramon Llull, MD, PhD</i>
2:15 - 3:15 pm	Panel Discussion - Clinical Innovations in Adipose Therapies Chair: <i>J. Peter Rubin, MD, FACS</i> Panelists: <i>Prof. Valerio Cervelli, Kotaro Yoshimura, MD, Daniel Del Vecchio, MD</i>
3:15 - 3:45 pm	Coffee Break and Exhibits
3:45 - 5:10 pm	Plenary Session 1: Clinical Soft Tissue Research I Moderators: <i>Sydney Coleman, MD & Jenneke Klein-Nulend, PhD</i>
3:45 pm	Introductory Lecture: Adipose Based Therapies for Craniofacial and Limb Reconstruction Speaker: <i>J. Peter Rubin, MD, FACS</i>
4:05 - 5:10 pm	Free Paper Presentations
5:10 - 5:35 pm	Plenary Lecture 1: Soft Tissue Research Transdifferentiation of ADSC into Motorneuron-like Cells for Cell Replacement Therapy of Spinal Cord Injury Speaker: <i>Jun Xu, MD, FACS</i>
5:35 - 6:00 pm	Plenary Lecture 2: Skeletal Tissue Research Treating Scleroderma of the Face and Hands Speaker: <i>Guy Magalon, MD, PhD</i>
6:00 pm	Adjourn





Friday, November 14, 2014

8:00 - 8:15 am	Introductory Remarks <i>Marco Helder, PhD</i>
8:15 - 9:00 am	INVITED SPEAKER LGR5 Stem Cells in Self-Renewal and Cancer <i>Hans Clevers, MD, PhD</i>
9:00 - 10:10 am	Plenary Session 2 - Clinical Cardiac Research Moderators: <i>Paul A. J. Krijnen, PhD & Hans W. M. Niessen, MD, PhD</i>
9:00 am	Introductory Lecture: From Bone Marrow to Adipose Derived Stromal Cells in Clinical Cardiology Speaker: <i>Jens Kastrup, MD, PhD</i>
9:20 - 10:00 am	Free Paper Presentations
10:00 - 10:30 am	Coffee Break and Exhibits
10:30 - 11:40 am	Plenary Session 3 - ASC Characterization Moderators: <i>Bruce Bunnell, PhD & Keith March, MD, PhD</i>
10:30 am	Introductory Lecture: ASCs with Enhanced Anti-Inflammatory Properties Demonstrate Improved Outcomes in the Murine EAE Model of Human Multiple Sclerosis Speaker: <i>Bruce Bunnell, PhD</i>
10:50 - 11:40 am	Free Paper Presentations
11:40 am - 1:30 pm	Lunch and Exhibits
1:30 - 2:50 pm	Concurrent Free Paper Session 1 - Soft Tissue Translational - St. John's Room Moderators: <i>Kacey Marra, PhD & Brian Johnstone, PhD</i>
1:30 pm	Introductory Lecture: Depletion of White Adipocyte Progenitors Induces Beige Adipocyte Differentiation and Suppresses Obesity Development Speaker: <i>Mikhail Kolonin, PhD</i>
1:50 - 2:50 pm	Free Paper Presentations
1:30 - 2:50 pm	Concurrent Free Paper Session 2 - Supportive Technologies - Amsterdam Room Moderators: <i>Keith March, MD, PhD & Jan Wolf, MD</i>
1:30 pm	Introductory Lecture: Three-Dimensional Bioprinting of Islet and Adipose Stromal Vascular Fraction Containing Spheroids Speaker: <i>Stuart K. Williams, PhD</i>
1:50 - 2:50 pm	Free Paper Presentations
1:30 - 2:50 pm	Concurrent Free Paper Session 3 - Wound Healing - Grand Ballroom Moderators: <i>Julie Fradette, PhD & Sydney Coleman, MD</i>
1:30 pm	Introductory Lecture: Differential Response of Human Adipose Tissue-Derived Mesenchymal Stem Cells, Dermal Fibroblasts and Keratinocytes to Burn Wound Exudates: Potential Role of Skin Specific Chemokine Ccl27 Speaker: <i>Lenie J. van den Broek, MS</i>
1:50 - 2:50 pm	Free Paper Presentations
2:50 - 4:00 pm	Concurrent Free Paper Session 4 - ASCs in Angiogenesis and Vasculogenesis: Basic Research - St. John's Room Moderators: <i>Dmitry Traktuev, PhD & Stuart Williams, PhD</i>
2:50 pm	Introductory Lecture: Vascular Actions and Plasticity of Perivascular Adipose Tissue Speaker: <i>Etto Eringa, PhD</i>
3:10 - 4:00 pm	Free Paper Presentations
2:50 - 4:00 pm	Concurrent Free Paper Session 5 - Soft Tissue Basic - Amsterdam Room Moderators: <i>Bruce Bunnell, PhD & Petra Bauer-Kreisel, PhD</i>
2:50 pm	Introductory Lecture: Distinguishing Adipose 'Stromal' Versus 'Stem' Cells In Vivo Utilizing 3-Dimensional Silk Scaffolds Speaker: <i>Jeffrey Gimble, MD, PhD</i>
3:10 - 4:00 pm	Free Paper Presentations



2:50 - 4:00 pm

Concurrent Free Paper Session 6 - Skeletal Tissues I - Grand Ballroom

Moderators: *Jenneke Klein-Nulend, PhD & Ivan Martin, PhD*

2:50 pm

Introductory Lecture: Regeneration of Damaged Intervertebral Discs by a Newly Designed Hydrogel System in Combination with AMSC in the Ovine Model

Speaker: *Hans J. Meisel, MD*

3:10 - 4:00 pm

Free Paper Presentations

4:00 - 4:30 pm

Coffee Break and Exhibits

4:30 - 6:00 pm

Concurrent Panel Discussion 1 - St. John's Room

Animal Models: Sense and Non-Sense of Animal Models and Bioreactors

Chair: *Theo Smit, PhD*

4:30 - 6:00 pm

Concurrent Panel Discussion 2 - Grand Ballroom

3D Bioprinting: Merging Cell and Biomaterial Technologies

Chair: *Jan Wolf, MD*

6:00 - 7:00 pm

Welcome Reception

Wintergarden - NH Grand Hotel Krasnapolsky

Saturday, November 15, 2014

8:00 - 8:05 am

Introductory Remarks - Marco Helder, PhD

8:05 - 8:50 am

Invited Speaker

Adipose Stem Cells in Cranio-Maxillofacial Reconstruction: Present Art and Future Direction

Speaker: *Györgi Sandor, MD, PhD*

8:50 - 9:40 am

Plenary Session 4 - Industry Innovations

Moderators: *Ivan Martin, PhD & Richard A. D'Amico, MD*

Free Paper Presentations

9:40 - 10:00 am

Plenary Lecture 3 - Skeletal Tissues Research

A Phase I Clinical Trial Evaluating a One-Step Surgical Procedure for Maxillary Bone Augmentation with Adipose Stem Cells and Calcium Phosphate Scaffolds

Speaker: *Marco Helder, PhD*

10:00 - 10:30 am

Coffee Break and Exhibits

10:30 am - 12:00 pm

Plenary Session 5 - Clinical Soft Tissue Research II

Moderators: *Ramon Llull, MD, PhD & Adam Katz, MD, FACS*

10:30 am

Introductory Lecture: To Collaborate, or Not to Collaborate? Importance and Challenges of Collaborations from an Academic Surgeon-Scientist Perspective

Speaker: *Adam Katz, MD, FACS*

10:50 am - 12:00 pm

Free Paper Presentations

12:00 - 2:00 pm

Lunch and Exhibits

2:00 - 2:20 pm

Plenary Lecture 4 - Skeletal Tissues Research

Engineered Osteogenic and Vasculogenic Grafts Using Adipose Derived Cells: From Fundamental Research to a Clinical Trial

Speaker: *Ivan Martin, PhD*



2:20 - 2:45 pm	Plenary Lecture 5 - Cardiac Tissue Research The Role of Adipose Derived Stem Cells in Cardiac Disease Speaker: <i>Hans Niessen, MD, PhD</i>
2:45 - 3:45 pm	Concurrent Free Paper Session 7 - Soft Tissue Basic - St. John's Room Moderators: <i>Jeffrey Gimble, MD, PhD & Kacey Marra, PhD</i>
2:45 pm	Introductory Lecture: Arteriogenesis and Inflammatory Cell Recruitment in a Murine Flap Delay Model Speaker: <i>Scott A. Seaman, MS</i>
3:05 - 3:45 pm	Free Paper Presentations
2:45 - 3:45 pm	Concurrent Free Paper Session 8 - ASCs in Angiogenesis and Vasculogenesis: Translational Research - Amsterdam Room Moderators: <i>Dmitry Traktuev, PhD & Brian Johnstone, PhD</i>
2:45 pm	Introductory Lecture: Xenotransplantation of Human Adipose Stromal Cells into Immunocompetent Rats as well as Mice, Significantly Reduces Inflammatory Markers and M1/M2 Macrophage Ratios in Cerulein-Induced Acute Pancreatitis Speaker: <i>Pamela I. Rogers, RLatg</i>
3:05 - 3:45 pm	Free Paper Presentations
2:45 - 3:45 pm	Concurrent Free Paper Session 9 - Soft Tissue Clinical - Grand Ballroom Moderators: <i>Ramon Lull, MD, PhD & J. Peter Rubin, MD, FACS</i>
2:45 pm	Introductory Lecture: Hair Follicle Stimulation by Stromal Vascular Fraction Enhanced Adipose Transplantation Speaker: <i>Eric J. Daniels, MD</i>
3:05 - 3:45 pm	Free Paper Presentations
3:45 - 4:15 pm	Coffee Break and Exhibits
4:15 - 5:15 pm	Concurrent Free Paper Session 10 - Soft Tissue Basic - St. John's Room Moderators: <i>Jeffrey Gimble, MD, PhD & Petra Bauer-Kreisel, PhD</i>
4:15 pm	Introductory Lecture: Qualitative and Quantitative Differences of Adipose Tissue-Derived Stromal Cells From Superficial and Deep Subcutaneous Lipoaspirates: A Matter of Fat Speaker: <i>Wanda Lattanzi, MD, PhD</i>
4:35 - 5:15 pm	Free Paper Presentations
4:15 - 5:25 pm	Concurrent Free Paper Session 11 - Skeletal Tissues II - Amsterdam Room Moderators: <i>Hans Jorg Meisel, MD, PhD & Jenneke Klein-Nulend, PhD</i>
4:15 pm	Introductory Lecture: Cytokines and Growth Factors, Present at the Site of Implantation of a Tissue Engineering Construct, Affect Osteogenic Differentiation of Adipose Tissue Derived Mesenchymal Stem Cells Speaker: <i>Astrid D. Bakker, PhD</i>
4:35 - 5:25 pm	Free Paper Presentations
4:15 - 5:25 pm	Concurrent Free Paper Session 12 - Soft Tissue Clinical - Grand Ballroom Moderators: <i>Etto Eringa, PhD & Susan Gibbs, PhD</i>
4:15 pm	Introductory Lecture: Preparation of SVF from Lipoaspirate Infranatant Speaker: <i>Robert E. Bowen, MD</i>
4:35 - 5:25 pm	Free Paper Presentations
7:00 pm	A Taste of Amsterdam - (Tickets required) <i>St. Olof Chapel - Barbizon Palace</i>



Sunday, November 16, 2014

8:00 - 8:05 am	Introductory Remarks - <i>Marco Helder, PhD</i>
8:05 - 8:35 am	Plenary Lecture 6 - Clinical Skeletal Tissue Research ADIPOA: MSC Based Therapy for Severe Osteoarthritis of the Knee. A Phase I Dose Escalation Trial Speaker: <i>Christian Jorgensen, MD, PhD</i>
8:35 - 9:45 am	Plenary Session 6 - Basic Cardiac and Vasculogenesis Research Moderators: <i>Paul A. J. Krijnen, PhD & Hans W. M. Niessen, MD, PhD</i>
8:35 am	Introductory Lecture: Adipose-Derived Stromal Cells (ADSC): the Good, the Bad and the Ugly in Myocardial Remodeling and Repair Speaker: <i>Martin C. Harmsen, PhD</i>
8:55 - 9:45 am	Free Paper Presentations
9:45 - 10:15 am	Coffee Break and Exhibits
10:15 - 10:35 am	Plenary Session 7 - Translational Soft Tissue Research Moderators: <i>Julie Fradette, PhD & Adam Katz, MD, FACS</i>
	Introductory Lecture: Enhancing Wound Healing of Full-Thickness Murine Skin Defects: Effects of Tissue-Engineered Biological Dressings Based on ASCs Speaker: <i>Julie Fradette, PhD</i>
10:35 - 11:45 am	Free Paper Presentations
11:45 am	Concluding Remarks and Farewell - <i>Marco Helder, PhD</i>





NOTES



NOTES



PROGRAM SCHEDULE

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

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Thursday, November 13, 2014

11:00 am - 6:00 pm	Registration
1:00 - 1:15 pm	Welcome Remarks Marco Helder, PhD - IFATS President
1:15 - 1:45 pm	Keynote Speaker 1 Developments in Clinical Fat Grafting Sydney Coleman, MD
1:45 - 2:15 pm	Keynote Speaker 2 Healing Parenchyma with Mesenchyma; A New Paradigm in Cell Surgery Ramon Llull, MD, PhD
2:15 - 3:15 pm	Panel Discussion - Clinical Innovations in Adipose Therapies Chair: J. Peter Rubin, MD, FACS Panelists: Prof. Valerio Cervelli - <i>The Use of Stromal Vascular Fraction, Platelet Rich Plasma and Insulin in Soft Tissue Defects</i> Kotaro Yoshimura, MD - <i>Our Strategy of Fat Grafting for Breast Augmentation and Reconstruction</i> J. Peter Rubin, MD, PhD - <i>Controversies in Clinical Use of Fat Grafting and Stem Cells</i>
3:15 - 3:45 pm	Coffee Break and Exhibits
3:45 - 5:10 pm	Plenary Session 1: Clinical Soft Tissue Research I Moderators: Sydney Coleman, MD & Jenneke Klein-Nulend, PhD
3:45 pm	1 Introductory Lecture: ADIPOSE BASED THERAPIES FOR CRANIOFACIAL AND LIMB RECONSTRUCTION Presenter: J. Peter Rubin, MD, FACS Affiliation: University of Pittsburgh Authors: Rubin JP
4:05 pm	Free Paper Presentations 2 ADIPOSE DERIVED STEM CELL THERAPY FOR THE TREATMENT OF REFRACTARY ERECTILE DYSFUNCTION Presenter: Alvaro H. Skupin, MD Affiliation: Mother Stem Institute Inc. Authors: Skupin AH, Hernandez-Serrano R, Alvarez N, Zevallos-Palma B
4:15 pm	3 USE OF AUTOLOGOUS ADIPOSE-DERIVED STROMAL VASCULAR FRACTION TO TREAT OSTEOARTHRITIS OF THE KNEE; 2 INDEPENDENT STUDIES USING SAME METHODS Presenter: William Cimino, PhD Affiliation: The GID Group Authors: Cimino W, Garza J, Santa Mariad D, Palomera T, Dumanian G, Dos-Anjos S, Fodor P, Paulseth S
4:25 pm	4 AN UPDATED EVIDENCE-BASED REVIEW OF ADIPOSE STEM CELL THERAPY IN CANCER RECONSTRUCTION Presenter: Michael Alperovich, MD, MSc Affiliation: New York University Langone Medical Center Authors: Alperovich M, Lee Zh, Chiu ES
NOT PRESENTED	
4:35 pm	5 STEM CELL ENRICHED FAT INJECTIONS TO THE BREAST: 5 YEARS EXPERIENCE AND THE EVOLUTION OF OUR TECHNIQUE Presenter: Tunc K. Tiriyaki, MD Affiliation: Cellest Plastic Surgery Clinic Authors: Tiriyaki TK, Isil E, Aksungur E, Tiriyaki D, Findikli N



4:45 pm

6

FIRST LONG TERM RESULTS OF LARGE VOLUME FAT GRAFTING BY BEAULI PROTOCOL

Presenter: Klaus Ueberreiter, MD

Affiliation: Park-Klinik Birkenwerder

Author: Ueberreiter K

4:55 pm

7

FEASIBILITY STUDY OF RENEVIA, A RESORBABLE MATRIX OR THE DELIVERY OF AUTOLOGOUS ADIPOSE DERIVED CELLS

Presenter: Aina Soler Mieras, MD

Affiliation: Stem Center

Authors: Soler Mieras A, Furr DA, Matas-Palau A, Erickson IE, Dos Anjos Vilaboa S, Tew WP, Lull R

5:10 - 5:35 pm

Plenary Lecture 1: Soft Tissue Research

Transdifferentiation of ADSC into Motorneuron-like Cells for Cell Replacement Therapy of Spinal Cord Injury

Presenter: Jun Xu, MD, FACS

Affiliation: Tongji University School of Medicine

Authors: Gao S, Chen X, Gao Z, Zhu H, Xu J

5:35 - 6:00 pm

Plenary Lecture 2: Skeletal Tissue Research

Treating Scleroderma of the Face and Hands

Presenter: Guy Magalon, MD, PhD

Affiliation: APHM

Author: Magalon G

6:00 pm

Adjourn





Friday, November 14, 2014

8:00 - 8:15 am

Introductory Remarks

Marco Helder, PhD

8:15 - 9:00 am

INVITED SPEAKER

LGR5 Stem Cells in Self-Renewal and Cancer

Hans Clevers, MD, PhD

9:00 - 9:20 am

Plenary Session 2 - Clinical Cardiac Research

Moderators: *Paul A. J. Krijnen, PhD & Hans W. M. Niessen, MD, PhD*

9:00 am

8

Introductory Lecture: FROM BONE MARROW TO ADIPOSE DERIVED STROMAL CELLS IN CLINICAL CARDIOLOGY

Presenter: Jens Kastrup, MD, PhD

Affiliation: Rigshospitalet

Author: Kastrup J

Free Paper Presentations

9:20 am

9

INTRA-MUSCULAR INJECTION OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF THROMBOANGIITIS OBLITERANS: A PROOF-OF-CONCEPT CLINICAL TRIAL

Presenter: EuiCheol Jeong, MD, PhD

Affiliation: SMG_SNU Boramae Medical Center

Authors: Jeong EC, Kim HS, Yoon KS, Seo JB, Jung IM, Baek SH

9:30 am

10

DEVELOPMENT OF RECOMBINANT COLLAGEN-BASED VEHICLES FOR ADSC DELIVERY AND RETENTION

Presenter: Mojtaba Parvizi, DVM

Affiliation: UMCU

Authors: Parvizi M, Plantinga JA, Van Spreuwel Goossens C, Van Dongen S, Kluijtmans SG, Harmsen MC

9:40 am

11

TOPICAL APPLICATION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS AMELIORATED RAT RENAL ISCHEMIA-REPERFUSION INJURY

Presenter: Ping Kuen Lam, PhD

Affiliation: Chinese University of Hong Kong

Authors: Lam PK, Lo A, Tong C, Kwong T, Ching D, Lau H, Lai P, Ng CF

9:50 am

12

ADIPOSE STEM CELLS (ASC) DELIVERED INTRAVENOUSLY ARE TARGETED TO THE ENDOTOXIN-INJURED LUNG

Presenter: Natalia V. Bogatcheva, PhD

Affiliation: Indiana University

Authors: Bogatcheva NV, Lu H, Poirier C, Traktuev DO, Cook T, Merfeld-Clauss S, Petrache I, March KL

10:00 - 10:30 am

Coffee Break and Exhibits



10:30 - 11:00 am

Plenary Session 3 - ASC Characterization

Moderators: *Bruce Bunnell, PhD & Keith March, MD, PhD*

10:30 am

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Introductory Lecture: ASCS WITH ENHANCED ANTI-INFLAMMATORY PROPERTIES DEMONSTRATE IMPROVED OUTCOMES IN THE MURINE EAE MODEL OF HUMAN MULTIPLE SCLEROSIS

Presenter: Bruce Bunnell, PhD

Affiliation: Tulane University School of Medicine

Authors: Bunnell B, Zhang X, Betancourt AM, Bowles A, Gimble JM

Free Paper Presentations

10:50 am

14

TIMING AND FREQUENCY OF ADIPOSE-DERIVED STEM CELL ADMINISTRATION FOR IMMUNOMODULATION IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Presenter: Riccardo Schweizer, MD

Affiliation: University Hospital Zurich

Authors: Plock JA, Schnider JT, Zhang W, Schweizer R, Tsuji W, Kostereva N, Fanzio P, Ravuri S, Solari M, Marra K, Gorantla VS

11:00 am

15

POTENTIAL MESENCHYMAL STEM CELLS SUBPOPULATION IDENTIFIED FROM ADIPOSE DERIVED STROMAL CELLS

Presenter: Wei Jing, MD

Affiliation: State Key Laboratory of Oral Diseases & National Engineering Laboratory for Oral Regenerative Medicine Sichuan University

Authors: Jing W, Xiao JG, Tian WD

11:10 am

16

ALTERATIONS OF GENE EXPRESSION AND PROTEINS SYNTHESIS IN CO-CULTURED ADIPOSE TISSUE-DERIVED STEM CELLS AND SQUAMOUS CELL-CARCINOMA CELLS

Presenter: Eva Koellensperger, MD

Affiliation: Clinic for Plastic and Reconstructive Surgery Aesthetic & Preventive Medicine at Heidelberg University Hospital

Authors: Koellensperger E, Gramley F, Preisner F, Germann G, Leimer U

11:20 am

17

THE MOLECULAR MECHANISM UNDERLYING THE PROLIFERATING AND PRECONDITIONING EFFECT OF VITAMIN C ON ADIPOSE-DERIVED STEM CELLS

Presenter: Seung Yong Song, MD, PhD

Affiliation: Yonsei University College of Medicine

Authors: Song SY, Sung JH

11:30 am

18

WITHDRAWN

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

Presenter: Dmitry O. Traktuev, PhD

Affiliation: Indiana University

Authors: Traktuev DO, Merfeld-Clauss S, Lupov IP, Cook T, March KL

11:30 am

NEW PRESENTATION

THE ROLE OF DONOR BONE MARROW STEM CELL NICHES IN VASCULARIZED COMPOSITE ALLOTRANSPLANT TOLERANCE

Presenter: Xin Xiao Zheng, MD

Affiliation: Tongji University, Shanghai East Hospital

Authors: Zheng XX

11:40 - 1:30 pm

Lunch and Exhibits



1:30 - 2:50 pm

Concurrent Free Paper Session 1 - Soft Tissue Translational - St. John's Room

Moderators: Kacey Marra, PhD & Brian Johnstone, PhD

1:30 pm

19

Introductory Lecture: DEPLETION OF WHITE ADIPOCYTE PROGENITORS INDUCES BEIGE ADIPOCYTE DIFFERENTIATION AND SUPPRESSES OBESITY DEVELOPMENT

Presenter: Mikhail Kolonin, PhD

Affiliation: Center For Metabolic and Degenerative Diseases

Authors: Daquinag AC, Tseng C, Salameh A, Zhang Y, Amaya-Manzanares F, Dabbín A, Florez F, Xu Y, Tong Q, Kolonin MG

Free Paper Presentations

1:50 pm

20

CHITOSAN SCAFFOLD CELLULARIZED BY STROMAL VASCULAR FRACTION FOR COLORECTAL TISSUE ENGINEERING IN SWINE MODEL: THE LAST STEP BEFORE CLINICAL APPLICATION

Presenter: Quentin Denost, MD, PhD

Affiliation: INSERM Bioingenierie Tissulaire

Authors: Denost Q, Buscail E, Pontallier A, Bareille R, Montembault A, Delmond S, David L, Bordenave L

2:00 pm

21

FAT GRAFTING SITE PREPARATION WITH EXTERNAL VOLUME EXPANSION IN CHRONIC SKIN FIBROSIS AFTER RADIATION EXPOSURE

Presenter: Jorge Lujan-Hernandez, MD

Affiliation: University of Massachusetts Medical School

Authors: Lujan-Hernandez J, Chin MS, Babchenko OO, Bannón E, Ignótz R, Lo YC, Fitzgerald TJ, Lalikos JF

2:10 pm

22

ADIPOSE-DERIVED STEM CELLS: A NOVEL, SHORTENED ISOLATION PROTOCOL YIELDING MULTIPOTENT CELLS FROM FAT

Presenter: Anna Wilson, MBChB, MRCS, MSc

Affiliation: University College London

Authors: Wilson A, Gareta-Garcia E, Butler P, Seifalian A

2:20 pm

23

THE PARACRINE EFFECT OF MESENCHYMAL STEM CELLS RESTORED HEARING IN AUTOIMMUNE SENSORINEURAL HEARING LOSS

Presenter: Tai June Yoo, MD, PhD, MBA

Affiliation: StemGen Therapeutic LLC

Authors: Yoo T, Zhou B, Du X, Cheng W, Zhou Y, Di Girolamo S, Barbieri M

2:30 pm

24

SUSTAINABLE FAT GRAFTING: OPTIMIZING FAT GRAFTING IN AN IN VIVO TISSUE ENGINEERING CHAMBER MODEL

Presenter: Heidi Debels, MD

Affiliation: UZ Brussel Belgium

Authors: Debels H, Han XL, Palmer J, Morrison W, Hamdi M, Abberton K

2:40 pm

25

SAFETY OF PERFORMING MANUAL ADIPOSE STEM CELL SEPARATION AT THE POINT OF CARE

Presenter: Reef Hardy, MD

Affiliation: Cedars Sinai Medical Center

Authors: Aronowitz JA, Hardy R, Hakakian CH



1:30 - 2:50 pm

Concurrent Free Paper Session 2 - Supportive Technologies - Amsterdam Room

Moderators: Keith March, MD & Jan Wolf, MD

1:30 pm

26

Introductory Lecture: THREE-DIMENSIONAL BIOPRINTING OF ISLET AND ADIPOSE STROMAL VASCULAR FRACTION CONTAINING SPHEROIDS

Speaker: Stuart K. Williams, PhD

Affiliation: University of Louisville

Authors: Williams SK, Touroo JS, Hoying JB, Hughes MG

Free Paper Presentations

1:50 pm

27

HUMAN ADIPOSE SUBSTITUTES ENGINEERED FROM ASCS: PERFORMANCE ANALYSIS OF GRAFTED TISSUES USING MAGNETIC RESONANCE IMAGING

Presenter: Maryse Proulx, MSc

Affiliation: Centre de Recherche en Organogenese Experimentale del Universite Laval

Authors: Proulx M, Aubin K, Lagueux J, Fortin MA, Fradette J

2:00 pm

28

CLINICALLY-FEASIBLE LARGE-SCALE MANUFACTURING OF ADIPOSE TISSUE-DERIVED STROMAL CELLS USING QUANTUM CELL EXPANSION SYSTEM AND HUMAN PLATELET LYSATE

Presenter: Mandana Haack-Soerensen, PhD

Affiliation: Rigshospitalet

Authors: Haack-Soerensen M, Brorsen SK, Follin B, Juhl M, Soendergaard RH, Kirchhoff M, Kastrup J, Ekblond A

2:10 pm

29

IMAGING OF HUMAN ADIPOSE-DERIVED STEM CELLS (ADSC) WITH ATOMIC FORCE MICROSCOPY (AFM): ASSESSING MORPHOLOGY AND SURFACE TOPOGRAPHY OF DIFFERENTIATED ADIPOCYTES

Presenter: Naghmeh Naderi, MSc

Affiliation: UCL

Authors: Naderi N, Griffin M, Francis W, Wilde C, Haque T, Francis L, Xia Z, Thornton CA, Mosahebi A, Seifalian AS, Whitaker IS

WITHDRAWN

2:20 pm

30

FIRST EXPERIENCE USING CONTRAST-ENHANCED ULTRASOUND (CEUS) TO EVALUATE VASCULARIZATION AND RESORPTION OF GRAFTED FAT

Presenter: Maria Wiedner, MD

Affiliation: Medical University Graz

Authors: Wiedner M, Weinke R, Kamolz LP, Parvizi D

2:30 pm

31

DEVELOPMENT OF A PHYSIOLOGICALLY RELEVANT CULTURE ENVIRONMENT OF TYPE II DIABETES MELLITUS FOR ADIPOCYTE CULTURE WITHIN A HOLLOW FIBER, THREE-DIMENSIONAL, DYNAMIC PERFUSION BIOREACTOR

Presenter: Danielle M. Minter, BS

Affiliation: University of Pittsburgh

Authors: Minter DM, Rubin JP, Gerlach JC, Marra KG

2:40 pm

32

ULTRASOUND EVALUATION OF ADIPOSE AND FAT GRAFTED TISSUE- A COST EFFECTIVE, POINT OF CARE TECHNOLOGY

Presenter: Ricardo L. Rodriguez, MD

Affiliation: Clinical Instructor Johns Hopkins

Authors: Rodriguez RL, Markelov A, Bhavani S



1:30 - 2:50 pm

Concurrent Free Paper Session 3 - Wound Healing - Grand Ballroom

Moderators: Julie Fradette, PhD & Sydney Coleman, MD

1:30 pm

33

Introductory Lecture: DIFFERENTIAL RESPONSE OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS, DERMAL FIBROBLASTS AND KERATINOCYTES TO BURN WOUND EXUDATES: POTENTIAL ROLE OF SKIN SPECIFIC CHEMOKINE CCL27

Speaker: Lenie J. van den Broek, MS

Affiliation: VU University Medical Center

Authors: van den Broek LJ, Kroeze KL, Waaijman T, Breetveld M, Sampat-Sardjoeppersad SC, Niessen FB, Middelkoop E, Scheper RJ, Gibbs S

Free Paper Presentations

1:50 pm

34

CELL SHEETS DERIVED FROM ADIPOSE-DERIVED STEM CELLS ACCELERATED CUTANEOUS WOUND HEALING

Presenter: Naichen Cheng, MD, PhD

Affiliation: National Taiwan University Hospital

Authors: Cheng N, Yu J

2:00 pm

35

FACTORS THAT MAY INFLUENCE NUMBER AND VIABILITY OF EXTRACTED ADRCS PER GRAM OF TISSUE

Presenter: Katarina Andjelkov, MD, PhD (Marcos Sforza, MD)

Affiliation: Private Clinic

Authors: Andelkov K, Sforza M, Zaccheddu R

2:10 pm

36

LOCALLY ADMINISTERED ADIPOSE-DERIVED STROMAL CELLS ACCELERATE PRESSURE ULCER WOUND HEALING IN YOUNG AND OLD MICE

Presenter: Amy L. Strong, PhD, MPH

Affiliation: Tulane University School of Medicine

Authors: Strong AL, Bowles AC, Maccrimmon CP, Frazier TP, Lee SJ, Katz AJ, Gawronska-Kozak B, Bunnell BA, Gimble JM

2:20 pm

37

ADIPOSE TISSUE-DERIVED STROMAL CELLS INHIBIT TGF-BETA₁-INDUCED DIFFERENTIATION OF HUMAN DERMAL FIBROBLASTS AND KELOID SCAR-DERIVED FIBROBLASTS IN A PARACRINE FASHION

Presenter: Maroesjka Spiekman, BS

Affiliation: University Medical Center Groningen

Authors: Spiekman M, Przybyt E, Plantinga JA, Gibbs S, Van Der Lei B, Harmsen MC

2:30 pm

38

EFFECTS OF LL-37-TREATED ASC CONDITIONED MEDIA ON THE WOUND HEALING OF HUMAN FIBROBLASTS

Presenter: Eunjung Yang, MD

Affiliation: Cheil General Hospital

Authors: Yang E, Yang YH, Yang YJ, Bang SI

2:40 pm

39

ADIPOSE DERIVED STEM CELLS AND SKIN INJURIES

Presenter: Nada M. Alaaeddine, PhD

Affiliation: University of St. Joseph

Authors: Alaaeddine NM, Saliba N, Atat O, Tarabey B, Hilal G, Hashem H



2:50 - 3:50 pm

Concurrent Free Paper Session 4 - ASCs in Angiogenesis and Vasculogenesis: Basic Research - St. John's Room

Moderators: *Dmitry Traktuev, PhD & Stuart Williams, PhD*

2:50 pm

40

Introductory Lecture: VASCULAR ACTIONS AND PLASTICITY OF PERIVASCULAR ADIPOSE TISSUE

Presenter: Etto C. Eringa, PhD

Affiliation: VU University Medical Centre

Authors: Eringa EC

Free Paper Presentations

3:10 pm

41

ADIPOSE-DERIVED STEM CELLS MITIGATE AORTIC ANEURYSM EXPANSION AND EXCESSIVE AORTIC INFLAMMATION IN AN ELASTASE-PERFUSED MURINE ABDOMINAL AORTIC ANEURYSM MODEL

Presenter: Keith March, MD, PhD (Jie Xie, MD)

Affiliation: Indiana University School of Medicine

Authors: Xie J, Feng D, Cook TG, Njoku VC, Babbey CM, March KL, Murphy MP

Changed Presenter

3:20 pm

42

ENGINEERING VASCULARIZED ADIPOSE TISSUE USING THE STROMAL VASCULAR FRACTION AND FIBRIN HYDROGELS

Presenter: Katharina Wittmann, MSc

Affiliation: University of Wuerzburg

Authors: Wittmann K, Dietl S, Berberich O, Storck K, Blunk T, Bauer-Kreisel P

3:30 pm

43

ASC DIFFERENTIATION TOWARDS SMOOTH MUSCLE CELL PHENOTYPE DIMINISHES THEIR VASCULOGENIC ACTIVITY THROUGH INDUCTION OF ACTIVIN A SECRETION

Presenter: Dmitry O. Traktuev, PhD (Keith March, MD, PhD)

Affiliation: Indiana University

Authors: Merfeld-Clauss S, Lease B, Lu H, March KL, Traktuev DO

Changed Presenter

3:40 pm

44

EFFECTS OF DONORS CIGARETTE SMOKING ON VASCULOGENIC ACTIVITY OF ADIPOSE STEM CELLS

Presenter: Daria Barwinska, BS

Affiliation: Indiana University School of Medicine

Authors: Barwinska D, Traktuev DO, Cook T, Merfeld Clauss S, Van Demark M, Petrache I, March KL

2:50 - 4:00 pm

Concurrent Free Paper Session 5 - Soft Tissue Basic - Amsterdam Room

Moderators: *Bruce Bunnell, PhD & Petra Bauer-Kreisel, PhD*

2:50 pm

45

Introductory Lecture: DISTINGUISHING ADIPOSE 'STROMAL' VERSUS 'STEM' CELLS IN VIVO UTILIZING 3-DIMENSIONAL SILK SCAFFOLDS

Presenter: Jeffrey Gimble, MD, PhD

Affiliation: Tulane University

Authors: Bowles A, Lee S, Abbott R, Kaplan D, Wang M, Strong A, Brown Q, Bunnell B, Gimble J

Free Paper Presentations

3:10 pm

46

COMPOSITION OF FATTY ACIDS IN HUMAN SUBCUTANEOUS ADIPOSE TISSUE

Presenter: Natalie I. Khramtsova, MA

Affiliation: Perm State Medical Academy

Authors: Khramtsova NI, Beskorovaynyi AV, Kotelev MS, Plaksin SA



3:20 pm

NEW Presenter

47

HOW MANY CELLS ARE THERE IN A GRAM OF LIPOASPIRATED ADIPOSE TISSUE?

Presenter: Severiano Dos Anjos Vilaboa Sr., MD (Ramon Llull, MD, PhD)

Affiliation: Stem Center SL

Authors: Dos Anjos Vilaboa Sr. S, Llull R

3:30 pm

WITHDRAWN

48

EFFECT OF NANOCOMPOSITE POLYMER FABRICATION ON PROLIFERATION AND MORPHOLOGY OF ADIPOSE DERIVED STEM CELLS

Presenter: Naghmeh Naderi, MSc

Affiliation: UCL

Authors: Naderi N, Kalaskar D, Whitaker IS, Mosahebi A, Thornton CA, Butler PE, Seifalian AM

3:40 pm

49

THREE-DIMENSIONAL FLOATING CULTURE OF ADIPOSE-DERIVED STROMAL CELLS (ASCS) IN NON-CROSS-LINKED HYALURONIC ACID (HA) GEL FOR PREPARATION OF THERAPEUTIC SPHEROIDS

Presenter: Jingwei Feng, BM

Affiliation: University of Tokyo School of Medicine

Authors: Feng J, Mineda K, Doi K, Kuno S, Kinoshita K, Kanayama K, Yoshimura K

3:50 pm

50

IS IT WORTH TO WASTE PART OF LIPOASPIRATE TO OBTAIN A GOOD FAT GRAFT?

Presenter: Severiano Dos Anjos Vilaboa Sr., MD

Affiliation: Stem Center SL

Authors: Dos Anjos Vilaboa Sr. S, Matas-Palau A, Llull R

2:50 - 4:00 pm

Concurrent Free Paper Session 6 - Skeletal Tissues I - Grand Ballroom

Moderators: *Jenneke Klein-Nulend, PhD & Ivan Martin, PhD*

2:50 pm

51

Introductory Lecture: REGENERATION OF DAMAGED INTERVERTEBRAL DISCS BY A NEWLY DESIGNED HYDROGEL SYSTEM IN COMBINATION WITH AMSC IN THE OVINE MODEL

Presenter: Hans J. Meisel, MD

Affiliation: BGKliniken Bergmannstrost

Authors: Friedmann A, Meisel HJ, Goehre F

Free Paper Presentations

3:10 pm

52

GENERATION OF A BONE ORGAN IN VIVO THROUGH ENDOCHONDRAL OSSIFICATION BY ADIPOSE-DERIVED STROMAL CELLS

Presenter: Arnaud Scherberich, PhD

Affiliation: University Hospital of Basel

Authors: Osinga R, di Maggio N, Allafi N, Barbero A, Schaefer DJ, Martin I, Scherberich A

3:20 pm

53

LUCIFERASE MEDIATED MONITORING OF ADIPOSE STEM CELLS IN THE GOAT INTERVERTEBRAL DISC

Presenter: Mirte Peeters, MSc

Affiliation: VU University Medical Centre

Authors: Peeters M, Van Rijn S, Vergroesen PP, Paul CP, Wurdinger T, Helder MN

3:30 pm

54

SYSTEMIC APPLICATION OF ADIPOSE DERIVED STEM CELLS ACCELERATES FUNCTIONAL PERIPHERAL NERVE REGENERATION

Presenter: Jonas Schnider, MD

Affiliation: University Hospital Zurich

Authors: Fanzio P, Tsuji W, Kostereva N, Schweizer R, Solari MG, Marra K, Plock JA, Gorantla VS



3:40 pm

55

THERAPEUTIC EFFECTS OF AUTOLOGOUS ADIPOSE DERIVED STEM CELL ON THE REGENERATION OF MUSCLE IN SARCOPENIA MODEL OF THE RAT

Presenter: Ji Ung Park, MD

Affiliation: Seoul National University Boramae Hospital

Authors: Park JU, Kwon ST, Hong JM

3:50 pm

56

SUPERCritical FLUID PROCESSED POROUS SCAFFOLDS SUPPORT THE ENDOTHELIAL DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS

Presenter: Sanna E. Pitkanen, MS

Affiliation: University of Tampere

Authors: Pitkanen SE, Kyllonen L, Paakinaho K, Ahola N, Kellomaki M, Miettinen S, Haimi S

4:00 - 4:30 pm

Coffee Break and Exhibits

4:30 - 6:00 pm

Concurrent Panel Discussion 1 - Grand Ballroom

EMA ATMP and FDA Regulatory Concepts: Regulatory Rationale, Biological Foundation, Translational Strategies and Industrial Impact

Moderators: J. Peter Rubin, MD & Ramon Llull, MD, PhD

Panelists: Hans Ovelgönne, PhD (Netherlands) - *CAT reflection paper on classification of advanced therapy medicinal products*

Jeff Gimble, MD, PhD (US) - *Exceptions for Homologous Use*

Valerio Cervelli, MD (Italy) - *On the concept of Product's "Homologous Use"*

J. Peter Rubin, MD (US) - *The FDA Perspective on Minimal Manipulation, Essential Function and Homologous Use*

4:30 - 6:00 pm

Concurrent Panel Discussion 2 - Amsterdam Room

Animal Models: Sense and Non-Sense of Animal Models and Bioreactors

Chair: Theo H. Smit, PhD

Panelists: Theo H. Smit, PhD (Netherlands) - *Why Animal Models?*

Ivan Martin, PhD (Switzerland) - *In Vitro 3D Stromal Tissue Models*

Hans Jorg Meisel, MD, PhD (Germany) - *Animal Models: Sense and Non-Sense of Animal Models and Bioreactors*

4:30 - 6:00 pm

Concurrent Panel Discussion 3 - St. John's Room

3D Bioprinting: Merging Cell and Biomaterial Technologies

Chair: Jan Wolff, MD

Panelists: Jan Wolff, MD (Netherlands) - *Medical 3D Printing*

Ernst Jan Bos, MD (Netherlands) - *Cell Printing Ear Cartilage for Burn Victims*

Gyorgi Sandor, MD, PhD (Finland) - *3D Bioprinting: Merging Cells and Biomaterial Technologies with Adipose-Derived Stem Cells*

6:00 - 7:00 pm

Welcome Reception

Wintergarden - NH Grand Hotel Krasnapolsky



Saturday, November 15, 2014

8:00 - 8:05 am **Introductory Remarks - Marco Helder, PhD**

8:05 - 8:50 am **Invited Speaker**

Adipose Stem Cells in Cranio-Maxillofacial Reconstruction: Present Art and Future Direction
Speaker: Györgi Sandor, MD, PhD

8:50 - 9:40 am **Plenary Session 4 - Industry Innovations**

Moderators: Ivan Martin, PhD & Richard A. D'Amico, MD

8:50 am

57
Lifecell Presentation: Innovation in Fat Processing
Presenter: William W. Cimino, PhD (Ramon Llull, MD, PhD)

Change Presenter

9:00 am

58
Musculoskeletal Transplant Foundation: Advancements in Adipose Derived Scaffolds
Presenter: Evangelia Chnari, PhD (Richard D'Amico, MD)

NEW Presenter

9:10 am

59
PALL: POWER ASSISTED LIPOSUCTION AND LIPOFILLING
Presenter: Saad Dibo, MD
Affiliation: MA Clinic
Author: Dibo SA

9:20 am

60
MORSI SUSPENSION MASTOPEXY TECHNIQUE
Presenter: Adel Morsi, MBBCh, MS, FRACS
Affiliation: Cleopatra Plastic Surgery and The Alfred Hospital
Authors: Morsi A, Hsieh YH

9:30 am

61
CHARACTERIZATION OF AN INNOVATIVE NON-ENZYMATIC CLOSED SYSTEM FOR MINIMAL MANIPULATION OF LIPOSUCTED ADIPOSE TISSUE: TRANSLATING THE STROMAL VASCULAR FRACTION INTO REGENERATIVE MEDICINE APPLICATIONS
Presenter: Claudia Cicione, PhD
Affiliation: Universit Cattolica del Sacro Cuore
Authors: Cicione C, Barba M, Di Taranto G, Bernardini C, Sciarretta FV, Baranzini M, Isgro MA, Di Stasio E, Michetti F, Lattanzi W

9:40 - 10:00 am

Plenary Lecture 3: Skeletal Tissue Research

A Phase I Trial for Maxillary Bone Augmentation with Adipose Stem Cells and Calcium Phosphate Scaffolds; Evaluation of a One-Step Surgical Procedure
Presenter: Marco N. Helder, PhD
Affiliation: VU University Medical Center
Author: Helder MN

10:00 - 10:30 am

Coffee Break and Exhibits

10:30 - 11:00 am

Plenary Session 5 - Clinical Soft Tissue Research II

Moderators: Sydney Coleman, MD & Adam Katz, MD, FACS

10:30 am

62
Introductory Lecture: TO COLLABORATE, OR NOT TO COLLABORATE? IMPORTANCE AND CHALLENGES OF COLLABORATIONS FROM AN ACADEMIC SURGEON-SCIENTIST PERSPECTIVE
Presenter: Adam Katz, MD, FACS
Affiliation: University of Florida
Author: Katz A



Free Paper Presentations

10:50 am	<p>63 IS WATER-ASSISTED LIPOSUCTION A VIABLE TECHNOLOGY FOR FACE FAT GRAFTING? Presenter: Jeffrey A. Ditesheim, MD Affiliation: Private Plastic Surgery Practice Author: Ditesheim JA</p>
11:00 am	<p>64 FATE OF ADIPOSE-DERIVED STROMAL VASCULAR FRACTION CELLS AFTER CO-IMPLANTATION WITH FAT GRAFTS: EVIDENCE OF CELL SURVIVAL AND DIFFERENTIATION IN ISCHEMIC ADIPOSE TISSUE Presenter: Fu Su, MD Affiliation: Breast Plastic and Reconstructive Surgery Center\Plastic Surgery Hospital\Chinese Academy of Medical Sciences\Peking Union Medical College Authors: Su F, Jie L, Minqiang X, Qian W, Ran X, Yunzhou G</p>
11:10 am	<p>65 STEM CELL ENHANCED FAT GRAFTING OF THE BREAST: A SIDE BY SIDE TRIAL Presenter: Joel A. Aronowitz, MD Affiliation: Cedars Sinai Medical Center Authors: Aronowitz JA, Hakakian CH</p>
11:20 am	<p>66 AUTOLOGOUS FAT INJECTION IS A SAFE AND EFFICACE ALTERNATIVE IN THE THERAPEUTIC ALGORITHM OF LEAKAGE AROUND TRACHEOESOPAHGEAL PUNCTURE Presenter: Giovanni Almadori, MD Affiliation: Catholic University of Sacred Hearth Authors: Almadori G, Parrilla C, Almadori A, Paludetti G, Salgarello M</p>
NOT PRESENTED	
11:30 am	<p>67 THE UTILIZATION OF CELLULAR THERAPIES FOR THE MODULATION OF BURN SCARS Presenter: Mehmet Bozkurt, MD Affiliation: Dr Lutfi Kirdar Kartal Training and Research Hospital Authors: Bozkurt M, Guvercin E, Filinte GT, Sirinoglu H, Temiz G</p>
11:40 am	<p>68 A NEW PARADIGM SHIFT IN THE MANAGEMENT OF COCCYXODYNIA: A PRELIMINARY STUDY USING FAT TRANSFER Presenter: Zeeshan Ahmad, BSc (Hons), MB BS, MRCS Affiliation: UHCW Coventry Authors: Ahmad Z, Park AJ</p>
11:50 am	<p>69 MAXIMIZING THE MINI FACE LIFT: HOW DID FACIAL FAT TRANSFER CHANGE EVERYTHING Presenter: Renato Zaccheddu, MD Affiliation: Dola Park Hospital Authors: Sforza M, Andjelkov K, Zaccheddu R</p>
12:00 - 2:00 pm	Lunch and Exhibits
2:00 - 2:20 pm	<p>Plenary Lecture 4 - Skeletal Tissues Research Engineered Osteogenic and Vasculogenic Grafts Using Adipose Derived Cells: From Fundamental Research to a Clinical Trial Presenter: Ivan Martin, PhD Affiliation: University Hospital Basel Authors: Martin I, Saxer F, Jakob M, Schaefer DJ, Scherberich A</p>



2:20 - 2:45 pm

Plenary Lecture 5 - Cardiac Tissue Research

The Role of Adipose Derived Stem Cells in Cardiac Disease

Presenter: Hans Niessen, MD, PhD

Affiliation: VU University Medical Center

Author: Niessen H

2:45 - 3:45 pm

Concurrent Free Paper Session 7 - Soft Tissue Basic - St. John's Room

Moderators: *Jeffrey Gimble, MD, PhD & Kacey Marra, PhD*

2:45 pm

70

Introductory Lecture: ARTERIOGENESIS AND INFLAMMATORY CELL RECRUITMENT IN A MURINE FLAP DELAY MODEL

Presenter: Scott A. Seaman, BS

Affiliation: University of Virginia

Authors: Seaman SA, Cao Y, Peirce SM

Free Paper Presentations

3:05 pm

71

IN SEARCH OF THE IDEAL NERVE CONDUIT, SUPPLEMENTATION OF DECELLULARIZED NERVE ALLOGRAFTS WITH STEM CELLS DERIVED FROM TWO DIFFERENT SOURCES

Presenter: Rezarta Kapaj, MD

Affiliation: National Trauma Center

Authors: Kapaj R, Alhan D, Uysal C, Akgun H, Nisanci M, Kurt B, Isik S

3:15 pm

72

METHACRYLATED GELATIN AND MATURE ADIPOCYTES: PROMISING COMPONENTS FOR ADIPOSE TISSUE ENGINEERING

Presenter: Birgit Huber, MD

Affiliation: University of Stuttgart

Authors: Huber B, Hoch E, Tovar G, Borchers K, Kluger PJ

3:25 pm

WITHDRAWN

73

TISSUE ENGINEERED BROWN ADIPOCYTE CONSTRUCTS

Presenter: Francisco Silva, BS

Affiliation: BioRestorative Therapies

Authors: Silva F, Vargas V, Holt D, Boudina S, Cho S, Atkinson D, Yockman J, Bull D, Patel A

3:35 pm

74

ADIPOSE STROMAL VASCULAR FRACTION; ARE MECHANICAL METHODS OF ISOLATION AS EFFECTIVE AS THE TRADITIONAL ENZYMATIC DIGESTION?

Presenter: Alexandra Conde-Green, MD

Affiliation: Johns Hopkins Bayview Medical Center & University of Maryland Medical Center

Authors: Conde-Green A, Rodriguez RL, Slezak S, Singh DP, Goldberg NH, Holton III L, Mclenithan J

2:45 - 3:45 pm

Concurrent Free Paper Session 8 - ASCs in Angiogenesis and Vasculogenesis: Translational Research - Amsterdam Room

Moderators: *Dmitry Traktuev, PhD & Brian Johnstone, PhD*

2:45 pm

75

Introductory Lecture: XENOTRANSPLANTATION OF HUMAN ADIPOSE STROMAL CELLS INTO IMMUNOCOMPETENT RATS AS WELL AS MICE, SIGNIFICANTLY REDUCES INFLAMMATORY MARKERS AND M1/M2 MACROPHAGE RATIOS IN CERULEIN-INDUCED ACUTE PANCREATITIS

Presenter: Pamela I. Rogers, RLATg

Affiliation: Indiana University

Authors: Rogers PI, Maxwell T, Serezani H, Feng D, Dey D, Gangaraju R, Murphy M, Zyromski N, Babbey C, March KL



Free Paper Presentations

3:05 pm 76
INTERACTION OF GELATION KINETICS AND NEEDLE SIZE ON DELIVERY OF SVF AND ADSCS USING RENEVIA: EFFECTS ON CELL SURVIVAL
 Presenter: Isaac E. Erickson, PhD
 Affiliation: Stem Center SL
 Authors: Matas-Palau A, Dos Anjos Vilaboa S, Onorato MV, Llull R, Zarembinski T, Erickson IE

3:15 pm 77
ROLE OF EXOSOMES IN ANGIOGENIC POTENTIAL OF HASC-DERIVED CONDITIONED MEDIUM
 Presenter: Yameena T. Jawed, MD
 Affiliation: Indiana University School of Medicine
 Authors: Jawed YT, Traktuev D, March KL

3:25 pm 78
PLATELET-RICH PLASMA (PRP) AUGMENTS THE PRO-ANGIOGENIC CAPACITY OF ADIPOSE TISSUE-DERIVED STROMAL CELLS (ADSC)
 Presenter: JCN Willemsen, MD
 Affiliation: UMCG Groningen
 Authors: Willemsen JCN, Van Der Lei B, Harmsen MC

3:35 pm 79
CHARACTERISATION OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS WITH ENHANCED ANGIOGENIC AND ADIPOGENIC PROPERTIES
 Presenter: Anne Therese Lauvrud, MD
 Affiliation: Norrland University Hospital Umeaa University
 Authors: Lauvrud AT, Wiberg M, Kingham PJ

2:45 - 3:45 pm **Concurrent Free Paper Session 9 - Soft Tissue Clinical - Grand Ballroom**
 Moderators: William Futrell, MD & J. Peter Rubin, MD, FACS

2:45 pm 80
Introductory Lecture: HAIR FOLLICLE STIMULATION BY STROMAL VASCULAR FRACTION ENHANCED ADIPOSE TRANSPLANTATION
 Speaker: Eric J. Daniels, MD
 Affiliation: Kerastem Technologies LLC
 Authors: Sforza M, Ball E, Perez-Meza D, Ziering C, Krishnan G, Daniels E

Free Paper Presentations

3:05 pm 81
AUTOLOGOUS FAT GRAFTING TO TREAT PENILE LICHEN SCLEROSUS
 Presenter: Aurora Almadori, MD
 Affiliation: Second University of Naples
 Authors: Almadori A, D Andrea F

3:15 pm 82
FAT GRAFTING WITH SILICONE CALF IMPLANTS: NEW RESULTS THAT WE COULD NEVER ACHIEVE BEFORE
 Presenter: Marcos Sforza, MD (Katarina Andjelkov, PhD)
 Affiliation: Private Clinic
 Authors: Andjelkov K, Sforza M, Zaccheddu R

3:25 pm 83
ADIPOSE VERSUS FASCIAL SLING FOR ANTERIOR SUBCUTANEOUS TRANSPOSITION OF THE ULNAR NERVE
 Presenter: Joseph M. Lombardi, MD
 Affiliation: Columbia University
 Authors: Lombardi JM, Verveld CJ, Danoff JR, Rosenwasser MP



3:35 pm

84

A RANDOMIZED PHASE II, DOUBLE-BLIND, DUAL ARM STUDY TO ASSESS THE EFFICACY OF ADIPOSE DERIVED STROMAL VASCULAR FRACTION (SVF)-ENRICHED AUTOLOGOUS FACIAL FAT GRAFTS, & CONCURRENT UPPER ARM FAT GRAFTS, ISOLATED VIA THE ANTRIA CELL PREPARATION PROCESS (ACPP)

Presenter: Shah Rahimian, MD, PhD

Affiliation: Antria Inc.

Authors: Rahimian S, Maliver L, Bizousky D, Tatarko B, Johnson T

3:45 - 4:15 pm

Coffee Break and Exhibits

4:15 - 5:25 pm

Concurrent Free Paper Session 10 - Soft Tissue Basic - St. John's Room

Moderators: *Jeffrey Gimble, MD, PhD & Petra Bauer-Kreisel, PhD*

4:15 pm

85

Introductory Lecture: QUALITATIVE AND QUANTITATIVE DIFFERENCES OF ADIPOSE TISSUE-DERIVED STROMAL CELLS FROM SUPERFICIAL AND DEEP SUBCUTANEOUS LIPOASPIRATES: A MATTER OF FAT

Presenter: Wanda Lattanzi, MD, PhD

Affiliation: Universit Cattolica del Sacro Cuore

Authors: Lattanzi W, Di Taranto G, Cicione C, Visconti G, Barba M, Baranzini M, Bernardini C, Michetti F, Salgarello M

Free Paper Presentations

4:35 pm

86

LIPOASPIRATE STORAGE TEMPERATURE AFFECTS YIELD AND VIABILITY OF ISOLATED SVF CELLS

Presenter: Michael A. Zieger, PhD

Affiliation: Indiana University School of Medicine

Authors: Zieger MA, Tholpady SS, Sood R, Gupta MP

4:45 pm

87

AUTOLOGOUS PLATELET RICH PLASMA: A BIOLOGICAL SUPPLEMENT TO ENHANCE ADIPOSE-DERIVED MESENCHYMAL STEM CELL EXPANSION

Presenter: Fatemeh Atashi, PhD

Affiliation: Geneva University

Authors: Atashi F, Jaconi M, Pittet-Cunod B, Modarressi A

4:55 pm

88

EFFECT OF PORE SIZE AND POROSITY ON ADIPOSE-DERIVED STEM CELL BEHAVIOUR ON NANOCOMPOSITE POLYMER SCAFFOLDS

Presenter: Naghmeh Naderi, MSc

Affiliation: UCL

Authors: Naderi N, Griffin M, Kalaskar D, Butler PE, Mosahebi A, Whitaker IS, Seifalian AM

5:05 pm

89

HUMAN ADIPOSE STEM CELLS CULTURED IN TENOGENIC DIFFERENTIATION MEDIUM ON BRAIDED POLYLACTIDE SCAFFOLD IS A POTENTIAL APPROACH FOR TENDON TISSUE ENGINEERING

Presenter: Kaisa Vuornos, MS

Affiliation: BioMediTech

Authors: Vuornos K, Bjorninen M, Talvitie E, Kellomaki M, Miettinen S, Haimi S



4:15 - 5:25 pm

Concurrent Free Paper Session 11 - Skeletal Tissues II - Amsterdam Room

Moderators: *Hans Jorg Meisel, MD, PhD & Jenneke Klein-Nulend, PhD*

4:15 pm

90

Introductory Lecture: CYTOKINES AND GROWTH FACTORS, PRESENT AT THE SITE OF IMPLANTATION OF A TISSUE ENGINEERING CONSTRUCT, AFFECT OSTEOGENIC DIFFERENTIATION OF ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

Presenter: Astrid D. Bakker, PhD

Affiliation: Academic Center for Dentistry

Author: Bakker AD

Free Paper Presentations

4:35 pm

91

AUTOLOGOUS ADIPOSE-DERIVED STEM CELLS FOR CRANIOFACIAL RECONSTRUCTION

Presenter: Sophie E. New, PhD

Affiliation: University College London

Authors: New SE, Guasti L, Ibrahim A, Bulstrode NW, Seifalian AM, Ferretti P

4:45 pm

92

BONE TISSUE ENGINEERING TO PROVIDE PERSONALISED IMPLANTS FOR TREATMENT OF CRITICAL BONE DEFECTS IN CHILDREN WITH CRANIOFACIAL SYNDROMES

Presenter: Amel Ibrahim, MBBS, BSc

Affiliation: UCL Institute of Child Health

Authors: Ibrahim A, New S, Bulstrode N, Britto J, Seifalian A, Ferretti P

4:55 pm

93

NOT PRESENTED

SURGICAL PROCEDURE FOR A MECHANICAL FAR LATERAL DISC HERNIATION MODEL IN SHEEP FOR EVALUATION OF REGENERATIVE THERAPY APPROACHES

Presenter: Stefan Schwan, MD

Affiliation: BGKliniken Bergmannstrost

Authors: Schwan S, Friedmann A, Meisel HJ

5:05 pm

94

EFFICIENT DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS TOWARDS ANNULUS FIBROSUS TISSUE IN VITRO IN BIOMIMETIC SCAFFOLDS

Presenter: Suvi Haimi, PhD

Affiliation: BioMediTech University of Tampere

Authors: Gebraad A, Blanquer SB, Miettinen S, Grijpma DW, Haimi SP

5:15 pm

95

IN VITRO MODEL USING BOVINE ARTICULAR CARTILAGE EXPLANT FOR INVESTIGATION OF ADIPOSE-DERIVED STEM CELL KINETICS WITH HYALURONIC ACID

Presenter: Peter Succar, PhD

Affiliation: Macquarie University

Authors: Succar P, Medynskyj M, Herbert B

4:15 - 5:25 pm

Concurrent Free Paper Session 12 - Soft Tissue Clinical - Grand Ballroom

Moderators: *Etto Eringa, PhD & Susan Gibbs, PhD*

4:15 pm

96

Introductory Lecture: PREPARATION OF SVF FROM LIPOASPIRATE INFRANATANT

Presenter: Robert E. Bowen, MD

Affiliation: The Center For Positive Aging

Author: Bowen RE



Free Paper Presentations

4:35 pm

97

RENEVIA(TM) SVF DELIVERY MATRIX: TEMPORAL GELATION CONTROL OF A NOVEL CELL RETENTION SYSTEM

Presenter: Aina Matas Palau, BS

Affiliation: Stem Center

Authors: Matas Palau A, Dos-Anjos Vilaboa S, Erickson IE, Zarembinski T, Llull R

4:45 pm

98

CULTURE EXPANSION OF ADIPOSE TISSUE-DERIVED STROMAL CELLS: MANUAL FLASK-BASED COMPARED WITH CLOSED, AUTOMATED QUANTUM CELL EXPANSION SYSTEM

Presenter: Sonja K. Brorsen

Affiliation: Rigshospitalet

Authors: Haack-Soerensen M, Juhl M, Brorsen SK, Follin B, Soendergaard RH, Kastrup J, Ekblond A

4:55 pm

99

THE EXTERNAL INTRAOPERATIVE SOFT-TISSUE EXPANSION SYSTEM (PALMPUMP) AN INNOVATIVE APPROACH FOR ENHANCED AUTOLOGOUS STRUCTURAL FAT GRAFTING

Presenter: Carlo M. Oranges, MD

Affiliation: University Hospital Basel

Authors: Oranges C, Tremp M, Largo RD, Schaefer DJ

5:05 pm

100

ENHANCEMENT OF DECELLULARIZED ADIPOSE TISSUE WITH LIPOASPIRATE AND ADIPOSE-DERIVED STEM CELLS

Presenter: Arta Kelmendi-Doko. MD, MSc (Kacey Marra, PhD)

Affiliation: University of Pittsburgh

Authors: Chnari E, Schilling B, Lannau B, Minteer D, Huang YC, Kelmendi-Doko A, Marra KG, Rubin JP

5:15 pm

101

HUMAN ADIPOSE SPHERE-DERIVED STEM CELLS INCREASE THE MESENCHYMAL POTENTIAL: THERAPEUTIC IMPLICATIONS

Presenter: Anna Barbara Di Stefano, PhD

Affiliation: Cellular and Molecular Pathophysiology Laboratory

Authors: Di Stefano AB, Giammona A, Leto Barone AA, Giunta G, Cordova A, Todaro M, Moschella F, Stassi G

7:00 pm

A Taste of Amsterdam - (Tickets required)
St. Olof Chapel - Barbizon Palace



Sunday, November 16, 2014

8:00 - 8:05 am **Introductory Remarks - Marco Helder, PhD**

8:05 - 8:35 am **Plenary Lecture 6 - Clinical Skeletal Tissue Research**

ADIPOA: MSC Based Therapy for Severe Osteoarthritis of the Knee. A Phase I Dose Escalation Trial

Presenter: Christian Jorgensen, MD, PhD

Affiliation: CHU Montpellier

Authors: Jorgensen C, Noeth U, Facchini A, Barry F, Sensebe L, Casteilla L, Noel D, Lisignoli G

8:35 - 9:45 am **Plenary Session 6 - Basic Cardiac and Vasculogenesis Research**

Moderators: Paul A. J. Krijnen, PhD & Hans W. M. Niessen, MD, PhD

8:35 am

102

Introductory Lecture: ADIPOSE-DERIVED STROMAL CELLS (ADSC): THE GOOD, THE BAD AND THE UGLY IN MYOCARDIAL REMODELING AND REPAIR

Speaker: Martin C. Harmsen, PhD

Affiliation: University of Groningen

Author: Harmsen MC

Free Paper Presentations

8:55 am

103

ADIPOSE TISSUE-DERIVED STEM CELLS ENHANCE ENDOTHELIAL SPROUTING IN FIBRIN SCAFFOLDS

Presenter: Ester Weijers, PhD

Affiliation: VU University Medical Center

Authors: Weijers E, Van Den Broek M, Tasev D, Van Den Broek LJ, Gibbs S, Van Hinsbergh VW, Koolwijk P

9:05 am

104

ACUTE MYOCARDIAL INFARCTION DOES NOT AFFECT FUNCTIONAL CHARACTERISTICS OF ADIPOSE DERIVED STEM CELLS IN RATS, BUT REDUCES THE NUMBER OF STEM CELLS IN ADIPOSE TISSUE

Presenter: Paul A. Krijnen, PhD

Affiliation: VU University Medical Center

Authors: Krijnen PA, Naaijken BA, Meinster E, Vo K, Musters RJ, Kamp O, Niessen HW, Juffermans LJ, Van Dijk A

9:15 am

105

ADIPOSE STEM CELL DIFFERENTIATION TOWARDS VASCULAR LINEAGES USING NOVEL 3D ELECTRICAL STIMULATION SYSTEM

Presenter: Miina Bjorninen, MSc

Affiliation: BioMediTech University of Tampere

Authors: Bjorninen M, Gilmore K, Pelto J, Kellomaki M, Miettinen S, Wallace G, Grijpma D, Haimi S

9:25 am

106

INFLUENTIAL ROLES OF HMGB1 PATHWAYS IN ACTIVATION OF ASCS AND ADIPOSE TISSUE REPAIR

Presenter: Shinichiro Kuno, MD

Affiliation: University of Tokyo

Authors: Kuno S, Kanayama K, Kinoshita K, Feng J, Yoshimura K

9:35 am

107

IN VITRO ASSESSMENT OF ADIPOSE STROMAL VASCULAR FRACTION CELL DELIVERY UTILIZING A PERFUSION BALLOON CATHETER

Presenter: Jeremy S. Touroo, MS

Affiliation: University of Louisville

Authors: Touroo JS, Khana A, Taylor JL, Williams SK



9:45 - 10:15 am

Coffee Break and Exhibits

10:15 - 11:45 am

Plenary Session 7 - Translational Soft Tissue Research

Moderators: *Julie Fradette, PhD & Adam Katz, MD, FACS*

10:15 am

108

Introductory Lecture: ENHANCING WOUND HEALING OF FULL-THICKNESS MURINE SKIN DEFECTS: EFFECTS OF TISSUE-ENGINEERED BIOLOGICAL DRESSINGS BASED ON ASCS

Presenter: Julie Fradette, PhD

Affiliation: Université Laval

Authors: Maux A, Morissette Martin P, Moulin VJ, Fradette J

Free Paper Presentations

10:35 am

109

INDUCTION OF ADIPOGENESIS IN VIVO BY MECHANICAL STIMULATION WITH EXTERNAL VOLUME EXPANSION

Presenter: Luca Lancerotto, MD

Affiliation: University of Padova

Authors: Lancerotto L, Lujan-Hernandez JR, Nabzdyk C, Hassan ZK, Khouri Jr. RK, Giatsidis G, Chin MS, Lalikos JF, Bassetto F, Orgill DP

10:45 am

110

XENOGENIC-FREE CULTURE SUPPLEMENTS FOR GMP-COMPLIANT EXPANSION OF MESENCHYMAL STROMAL CELLS

Presenter: Karen Bieback, PhD

Affiliation: Institute of Transfusion Medicine and Immunology

Authors: Bieback K, Kinzebach S, Hecker A, Uhlig S, Elvers-Hornung S, Klier H

10:55 am

111

ADIPOSE TISSUE-DERIVED STEM CELL SHEETS TO PROMOTE WOUND REPAIR

Presenter: Panithi Sukho, DVM

Affiliation: Utrecht University

Authors: Sukho P, Kirpensteijn J, Verseijden F, Bastiaansen-Jenniskens YM

11:05 am

112

FREE ADIPOCYTE ISOLATION TECHNIQUES AND HARVEST (FAITH) STUDY: PROSPECTIVE DIRECT COMPARISON OF COLEMAN VS. CYTORI FAT GRAFT HARVESTING TECHNIQUES

Presenter: Edward I. Chang, MD

Affiliation: MD Anderson Cancer Center

Authors: Chang EI, Scaglioni MF, Zhang LQ, Iyyanki TS, Butler CE, Beahm EK

11:15 am

113

DEVELOPING ANIMAL MODEL FOR LOCAL BREAST CANCER RECURRENCE IN AUTOLOGOUS FAT GRAFTING

Presenter: Wakako Tsuji, MD, PhD

Affiliation: University of Pittsburgh

Authors: Tsuji W, Valentin J, Marra K, Donnenberg A, Donnenberg V, Rubin JP

11:25 am

NOT PRESENTED

114

CHARACTERIZATION OF ADIPOSE DERIVED CELLS FROM HUMAN ESCHAR TISSUE

Presenter: Zeni Alfonso, PhD

Affiliation: Cytore Therapeutics

Authors: Alfonso Z, Foubert P, Zhao S, Hicok K, Arm D, Tenenhaus M, Fraser J



11:35 am

115

THE COMBINED USE OF ENHANCED STROMAL VASCULAR FRACTION AND PLATELET-RICH PLASMA IMPROVES FAT GRAFTING MAINTENANCE IN BREAST RECONSTRUCTION: CLINICAL AND INSTRUMENTAL EVALUATION

Presenter: Pietro Gentile, MD, PhD

Affiliation: University of Rome Tor Vergata

Authors: Gentile P, Cervelli V

11:45 am

Concluding Remarks and Farewell - *Marco Helder, PhD*







PLENARY LECTURES

in numerical order



Lecture 1

TRANSDIFFERENTIATION OF ADSC INTO MOTONEURON-LIKE CELLS FOR CELL REPLACEMENT THERAPY OF SPINAL CORD INJURY

Presenter: Jun Xu, MD, FACS

Authors: Gao S, Chen X, Gao Z, Zhu H, Xu J

Tongji University School of Medicine

Human adipose-derived stem cells (hADSCs) are increasingly presumed to be an ideal stem cell sources as an alternative to ESCs and iPSCs for cell-replacement therapies. In the present study, we have developed a stepwise hADSCs trans-differentiation protocol with retinoic acid (RA), sonic hedgehog (SHH) and neurotrophic factors. Our protocol can efficiently trans-differentiate hADSCs into electrophysiologically active motoneuron-like cells (hADSC-MNs) which expressed both a cohort of pan neuronal markers and various motor neuron specific markers. Importantly, when RA- and SHH-preconditioned hADSCs were transplanted to a SCI mouse model, hADSC-MNs survived well in mouse spinal cord and fully integrated into the host tissue. Except for a small subset stained GFAP positive, the transplanted cells persisted as MAP2 positive neurons with classical neuronal morphology. As well, Transplanted hADSC-MNs largely prevented the formation of injury-related cavities and exerted obvious immune-suppressive effect at the injured site. As such, the SCI mice from hADSC-MN transplanted group survived better than the PBS control group and gradually gained significant functional recovery. Assessment of hindlimb locomotor function by BMS indicated a dramatic behavior improvement in SCI-hADSC-MN group after transplantation. Our work suggests that hADSCs can be readily transformed into motoneuron like cells in vitro and stay viable in spinal cord when transplanted into mouse SCI model and exert therapeutic effect for spinal cord injury by rebuilding the broken circuit and suppressing the injury-induced inflammation.

Lecture 2

TREATING SCLERODERMA OF THE FACE AND HANDS

Presenter: Guy Magalon, MD, PhD

Author: Magalon G

APHM

Background: Since 2009, we have treated systemic sclerosis patients. Systemic scleroderma is an autoimmune disease characterized by varying degrees of fibrosis in the skin and other tissues.

Materials & Methods: We treated the faces of 14 patients using micro-injection with a minimally invasive closed filtration system, aiming at volumetric and trophic effects. We used 16 to 22 cc of fat., which was harvested with 14 gauge or 2mm cannulae, and reinjected with 21 gauge or 0.8mm cannulae. In addition, we treated 12 patients (24 hands) with the Stromal Vascular Fraction, aimed at an angiogenic and anti-fibrotic effects. We harvested 135-270g of fat which allowed us to get 5 cc of stromal vascular fraction with the Celution system. We got on average 50×10^6 cells which were divided into 10 doses of 1 cc. A subcutaneous injection was performed in the patient's every finger with 25 gauge or 0.5mm cannulae. Both facial and finger procedures were performed under local anaesthesia

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

Conclusions: We conclude that microfat grafting on the face is efficient to treat functional and aesthetic disorders. The injection of stromal vascular fraction in fingers triggers an obvious functional improvement in every day life activities. Overall, these safe and minimally invasive techniques provide an important benefit in terms of aesthetic and functional improvements.



Lecture 3

A PHASE I TRIAL FOR MAXILLARY BONE AUGMENTATION WITH ADIPOSE STEM CELLS AND CALCIUM PHOSPHATE SCAFFOLDS; EVALUATION OF A ONE-STEP SURGICAL PROCEDURE

Presenter: Marco N. Helder, PhD

Author: Helder MN

VU University Medical Center

Patients with insufficient maxillary bone height may require maxillary sinus floor elevation (MSFE) prior to dental implant placement. Currently, bone substitutes are used as an alternative for the 'gold standard', i.e. autologous bone. However, bone substitutes only allow osteoconduction, since viable osteogenic cells are lacking. Cell-based bone tissue engineering is a promising technique to improve the bone forming capacity of bone substitutes. In a government-sponsored phase I trial, we evaluated feasibility, safety and efficacy of combining a calcium phosphate (CaP) as bone substitute with freshly isolated adipose stem cells during a one-step surgical procedure for MSFE.

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

So far, we included 10 patients. All patients uneventfully underwent an MSFE procedure and no adverse effects were reported during 2 year follow-up. Bone as well as osteoid percentage were higher in bone biopsies taken from study sides than control sides throughout the complete biopsies, suggesting that bone formation does not only occur from the pre-existing sinus floor, and that adipose stem cells may stimulate bone formation.

This study demonstrated for the first time the feasibility, safety and potential efficacy of freshly isolated adipose stem cells with a calcium phosphate for MSFE, and provides the first step towards a novel treatment concept that might offer broad potential for cell-based regenerative medicine applications.

Lecture 4

ENGINEERED OSTEOGENIC AND VASCULOGENIC GRAFTS USING ADIPOSE DERIVED CELLS: FROM FUNDAMENTAL RESEARCH TO A CLINICAL TRIAL

Presenter: Ivan Martin, PhD

Authors: Martin I, Saxer F, Jakob M, Schaefer DJ, Scherberich A

University Hospital Basel

The stromal vascular fraction (SVF) of adipose tissue contains mesenchymal and endothelial lineage cells, which have been used by our group to engineer osteogenic and vasculogenic grafts [1]. The endothelial component, which could be functionally preserved using a 3D culture system in a perfusion-based bioreactor, was shown to improve cell survival within upon implantation of scaled-up constructs, ultimately resulting in a more efficient and uniform bone tissue formation throughout the engineered grafts [2].

In a parallel series of studies, we demonstrated that the osteogenic and vasculogenic properties of human SVF cells could also be maintained within a single-stage processing, compatible with an intra-operative setting [3,4].

This lecture will present recent pre-clinical studies validating the intraoperative use of human SVF cells to support repair a segmental bone defect in nude rats. Finally, early clinical observations in the treatment of proximal humeral fractures in elderly individuals using autologous, freshly harvested SVF cells will be communicated (clinical trial ROBUST (<http://clinicaltrials.gov/show/NCT01532076>).

[1] Scherberich A, Galli R, Jaquiere C, Farhadi J, Martin I. 3D perfusion culture of human adipose tissue-derived endothelial and osteoblastic progenitors generates osteogenic constructs with intrinsic vascularization capacity. *Stem Cells* 25:1823-1829 (2007)

[2] Gueven S, Mehrkens A, Saxer F, Schaefer DJ, Martinetti R, Martin I, Scherberich A. Engineering of large osteogenic grafts with rapid engraftment capacity using mesenchymal and endothelial progenitors from human adipose tissue. *Biomaterials* 32:5801-5809 (2011)

[3] Mueller AM, Mehrkens A, Schaefer DJ, Jaquiere C, Gueven S, Lehmicke M, Martinetti R, Farhadi J, Jakob M, Scherberich A, Martin I. Towards an intraoperative engineering of osteogenic and vasculogenic grafts from the stromal vascular fraction of human adipose tissue. *Eur Cell Mater* 19:127-135 (2010)

[4] Mehrkens A, Saxer F, Gueven S, Hoffmann W, Mueller AM, Jakob M, Weber FE, Martin I, Scherberich A. Intraoperative engineering of osteogenic grafts combining freshly harvested, human adipose-derived cells and physiological doses of bone morphogenetic protein-2. *Eur Cells Mater* 24:308-19 (2012)



Lecture 5

THE ROLE OF ADIPOSE DERIVED STEM CELLS IN CARDIAC DISEASE

Presenter: Hans Niessen, MD, PhD

Author: Niessen H
VU University Medical Center

Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality in western society. Death of cardiomyocytes is not only induced by the ischemic event itself but also by inflammation-induced necrosis post-AMI. These lost cardiomyocytes are then replaced by non-contractile scar tissue, facilitating cardiac impairment, eventually resulting in heart failure. This loss of cardiomyocytes is not only found in AMI but also in other pathological conditions of the heart, e.g. (viral) myocarditis.

Live-saving therapies however do not actively restore or degenerate the damaged myocardial tissue. Therefore replenishing lost cardiomyocytes using stem cell therapy was hypothesized to be an ideal solution to retain cardiac function and prevent heart failure development.

The first adult mesenchymal stem cells used in therapy post-AMI in preclinical and clinical studies were isolated from bone marrow (BMSC). In 2002 it was shown that adipose tissue is another interesting source of adult mesenchymal stem cells (ASC). In contrast to bone marrow, adipose tissue can be obtained via a lesser invasive method resulting in lower patient discomfort and risk. Even more adipose tissue provides more stem cells compared with bone marrow. In rat studies it was shown that ASC have a higher capacity to reduce the infarcted area post-AMI than BMSC.

In this lecture different aspects of preclinical studies using ASC as therapy in cardiac disease will be discussed, including features that are important for future successful clinical application of ASC in cardiac disease.

Lecture 6

ADIPOA: MSC Based Therapy for Severe Osteoarthritis of the Knee. A Phase 1 Dose Escalation Trial

Presenter: Christian Jorgensen, MD, PhD

Authors: Jorgensen C, Noeth U, Facchini A, Barry F, Sensebe L, Casteilla L, Noel D, Lisignoli G

CHU Montpellier

Among the degenerative diseases associated with aging, osteoarthritis is the most common pathology and affects 16% of the female population over 65 years. The ADIPOA project started in January 2010 with the goal to develop a new cell based strategy for patients suffering from knee osteoarthritis (OA). Up to now, no therapeutic option exists to obtain a sustainable improvement of joint function beside knee arthroplasty. This prompted us to propose adipose derived stem cells as a possible cell therapy.

Adipose derived mesenchymalstromal cells (ASC) are adult stem cells exhibiting functional properties that have opened the way for cell-based clinical therapies. Primarily, their capacity of multilineage differentiation has been explored in a number of strategies for skeletal tissue regeneration. More recently, MSCs have been reported to exhibit immunosuppressive as well as healing capacities, to improve angiogenesis and prevent apoptosis or fibrosis through the secretion of paracrine mediators. We performed 2 pre-clinical models of osteoarthritis, and showed that a local injection of ASC showed a reduction of synovitis, reduction of osteophytes, joint stabilization, reducing the score of cartilage lesions. This work was completed by toxicology data showing the excellent tolerance of the local injection of ADSC and biodistribution showing the persistence of cells after 6 months in murine models. In addition, quality control and tolerability of the injection of adipose derived mesenchymal cells led to the approval by AFSSAPS in France and in Germany by the PEI to conduct the clinical trial phase I.

In this open-label phase 1 trial we included 18 patients with severe osteoarthritis of the knee in failure of conventional therapies (62.5% were KL IV) at two sites, Montpellier and Wurzburg. Mean age was 61 years, with a 10 years history of knee OA. The patient received a single injection of autologous ASC 15 days after lipoaspiration ($2 \cdot 10^6$, 10^7 or $5 \cdot 10^7$) through intra-articular injection. The primary outcome measure of effectiveness was patient-reported WOMAC pain subscores by VAS in the affected knee at week 12. Secondary outcome measures included Outcome Measures in Rheumatology Clinical Trials and Osteoarthritis Research Society International (OMERACT OARSI) responses. We observed a decrease of the VAS Pain (73 ± 11 mm day 0 to 32 ± 23 month 3), and of WOMAC (50 ± 18 to 25 ± 7 month 3). This study confirms the feasibility and safety of local injection of autologous cells from adipose tissue and suggested that the most effective dose was 10^7 autologous cells.

The ADIPOA research teams performed successfully the phase 1 clinical trial in France and Germany. A phase 2B controlled trial is scheduled to confirm the clinical benefit of this strategy.



PAPER PRESENTATIONS

in numerical order



1

ADIPOSE BASED THERAPIES FOR CRANIOFACIAL AND LIMB RECONSTRUCTION

Presenter: J. Peter Rubin, MD
University of Pittsburgh

2

ADIPOSE DERIVED STEM CELL THERAPY FOR THE TREATMENT OF REFRACTARY ERECTILE DYSFUNCTION

Presenter: Alvaro H. Skupin, MD
Authors: Skupin AH, Hernandez-Serrano R, Alvarez N, Zevallos-Palma B

Mother Stem Institute Inc

Background: Erectile Dysfunction (ED) is a sexual pathology caused by numerous comorbidities. In Latin America according to DENSA study, 54% of the patients in Colombia, Ecuador and Venezuela, were diagnosed with this pathology. The USA National Institute of Health reports that 30 million men experience chronic ED and the incidence of the disorder increases with age. Transient ED and inadequate erection affect as many as 50% of men between ages of 40-70. Several studies done in rats have shown the effectiveness of mesenchymal stem cells in the treatment of ED. In this study we are working with adipose derived stem cells (ADSc) which are multipotent and exhibit capabilities of differentiation and regeneration of myogenic, neurogenic and vascular cells as well as repair mechanisms that increase the synthesis of NO in the penis.

Methods: A retrospective survey of 79 patients that were treated with ADSc for ED between 2012 and 2014 was done. All of the patients had comorbidities including diabetes, hypertension, coronary artery disease, obesity and consumption of alcohol and tobacco. The StemProCell Protocol was used to harvest, isolate and re-inject the stromal vascular fraction (SVF) into the corpus cavernosum of the men treated. Platelet rich plasma was also isolated and injected with the SVF.

Results: Of the 79 patients that were treated in the clinic, we followed up with 41 patients, of which 69% showed improvement of sexual function of more than 51% after the treatment with ADSc and 31% reported no improvement. Of this latter group, some had low testosterone levels which might account to the non-responsiveness of the treatment. The patients that reported improvement, 60% had hypertension, 36% had DM Type II, 20% were smokers, 53% consumed alcohol and 6% had coronary artery disease.

Conclusions: ADSc is an innovative and alternative way to treat patients with ED, even those with comorbidities. The stem cells can regenerate tissue that has been injured due to vascular disease or compromised by surgical procedures in the pelvis. There is a surge to create a multicenter prospective study of new cases with an established protocol and classification system that will allow standard research criteria and valid and reliable results.



3

USE OF AUTOLOGOUS ADIPOSE-DERIVED STROMAL VASCULAR FRACTION TO TREAT OSTEOARTHRITIS OF THE KNEE; 2 INDEPENDENT STUDIES USING SAME METHODS

Presenter: William Cimino, PhD

Authors: Cimino W, Garza J, Santa Mariad D, Palomera T, Dumanian G, Dos-Anjos S, Fodor P, Paulseth S

The GID Group

Introduction: Autologous adipose-derived stromal vascular fraction (SVF) was used to treat 10 osteoarthritic knees of grade II or III (K-L scale) (Study 1), and 10 additional osteoarthritic knees of grades I-III (K-L scale), including MRI imaging (Study 2).

Questions/Purposes: (1) Can adipose-derived SVF be safely used for intra-articular injection of the knee? (2) Does intra-articular injection of adipose-derived SVF provide relief of pain and increase in mobility in osteoarthritic knees? (3) Does MRI imaging of the knee show changes in knee tissues corresponding to any measured changes in pain or mobility (Study 2)?

Patients and Methods: Patient ages ranged from 52 - 69 years with a mean of 59 years (Study 1) and 51 - 68 years with a mean of 59 years (Study 2). SVF was obtained through disaggregation of lipoaspirate and resuspension of the SVF pellet in 3 ml of Lactated Ringer's Solution, with a mean of 37 million viable nucleated SVF cells injected per knee (Study 1) and a mean of 17 million viable nucleated SVF cells injected per knee (Study 2).

Results: (1) No infections, acute pain flares, or other adverse events were reported related to intra-articular injection of adipose-derived SVF in the knee (Study 1 and 2). (2) At 12 weeks post-op all 10 knees showed decreased pain and increased mobility, both statistically significant ($= .01$) and nine of ten knees reported either maximum possible or very significant decrease in pain (Study 1). At 12 weeks post-op 8 of 10 knees showed decreased pain and increased mobility, both statistically significant ($= .01$) (Study 2). (3) At 12 weeks post-op MRI imaging of knee tissues showed increase in cartilage thickness (Study 2).

Conclusions: Two independent studies using autologous SVF to treat OA of the knee showed statistically significant reduction in pain and increase in mobility. MRI imaging showed thickening of the cartilage of the knee. Use of autologous SVF in the knee is a promising cell-based therapy that addresses a significant clinical need with no known regenerative solution.

4

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

Presenter: Michael Alperovich, MD, MSc

Authors: Alperovich M, Lee ZH, Chiu ES
New York University Langone Medical Center

NOT PRESENTED



5

STEM CELL ENRICHED FAT INJECTIONS TO THE BREAST: 5 YEARS EXPERIENCE AND THE EVOLUTION OF OUR TECHNIQUE

Presenter: Tunc K. Tiryaki, MD

Authors: Tiryaki TK, Isil E, Aksungur E, Tiryaki D, Findikli N

Cellest Plastic Surgery Clinic

Introduction: Fat injection to the breast is becoming a routine reconstructive/cosmetic procedure. However, the need of preoperative preparations like BRAVA, adequate graft sustainability and postoperative dystrophic calcifications due to fat necrosis raise concerns about this procedure. On the other hand, stem cell enriched tissue (SET) grafting is shown to increase the revascularisation of the graft, possibly reducing the need of any preparation and these mentioned risks.

Method: The first part of the lipo-aspirated fat was transferred to the isolation facility in the OR. Using routine cell separation techniques, the stromal vascular fraction (SVF) was acquired, and the non-stem cells in the SVF were negatively selected by a manual MACS cell separation system. While the cell separation took place, the major fat aspiration was finished. Once obtained, the regenerative cells were mixed with the harvested and washed fat, and injected to the breast using 2-3 mm cannulae.

Results: We present 61 patients treated with single session SET injections for reconstructive/cosmetic breast augmentation between December 2008 and December 2013 with a follow up changing from 8 months to 32 months. Mean volume of injections was 390 cc. Mean volume for unilateral cases was 210 cc and bilateral cases 530 cc. Mean age of patients was 42. In 5 cases breast prosthesis was implanted simultaneously. The results were evaluated by clinical examination, mammographies, patient photos and Vectra 3D Surface Imaging. The mean graft uptake was 55 percent without any ancillary preparation and the result did not change after 8 weeks. Four patients were not satisfied with the outcome. One patient developed relapse of her malignancy and 2 patients had postoperative sustained calcifications. No major complication was observed.

Conclusion: Our results suggest that SET injections can be used successfully to augment or reconstruct the breast. By increasing the neo-angiogenesis, enrichment seems to eliminate the need of ancillary preoperative procedures like BRAVA, decrease the number of the operative sessions as well as the frequency of dystrophic calcifications.

6

FIRST LONG TERM RESULTS OF LARGE VOLUME FAT GRAFTING BY BEAULI PROTOCOL

Presenter: Klaus Ueberreiter, MD

Author: Ueberreiter K

Park-Klinik Birkenwerder

During the years 2007 to 2010 85 patients underwent breast augmentation for aesthetic purpose using the BEAULI protocol. Of these patients 36 underwent MRI breast investigation before and after 6 months. The obtained results were analysed and could show a take rate of the grafted fat of 76 \pm 11%. They were published 2010 in Hamipla.

To follow up the long term outcomes we started to call back the patients and had them reinvestigated and new MRI's carried out. As the recruiting lasted 2,5 years, the follow up study will not be finished until 2015. Nevertheless we are able to show first results. They mainly consist in a significant change in breast form; the volume is stable or even increased.



7

FEASIBILITY STUDY OF RENEVIA, A RESORBABLE MATRIX OR THE DELIVERY OF AUTOLOGOUS ADIPOSE DERIVED CELLS

Presenter: Aina Soler Mieras, MD

Authors: Soler Mieras A, Furr DA, Matas-Palau A, Erickson IE, Dos Anjos Vilaboa S, Tew WP, Llull R

Stem Center

Introduction: Renevia™ is a resorbable, biocompatible hydrogel that mimics the natural extracellular matrix of soft tissues. It is designed for the delivery of autologous adipose derived cells for the treatment of subcutaneous lipoatrophies. Renevia™ has demonstrated suitable biocompatibility through standard pre-clinical testing for an implantable medical device (Table 1).

Methods: The purpose of this prospective, non-randomized, interventional, consecutive series, open label, feasibility study in healthy subjects was to determine the safety, tolerability, and acceptance of Renevia™. The primary endpoint was absence of adverse events (AE) such as erythema, blistering, or dyschromia greater than 10mm and/or no symptomatic seroma, ulceration, necrosis or infusion edema. Secondary endpoints included rate and type of AE and subject reported pain post-procedure and during the follow-up period (4 weeks).

Results: Ten healthy subjects, male and female, between 18-55 years old were consented and enrolled in this safety study. All received a single subcutaneous injection of 0.2cc of Renevia™ (without stromal vascular cells) on the retro-auricular area. All subjects completed follow-up and met the primary endpoint (Figure 2). In addition, no unanticipated or serious adverse device effects were observed. All reported AE were mild and self-resolved in the study period. Assessment of pain was recorded using a VAS pain score. Subjects exhibited VAS pain score of (0-2 cm) and recovered within the first 48h.

Conclusions: The Renevia™ device met the safety objectives of this feasibility study and was well tolerated and accepted by all subjects. Renevia™ offers the plastic and reconstructive surgeon a safe and consistently uniform product to deliver minimally manipulated, autologous stromal vascular cells for tissue augmentation procedures and for the treatment of contour defects currently treated with cell assisted lipotransfer. The data obtained have been used to provide a basis for designing a more comprehensive pivotal study (a randomized, evaluator-blinded, delayed-treatment-controlled study of the effectiveness and safety) to treat HIV facial lipoatrophy.

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FEASIBILITY STUDY OF RENEVIA, A RESORBABLE MATRIX OR THE DELIVERY OF AUTOLOGOUS ADIPOSE DERIVED CELLS

Biocompatibility Test	Result
Cytotoxicity	"non-cytotoxic"
Maximization Sensitization Test	"non-sensitizing"
Intracutaneous Reactivity	"met test criteria" **
Acute systemic Toxicity	"non-toxic"
Subchronic Toxicity	"non-toxic"
Pyrogenicity in Rabbits	"non-pyrogenic"
Hemocompatibility - Hemolysis Test	"non-hemolytic"
Genotoxicity - Ames Test	"non-mutagenic"
Genotoxicity - In Vivo Mouse Micronucleus	"non-mutagenic"
Genotoxicity - Chromosome Aberration	"non-genotoxic"
Intramuscular Implant (2, 4, 8, 13, 16, 20 wks)	"non-irritant" **

Table 1 Renevia pre-clinical biocompatibility test findings. Renevia has demonstrated safety in numerous ISO standard tests for biocompatibility of implantable devices. **For muscle and skin implantation tests, an approved device for subcutaneous implantation - Restylane was used by way of comparison.

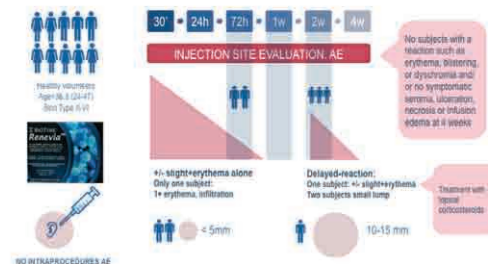


Figure 1 Safety, tolerability and acceptance of Renevia™ in humans. Ten healthy volunteers received 0.2cc subcutaneous injections of Renevia and were followed for 4 weeks. No serious adverse events or unanticipated reactions were observed. With pre-clinical and human clinical safety data in hand, the next clinical trial will incorporate autologous stromal vascular cells to assess effectiveness and safety in treating HIV facial lipoatrophy.



8

FROM BONE MARROW TO ADIPOSE DERIVED STROMAL CELLS IN CLINICAL CARDIOLOGY

Presenter: Jens Kastrup, MD, DMSc

Author: Kastrup J

Rigshospitalet

Introduction: With more than 17 million deaths worldwide each year, ischemic heart disease (IHD) caused by coronary artery disease is the most common cause of death and a major cause of hospital admissions in industrialised countries. Therefore there are unmet needs for novel, effective treatments for cardiac disease to improve patient survival rates and quality of life and reduce health care costs. A promising therapeutic concept is stem cell therapy. Most clinical trials are using bone marrow derived cell solutions, but more focus is now on using adipose tissue-derived stromal cells.

Method: We have established an in-house cultivation facility for cultivation of stem cells for clinical use approved by the national competent authorities. We have used culture expanded mesenchymal stromal cells isolated from bone marrow and abdominal tissue in clinical trials in patients with severe IHD with and without heart failure.

Results: Methodological cultivation methods and results from the clinical trials will be presented and discussed, in addition with the arguments to move from bone marrow to adipose tissue derived stem cell solutions.

Conclusion: Mesenchymal stromal cells from both bone marrow and adipose tissue are available for clinical trials in patients with ischemic heart disease and the results are promising.

9

INTRA-MUSCULAR INJECTION OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF THROMBOANGIITIS OBLITERANS: A PROOF-OF-CONCEPT CLINICAL TRIAL

Presenter: Eui Cheol Jeong, MD, PhD

Authors: Jeong EC, Kim HS, Yoon KS, Seo JB, Jung IM, Baek SH

SMG_SNU Boramae Medical Center

Background and Objectives: Most patients with Thromboangiitis obliterans (TAO) are unsuitable for revascularization surgery or radiologic intervention at diagnosis. ¹ The aim of this study was to assess the safety and feasibility of intramuscular autologous adipose derived mesenchymal stem cell (ADMSC) injection in the patients.

Methods and Results: A total of 17 patients (all male, median age 42.59 years, range 24-60) with CLI were enrolled in this phase I/II trial. All patients were considered ineligible for further revascularization and applicable to Rutherford class II/III. We injected 1×10^7 ADMSCs per single dose intramuscularly into the affected limb. The primary end points of safety were occurrence of adverse reactions and improvement of symptoms/clinical parameters (treadmill walking distance, ankle-brachial index, and pain-free walking distance, etc). Angiogenesis was measured with conventional angiography and scored by an independent reviewer. There were no significant adverse events in the patients. Abnormal results of laboratory parameters were not detected in any patients. The treadmill walking distance and pain-free walking distance was significantly improved, and the ischemic pain (claudication) was also decreased by the patient self-measured visual analog scale of pain.

Conclusions: This phase I/II study demonstrates that intramuscular ADMSC injection is a safe and well tolerated treatment for patients with TAO.



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DEVELOPMENT OF RECOMBINANT COLLAGEN-BASED VEHICLES FOR ADSC DELIVERY AND RETENTION

Presenter: Mojtaba Parvizi MP, DVM

Authors: Parvizi M, Plantinga JA,
Van Spreuwel Goossens C, Van Dongen S,
Kluijtmans SG, Harmsen MC

UMCG

Introduction: Stem cell therapy is a promising approach for repair, remodeling and even regenerate tissue of otherwise irreparable damage, such as after myocardial infarction (aMI). A severe limitation of cardiac stem cell therapy is the generally poor retention of administered cells in the target tissue. A goal of the ICARUS consortium was to improve the retention and maintain the function of stem cells in cardiac stem cell therapy. In tissue repair the main mode of action of adipose tissue-derived stem cells (ADSC) is the production of various growth factors, cytokines, anti-inflammatory and anti-apoptotic factors that together augment repair, remodeling and regeneration. We argued that a strong integrin-ECM binding could be exploited to deliver ADSC to damaged myocardium, while improving their retention. For this, we used recombinant gelatin with additional integrin-binding motives. Formulated as 50mm microspheres with bound ADSC, we hypothesized that this would improve ADSC retention and function.

Methods & Materials: Human ADSC were isolated from healthy donors. Cross-linking was with chemical cross-linkers (A and B) at high and low concentrations or by thermal treatment (DHT). ADSC adhesion, proliferation, apoptosis/necrosis and gene expressions were analyzed. In addition, the effect of ADSC conditioned medium (ADSC-CM) on pro-apoptotic/sprouting HUVEC was examined.

Results: Our results show that all materials support cell adhesion in short time point, however, cross-linker A used at high concentration induced ADSC apoptosis/necrosis. Gene expression results revealed down regulation of proinflammatory genes in chemical cross-linker materials, despite cross-linker A with higher concentration the pro-inflammatory genes expressions were similar or higher than TCPC or even higher. In addition, cultured ADSC on thermally cross-linked gelatin showed a proinflammatory phenotype compared to TCPS. Sprouting assay results confirmed the protective effect of ADSC-CM TCPS and chemical cross-linker B with high concentration on pro-apoptotic HUVEC.

Conclusion: We conclude that ADSC adhere to the materials and maintain their therapeutic profile. Currently, the therapeutic benefit of ADSC-loaded B type microspheres is employed in a pig model for aMI.

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TOPICAL APPLICATION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS AMELIORATED RAT RENAL ISCHEMIA-REPERFUSION INJURY

Presenter: Ping Kuen Lam, PhD

Authors: Lam PK, Lo A, Tong C, Kwong T, Ching D,
Lau H, Lai P, Ng CF

The Chinese University of Hong Kong

Introduction: Ischemia reperfusion injury (IRI) is a leading cause of acute kidney injury.

Objectives: We aim to investigate the renoprotective effects of topically applied MSCs in renal IRI.

Methods: Ischemia-reperfusion injury (IRI) was induced by clamping bilateral renal pedicles simultaneously for 60 minutes using bulldog clamps in 72 SD rats which were randomized into 6 groups. 3×10^6 MSC (Group I) and 1×10^6 (Group II) derived from the adipose tissue of transgenic GFP rats were topically applied to the surface of both kidneys of rats. The MSCs were fixed in position with a thin layer of fibrin. Either fibrin (Group III) or MSC (Group IV) was applied to both kidneys. 1×10^6 MSCs were injected into the tail vein (Group V). No treatment was given to the control (Group VI).

Results: 3 days post topical application, few GFP+ve cells were found in the renal parenchyma in Group I. The serum creatinine levels ($\mu\text{mol/L}$) at day 3 were 142 ± 60 ; 313 ± 146 ; 535 ± 127 ; 574 ± 229 ; 319 ± 87 and 516 ± 56 in Group I to VI respectively. The value of Group I was significantly lowered than other groups. ($p < 0.05$) At day 7, the survivals of animals were 85%, 77%, 63%, 50%, 71% and 56%. There were less tubular necrosis and inflammation in the kidney treated by topical MSCs. RT-PCR showed down-regulation of Caspase-3 (indicator of apoptosis) and Endothelin-1 (index of endothelial damage) and up-regulation of NQO-1 (anti-oxidative) ($p < 0.05$) in group I.

Conclusion: Topically applied adipose tissue-derived mesenchymal stem cells could ameliorate renal ischemia-reperfusion injury.



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ADIPOSE STEM CELLS (ASC) DELIVERED INTRAVENOUSLY ARE TARGETED TO THE ENDOTOXIN-INJURED LUNG

Presenter: Natalia V. Bogatcheva, PhD

Authors: Bogatcheva NV, Lu H, Poirier C, Traktuev DO, Cook T, Merfeld-Clauss S, Petrache I, March KL

Indiana University

Intravenously (IV) delivered cells and particles are known to be retained by the pulmonary capillary bed; however, the effect of lung inflammation on stem cell homing toward lung and other organs is not studied at depth. In this work, we assessed the dynamics of IV-delivered ASC distribution to lungs, spleen, liver, kidney, heart, and brain of animals with endotoxin-induced lung injury. The injury was induced by endotoxin delivery directly to lungs by oropharyngeal aspiration. 4h later, when mice manifested the peak of hypothermic shock, severe drop in cardiac output, and the onset of neutrophilic infiltration into airspaces, they were intravenously injected with 300,000 CRE positive ASC mixed with fluorescent agent. Efficacy of IV injection was controlled by the assessment of blood plasma fluorescence 10 min after injection. Distribution of CRE+ cells was assessed by the analysis of genomic DNA isolated from the organ of interest with quantitative PCR. Of the ASC administered via tail vein, 10% were immediately retained in the lungs of saline-instilled animals compared to ~2% cells in endotoxin-challenged lungs. In contrast, after 48h no ASC could be detected in control lungs, whereas endotoxin-challenged lungs retained ~3% of the administered ASC. These dynamics likely represent an interplay between an initial stem cell retention by the pulmonary vascular bed and a subsequent inflammation-induced stem cell homing to injured lung. Comparing to lung, other organs retained only minor amount of cells with the highest levels detected within the first 2h of injection. No specific re-distribution of ASC from lung to other organs was found at either time-point. Altogether, these data demonstrate that ASC persist in endotoxin-damaged lungs for at least 48h, thereby providing a source of continuing therapeutic effect for the suppression of lung injury and the lung rescue. On the other hand, when targeting of spleen, liver, kidney, heart, and brain is desirable, intravascular cell delivery, either arterial or retrograde venous, may be a valid technique to explore. This work was supported by Indiana University Health - Indiana University School of Medicine Strategic Research Initiative and VC-CAST.

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ASCS WITH ENHANCED ANTI-INFLAMMATORY PROPERTIES DEMONSTRATE IMPROVED OUTCOMES IN THE MURINE EAE MODEL OF HUMAN MULTIPLE SCLEROSIS

Presenter: Bruce A. Bunnell, PhD

Authors: Bunnell BA, Zhang X, Betancourt AM, Bowles A, Gimble JM

Tulane University School of Medicine

Adipose-derived stem cells (ASCs) are very attractive candidates for use as treatments in autoimmune or inflammatory diseases. Preclinical animal studies and small numbers of clinical trials have demonstrated that human ASCs can be safely administered in vivo and that they reduce the levels of inflammation by modification of the inflammatory processes. The laboratory of Dr. Betancourt developed a novel method that optimizes the anti-inflammatory effects of bone marrow-derived mesenchymal stem cells (BMSCs). The technique involves the stimulation of the Toll-like Receptor 3 (TLR3) by exposing cells to specific TLR3 agonists in culture. The cells prepared by this method have been termed MSC2 and have been shown to have enhanced anti-inflammatory effects in vivo. In this study, we determined if ASCs treated with TLR3 priming agents were more uniformly anti-inflammatory and assessed the impact of ASC2-based therapies in the murine EAE model of Multiple Sclerosis (MS). The method for induction of ASC2 involves short-term (1 hour) stimulation of the ASCs with small amounts of poly I:C (a TLR 3 agonist) in culture. The resultant ASC2 cells were defined by the increased production of anti-inflammatory molecules, including IL-10, TGF-beta, prostaglandin E2 (PGE2) and indoleamine 2,3 dioxygenase (IDO) as detected by RT-PCR analysis. The resultant ASC2 also demonstrated enhanced migration and invasion, at levels similar to those reported for BMSC2. The assessment of ASC2 in the EAE model demonstrated that anti-inflammatory ASC2-treatment is safe and effective with no overt adverse effects. Compared to vehicle and conventional ASC, ASC2 treated mice had significant improvement in their clinical course of disease as indicated by a 50% reduction in the clinical severity, improved neuromotor activity and histopathologic outcomes. These findings indicate that ASC2 may be an important anti-inflammatory treatment for MS and other inflammation-based diseases states.



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TIMING AND FREQUENCY OF ADIPOSE-DERIVED STEM CELL ADMINISTRATION FOR IMMUNOMODULATION IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Presenter: Riccardo Schweizer, MD

Authors: Plock JA, Schnider JT, Zhang W, Schweizer R, Tsuji W, Kostereva N, Fanzio P, Ravuri S, Solari M, Marra K, Gorantla VS

University Hospital Zurich

Introduction: Vascularized composite allotransplantation (VCA) is a reality. The pursue for an alternative to life-long drug immunosuppression unveiled encouraging outcomes with cellular therapies, especially using adipose-derived stem cells (AD-MSCs). Clinical translation of these therapies must consider collateral effects of depletional drugs such as anti-lymphocyte serum (ALS). We investigated the influence of timing and frequency of AD-MSC administration on allograft survival and immunological outcome.

Methods: Brown Norway (BN) rat AD-MSCs (CD29+CD73+CD90+CD45-) were assessed in vitro for proliferation/viability under the influence of ALS, as well as for immunosuppressive function in mixed lymphocyte reaction assays. White blood cells (WBCs) were assessed repetitively in naive Lewis rats (LEW) after ALS injection to investigate its effect in vivo. LEW rats received full-mismatched BN limbs (day 0). The ideal AD-MSC injection timepoint was assessed treating recipients with 1×10^6 cells, on postoperative day (POD) 1, POD 4 or repeatedly on PODs 4, 8, and 15. All animals received ALS conditioning on days -4 and +1 and daily immunosuppression for 21 days (tacrolimus). Microchimerism and regulatory T-cell function were investigated in long-term survivors.

Results: ALS has detrimental effects on AD-MSCs co-cultured over 7 days. WBCs decreased within 24h after ALS injection ($p < 0.01$) and recovered within 10 days. AD-MSCs showed a strong dose-dependent immunosuppressive function in-vitro ($p < 0.001$). Single early and repetitive AD-MSC injection resulted in 50% long-term graft acceptance, whereas single treatment on POD 4 resulted in rejection comparable to untreated controls ($< \text{POD } 50$). All treated animals revealed peripheral blood chimerism at 4 weeks, most pronounced following repetitive cell administration (12.92% vs. 5.03% [group 1] and 6.31% [group 2]; $p < 0.05$). Chimerism levels were associated with induction of regulatory T-cells (CD4+CD25^{high}FoxP3+).

Conclusion: Immunomodulatory effects of AD-MSCs could reduce intensity, frequency and duration of immunosuppressive drug-regimens in VCA. Given their immunomodulatory efficacy, high cell yields and pro-neuroregenerative benefits, AD-MSCs are a promising cell ripe for translation into clinical VCA.

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POTENTIAL MESENCHYMAL STEM CELLS SUBPOPULATION IDENTIFIED FROM ADIPOSE DERIVED STROMAL CELLS

Presenter: Wei Jing, MD

Authors: Jing W, Xiao JG, Tian WD

State Key Laboratory of Oral Diseases and National Engineering Laboratory for Oral Regenerative Medicine, Sichuan University

Introduction: Adipose-derived stromal cells have been defined as cells that undergo multilineage differentiation potential. However, the purification of adipose derived stem cells has proved elusive due to the lack of specific markers and poor understanding of their physiological roles. In this study, we used stem cell related markers including Sca-1, Lin and CD271 trying to identify and isolate stem cells from stromal cells.

Method: Murine Adipose derived stromal cells were harvested and classified into Sca-1(-) and Sca-1(+) subpopulations. In each population adipogenic genes and osteogenic genes were both examined. Chemical induction was also conducted to determine the differentiation potential. After that, Lin-negative cells were separated from the stromal cells by using Lineage Cell Depletion Kit and a magnetic cell separation magnet. Lin-positive cells were removed. Lin(-)CD271(+)Sca-1(+) cells were isolated on the basis of the cell-surface markers CD271 and Sca-1, and cultured immediately after isolation. Proliferation, CFU-F activity and differentiation potential were examined to test the stemness of the separated populations.

Results: A group of cells that expressed both osteogenic and adipogenic characters were identified from murine adipose stromal cells. Osteogenic induction enhanced expression of osteogenic genes and inhibited expression of adipogenic genes, while adipogenic induction enhanced expression of adipogenic genes and inhibited expression of osteogenic genes. We also isolated and identified a restricted homogeneous subpopulation of stem cells from murine adipose tissues, and expanded them in medium supplemented with fibroblast growth factor-2 and leukemia inhibitory factor without loss of adipogenic and osteogenic differentiation capacity. These cells can differentiate into adipocytes and osteoblasts, respectively, in adipogenic and osteogenic microenvironment in vivo.

Conclusions: This study suggests that progenitor cells could be enriched in the Sca-1(-) population and express both osteogenic and adipogenic genes, and osteoblasts and adipocytes might share a common predecessor in murine adipose stromal cells. Also we proved that Lin, CD271, and Sca-1 could be used as the specific markers to purify stem cells from adipose tissue.



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ALTERATIONS OF GENE EXPRESSION AND PROTEINS SYNTHESIS IN CO-CULTURED ADIPOSE TISSUE-DERIVED STEM CELLS AND SQUAMOUS CELL-CARCINOMA CELLS

Presenter: Eva Koellensperger, MD

Authors: Koellensperger E, Gramley F, Preisner F, Germann G, Leimer U

Heidelberg University Hospital

Introduction: This is the first study evaluating the interactions of human adipose tissue derived stem cells (ADSCs) and human squamous cell carcinoma cells (SCCs), with regard to a prospective cell-based skin regenerative therapy and a thereby unintended co-localization of ADSCs and SCCs.

Methods: ADSCs were co-cultured with A431-SCCs and primary SCCs (pSCCs) in a transwell system, and cell-cell-interactions were analyzed by assessing doubling time, migration and invasion, angiogenesis, quantitative real time PCR of 229 tumor associated genes, and multiplex protein assays of 20 chemokines and growth factors and eight matrix metalloproteinases (MMPs). Results of co-culture were compared to those of the respective mono-culture.

Results: ADSCs' proliferation on the plate was significantly increased when co-cultured with A431-SCCs ($P = 0.038$). pSCCs and ADSCs significantly decreased their proliferation in co-culture if cultured on the plate ($P < 0.001$ and $P = 0.03$). The migration of pSCC was significantly increased in co-culture ($P = 0.009$), as well as that of ADSCs in A431-SCC-co culture ($P = 0.012$). The invasive behavior of pSCCs and A431-SCCs was significantly increased in co-culture by a mean of 33% and 35% respectively ($P = 0.038$ and $P < 0.001$). Furthermore, conditioned media from co-cultured ADSC-A431-SCCs and co-cultured ADSCs-pSCCs induced tube formation in an angiogenesis assay in vitro. The expression of multiple different tumor-associated genes was highly up- or down-regulated in ADSC-SCCs-co-culture compared to mono-culture. Protein expression analysis revealed that three proteins were exclusively produced in co-culture (CXCL9, IL-1b, and MMP-7). Furthermore, the concentration of multiple different proteins was considerably increased in co-culture compared to the respective mono-cultures.

Conclusions: This is the first study evaluating the possible interactions of primary human ADSCs with human SCCs, pointing towards a doubtlessly increased oncological risk, which should not be neglected when considering a clinical use of isolated human ADSCs in skin regenerative therapies.

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THE MOLECULAR MECHANISM UNDERLYING THE PROLIFERATING AND PRECONDITIONING EFFECT OF VITAMIN C ON ADIPOSE-DERIVED STEM CELLS

Presenter: SeungYong Song, MD, PhD

Authors: Song SY, Sung JH

Yonsei University College of Medicine

Introduction: Although adipose-derived stem cells (ASCs) show promise for cell therapy, there is a tremendous need for developing ASC activators. In the present study, we investigated whether or not vitamin C increases the survival, proliferation, and hair-regenerative potential of ASCs. In addition, we tried to find the molecular mechanisms underlying the vitamin C-mediated stimulation of ASCs.

Methods: Cell proliferation and cell survival assays were conducted with ASCs treated with vitamin C. Signaling pathways were investigated using phosphor-kinase array and its inhibitor. Target genes were identified with microarray. Growth factors secreted by activated ASCs were analyzed by growth factor antibody array. Preconditioned ASCs were treated on the shaved skin of C3H/HeN mice and growth was measured. Conditioned medium from vitamin C-treated ASCs was treated on hair organ culture and hair length was measured.

Results: Sodium-dependent vitamin C transporter 2 (SVCT2) is expressed in ASCs, and mediates uptake of vitamin C into ASCs. Vitamin C increased the survival and proliferation of ASCs in a dose-dependent manner. Vitamin C increased ERK1/2 phosphorylation, and inhibition of the mitogen-activated protein kinase (MAPK) pathway attenuated the proliferation of ASCs. Microarray and quantitative polymerase chain reaction showed that vitamin C primarily upregulated expression of proliferation-related genes, including Fos, E2F2, Ier2, Mybl1, Cdc45, JunB, FosB, and Cdc45, whereas Fos knock-down using siRNA significantly decreased vitamin C-mediated ASC proliferation. In addition, vitamin C-treated ASCs accelerated the telogen-to-anagen transition in mice, and conditioned medium from vitamin C-treated ASCs increased the hair length and the Ki67-positive matrix keratinocytes in hair organ culture. Vitamin C increased the mRNA expression of HGF, IGFBP6, VEGF, bFGF, and KGF, which may mediate hair growth promotion.

Conclusions: Vitamin C is transported via SVCT2, and increased ASC proliferation is mediated by the MAPK pathway. In addition, vitamin C preconditioning enhanced the hair growth promoting effect of ASCs. Because vitamin C is safe and effective, it could be used to increase the yield and regenerative potential of ASCs.



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

Presenter: Dmitry O. Traktuev, PhD

Authors: Traktuev DO, Merfeld-Clauss S, Lupov IP,
Cook T, March KL

Indiana University

WITHDRAWN

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**DEPLETION OF WHITE ADIPOCYTE PROGENITORS
INDUCES BEIGE ADIPOCYTE DIFFERENTIATION AND
SUPPRESSES OBESITY DEVELOPMENT**

Presenter: Mikhail Kolonin, PhD

Authors: Daquinag AC, Tseng C, Salameh A,
Zhang Y, Amaya-Manzanares F, Dadbin A,
Florez F, Xu Y, Tong Q, Kolonin MG

Center For Metabolic and Degenerative Diseases

Overgrowth of white adipose tissue (WAT) in obesity occurs as a result of adipocyte hypertrophy and hyperplasia. Expansion and renewal of adipocytes relies on proliferation and differentiation of white adipocyte progenitors (WAP), however, the requirement of WAP for obesity development has not been proven. Here, we investigate whether depletion of WAP can be used to prevent WAT expansion. We test this approach by using a hunter-killer peptide designed to induce apoptosis selectively in WAP. We show that targeted WAP cytoablation results in a long-term WAT growth suppression despite increased caloric intake in a mouse diet-induced obesity model. Our data indicate that WAP depletion results in a compensatory population of adipose tissue with beige adipocytes. Consistent with reported thermogenic capacity of beige adipose tissue, WAP-depleted mice display increased energy expenditure. We conclude that targeting of white adipocyte progenitors could be developed as a strategy to sustained modulation of WAT metabolic activity.



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CHITOSAN SCAFFOLD CELLULARIZED BY STROMAL VASCULAR FRACTION FOR COLORECTAL TISSUE ENGINEERING IN SWINE MODEL: THE LAST STEP BEFORE CLINICAL APPLICATION

Presenter: Quentin Denost, MD, PhD

Authors: Denost Q, Buscail E, Pontallier A, Bareille R, Montembault A, Delmond S, David L, Bordenave L

INSERM Bioingenierie tissulaire

Objective: Tissue engineering may provide a new surgical tool for colorectal surgery. It consists mainly of two components: scaffold from which the extracellular matrix is organized in neotissue and cells seeded on the scaffold. The aim of this study was to demonstrate the interest of scaffold cellularization for colorectal tissue engineering.

Methods: A 2x3 cm colonic wall defect was performed in 20 swines. Repair was done by suturing a chitosan patch. Animals were divided in 2 groups: chitosan scaffold alone (Group A, n=10) vs. chitosan scaffold cellularized with autologous Stromal Vascular Fraction, SVF (Group B n=10). During the same surgical procedure, fat tissue was harvested to obtain SVF. Cells were mixed in composite chitosan-fibrin glue for seeding. Graft areas were explanted at 8 weeks. Endpoints were technical feasibility, scaffold behavior and quality of tissue regeneration.

Results: All scaffolds were successfully implanted. One animal died in Group A without graft area leakage. Animals tolerated their oral intake without difference about the gained weight between the 2 groups. A complete coverage of the patched area was observed with an ad integrum regeneration of the colonic wall including smooth muscle cells layer around the fibrosis scar. Immunohistochemical examination confirmed this smooth muscle layer regeneration. A significant lower fibrosis rate ($p=0.01$) was observed in the cellularized scaffold group (Group B).

Conclusion: Our data confirmed in a large animal model the healing effect of chitosan on colorectal tissue. Chitosan scaffold implantation seeded by autologous adiposed cells in large animal was mandatory to promote colorectal tissue engineering before any human uses.

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FAT GRAFTING SITE PREPARATION WITH EXTERNAL VOLUME EXPANSION IN CHRONIC SKIN FIBROSIS AFTER RADIATION EXPOSURE

Presenter: Jorge Lujan-Hernandez, MD

Authors: Lujan-Hernandez J, Chin MS, Babchenko OO, Bannon E, Ignatz R, Lo YC, Fitzgerald TJ, Lalikos JF

University of Massachusetts Medical School

Introduction: External Volume Expansion (EVE) prepares breasts for fat grafting. It has gained popularity but, for patients receiving radiotherapy (XRT), results with EVE vary. The intense fibrosis after XRT is evident and prevents full tissue expansion. However the effects of EVE in damaged and hypo-perfused tissue post-radiotherapy remains unexplored. Based on experience with both EVE and XRT, we developed a new translational model to investigate the effects in chronic skin fibrosis after radiation exposure.

Methods: n=28 SKH1E mice received bilateral 50Gy of beta-radiation (one per flank). Animals were monitored for perfusion changes with Hyperspectral Imaging (HSI) and clinical inspection until chronic radiation fibrosis developed (8 weeks). EVE was then applied at -25mmHg to one side for 6 hrs during 5 days. The opposite side served as control. Perfusion changes were assessed with HSI before and after EVE. Mice were sacrificed at 5 (n=14) and 15 days (n=14) after last EVE application and biopsied. Tissue samples were stained with H&E for structural analysis, CD31 (IHC) for vascularity and Picro-Sirius red to analyze collagen composition by scar index.

Results: All animals developed skin fibrosis 8 weeks post-radiation and became hypo-perfused per HSI. EVE induced edema on treated sides that was visible immediately after stimulation and persisted to a minor extent by day five as seen histologically. At this point HSI showed both sides hypo-perfused at comparison with pre-irradiation levels; with the EVE side 13% more ischemic than the untreated ($p<0.001$). Perfusion returned to control-side levels by day 15. Vascularity increased 25% by day 5 ($p<0.001$) and 37% by day 15 ($p<0.01$) in EVE versus control respectively. Collagen composition showed no difference in scar index analysis.

Conclusion: EVE temporarily augments radiation-induced hypo-perfusion, likely due to transient edema. Pro-angiogenic EVE effects in irradiated tissues are seen similarly as in non-irradiated tissue, although perfusion seems to remain as untreated side at the time points analyzed. No changes in the fibrosis after radiotherapy were seen. This suggests that EVE still could positively influence the success of fat grafting in irradiated tissues if intense fibrosis and soft tissue contractures can be counterbalanced.



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ADIPOSE-DERIVED STEM CELLS: A NOVEL, SHORTENED ISOLATION PROTOCOL YIELDING MULTIPOTENT CELLS FROM FAT

Presenter: Anna Wilson, MBChB, MRCS, MSc

Authors: Wilson A, Gareta-Garcia E, Butler P, Seifalian A

University College London

Introduction and Aims: The use of adipose-derived stem cells (ASCs) as an autologous and self-replenishing source of tissue provides much promise in reconstructive surgery. Multiple methods of extraction of multipotent ASCs from lipoaspirated tissue have been described. The aims of this research was the development of a shortened (45 minutes) ASC isolation protocol, which can be applied for use in the intraoperative timeframe to ameliorate results in reconstructive surgery, and to demonstrate its effectiveness.

Materials and Methods: Six patients undergoing free fat transfer procedures donated surplus adipose tissue collected by the Coleman method from the abdomen for isolation and characterisation of ASCs. 3 isolation times were trialled per patient (5g fat each): 45 minutes, 75 minutes, and 135 minutes total. Cells were characterised using flow cytometry for cell markers CD14, CD45, CD73, CD90, CD105 and HLA-DR. Cell differentiation was induced to adipogenic, chondrogenic and osteogenic lineages. Cell numbers were compared at day 0 and day 5. Cell activity was compared using Alamar Blue assay at day 5.

Results: ASCs were isolated from all 3 protocols. Cultured cells largely stained positive for CD73, CD90 and CD105, as expected from ASCs, and negative for CD14 and CD45. ASCs from all protocols differentiated into adipogenic, chondrogenic and osteogenic lineages. Cell numbers and viability were shown to be compromised during the 45 minute protocol, but no statistically significant difference was found between the 75 and 135 minute protocols.

Conclusion: An ASC isolation protocol suitable for use within the intraoperative time frame has been identified and shortened further to 45 minutes. The shortest protocol for optimal cell numbers and viability stands at 75 minutes. We have demonstrated its comparability to established, longer protocols. Further work will include the comparison of ASCs' properties as obtained from different patient groups.

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THE PARACRINE EFFECT OF MESENCHYMAL STEM CELLS RESTORED HEARING IN AUTOIMMUNE SENSORINEURAL HEARING LOSS

Presenter: Tai June Yoo, MD, PhD, MBA

Authors: Yoo TJ, Zhou B, Du X, Cheng W, Zhou Y, Di Girolamo S, Barbieri M

StemGen Therapeutic LLC

The aim of this study was to examine the activities of hASCs on autoimmune hearing loss (EAHL) and how human stem cells regenerated mouse cochlea cells. BALB/c mice underwent to develop EAHL; mice with EAHL were given hASCs intraperitoneally once a week for 6 consecutive weeks. ABR were examined over time. The helper type 1 autoreactive responses and Treg cells were examined. H&E staining or immunostaining with APC conjugated anti-HLA-ABC antibody were conducted. The organ of Corti, stria vascularis, spiral ligament and spiral ganglion in stem cell group are normal. In control group, without receiving stem cells, the organ of Corti is replaced by a single layer of cells, atrophy of stria vascularis. Systemic infusion of hASCs significantly improved hearing function and protected hair cells in established EAHL. The hASCs decreased the proliferation of antigen-specific Th1/Th17 cells and induced the production of anti-inflammatory cytokine interleukin-10 in splenocytes. They also induced the generation of antigen-specific CD4⁺CD25⁺ Foxp3⁺ T reg cells. The experiment showed the restoration is due to the paracrine activities human stem cells, since there are newly regenerated mice spiral ganglion cells, not human mesenchymal stem cells given by intra peritoneal transfer.



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SUSTAINABLE FAT GRAFTING: OPTIMIZING FAT GRAFTING IN AN IN VIVO TISSUE ENGINEERING CHAMBER MODEL

Presenter: Heidi Debels, MD

Authors: Debels H, Han XL, Palmer J, Morrison W, Hamdi M, Abberton K

UZ Brussel Belgium

Purpose: Fat grafting or lipofilling is used in various body contour procedures, such as breast reconstruction. One of the main concerns is graft resorption over time, raising questions of sustainability. In a previous study we have demonstrated that fat graft success seems to be based on regeneration of dying adipocytes, rather than on adipocyte survival. The aim of this study is to improve long term clinical outcome by adding a novel adipose derived acellular gel (Adipogel) to the fat graft.

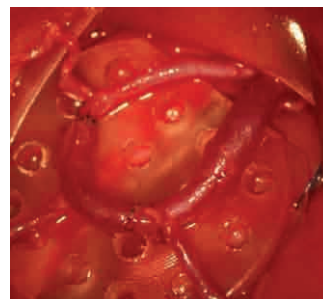
Methodology: A known rat tissue engineering model is used. An arteriovenous loop, microsurgically created from the femoral vessels, is positioned inside a 2 ml hemispheric perforated chamber in the groin. 1 mL minced autologous fat, the novel Adipogel or a combination of both is inserted within the chamber. The constructs are morphologically and histologically examined at 6 (n=3) and 12 weeks (n=6).

Results: In groups where fat tissue was inserted, the volume of grafted tissue remained stable at 6 weeks, however histologically most of the inserted adipocytes appeared dead (Perilipin immunostaining). In all groups, the amount of viable adipocytes rose between 6 and 12 weeks, indicating a period of neoadipogenesis. At 12 weeks, the Adipogel group showed similar results to the fat graft group, whereas combining both resulted in significantly better results (2.5 times more viable fat). In this group the fat graft was almost entirely regenerated.

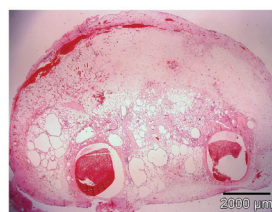
Conclusion: The mechanisms behind fat grafting seem to be based on regeneration of adipocytes, rather than on adipocyte survival. At 12 weeks, excellent adipogenesis was seen when the acellular Adipogel was added to the fat graft. We believe that the use of Adipogel has a great potential in clinical fat grafting. Moreover, we demonstrated that a pedicled fat flap can be grown in vivo, offering perspectives for larger reconstructive flap surgery as well.

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SUSTAINABLE FAT GRAFTING: OPTIMIZING FAT GRAFTING IN AN IN VIVO TISSUE ENGINEERING CHAMBER MODEL



Histological cross section(H&E) of tissue within the chamber at 12weeks



Fat alone group



Fat + ADM group



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SAFETY OF PERFORMING MANUAL ADIPOSE STEM CELL SEPARATION AT THE POINT OF CARE

Presenter: Reef Hardy, MD

Authors: Aronowitz JA, Hardy R, Hakakian CH
Cedars Sinai Medical Center

Background: Evidence suggests that ADSCs improve engraftment and long term adipose tissue regeneration when used to enrich fat graft. The Cell assisted lipotransfer (CAL) strategy is based on the observation that lipoaspirate is depleted in stem cells 1. Point of care (POC) separation of cells from lipoaspirate is required to allow the immediate use of viable cells free of risks associated with offsite transport of fat tissue and storage of cells for later injection. Collagenase POC separation processes with manual and automated devices increase cell recovery by a factor of 103 over any mechanical, non enzymatic method. Parameters such as cell characterization, cost, and residual enzyme level have been reported but valid questions of clinical safety still present a barrier to adoption of this emerging technology. This study investigates the safety and cost of a manual separation to isolate ADSCs from lipoaspirate at POC for immediate reinjection with fat grafting.

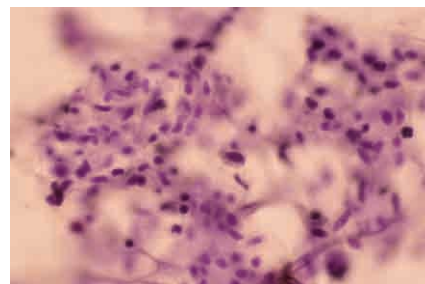
Methods: A non-automated, collagenase based process was performed on 110 patients undergoing CAL. ADSCs were restored to lipoaspirate fat graft for reinjection using a microcannula, layered technique. Detailed data concerning subject health, fat harvest, separation technique, process time and postoperative outcomes were collected.

Results: CAL was performed for a variety of indications. The mean length of the separation process was 92 min. Due to frequent concomitant procedures the separation process extended the average operating room time by only 12 minutes. The mean follow-up time was 1.5 years. There were no major complications.

Conclusions: This series of 110 CAL cases demonstrates that cell isolation using a manual, collagenase based process can be performed at the POC without added risk over fat grafting alone. The risk of oil cyst and nodule formation is typical of unenhanced fat grafting. No adverse sequelae potentially related to residual collagenase activity were noted. These results support the use of this emerging technology in the context of a clinical research environment within ASPS guidelines.

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SAFETY OF PERFORMING MANUAL ADIPOSE STEM CELL SEPARATION AT THE POINT OF CARE



Parameter	
Patient Demographics	
Age	54.9 (Range 20-77)
BMI	23.3 (Range 14-31)
Indication for Fat Grafting	
Breast Augmentation	44
Breast Reconstruction	41
Facial Aging	10
Scars and Chronic Wounds	8
Lipodystrophy/Contour Defect	5
Buttock Enhancement	2
Harvest Location	
Abdomen	65
Flanks	41
Thighs	25
ADSC Isolation Process	
Mean lipoaspirate volume digested	163cc
Number of washes	3
Total Process Time	92 min
Volume of final SVF suspension	8 cc
Volume of lipoaspirate injected	258cc
Cost	
Location of Injection	
Breast	86
Face	11
Abdomen	1
Buttock	2
Lower Extremity	8
Upper Extremity	1
Perianal fistula	1
Outcomes	
Follow-Up Time (months)	
Average	19.8
Range	1-55
Complications	
Oil cysts	3
Unrelated complications	3



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THREE-DIMENSIONAL BIOPRINTING OF ISLET AND ADIPOSE STROMAL VASCULAR FRACTION CONTAINING SPHEROIDS

Presenter: Stuart K. Williams, PhD

Authors: Williams SK, Touroo JS, Hoying JB, Hughes MG

University of Louisville

Introduction: The transplantation of isolated islets remains a significant goal toward the treatment of diabetes. A significant technical barrier to the success of this technology is the poor viability of islets following transplantation due to the lack of efficient revascularization. We have developed a strategy to utilize 3D Bioprinting technology to create spheroids that contain both islets and adipose stromal vascular fraction cells. The adipose SVF provide a neovasculature for the islets with a goal of increasing islet function through accelerated revascularization following transplantation.

Methods: Islets were isolated from human pancreata using enzymatic digestion and stored in Hanks buffered saline prior to bioprinting. Simultaneous with islet preparation, stromal vascular fraction cells were isolated from the peri-pancreatic fat using enzymatic methods. A sample of the peri-pancreatic fat was prepared for histologic evaluation. The isolated islets were co-isolated with adipose SVF and suspended in alginate and transferred to a bioprinting pen. Spheroids of islets and adipose SVF were bioprinted to obtain spheroids of varying sizes based on adjustment in pen inner diameters.

Results: The simultaneous preparation of human islets and human adipose stromal vascular fraction cell populations can be performed using the peri-pancreatic fat as a source of the SVF. A 3D Bioprinter, based on microfluidic direct-write extrusion pens, was used to create spheroids of uniform size. Evaluation of the spheroids established the presence of islets and stromal vascular fraction cells within the spheroids. The distribution of islets and SVF cells was homogeneous throughout the spheroids

Conclusions: The use of 3D Bioprinters to create organoids has seen increased evaluation. We show that a 3D bioprinter can be modified to permit co extrusion of isolated islets and vascular cells to form spheroids of uniform size and composition. Peri-pancreatic adipose tissue represents an excellent source of adipose stromal vascular fraction for construction of islet-SVF spheroids. The presence of adipose SVF within these spheroids is predicted to accelerate islet revascularization.

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HUMAN ADIPOSE SUBSTITUTES ENGINEERED FROM ASCS: PERFORMANCE ANALYSIS OF GRAFTED TISSUES USING MAGNETIC RESONANCE IMAGING

Presenter: Maryse Proulx, MSc

Authors: Proulx M, Aubin K, Lagueux J, Fortin MA, Fradette J

Centre de Recherche en Organogenese Experimentale de l'Universite Laval

Adipose tissue substitutes are being developed to answer the strong demand for reconstructive surgery applications and tissue engineering represents a promising approach for their production. In this study, tissue-engineered adipose substitutes featuring natural human extracellular matrix components were produced from adipose-derived stromal cells using the self-assembly approach. A total of three series of substitutes were implanted subcutaneously onto the muscle of athymic mice in two independent graft experiments. Magnetic Resonance Imaging (MRI) was used to study the fate of grafted adipose substitutes since this technique is one of the most powerful tools to validate their performance non-invasively (1 Tesla, M2 Compact High-Performance MRI System, Aspect Imaging). In vivo, reconstructed adipose substitutes were clearly visualized by MRI to enable efficient volume delineation. Their mean initial volume was $160.2 \pm 34.4 \text{ mm}^3$ for a mean surface of $1.41 \pm 0.36 \text{ cm}^2$. The graft volume retention was followed weekly after implantation, revealing a gradual resorption rate averaging at 65% and 44% of initial substitutes volumes at three and six weeks after grafting, respectively. In addition, vascular perfusion measured by dynamic contrast-enhanced (DCE)-MRI using the Gadomer 17 contrast agent confirmed the graft's vascularization post-implantation (day 14 and 21 after grafting). At the time of harvest, the grafted tissues revealed a healthy macroscopic aspect. Blood vessel presence was also observed on histological cross-sections after Masson's trichrome staining. Importantly, histological analysis revealed the persistence of numerous adipocytes without evidence of cysts or tissue necrosis at three and six weeks after implantation. This study describes the grafting and monitoring of human adipose substitutes devoid of exogenous matrix components and provides a practical MRI-based assessment that will facilitate the integration of this powerful imaging modality into tissue engineering preclinical studies. Supported by CIHR.



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CLINICALLY-FEASIBLE LARGE-SCALE MANUFACTURING OF ADIPOSE TISSUE-DERIVED STROMAL CELLS USING QUANTUM CELL EXPANSION SYSTEM AND HUMAN PLATELET LYSATE

Presenter: Mandana Haack-Soerensen, PhD

Authors: Haack-Soerensen M, Brorsen SK, Follin B, Juhl M, Soendergaard RH, Kirchhoff M, Kastrup J, Ekblond A

Rigshospitalet

Background: In vitro expanded adipose tissue-derived stromal cells (ASCs) are a useful resource for tissue regeneration. Translation of small-scale autologous cell production into large-scale, allogeneic clinical applications necessitates wise selection of media and cell culture platform. We compare the use of clinical-grade human platelet lysate (hPL) (heparin-free, COOK Medical) and Fetal Bovine Serum (FBS) (Life Technology) as growth supplements in the automated functionally closed hollow fiber bioreactor-based Quantum Cell Expansion System (Terumo BCT) in pursuit of safe and feasible large-scale production of ASCs.

Methods: Stromal vascular fraction (SVF) was isolated from abdominal fat. 100×10^6 cells were suspended in -MEM with 10% FBS or 5% hPL and loaded into a bioreactor coated with cryoprecipitate. ASC Po was harvested and 30×10^6 ASC from Po were reloaded for continued expansion (Pi). Metabolic monitoring guided feeding rate and time of harvest. Viability, sterility, purity, differentiation capacity and genomic stability of ASCs were determined.

Results: Each passage of this two-passage process demonstrates that hPL supports proliferation of ASCs to a greater extent than FBS. Cultivation of SVF in hPL-media for in average 9 (7-11) days yields in average 546×10^6 ASCs. Cultivation of the SVF in FBS-media requires 8 days more to yield 111×10^6 . ASCs Pi, yields in average 800×10^6 cells (PD (population doublings): 5.2) after 6 days in hPL-media compared to 100×10^6 (PD: 2.2) cells in FBS-media after 21 days. ASCs fulfil ISCT criteria in both media types (immunophenotype and triple differentiation). Comparative genomic hybridization demonstrates genomic stability. Sterility, mycoplasma and endotoxin tests were all negative.

Conclusion: The closed automated hollow fiber Quantum Cell Expansion System provides a reliable and efficient mean of manufacturing ASCs while minimising manipulation and risk of contamination. HPL serves as an effective growth supplement for expansion of clinical-grade ASCs. With combination of Quantum bioreactors and hPL we achieved 5 times more ASCs Po and 8 times more ASCs Pi relative to the same process using FBS. These yields exhibit no chromosomal aberrations and meet all release criteria, indicating a clinically-feasible

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IMAGING OF HUMAN ADIPOSE-DERIVED STEM CELLS (ADSC) WITH ATOMIC FORCE MICROSCOPY (AFM): ASSESSING MORPHOLOGY AND SURFACE TOPOGRAPHY OF DIFFERENTIATED ADIPOCYTES

Presenter: Naghmeh Naderi, MSc

Authors: Naderi N, Francis W, Wilde C, Haque T, Francis L, Xia Z, Thornton CA, Mosahebi A, Seifalian AS, Whitaker IS

UCL

WITHDRAWN



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FIRST EXPERIENCE USING CONTRAST-ENHANCED ULTRASOUND (CEUS) TO EVALUATE VASCULARIZATION AND RESORPTION OF GRAFTED FAT

Presenter: Maria Wiedner, MD

Authors: Wiedner M, Weinke R, Kamolz LP, Parvizi D
Medical University Graz

Background: Over the past 20 years autologous fat grafting became very popular in plastic, aesthetic and reconstructive surgery. It is an easy available filler substance which is tolerated excellently. The main problem in using lipofilling is the unpredictable resorption process and mostly the need of several procedures. Neither the amount of resorption nor the best time point for the second fat grafting procedure is known yet. To date there have been no reports on imaging of vascularization of grafted fat with modern noninvasive imaging techniques such as contrast-enhanced ultrasound (CEUS). In this work we want to show CEUS as a reliable and easy to use method to figure out the time of highest vascularization in the grafted fat area and the velocity of resorption. We started this examination according to our experience in using CEUS to follow up sixteen patients with acellular dermal matrices.

Methods: First application of CEUS in the follow up of lipofilling one day, two and six weeks postoperatively. The investigation is ongoing in a case of partial breast reconstruction with latissimus dorsi flap and lipofilling with 90 ml fat (not centrifugated) in a 42 year old woman. The used contrast agent is a blood-pool perfluoro gas agent consisting of microbubbles of sulfur hexafluoride stabilized by a phospholipid shell. 2.5 - 3ml of contrast media in isotonic saline was administered directly via a two-way stopcock in a peripheral vein as a bolus, 10ml isotonic saline as a post-bolus. Continuous scanning was performed immediately after injection of the contrast agent and lasted for six min.

Results: No contrast agent allergies or side effects were reported for the ultrasound examination. After contrast agent injection, at day one no vascularization was seen in the grafted fat, after two and six weeks a continuous rise in capillary ingrowth was seen. Using CEUS, 85 ml of fat volume was detected one day postoperatively and only few resorption after two weeks. Six weeks postoperatively two thirds of the volume still was visible (57 ml).

Conclusion: Based on our preliminary experience, we conclude that CEUS is a non invasive, objective real time imaging, to evaluate vascularisation and resorption of fat grafting without side effects.

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DEVELOPMENT OF A PHYSIOLOGICALLY RELEVANT CULTURE ENVIRONMENT OF TYPE II DIABETES MELLITUS FOR ADIPOCYTE CULTURE WITHIN A HOLLOW FIBER, THREE-DIMENSIONAL, DYNAMIC PERFUSION BIOREACTOR

Presenter: Danielle M. Minteer, BS

Authors: Minteer DM, Rubin JP, Gerlach JC, Marra KG
University of Pittsburgh

Introduction: We have developed an in vitro three-dimensional perfusion model to study adipose tissue function, resulting in long-term culture (>70 days) of metabolically active adipose tissue derived from patients with type II diabetes mellitus and patients without. Type II diabetes mellitus is characterized by insulin resistance resulting in elevated levels of glucose in the bloodstream caused by inhibition of glucose uptake by adipose and muscle tissues. Current protocols for adipose-derived stem cell (ASC) culture involve DMEM-based mediums with glucose concentrations 2-4 times higher than physiological blood glucose levels. To more accurately investigate metabolism of adipose, a more relevant culture condition must be optimized and an environment in which insulin-stimulated glucose uptake by adipose tissue is inhibited comparable to patients with type II diabetes mellitus should be developed.

Methods: Our established bioreactor culture system was inoculated with ASCs isolated from discarded adult human adipose tissue. Cells were expanded and adipogenic differentiation initiated through modifying the medium perfusate composition consisting of physiological glucose concentrations, then an insulin-dependent glucose uptake-hindered model was established through the utilization of sphingolipid C2-ceramide. Measurements include daily glucose consumption, lactate production, and end-point histological/immunohistochemical assessment over 70 days of culture.

Results: Mature adipocytes were generated within both groups of bioreactor culture systems exposed to high and low glucose concentrations for 70 days ex vivo, as confirmed by FABP4 immunohistochemical analysis and AdipoRed lipid accumulation. C2-ceramide did not result in a notable hindrance of glucose uptake by cells after a seven-day period.

Conclusions: Future enhancement of this model of human adult adipose tissue will be perfused with various drugs to test potential therapeutic effects addressing type II diabetes mellitus and fat graft volume retention.



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ULTRASOUND EVALUATION OF ADIPOSE AND FAT GRAFTED TISSUE - A COST EFFECTIVE, POINT OF CARE TECHNOLOGY

Presenter: Ricardo L. Rodriguez, MD

Authors: Rodriguez RL, Markelov A, Bhavani S

Johns Hopkins

Introduction: Ultrasound imaging has been used successfully in many areas of medicine as a non-invasive and cost effective point of care diagnostic tool and therapeutic guide. Nevertheless its role in the field of Regenerative Medicine has not been well defined. Discriminating clinical conditions of adipose tissue from ultrasound images is a difficult task due to poor image quality, speckle noise, illumination, and variations in probe orientation. We propose an automated ultrasound image analysis method to quantitate adipose tissue under different clinical conditions (preoperative, after saline injection, and at several time points after fat grafting).

Materials and Methods: 30 Patients were analysed. Representative 2D ultrasound images were obtained from the 4 quadrants of each buttock to 6cm depth at different time points. These were: during surgery prior to injection of saline, after saline injection, and immediately after fat grafting. Follow up images of the fat grafted area were obtained at 5 days and 4 weeks after surgery.

Our automated method extracts features from local regions of interest (ROI's) that are scale and orientation invariant in the images. These ROIs are reduced to a consensus set of "visual words" across all images. This defines a vocabulary a statistical distribution of "visual words" for each image and clinical condition. Finally, an ensemble of SVMs (Support Vector Machines- supervised learning models with associated learning algorithms that analyze data and recognize patterns) are used to classify the conditions for each patient.

Results: The experimental results show that the proposed method is reliable and predictive. We were able to accurately identify and discriminate between all clinical conditions imaged and compare the evolution of grafted tissue over time.

Conclusion: Our automated image analysis method is a significant improvement over traditional ultrasound imaging because it identifies subtle visual features in ultrasound images that are not apparent to the naked eye but are discriminative of clinical conditions. It will bring sophisticated and accurate soft tissue analysis to the bedside cost effectively and may even help define previously unrecognized soft tissue transformations.

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DIFFERENTIAL RESPONSE OF HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS, DERMAL FIBROBLASTS AND KERATINOCYTES TO BURN WOUND EXUDATES: POTENTIAL ROLE OF SKIN SPECIFIC CHEMOKINE CCL27

Presenter: Lenie J. van den Broek, MS

Authors: van den Broek LJ, Kroeze KL, Waaijman T, Breetveld M, Sampat-Sardjoeppersad SC, Niessen FB, Middelkoop E, Scheper RJ, Gibbs S

VU University Medical Center

Introduction: Cell-based regenerative medicine strategies towards tissue-engineered constructs are currently explored. Whereas cell-cell and cell-biomaterial interactions are extensively investigated, there are few studies which address how cells will respond to factors present within the wound bed. The aim of this study was to determine how cells that are currently used in skin tissue engineering applications (adipose tissue-derived mesenchymal stem cells (ASC), dermal fibroblasts and keratinocytes) will react when they come in contact with the deep cutaneous burn wound bed.

Method: The profile of wound healing mediators present in burn wound exudates (BWE) was identified by ELISA. The ability of BWE to activate mono-cultures of ASC, fibroblasts and keratinocytes and skin substitutes consisting of a reconstructed epidermis on a ASC or fibroblast populated dermis to release mediators involved in wound healing (VEGF, IL-6, CXCL1, CXCL8, CCL2, CCL5 and CCL20) was assessed by ELISA. To further study our observations, the effect of skin-specific CCL27 (present in BWE) was studied on skin cells.

Results: BWE contained a wide variety of cytokines and growth factors related to inflammation and wound healing. BWE activated both ASC and fibroblasts, but not keratinocytes, to increase secretion of CXCL1, CXCL8, CCL2 and CCL20. Only ASC showed significant increased secretion of VEGF and IL-6. When ASC and fibroblast were incorporated in bi-layered skin substitutes these differences were less pronounced. A similar discrepancy between ASC and fibroblast mono-cultures was observed when rh-CCL27 was used instead of BWE. Both CCL27 and BWE did not stimulate keratinocytes to increase secretion of any of the wound healing mediators studied. Although keratinocytes, in contrast to ASC and fibroblast, did showed increased proliferation and migration upon rh-CCL27 stimulation.

Conclusions: Taken together, these results indicate that fibroblasts and in particular ASC respond vigorously to factors present in the wound bed leading to increased secretion of inflammatory, angiogenic and granulation tissue formation related factors. Our findings indicate the importance of taking into account interactions with the wound bed when developing therapies for difficult-to-close cutaneous wounds.



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CELL SHEETS DERIVED FROM ADIPOSE-DERIVED STEM CELLS ACCELERATED CUTANEOUS WOUND HEALING

Presenter: Naichen Cheng, MD, PhD

Authors: Cheng N, Yu J

National Taiwan University Hospital

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

We stimulated extracellular matrix (ECM) secretion of ASCs and fabricated cell sheets by treatment with a stable form of ascorbic acid, ascorbate 2-phosphate (A2-P). Interestingly, we found enhanced expression of stemness markers (Nanog, Oct4 and Sox2) in ASCs within cell sheets. We further demonstrated that ASCs within cell sheets exhibited significantly enhanced neurogenic (ectoderm) and hepatogenic (endoderm) transdifferentiation capabilities comparing to monolayer ASCs, demonstrated by upregulated Nestin and Albumin genes when cultured in appropriate induction media. In the mean time, adipogenic and osteogenic (mesoderm) differentiation capacities of ASCs were still maintained after cell sheet formation. Moreover, treatment with two types of collagen synthesis inhibitors, l-2-azetidine carboxylic acid and cis-4-hydroxy-d-proline, significantly inhibited the A2-P-mediated stemness enhancement, whereas treatment with the antioxidant N-acetyl cysteine showed no effect. These findings demonstrated that ascorbic acid enhances stemness and differentiation capabilities of ASCs through ECM synthesis, thus creating an in vitro ASC niche.

Using a murine model of healing-impaired cutaneous wound, faster wound healing was noted in the group that received ASC sheet treatment, and we observed significantly more engrafted ASCs. Immunofluorescent staining of the wound sections further revealed more co-localization of ASC with CD-31 and pancytokeratin immunofluorescence in the wounds treated with ASC sheets, suggesting enhanced plasticity of ASC sheets to contribute to endothelial and epithelial cell populations within healing cutaneous wounds. Therefore, A2-P-mediated ASC sheet formation enhanced ASC stemness and transdifferentiation capabilities, thereby representing a promising approach for applications in cutaneous wound healing.

CHANGED PRESENTER

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FACTORS THAT MAY INFLUENCE NUMBER AND VIABILITY OF EXTRACTED ADRCS PER GRAM OF TISSUE

Presenter: Katarina Andjelkov, MD, PhD
(Marcos Sforza, MD)

Authors: Andjelkov K, Sforza M, Zaccheddu R
Private Clinic

Introduction: Human adipose tissue has been shown to contain a population of cells that possess extensive proliferative capacity and the ability to differentiate into multiple cell lineages. Cells isolated from the fatty portion are termed processed lipoaspirate cells and contain adipose-derived regenerative stromal cells (ADRSCs). ADRSCs are most conveniently extracted from tissue removed during an elective cosmetic liposuction procedure but may also be obtained from resected adipose tissue. This study aims to verify if there are differences on the number and viability of cells obtained from patients with different age, sex, Body Mass index (BMI), smoking and physical activity, within the same amount of processed fat.

Method: We analyzed 20 patients, both sexes, who filled questionnaire about their age and habits (sport activity and smoking). They were divided in two groups. Group 1, patients with normal BMI range from 18,5 to 24.9 (n=10) and Group 2 comprising overweight patients with BMI index 25 to 29.9 (n=10). There were no underweight or obese patients. All twenty patients had the ADRSCs extrated for therapeutical use, but with different indications. All of them had liposuction done by the author and in the same manner (tumescet technique, same cannula) to obtain 150ml of fat. The cells were purified using a commercially available fat filtration system (Puregraft) and then were digested to obtain ADRSCs (Cellution 800, Cytori Therapeutics). The cell counting was performed using a Nucleocounter (NC100, Chemotec).

Results/Complications: Number of ADRSCs obtained, cells' viability, viable ADRCS per g of fat processed, ADRSCs for active dose and total volume for cell therapy were statistically analyzed in correlation with patients' BMI, sport activity, sex, age and smoking habits and results are presented.

Conclusion: The authors could not statistically prove that any of the influencing factors studied could promote a difference on the number of cells obtained in our study group. There doesn't seem to be any statistical significance on the results that allow us to predict the number of ADRSCs that can be obtained from different subjects with diverse constitution or social habits.



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LOCALLY ADMINISTERED ADIPOSE-DERIVED STROMAL CELLS ACCELERATE PRESSURE ULCER WOUND HEALING IN YOUNG AND OLD MICE

Presenter: Amy L. Strong, PhD, MPH

Authors: Strong AL, Bowles AC, Maccrimmon CP, Frazier TP, Lee SJ, Katz AJ, Gawronska-Kozak B, Bunnell BA, Gimble JM
Tulane University School of Medicine

Pressure ulcers are localized injuries to the skin and underlying tissue that commonly occur over bony prominences. Risk factors for pressure ulcers include conditions that deteriorate with advanced age. As the world's population lives longer, the number of individuals at risk for pressure ulcers will increase considerably in the coming decades. Current standard of care for pressure ulcers relies on traditional surgical debridement and hyperbaric oxygen treatment; however, these treatments are not entirely effective. Adipose-derived stromal/stem cells (ASCs) offer an innovative therapeutic approach to the treatment of this condition. Recent studies have shown that ASCs effectively heal diabetes-related pressure ulcers. Herein, we investigate the efficacy of ASCs in healing pressure ulcers in aged mice. ASCs were isolated from inguinal white adipose tissue harvested from transgenic C57BL/6-Tg(UBC-GFP)^{30Scha/J} mice. The dorsal skin of young (2-3 month old) and old (20-22 month old) female C57BL/6 mice was sandwiched between two circular magnets (12-mm diameter) for 12-hours on and 12-hours off, repeated over two successive days to induce pressure ulcer formation. The following day, wounds were treated with PBS or ASCs. ASC treatment expedited the healing process in both young and old mice by accelerating the rate of wound closure. Histological staining with H&E demonstrated a significant reduction in the number of infiltrating leukocytes at the wound site in young and old mice treated with ASCs. Furthermore, skin of both ages showed excessive dermal and epidermal hypertrophy on day 20 following PBS treatment, yet these skins layers were reduced following ASC treatment. Histological staining with Masson's trichrome revealed increased collagen deposition following treatment with ASCs. These histological findings suggest that ASC treatment reduced fibrosis and scarring and aid in the regeneration of skin following pressure ulcer insult in both age groups. Moreover, these results corresponded to increased expression of TGF β ₁, PDGF β , VEGF, HGF, MMP9 and MMP13 following ASC treatment compared to PBS treatment. Together, these findings document the efficacy of ASCs in healing pressure ulcer wounds in young and aged mice.

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ADIPOSE TISSUE-DERIVED STROMAL CELLS INHIBIT TGF-BETA₁-INDUCED DIFFERENTIATION OF HUMAN DERMAL FIBROBLASTS AND KELOID SCAR-DERIVED FIBROBLASTS IN A PARACRINE FASHION

Presenter: Maroesjka Spiekman, BS

Authors: Spiekman M, Przybyt E, Plantinga JA, Gibbs S, van der Lei B, Harmsen MC
University Medical Center Groningen

Background: Adipose tissue-derived stromal cells (ADSC) augment wound healing and regeneration of the skin. Currently, it is unknown if and how ADSC can also influence dermal scarring. We hypothesized that ADSC inhibit adverse differentiation of dermal fibroblasts induced by the pivotal factor in scarring i.e. Transforming Growth Factor-beta₁ (TGF-beta₁).

Methods: TGF-beta₁-treated adult human dermal fibroblasts (HDFa) and keloid scar-derived fibroblasts (KLF), were incubated with ADSC conditioned medium (ADSC CM) and assessed for proliferation and differentiation in particular the production of collagen, expression of SM22alpha and development of hypertrophy and contractility.

Results: TGF-beta₁-induced proliferation of HDFa was abolished by ADSC CM. Simultaneously, ADSC CM reduced SM22alpha gene and protein expression of TGF-beta₁-treated HDFa, while their contractility was reduced too. Furthermore, ADSC CM strongly reduced transcription of collagen I and III genes as well as their corresponding proteins. On the other hand the ADSC CM tipped the balance of matrix turnover to degradation through stimulating gene expression of MMP-1, -2 and -14, while MMP-2 activity was upregulated too. Even in end stage myofibroblasts i.e. KLF, ADSC CM suppressed TGF-beta₁-induced myofibroblast contraction, and collagen III gene expression.

Conclusion: In this study we show that ADSC inhibit TGF-beta₁-induced adverse differentiation and function of HDFa and TGF-beta₁-induced contraction in KLF, in a paracrine fashion.



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EFFECTS OF LL-37-TREATED ASC CONDITIONED MEDIA ON THE WOUND HEALING OF HUMAN FIBROBLASTS

Presenter: Eunjung Yang, MD

Authors: Yang E, Yang YH, Yang YJ, Bang SI
Cheil General Hospital

Background: Adipose stem cell-conditioned media (ASC-CM) can promote human dermal fibroblasts (HDFs) proliferation and migration by directly contacting cells and activating paracrine peptides in the re-epithelization phase of wound healing.

Human antimicrobial peptide LL-37 is upregulated in skin epithelium as a normal response to injury. Recently, LL-37 has also been recognized as a chemoattractant and activator of stem cells.¹ Because the effects of LL-37-treated ASC-CM on cutaneous wound healing are not completely understood, our goal was to determine how ASCs would react to an LL-37-rich microenvironment and whether LL-37-treated ASC-CM influence to the migration of HDFs.

Methods: A migration assay of the HDFs with ASC-CM activated by LL-37 was examined. CXC chemokine receptor 4 (CXCR4), which controls the recruitment of HDFs, was analyzed at the mRNA and protein levels. To further characterize the stimulatory effect of LL-37 to ASC-CM, expression of stromal cell-derived factor-1 α (SDF-1 α), a CXC chemokine, was also investigated.

Results: LL-37- treated ASC-CM induced the migration of HDFs in a time and dose dependent manner with a maximum difference of migration at 24h after stimulation with LL-37 concentration of 10 ug/ml. The expression of CXCR4 was markedly increased by treatment of LL-37-treated ASC-CM in fibroblast, compared to non-treated ASC-CM. SDF-1 α expression was markedly increased in ASC-CM with LL-37 pretreatment. SDF-1 α blockade was significantly reduced HDFs migration.

Conclusion: These findings suggest the feasibility of LL-37-treated ASC-CM administration as an enhanced therapy for wound healing.

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ADIPOSE DERIVED STEM CELLS AND SKIN INJURIES

Presenter: Nada M. Alaaeddine, PhD

Authors: Alaaeddine NM, Saliba N, Atat O, Tarabey B, Hilal G, Hashem H

University of St. Joseph

Adipose Derived Stem cells (ADSC) are being used therapeutically in many diseases but also extensively in plastic surgery. Scars in the faces or stretch marks are an additional problem nowadays where appearances play a major role and have psychological and social impact. No available permanent and effective treatments are known to drastically reduce or eliminate scars or wounds. Our objectives were to evaluate the regenerative and repair capacity of adipose-derived stromal vascular fraction (SVF) and stem cells (ADSC) on stretch mark treatment, scar, wound healing and face lipofilling.

Methods: ADSC were isolated, purified, and cultured in vitro from lipoaspirates using a well-established protocol. The immunophenotypic properties of freshly isolated human adipose tissue derived stromal vascular fraction (SVF) and serial passaged ADSC (P0-P4) were observed by flow cytometry. After patient consent the synovial vascular fractions (SVF) and cultured AD-MSc were injected subcutaneously on the site of injury.

Results: SVF and ADSC were positive for CD 29, CD 44, CD 73, CD 90, and CD 105, and they were negative for CD 31, CD34, CD45 and CD 106. 32 patients were injected with SVF and or AD-MSc for the following indications, 6 patients for Scar reduction, 4 for wound healings, 10 for stretch marks, two for burns, and 10 for facial rejuvenation. After 40 days of follow up all the patients were satisfied of the results and consented to be followed for 12 month. 8 patients out of 10 had their stretch mark disappeared completely, the average recovery volume of wound and scars were 75% at 12 weeks, The patients with hair problems had hair growth on as early as two weeks. No adverse effects were seen for all patients.

Conclusions: The use of AD-MSc can be safe and effective treatment for soft tissue injury and hold a promising future in reconstructive surgery.



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VASCULAR ACTIONS AND PLASTICITY OF PERIVASCULAR ADIPOSE TISSUE

Presenter: Etto Eringa, PhD

Author: Eringa EC
VU University Medical Centre

A small amount of adipose tissue associated with small arteries and arterioles is encountered both in mice and man. This perivascular adipose tissue (PVAT) has a paracrine effect on the vascular tone regulation, and expands in obesity and in diabetes. This expansion not only involves enlargement of fat cells, but also accumulation of inflammatory cells and preadipocytes from bone marrow and a shift in the production of adipokines and cytokines. This effect is illustrated by the effect of PVAT-derived factors on insulin-mediated vasoregulation in mouse resistance arteries. Insulin sensitivity of endothelial cells is also involved in the insulin-mediated regulation of muscle glucose uptake by activating different signaling pathways regulating NO and endothelin-1 release. This process is influenced by various adipokines and inflammatory mediators released from PVAT, and is affected by the degree of expansion and content of inflammatory cells. It is modulated by adiponectin (via 5' adenosine monophosphate-activated protein kinase, AMPK), TNF α (via c-jun N-terminal kinase) and free fatty acids (via protein kinase C- θ). PVAT thus provides an important site of control of vascular (dys) function in obesity and type 2 diabetes. An altered profile of adipokine and cytokine production by PVAT of resistance arteries may also contribute to or modulate hypertension, but a causal role in hypertension is still under investigation.

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ADIPOSE-DERIVED STEM CELLS MITIGATE AORTIC ANEURYSM EXPANSION AND EXCESSIVE AORTIC INFLAMMATION IN AN ELASTASE-PERFUSED MURINE ABDOMINAL AORTIC ANEURYSM MODEL

Presenter: Keith March, MD, PhD (Jie Xie, MD)

Authors: Xie J, Feng D, Cook TG, Njoku VC, Babbey CM, March KL, Murphy MP

Indiana University School of Medicine

Introduction: Pathogenesis of abdominal aortic aneurysm (AAA) formation encompasses multiple inflammatory cascades leading to local tissue degradation and eventual expansion. Our study aims to explore easily accessible human Adipose Stem Cell (ASC) as a novel cell therapy to mitigate excessive aortic inflammation, thereby inhibiting AAA development.

Methods: AAA was induced in C57BL/6 mice by elastase perfusion of the abdominal aorta. Mice received intravenous injection of 106 human ASCs or phosphate buffered saline (PBS) within 24 hours of perfusion. Inflammatory cell infiltration in aorta was evaluated using flow cytometry and immunohistochemistry (IHC).

Results: Early-stage ASC i.v. immediately following elastase treatment results in a significant decrease in lymphocytes (CD4+ or CD8+), and neutrophils (Ly6G/C+) (N=2-4, p<0.05, t test). IHC staining of murine aorta demonstrated an increase in the percentage of regulatory T-cells (CD3+/FoxP3+) in peri-aortic cellular infiltrate, suggesting a role of regulatory T-cells in mitigating the inflammatory responses. Measurements of abdominal aorta diameter using video micrometry at day 0 and 14 post-elastase perfusion indicated a reduction in aortic diameter with ASC injections compared to PBS (n=3, p<0.05, t test).

Discussion: Our results indicate that systemic administration of ASCs can effectively mitigate AAA formation most likely by attenuating aortic inflammatory cell infiltration and upregulation of anti-inflammatory regulatory T-cells. Thus ASC may be employed as a novel therapy for early intervention of AAA progression in high risk patients.



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ENGINEERING VASCULARIZED ADIPOSE TISSUE USING THE STROMAL VASCULAR FRACTION AND FIBRIN HYDROGELS

Presenter: Katharina Wittmann, MSc

Authors: Wittmann K, Dietl S, Berberich O, Storck K, Blunk T, Bauer-Kreisel P

University of Wuerzburg

Introduction: Adipose tissue engineering seeks to provide novel solutions in order to improve treatment options for soft tissue defects and pathologies. Adipose-derived stem cells (ASC) have been widely applied for regenerative purposes, yet the development of coherent and vascularized adipose tissue remains challenging. In this study, as an alternative cell source, the potential of the stromal vascular fraction of adipose tissue (SVF) was evaluated in vivo. Adipogenic precultivation in vitro prior to implantation and different fibrin gels as cell carrier were investigated regarding their influence on adipose development by SVF cells.

Material and Methods: SVF cells were isolated from human aspirated fat and seeded in fibrin gel. Two fibrin formulations were used, a long-term stable fibrin gel developed by our group and a commercial fibrin sealant (TissuCol, Baxter). Seeded constructs were either implanted directly or were subjected to adipogenic as well as non-adipogenic precultivation for 7 days prior to implantation. Before being implanted, constructs were characterized regarding cell viability, adipogenesis and prevascularization. Constructs were placed subcutaneously in nude mice. After 4 weeks, adipose development and vascularization of grafts was assessed by histology, immunohistochemistry and histomorphometrical evaluation. Tissue architecture was visualized by whole-mount staining.

Results: Prior to implantation, adipogenic differentiation of cells and the formation of prevascular structures under adipogenic and non-induced conditions, respectively, were shown in vitro. Adipogenic precultivation of SVF cells seeded in stable fibrin gels was superior in comparison to non-adipogenic culture, resulting in mature adipocytes of human origin in vivo. In constructs implanted directly without precultivation, the SVF displayed only a weak differentiation capability in stable fibrin gels. In contrast, in TissuCol gels coherent and well-vascularized adipose tissue, largely from human origin, was formed. Whole-mount staining highlighted the native-like tissue architecture.

Conclusion: The SVF was successfully shown to promote adipose tissue formation and vascularization in fibrin gel in vivo and is suggested as promising cell source for adipose tissue engineering.

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ASC DIFFERENTIATION TOWARDS SMOOTH MUSCLE CELL PHENOTYPE DIMINISHES THEIR VASCULOGENIC ACTIVITY THROUGH INDUCTION OF ACTIVIN A SECRETION

Presenter: Dmitry O. Traktuev, PhD
(Keith March, MD, PhD)

Authors: Merfeld-Clauss S, Lease B, Lu H, March KL, Traktuev DO

Indiana University

Multipotent human adipose stromal cells (ASC), an attractive cell source for therapeutic applications, share multiple properties with pericytes and support endothelial cell (EC) vasculogenesis in vitro and in vivo through paracrine and direct contact communications. ASC easily undergo differentiation into smooth muscle cells (SMC) which prompted the idea of their use to fabricate large diameter vessels, where pre-differentiated ASC should establish second, tunica media, layer of the vessel wall. However, the effect of ASC lineage commitment on their potency to support EC bioactivities has not been evaluated. To address it, a comparative evaluation of vasculogenic properties of human ASC in progenitor stage (psASC) versus ASC differentiated towards SMC phenotype (smASC) was performed. ASC were exposed to 10 ng/ml of TGF β 1 or 25 ng/ml of activin A for six days which induced expression of SMC markers in cells, including SM22, SMA, and calponin. Analysis of ASC vasculogenic activities, after stimuli were withdrawn, revealed that smASC had diminished potency to support EC vasculogenesis in vitro compared to psASC. smASC conditioned media (CM) had lower ability to support EC survival in growth factor poor media. smASC had lower mitogenic response to EC CM as well as to recombinant PDGF-BB, bFGF, and EGF (factors secreted by EC). Analysis of mRNA expression of angiogenic factors in ASC revealed a fourfold increase in VEGF, a fourfold decrease in HGF, and a 40-fold increase in Inhibin BA (monomer of activin A). Diminished potency of smASC to support EC vasculogenesis was not improved by enriching the incubation media with VEGF or HGF. However, supplementing incubation media with neutralizing IgG to activin A restored the degree of vascular network formation by EC on smASC monolayer to the level observed for psASC. Similar to in vitro findings, in vivo vasculogenesis in subcutaneously implanted semisolid matrices carrying vasculogenic cell mixtures, EC+ASC, was diminished by 50% when EC were premixed with smASC instead of psASC. This study suggests that the use of SMC-phenotype pre-committed ASC instead of ASC in their progenitor state can substantially decrease the efficiency of tissue revascularization and success in fabrication of multilayered vessel constructs.



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EFFECTS OF DONORS CIGARETTE SMOKING ON VASCULOGENIC ACTIVITY OF ADIPOSE STEM CELLS

Presenter: Daria Barwinska, BS

Authors: Barwinska D, Traktuev DO, Cook T, Merfeld-Clauss S, Van Demark M, Petrache I, March KL

Indiana University School of Medicine

Introduction: The potential use of progenitor cells to treat ischemic as well as pulmonary disorders is an area of significant clinical interest. Since adipose stem cells (ASC) can be easily isolated in therapeutic amounts immediately following tissue collection, they may represent practical cells of choice when autologous therapy is desired. While it is known that cigarette smoking (CS) of patients results in a dysfunctional angiogenic activity of their endothelial progenitor cells, and lowers the number of bone marrow hematopoietic stem cells, the effect of CS on ASC vasculogenic activity has not yet been studied. Here we examined the effect of CS on therapeutic vasculogenic properties of human adipose stem cells (ASC).

Methods: Human ASC were isolated from subcutaneous fat of smokers and non-smokers (men and women), expanded, and evaluated for their ability to support vascular network formation (VNF) by human endothelial cells (EC). To analyze how the CS exposure modifies the secretome of ASC in regards to vasculogenic activity, conditioned media (CM) collected from the ASC cultures were tested in their ability to promote EC survival and vasculogenic activity in EC-normal human fibroblasts co-culture model. CM was generated by culturing ASC in EBM -2/5% FBS for 48 hours.

Results: We have shown that cultured ASC from smoking women have significantly diminished activity to support VNF compared to ASC harvested from non-smoking women. At the same time, cultured ASC from smoking and non-smoking men showed no significant difference in their pro-VNF activity. Further evaluation revealed that CM generated by ASC from smoking women induce less dense vascular networks of EC-fibroblast co-cultures compared to the ASC CM of healthy donors ($p=0.001$).

Conclusions: Our data suggest that long term smoking leads to a decrease in vasculogenic activity of human ASC, indicative by the diminished support of EC vasculogenesis by ASC and their conditioned media. Our findings demonstrate that ASC from patients with the history of smoking have limited therapeutic capacity. This information should be considered when developing ASC as a therapeutic product for autologous applications.

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DISTINGUISHING ADIPOSE “STROMAL” VERSUS “STEM” CELLS IN VIVO UTILIZING 3-DIMENSIONAL SILK SCAFFOLDS

Presenter: Jeffrey Gimble, MD, PhD

Authors: Bowles A, Lee S, Abbott R, Kaplan D, Wang M, Strong A, Brown Q, Bunnell B, Gimble J

Tulane University

Progenitors derived from white adipose tissue (WAT) possess the ability to form clonal populations and differentiate along multiple lineage pathways. However, the literature continues to vacillate between defining adipocyte progenitors as “stromal” vs. “stem” cells. We hypothesized that a labeled adipose progenitor could be sorted based on the expression of CD146, CD34, and/or CD29 and, when placed in vivo, could persist, proliferate, and generate a functional fat pad over serial transplants. Stromal vascular fraction (SVF) cells and culture expanded adipose stromal/stem cells (ASC) ubiquitously expressing the GFP transgene (GFP-Tg) were fractionated by live cell sorting with flow cytometry. Both freshly isolated SVF and ASC were seeded in 3-dimensional silk protein scaffolds, implanted subcutaneously in wild type hosts, and serially transplanted. WAT constructs were removed and evaluated for the presence of GFP-Tg adipocytes and repopulating stem cells. Morphological characterization was performed based on immunofluorescent, histochemical and confocal microscopy. Functionality was evaluated via glycerol secretion and glucose uptake. Constructs seeded with GFP-Tg SVF cells or ASC exhibited enhanced vascularization, higher SVF yields from digested tissue, and higher construct weights. Flow cytometry, quantitative polymerase chain reaction, and confocal microscopy demonstrated GFP-Tg cell persistence, proliferation, and expansion, respectively. CD146- CD34+ -enriched ASC populations exhibited higher %Hb saturation, and higher GFP+ populations than unsorted and CD29+ ASC counterparts. These data demonstrated successful serial transplantation of non-pericytic adipose derived progenitors that can constitute adipose tissue as a solid, functional organ. Such findings have the potential to provide new insights regarding the stem cell identity of adipose progenitor cells.



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COMPOSITION OF FATTY ACIDS IN HUMAN SUBCUTANEOUS ADIPOSE TISSUE

Presenter: Natalie I. Khramtsova, MA

Authors: Khramtsova NI, Beskorovaynyi AV, Kotelev MS, Plaksin SA

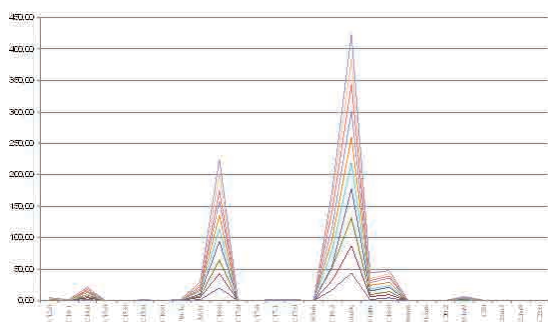
Perm State Medical Academy

Introduction: Human adipose tissue in recent years have been increasingly examined for stem cells. However, the composition of the fatty acids of human fat is not sufficiently studied.

Method: 10 samples of subcutaneous adipose tissue of different individuals from different anatomical regions have been studied. Adipose tissue was collected during liposuction, then it was centrifuged. The subject of the analysis was the upper part of lipoaspirate - pure fat that was released from the destroyed adipocytes. Pure fat samples were centrifuged and methylated by NaOH in methanol solution. Methyl esters were extracted by hexane and MTBE then analyzed on GC-MS Thermo Scientific Trace GC Ultra DSQ. The material was examined for 27 fatty acids.

Results: In samples dominated fat oleic acid methyl ester C18:1n7c (42.3±2.2%) and palmitic acid C16:0 (22.5±3.4%). In less percent linoleic C18:2 (17.5±6.5%), stearic C18:0 (4.8±1.0%), palmitoleic C16:1 (3.0±0.6%), myristic C14:0 (2.2±0.5%) and eicosenoic C20:1n7 (0.8±0.2%) acids observed.

Conclusions: In human subcutaneous fatty tissue, regardless of the anatomic zones are more common oleic, methyl ester, palmitic, stearic and linoleic acids.



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HOW MANY CELLS ARE THERE IN A GRAM OF LIPOASPIRATED ADIPOSE TISSUE?

Presenter: Ramon Llull, MD, PhD

Authors: Dos Anjos Vilaboa Sr. S, Llull R

Stem Center SL

Introduction: The adipose tissue (AT) is an easily accessible tissue that has been long used as a soft-tissue filler and source of adult autologous cells. Besides adipocytes (triglyceride storage), adipose tissue contains a variety of regenerative cells comprising the stromal vascular fraction (SVF). Currently, there is no precise knowledge of how many nucleated cells can be found per gram of adipose tissue. We establish the hypothesis that collagenase enzymatic digestion is inefficient in extracting most stromal vascular fraction cells existing in a given volume of AT. To prove this hypothesis we have calculated the theoretical number of nucleated cells per gram of tissue based on DNA isolation studies and other biochemical and volumetric approaches.

Method: The DNA isolation was performed using the E.Z.N.A. Tissue DNA Kit (OMEGA bio-tek) following the manufacturers recommendations. The yield of nucleated cells per gram of adipose tissue is based on the constant and known amount of DNA present in every eukariotic human cell. Briefly, we have performed DNA isolations from known concentrations of SVF cultured cells, fresh SVF cells (technical validation) and lipoaspirated adipose tissue samples (30-80 mg). Other methodological and theoretical approaches, such as geometrical, histological, enzymatic or biochemical were also used to estimate the theoretical number of nucleated cells per gram of AT.

Results: After setting up the technique for an accurate assessment of DNA content (digestion time, elution, etc), we determined a standard curve with known cell numbers that was reliable and followed linear behaviour. This allowed us to correlate a given amount of DNA extracted with a theoretical number of nucleated cells consistently. Based on DNA isolation, we suggest that every gram of washed and strained lipoaspirated AT (single adipocytes removed) has around 8-12 million nucleated cells. Around 10 percent of the total population would be comprised by adipocytes (1 million cells per gram).

Conclusions: Adipose tissue contains a huge amount of nucleated SVF cells that might be isolated by different processing methods (enzymatic, mechanical, etc). The best way to achieve the maximum amount of isolated viable nucleated cells from AT is still to be determined.



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EFFECT OF NANOCOMPOSITE POLYMER FABRICATION ON PROLIFERATION AND MORPHOLOGY OF ADIPOSE DERIVED STEM CELLS

Presenter: Naghmeh Naderi, MSc

Authors: Naderi N, Kalaskar D, Whitaker IS, Mosahebi A, Butler PE, Seifalian AM

UCL

WITHDRAWN

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THREE-DIMENSIONAL FLOATING CULTURE OF ADIPOSE-DERIVED STROMAL CELLS (ASCs) IN NON-CROSS-LINKED HYALURONIC ACID (HA) GEL FOR PREPARATION OF THERAPEUTIC SPHEROIDS

Presenter: Jingwei Feng, BM

Authors: Feng J, Mineda K, Doi K, Kuno S, Kinoshita K, Kanayama K, Yoshimura K

University of Tokyo School of Medicine

Background: 3D culture of adipose-derived stromal cells (ASCs) for spheroid formation is known to enhance ASCs' therapeutic potential in regenerative medicine. Here we established a new method of spheroid formation using non-cross-linked HA gel, and we examined ASC-spheroids' biological traits in vitro and therapeutic efficacy in vivo.

Method: Monolayer cultured human ASCs were placed in 1, 2, 3, 4, 5 or 10% of non-cross-linked HA gel up to 7 days to optimize spheroid formation. The spheroids were then analyzed with microarray. Pluripotency was assessed by immunocytology for pluripotent markers. ELISA was also used to detect the released angiogenic growth factor. Finally, hASC spheroids, dissociated hASCs or saline were injected into the adipose tissue which underwent ischemia-reperfusion SCID mice, and fat regeneration was evaluated at multiple time points.

Results: After a preliminary experiment, we decided to use 48 hours of culture in 4% HA gel for spheroid preparation. Some ASCs aggregated into 20-30 μ m spheroids. Among all spheroids, 37% were SSEA-3(+) and considered multi-lineage differentiating stress enduring (Muse) cell-rich spheroids, which were also positive for other pluripotent markers (Nanog, Oct3/4 and Sox-2). In contrast with dissociated ASCs, spheroids have a boost of VEGF and HGF secretion in ELISA. Microarray also demonstrated that spheroids' substantial upregulation involves many growth factors and nucleic acid-binding transcriptional factors, while some proliferation-related genes were suppressed. Reperfusion injury mice models which received local hASC spheroids injection, suffered the least fat atrophy (1.6%) comparing with those treated with dissociated hASCs (14.3%) or saline (20.3%) 28 days post injury; h-Golgi antibody staining confirmed that some hASCs differentiated into capillaries in situ in both spheroids and dissociated ASCs group; even though spheroids-treated group held highest capillary density in the first week, this change was no longer significant at 4 weeks.

Conclusion: Compared with monolayer cultured ASCs, ASC spheroids formed within non-cross-linked HA gel maintained better pluripotency and promoted angiogenesis in the process of post-injury adipose tissue repair.



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IS IT WORTH TO WASTE PART OF LIPOASPIRATE TO OBTAIN A GOOD FAT GRAFT ?

Presenter: Severiano Dos Anjos Vilaboa Sr., PhD

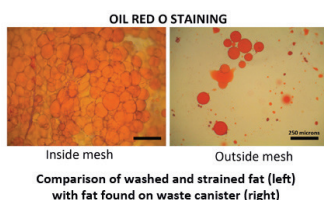
Authors: Dos Anjos Vilaboa Sr. S, Matas-Palau A, Llull R
Stem Center SL

Background: Autologous fat grafting is a well-known procedure utilized by plastic surgeons to restore soft tissue volume deficits. There is significant evidence that adipose derived stromal vascular fraction cells are responsible for the long-term fat graft retention and improved clinical results. However the best method to prepare and standardize the fat grafts before implantation is still not established. In this study we characterize the adipose tissue quality and stromal content of adipose tissue grafts prepared using an innovative point of care medical device.

Method: Raw lipoaspirates were harvested into GID700 medical devices using suction assisted liposuction. Briefly, the adipose tissue was washed using warmed Lactated Ringer following manufacturer's instructions. Oil, aqueous and tissue phases were analyzed on washed and strained fat obtained and also on waste canister. Aliquots from tissue phases were processed for phase contrast imaging, oil red O staining and cell yield studies.

Results: Washed and strained adipose tissue grafts have a consistent aqueous phase (10-20%), around 80% adipose and scarce or no oil. Wasted material -lost during processing- contains a significant amount of oil (40%), 40% of tissue and around 20% aqueous phase. The adipose tissue retained inside the mesh maintain cellular integrity, and most of the natural scaffold (extracellular matrix and vasculature) with the stromal vascular fraction cells (SVF) abundantly distributed throughout the tissue. In contrast, the tissue phase found in the waste canister is mainly composed of single or small groups of adipocytes. The native adipose tissue organization is altered, oil present and lacks most of the stromal support and extracellular scaffold. The number of nucleated SVF cells isolated by collagenase digestion per gram of adipose tissue retained in the mesh was $1.073.715 \pm 653.526$ cells (mean \pm SD), whereas in the tissue lost on waste canister was 178.775 ± 91335 cells per gram.

Conclusions: Adipose tissue processed by washing and straining has consistent aqueous phase, no oil and high stromal support (SVF yield) compared to tissue wasted during processing. This process yields adipose tissue grafts with optimal physiological conditions for fat grafting.



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REGENERATION OF DAMAGED INTERVERTEBRAL DISCS BY A NEWLY DESIGNED HYDROGEL SYSTEM IN COMBINATION WITH AMSC IN THE OVINE MODEL

Presenter: Hans J. Meisel, MD

Authors: Friedmann A, Meisel HJ, Goehre F
BG Kliniken Bergmannstrost

Introduction: Due to changed ways of life and the increasing life expectancy, the therapy of disc degeneration and its secondary diseases belongs to the most important sociomedical problems. Until today, the therapy of intervertebral disc disease is mainly based on the surgical removal of the cartilage tissue, protruding between the vertebrae. Such a sequestrectomy means a permanent loss of tissue. Regenerative implants on the basis of cell transfer, can offer a promising alternative to conventional therapies. From a clinical point of view, it is assumed that a self-regenerative implant based adipose mesenchymal stem cells (AMSC), combined with an injectable scaffold material, represents a crucial improvement of the mechanical long-term stability of damaged intervertebral disc segments.

Method: The intervertebral discs of adult female sheep, 2 years of age, were mechanically damaged. Subsequently, approximately 3g of adipose tissue was taken from each sheep. From this tissue, the AMSC were recovered through outgrowing and expansion in a cell culture system, up to a number of approximately 10^7 cells. The addition of growth factors was omitted. After scar tissue has formed over the damaged annulus fibrosus, the cell differentiation was induced in combination with a collagen based hydrogel in the disc. The sheep were killed after a standing time of 3, 6, 12 months to display the regeneration progress, as examined by CT, μ CT and histologically.

Results: Approximately 6 weeks after inducing damage, a dense scar tissue over the defected site was observed. The injected mixture of cells and the hydrogel remains within the nucleus pulposus. The damage to the cells throughout the injection is minimal. At all three points throughout the study, an even distribution of cells in the nucleus tissue is present. Typical signs of degradation, characteristic of the formation of cell clusters, could not be observed. It is observed that in comparison to the negative controls, a definite decrease in disc height could be stopped through treatment. The formation of tumors could not be observed over the period of one year.



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GENERATION OF A BONE ORGAN IN VIVO THROUGH ENDOCHONDRAL OSSIFICATION BY ADIPOSE-DERIVED STROMAL CELLS

Presenter: Arnaud Scherberich, PhD

Authors: Osinga R, di Maggio N, Allafi N, Barbero A, Schaefer DJ, Martin I, Scherberich A

University Hospital of Basel

Introduction: Bone marrow derived stromal cells (BMSC) form bone ectopically either through intramembranous ossification, by direct mineralization of a BMSC-laid matrix or through endochondral ossification, by forming a cartilaginous matrix remodelled into bone. Adipose-derived stromal cells (ASC) have yet only been shown to form bone through intramembranous ossification. The goal of this study was to investigate if ASC can form bone through endochondral ossification.

Methods: Stromal vascular fraction (SVF) cells were isolated by collagenase digestion of human adipose tissue from three healthy donors and cultured as monolayer. Expanded ASC were then either centrifuged to create micromass pellets or seeded on 4 mm-diameter, 1 mm-thick collagen-based cylindrical scaffolds (Ultrafoam™). They were then cultured in serum free medium in the presence of TGFβ-3, dexamethasone, ascorbic acid and BMP-6 for 4 weeks (chondrogenic medium). Half of the constructs were further cultured for 2 weeks in serum free medium supplemented with β-glycerophosphate, L-thyroxine and IL1β (hypertrophic medium). All constructs were implanted subcutaneously in nude mice and harvested after 4 and 8 weeks, followed by histological analysis.

Results: In vitro, deposition of a cartilaginous matrix, positive for glycosaminoglycans (GAG) and collagen type II was shown in pellets and scaffolds. Upon induction of hypertrophy, gene expression analysis showed up-regulation of collagen type X, BSP and MMP13, confirmed at protein level.

In vivo, ASC formed bone tissue in pellets and in scaffolds, in chondrogenic or hypertrophic conditions. In pellets, bone was formed after 4 weeks, whereas in scaffolds it was only observed after 8 weeks. All constructs still displayed areas with chondrocytes, GAG and collagen type II. Adjacent areas with bone tissue contained osteocytes, surrounded by a matrix with collagen type X, BSP and MMP13. Osteoclasts were found at the rim of the constructs, indicating matrix remodelling. Bone was vascularized and included bone marrow at 8 weeks. In situ hybridization for human-specific sequences identified osteocytes and osteoblasts derived from the implanted ASC.

Conclusion: These data indicate bone organ formation by human ASC through an endochondral ossification program, characterized by vascularized bone tissue homing bone marrow.

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LUCIFERASE MEDIATED MONITORING OF ADIPOSE STEM CELLS IN THE GOAT INTERVERTEBRAL DISC

Presenter: Mirte Peeters, MSc

Authors: Peeters M, Van Rijn S, Vergroesen PP, Paul CP, Wurdinger T, Helder MN

VU University Medical Centre

Introduction: Adipose stem cells (ASCs) have been shown to be promising candidates for cellular therapies, a.o. for the regeneration of mildly degenerated intervertebral discs (IVDs). However, despite many studies, the fate and survival of ASCs inside the IVD is still unclear. The purpose of this study was to determine the feasibility of ex vivo bioluminescence imaging (BLI) to monitor the temporal behavior of luciferase modified MSCs transplanted in goat IVDs which are cultured in the loaded disc culture system (LDCS)1.

Material and Methods: Cultured ASCs, transduced with lentivectors encoding either Firefly or Gaussia Luciferase (respectively Fluc, Gluc), were injected in caprine IVDs. IVDs were cultured in the LDCS for 7 days under simulated physiological loading conditions to maintain native properties of the IVD. Cells were monitored using BLI on 1, 3, 5 and 7 days post cell injection. After 7 days the IVDs were processed to detect the injected luciferase modified ASCs by H&E staining.

Results: ASCs were efficiently and stably transduced with lentivectors encoding Fluc and Gluc, and no negative effects on cell morphology, viability and proliferation rate were observed. Gluc transfected ASCs injected in the IVD showed a stable BLI signal for 7 days (Fig 1). The intensity of the BLI signal of Fluc modified ASCs was very low and hard to detect (Fig 2). Upon microscopic analysis of the IVD, the injected ASCs could be visualized using H&E staining, thus confirming their location within the nucleus pulposus of the IVD (Fig 3). Currently ongoing in vitro experiments are evaluating the effects of repeated substrate additions and the effects of hypoxia on the Fluc and Gluc BLI signals.

Conclusions: We showed the feasibility of ex vivo BLI to monitor luciferase labeled ASCs transplanted in the goat IVD. To our knowledge, this is the first study using BLI to study cell behavior inside an IVD. This technique, although further development is required, provides the ability to ex vivo investigate the survival and distribution of ASCs in the treatment of IVD degeneration prior to in vivo experimentations.

LUCIFERASE MEDIATED MONITORING OF ADIPOSE STEM CELLS IN THE GOAT INTERVERTEBRAL DISC

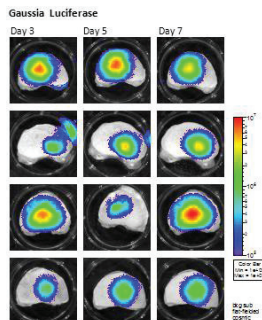


Figure 1 | BLI signal of 4 IVDs injected with Gluc-transfected ASCs, imaged 1,3,5 and 7 days post cell injection

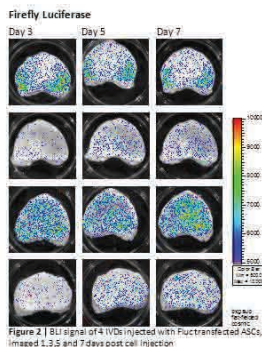


Figure 2 | BLI signal of 4 IVDs injected with Firefly-transfected ASCs, imaged 1,3,5 and 7 days post cell injection

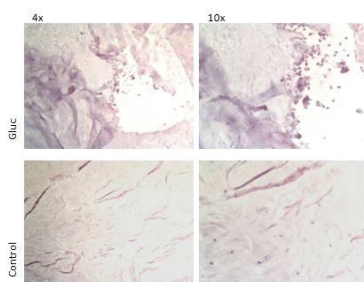


Figure 3 | Visualization of the injected Gluc cells in the nucleus pulposus of the IVI

SYSTEMIC APPLICATION OF ADIPOSE DERIVED STEM CELLS ACCELERATES FUNCTIONAL PERIPHERAL NERVE REGENERATION

Presenter: Jonas Schnider, MD

Authors: Fanzio P, Tsuji W, Kostereva N, Schweizer R, Solari MG, Marra K, Plock JA, Gorantla VS

University Hospital Zurich

Introduction: Complete disruption of peripheral nerves leads to total loss of motor or sensory function and often results in unsatisfactory regeneration. Improvement of nerve regeneration after local application of adipose derived stem cells (AD-MSCs) has been described recently. Possible mechanisms include transdifferentiation of AD-MSCs into Schwann cells (SC) as well as paracrine effects. The aim of this study was to evaluate the functional outcome after systemic application of AD-MSCs.

Methods: Lewis rats underwent sciatic nerve resection (RES, n=10), transection and repair (TSR, n=10), TSR + ASCs (TSRA, n=12). AD-MSCs (1×10^6) were administered intravenously on postoperative day one. Functional outcome was evaluated on a weekly basis with swim test and static sciatic index (SSI) over 6 weeks (TSR, TSRA). Sciatic nerve and gastrocnemius muscle were harvested at 2, 4, (n=2 per group) and at 6 weeks (Res / TSR n=6; TSRA, n=8) for histological and histomorphometrical analysis.

Results: The swim test could detect the intervention in all groups and parameters as well as an early improvement for TSR and TSRA in terms of # of strokes and performance score at week 2-3 followed by an improvement in time and angle at 4 weeks. RES did not improve in any of these parameters. Interestingly animals treated with AD-MSCs showed a superior outcome regarding time and performance score at 6 weeks and an earlier onset of improvement in # of strokes ($P < 0.01$ vs. TSR). SSI and histomorphometry revealed a significant improved recovery for TSRA compared to TSR at 6 weeks. Gastrocnemius ratios did not show a significant difference between TSR and TSRA.

Conclusion: Systemic administration of AD-MSCs after peripheral nerve transection and repair has the potential to enhance motor functional recovery and can be detected by static and functional tests, as well as histomorphometry. Systemic application of AD-MSCs appears to be a promising approach in cases where multiple peripheral nerves are involved or in order to avoid direct access to the nerve as necessary in local application.



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THERAPEUTIC EFFECTS OF AUTOLOGOUS ADIPOSE DERIVED STEM CELL ON THE REGENERATION OF MUSCLE IN SARCOPENIA MODEL OF THE RAT

Presenter: Ji Ung Park, MD

Authors: Park JU, Kwon ST, Hong JM

Seoul National University Boramae Hospital

Introduction: Sarcopenia in aging muscle was mainly caused by denervation-reinnervation process and brings about functional impairment. The purpose of this study was to demonstrate the therapeutic effect of autologous adipose derived stem cells and clarify the mechanism in an in-vivo sarcopenia rat model by nerve denervation.

Method: 1. Rat sarcopenia model and injection of ADSCs. A total of 14 male Sprague-Dawley rats were used and divided into two groups. Sarcopenia was induced by severing the common tibial nerve. In the experimental group, precultured 3×10^6 ADSCs which obtained from autologous inguinal fat pad were injected, whereas in the control group, 1cc of normal saline was injected (Fig. 1). After 2 weeks, gross examination, histologic staining (H&E, M-T staining), immunohistochemistry (MHC-s, MHC-f, amylase-PAS) and immunofluorescent staining (alpha-bugarotoxin) were performed.

2. Tracing of in-vivo injected ADSCs using Fluorescence (GFP) tagging by lenti-virus transfection. The fate of injected ADSCs into the muscle was observed through fluorescence microscopy.

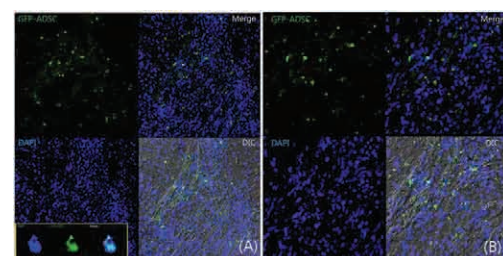
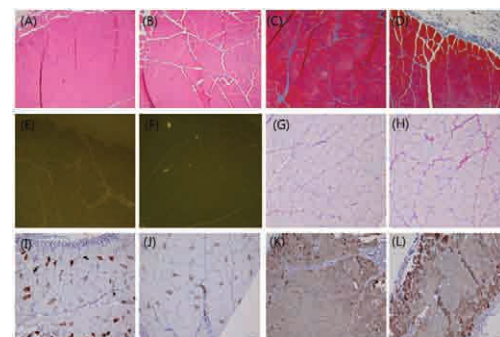
Results: 1. Analysis of histologic staining, immunohistochemistry and immunofluorescent staining. A result of H&E staining revealed that distortion of muscle fiber arrangement and muscle atrophy were less severe in the experimental group. In addition, the cross sectional area of muscle was greater in the experimental group. Through M-T staining, proliferation of connective tissue were more prominent in the control group. Alpha-bugarotoxin binding in neuromuscular junction was analyzed and the experimental group exhibited a significantly higher number of neuromuscular junctions than the control group. Capillary density, measured with amylase-PAS staining, was higher in the experimental group. The results showed no significant difference between two groups in the proportion of MHC-s, MHC-f and co-expression of two isoforms (Fig. 2).

2. Tracing of fluorescently labeled ADSCs. Injected ADSCs after transfection with Fluorescence (GFP) were observed in the leg muscle after 2 weeks (Fig. 3).

Conclusions: In this study, we suggested that autologous ADSCs prevent atrophy of a denervated muscle, and may be great modality for rejuvenation of aging muscle.

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THERAPEUTIC EFFECTS OF AUTOLOGOUS ADIPOSE DERIVED STEM CELL ON THE REGENERATION OF MUSCLE IN SARCOPENIA MODEL OF THE RAT





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SUPERCritical FLUID PROCESSED POROUS SCAFFOLDS SUPPORT THE ENDOTHELIAL DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS

Presenter: Sanna E. Pitkanen, MS

Authors: Pitkanen SE, Kyllonen L, Paakinaho K, Ahola N, Kellomaki M, Miettinen S, Haimi S

University of Tampere

Introduction: One of the major challenges in bone tissue engineering is to ensure blood supply for cells after implantation. Vascularization is essential for the viability of implanted cells especially in large tissue constructs [1]. Adipose stem cells (ASCs) have been shown to have potential as a single cell source in the engineering of vascularized bone [2]. In the current study, the aim was to evaluate proliferation and endothelial differentiation of human ASCs (hASCs) on porous composite scaffolds engineered for bone tissue engineering.

Materials and Methods: Supercritical carbon dioxide (ScCO₂) processing was used to manufacture scaffolds with the average pore size of 570±220 µm from poly (lactide-co-ε-caprolactone) 70L/30CL and βx-tricalcium phosphate (β-TCP, 40 wt-%, max. particle size 40 µm). hASCs were cultured in maintenance medium (MM), osteogenic medium (OM) or endothelial medium (EM) for 3 weeks. Cell number was studied by analyzing the total DNA amount. Endothelial differentiation was evaluated using typical endothelial markers (PECAM-1, VWF and CDH5) on gene and protein level by qRT-PCR technique and immunocytochemical staining, respectively.

Results: Proliferation of hASCs was the most pronounced in OM in comparison to MM and EM. In immunocytochemical staining all studied endothelial markers were positive only in EM. Furthermore, the endothelial marker expression in EM was also evident according to the qRT-PCR results.

Discussion and Conclusions: The hASCs were able to differentiate towards the endothelial lineage when seeded onto the composites and cultured in EM. As a conclusion, ScCO₂ processed porous PLCL/β-TCP composites together with EM differentiated hASCs are a promising combination in the engineering of vascularized bone.

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PALL: POWER ASSISTED LIPOSUCTION AND LIPOFILLING

Presenter: Saad Dibo, MD

Author: Dibo SA

Ma Clinic

Introduction: The purpose is to share the authors' five years experience with mega volume fat grafting in the breast, buttocks and arms using the power assisted liposuction technology.

Material and Methods: The Power assisted liposuction technology is used for all the steps of the surgical procedure, namely harvesting and injection of fat as well as preparation of the recipient site. Fat is harvested using a 3 mm multi-hole-cannula. The lipoaspirate is allowed to decant then is filled into 60 ml syringes. Following that, preparation of the recipient site is achieved through multidirectional and multilayered tunneling through multiple access points, in a way to fashion a matrix for fat grafting. Lipofilling is carried out in multiple planes with a custom-made V-shaped 3 mm multi-hole-cannula, enabling simultaneous vibration and tunnelization of the recipient site during fat injection.

Results: The technique was applied for a total of 330 patients over a 5 years period. Patient population included 50, 80 and 200 patients for arms, buttocks and breast lipofilling respectively. The latter comprised cases of breast augmentation, breast reconstruction and immediate exchange of implant with fat. The injected volumes of fat per side and per session ranged from 200 to 600 ml for the breast group, 150 to 250 ml for the arms group and 300 to 700 ml for the buttocks group. The operative time ranged between 50 to 80 min. One to two injection sessions were required to achieve the desired final outcome. The follow up period ranged between 12 and 40 months. Complications included 6.25% fat cysts for the breast lipofilling group, and 1.5% recipient site infection manifested as cellulitis.

Conclusion: The proposed grafting strategy holds the following keypoints: -Exploiting the recipient site as a matrix by performing multilayered multiaxial subcutaneous tunnelization using the power assisted liposuction technology to provide a larger space capacity and thus increase the volumes of fat transfer. -Simultaneous vibration and tunnelization in the recipient site during fat grafting, optimizing diffusion and dispersion of the fat in the recipient site while respecting utmost contact between the grafted fat lobules and recipient site.



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MORSI SUSPENSION MASTOPEXY TECHNIQUE

Presenter: Adel Morsi, FRACS

Authors: Morsi A, Hsieh YH

Cleopatra Plastic Surgery and The Alfred Hospital

Introduction: Mastopexy is a commonly performed, yet, a challenging operation in Plastic Surgery. In modern techniques, combining breast mound reshaping and tightening of skin brassiere provide superior aesthetic results. However, soft tissue can migrate caudally under the effect of gravity with time, resulting recurrent ptosis and patient dissatisfaction. Morsi Suspension Mastopexy (MSM) is designed to defy gravity. Breast mounds are not only reshaped and repositioned, but also suspended to clavicles creating an internal brassiere system, therefore achieving longevity.

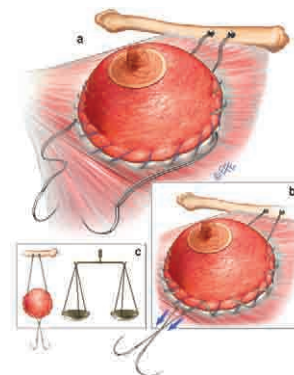
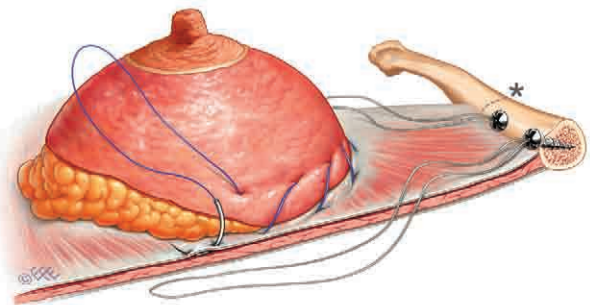
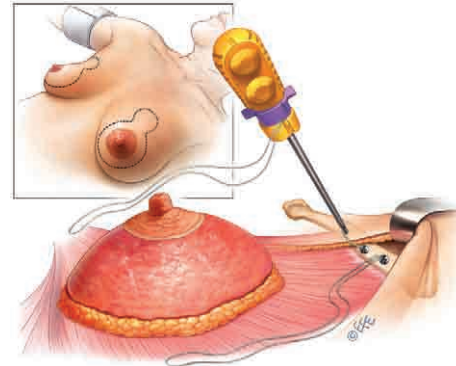
Methods: The technique involves creating a central pedicle, recruiting lateral chest roll tissue to increase breast volume, reshaping of the breast parenchyma using a purse string suture around the central pedicle with 3/0 Ethibond. Suspending the reshaped breast parenchyma to the clavicle using twin-fix anchor and its suspension suture. Vertical scar design will allow re-draping of the skin over the well projected, adequately positioned breast as well as reduction of lateral chest roll. Leaves a vertical or a T-shaped scar if conversion is needed.

Results: 10 patients were recruited, 20 breasts were operated. Mean age = 40 (22-56), average suprasternal notch-to-nipple distance = 27.4 cm (23-31). All patients have grade 2 breast ptosis or above. Mean duration of follow up is 21.7 months (7-28). Post operatively; 2 patients developed minor wound complications; 1 patient (1 breast) developed seroma, needing 1 aspiration on 2 occasions.

Conclusions: Morsi Suspension Mastopexy is versatile and reliable. It can be applied to both small and large ptotic breast, as well as patient with massive weight loss. Although short-term result is promising, long-term follow up would be the judge on this revolutionary technique.

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MORSI SUSPENSION MASTOPEXY TECHNIQUE





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CHARACTERIZATION OF AN INNOVATIVE NON-ENZYMATIC CLOSED SYSTEM FOR MINIMAL MANIPULATION OF LIPOSUCTED ADIPOSE TISSUE: TRANSLATING THE STROMAL VASCULAR FRACTION INTO REGENERATIVE MEDICINE APPLICATIONS

Presenter: Claudia Cicione, PhD

Authors: Cicione C, Barba M, Di Taranto G, Bernardini C, Sciarretta FV, Baranzini M, Isgro MA, Di Stasio E, Michetti F, Lattanzi W

Universit Cattolica del Sacro Cuore

Introduction: The adipose stromal vascular fraction (SVF) is a rich and promising source of adipose derived stem cells (ASC). Available procedures for SVF and ASC isolation are mainly based on tissue fractionation and enzymatic digestion, requiring multiple hours of uninterrupted work, which is not suitable for direct surgical applications. The development of one-step procedures based on freshly harvested SVF can overcome this problem. Here, we present an innovative device (MyStem EVO®), allowing GMP-proof non-enzymatic tissue separation and cellular enrichment, for the rapid isolation of SVF from human lipoaspirates, within a closed sterile system.

Methods: Adipose tissue (AT) from 5 donors was liposucted using the cannula contained in the kit and alternatively processed to isolate: ASCs through tissue enzymatic digestion (PLA), cells through lipoaspirate fluid protocol (LAF) and cells through MyStem protocol. All isolated cells were comparatively analyzed to assess: cell isolation yield by counting viable cells, morphological features through light microscopy and histological stainings, immunophenotype by citometry. The SVF isolated by the MyStem device was further used for in vitro osteogenic and adipogenic differentiation assays and co-culture experiments. Staining techniques and real time PCR were used to analyze the outcome in differentiation experiments.

Results: AT harvested using the MyStem device comprised small lobules, with intact cell membranes and structurally integer adipocytes. Histology showed the presence of cells without lipid content, placed in clusters along lobules stromal axis. Cells isolated with the three alternative protocols displayed a spindle shaped, bipolar morphology, comparable expression of surface antigens and differentiation capacity. Co-culture assays indicated that the SVF isolated through the novel device retained a strong osteogenic potential and osteoinductive properties.

Conclusions: These results provided the first proof of principle on the feasibility of using a closed non-enzymatic device to collect intact AT and separate a SVF enriched with ASC from liposucted adipose tissue, which can be perfectly suitable for diverse regenerative medicine applications.

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TO COLLABORATE, OR NOT TO COLLABORATE? IMPORTANCE AND CHALLENGES OF COLLABORATIONS FROM AN ACADEMIC SURGEON-SCIENTIST PERSPECTIVE

Presenter: Adam Katz, MD, FACS

Author: Katz A

University of Florida



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IS WATER-ASSISTED LIPOSUCTION A VIABLE TECHNOLOGY FOR FACE FAT GRAFTING?

Presenter: Jeffrey A. Ditesheim, MD

Author: Ditesheim JA

Private Plastic Surgery Practice

Introduction: Water-assisted liposuction (WAL) is a predictable method of fat transfer for the breast, but to date no study has evaluated WAL as an effective technique for face fat grafting (FFG). Most surgeons prefer manual fat harvest for the face because of the smaller quantities of fat. Lack of standardization of grafted fat (collected, decanted, dry) has led to an inability to validate a particular method.

Methods: WAL fat was collected with a Lipocollector using a standard protocol (0.5atm, 3.8mm cannula, range:1,2). WAL fat was dried on gauze and “% fat grafted” (of collected fat solution) was recorded. Time and quantity of fat grafted and regional amounts by facial thirds was noted. WAL and non-WAL procedures were compared. Standard view patient photographs were reviewed, including split images at time intervals of at least three months.

Results: 193 consecutive WAL procedures in 173 patients included 28 FFG. Comparison was made with a non-WAL group (n=19). (Age: 60yr (8), Gender: 41 female, 6 male; BMI: 24.6 (4.6). Total time for FFG (mean (st. dev) was 154 min (61); harvest 39 min (34), process 30.7 min (11), injection 87 min (39.5). Quantity of total FFG: 60cc (28); upper face: 13.4cc (13), midface: 30.3cc (16), lower face: 16cc (11). Follow-up: 9.4 months (8.4). Major complications included depression, facial swelling >2 weeks. Based on patient photographs, fat survival was present in all patients in the midface, to a less degree in the lower and least in the upper face. This held true for WAL and non-WAL patients.

Conclusion: 1. Whether used alone or in combination with aesthetic surgical procedures, WAL is an effective technique for FFG in facial rejuvenation. 2. Standardizing FFG permits evaluation of different patients using one method and comparison of different FFG methods.



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FATE OF ADIPOSE-DERIVED STROMAL VASCULAR FRACTION CELLS AFTER CO-IMPLANTATION WITH FAT GRAFTS: EVIDENCE OF CELL SURVIVAL AND DIFFERENTIATION IN ISCHEMIC ADIPOSE TISSUE

Presenter: Fu Su, MD

Authors: Su F, Jie L, Minqiang X, Qian W, Ran X, Yunzhou G

Breast Plastic and Reconstructive Surgery Center\Plastic Surgery Hospital\Chinese Academy of Medical Sciences\Peking Union Medical College

Background: Some studies have suggested that adipose-derived stromal vascular fraction is a potential cell source responsible for the improved quality and long-term retention of fat grafts, but studies that have clearly demonstrated the survival and differentiation potential of the implanted stromal vascular fraction cells as being dynamic phenomena have not been widely reported.

Methods: The authors isolated stromal vascular fraction cells from C57BL/6JGFP mice. Green fluorescence protein–positive stromal vascular fraction cells were mixed with minced inguinal adipose tissue harvested from C57BL/6J mice and then co-implanted into BALB/c nude mice. The survival of implanted green fluorescence protein–positive stromal vascular fraction cells was tracked by in vivo fluorescence imaging for 56 days. Immunofluorescence staining was used to analyze the differentiation of green fluorescence protein–positive stromal vascular fraction cells occurring in ischemic adipose tissue at 7, 14, 28, 35, 42, or 56 days.

Results: The fluorescence signal intensity fell drastically within the first 14 days after co-implantation and continued to decrease thereafter, with 17.3 percent signal intensity (relative to day 1) at 56 days. Immunofluorescence staining revealed that some green fluorescence protein–positive cells can spontaneously differentiate into adipocytes from day 7, and some of the implanted stromal vascular fraction cells can incorporate into new blood vessels.

Conclusions: The authors show convincing evidence for dynamic changes of stromal vascular fraction cells after co-implantation with fat grafts. The results prove the principle that implanted stromal vascular fraction cells can survive in the ischemic microenvironment of fat grafts and participate in the process of adipogenesis and angiogenesis.



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STEM CELL ENHANCED FAT GRAFTING OF THE BREAST: A SIDE BY SIDE TRIAL

Presenter: Joel A. Aronowitz, MD

Authors: Aronowitz JA, Hakakian CH
Cedars Sinai Medical Center

Background: Fat grafting is widely performed but still characterized by inconsistent long term volume retention. Yoshimura proposed ADSC enrichment as a strategy to improve permanent engraftment and tissue regeneration due to the fact that ADSCs are depleted by lipoaspiration. Safety and practicality ADSC enrichment of lipoaspirate is established but conclusive evidence of improved long term volume retention is elusive. This is a prospective, blind study of women undergoing elective breast fat grafting with unmodified lipoaspirate to one breast and ADSC enriched fat graft on the opposite breast.

Methods: 12 volunteers underwent liposuction fat graft harvest. A manual, collagenase process was utilized to isolate an average of 9.3×10^6 viable ADSCs from 200cc of lipoaspirate. Each breast received an equal volume of fat graft, one with cell enriched fat and the control with unmodified fat. Breast volume was compared at regular intervals using a quantitative volume estimation formula with existing asymmetry controlled to allow comparison.

Results: At 1 month postop, quantitative measurements showed similar volume increase in each breast, enriched volume/control volume was $1.12/1.00=1.20$. At 3 months the enriched breast had 1.40 times the retained volume compared to the control and at 6 months the enriched breast volume increased by 1.73 times. Blinded observer and patient self-evaluation correctly assessed the control vs enriched breast 96 and 88% respectively. No complications reported.

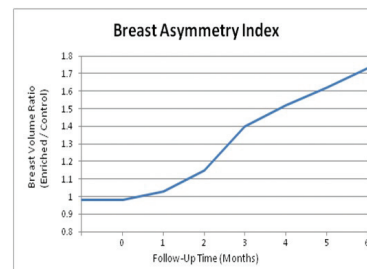
Conclusions: The design of this study overcomes some limitations of prior reports by allowing direct comparison of the effect of ADSCs on retained volume without confounding variables. Breasts injected with ADSC enriched fat began to show greater volume than the control beginning 2 months postop. The enrichment effect persisted and widened to a 73% difference in retained volume at 6 months. Blinded observer and subject evaluations were in agreement with the quantitative measurements. These results confirm the efficacy of CAL. Further studies are needed to quantify the number and subpopulation of cells needed to achieve optimal results.

References:

1. Yoshimura K et.al. CAL for cosmetic breast aug: supportive use of ADSCs. Aest Plast Surg. 2008

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STEM CELL ENHANCED FAT GRAFTING OF THE BREAST: A SIDE BY SIDE TRIAL





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AUTOLOGOUS FAT INJECTION IS A SAFE AND EFFICACE ALTERNATIVE IN THE THERAPEUTIC ALGORITHM OF LEAKAGE AROUND TRACHEOESOPHAGEAL PUNCTURE

Presenter: Giovanni Almadori, MD

Authors: Almadori G, Parrilla C, Almadori A, Paludetti G, Salgarello M

Catholic University of Sacred Heart

NOT PRESENTED

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THE UTILIZATION OF CELLULAR THERAPIES FOR THE MODULATION OF BURN SCARS

Presenter: Mehmet Bozkurt, MD

Authors: Bozkurt M, Guvercin E, Filinte GT, Sirinoglu H, Temiz G

Dr Lutfi Krdar Kartal Training and Research Hospital

Introduction: The long-term morbidity of chronic burn trauma is devastating and patients concern about their aesthetic, social, psychologic and functional problems which enforce the plastic surgeons to improve their skills and knowledge about the burn trauma. This article includes the outcomes of the utilization of fat grafts on the severe burn sequels combining with various types of stem cell sources.

Materials and Methods: Ten patients with an average burn area of 21% were treated using processed and combined fat grafts. Eight patients had burn scars in head and neck region, five in the upper extremity, three in the trunk region and two in the lower extremity. In patients who have enough subcutaneous fat tissue; fat grafts are enriched with platelet rich plasma (PRP) thrombin and stromal vascular fraction (SVF) cells. SVF are prepared using the Cytori's Celution® System. In pediatric patients bone marrow derived mesenchymal stem cells (BMDSC's) are used in the first operation followed by the utilization of thrombin with only minimal fat grafting in the second operation after three months. For the preparation of BMDSC's, 30 cc of bone marrow is harvested from the anterior iliac crest in adults and from anteromedial face of the tibia in children younger than 2 years of age and processed using the SmartPreP BMAC® system. Postoperative results were evaluated using a standardized questionnaire and photographic documentation.

Results: Average follow-up period was 10 months. Average operation number per patient was 4.7. A considerable improvement in skin softness, thickness, elasticity, color was obtained in all patients according to the results of the questionnaire.

Conclusion: Autologous fat grafting may improve the skin quality in burn scars and is a useful addition to the conventional surgical methods. The results of the study demonstrate that combining the fat grafts with various types of stem cell sources such as PRP, thrombin, SVF and BMDSC's improves the outcomes of the fat grafting procedures and creates more favorable and permanent results.



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A NEW PARADIGM SHIFT IN THE MANAGEMENT OF COCCYXODYNIA: A PRELIMINARY STUDY USING FAT TRANSFER

Presenter: Zeeshan Ahmad, BSc (Hons), MB, BS, MRCS

Authors: Ahmad Z, Park AJ

UHCW Coventry

Coccyxodynia is a debilitating and often underdiagnosed condition, where management options ranging from nerve modulation therapies to pharmacological intervention as well as surgical procedures are unpredictable. The authors present their early experience of managing coccyxodynia using the technique of local fat transfer adjacent to the coccyx. Although the exact mechanism of this is not fully understood, the authors have noticed an objective improvement in patient outcome scores (Oswestry Pain Questionnaire). A total of 15 patients have so far undergone treatment and 12/15 have had symptomatic improvement. We strongly feel that this technique in this particular patient cohort with recalcitrant pain and incapacity warrants further detailed study and may prove to be a useful tool in managing this restrictive and disabling problem.

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MAXIMIZING THE “MINI” FACE LIFT: HOW DID FACIAL FAT TRANSFER CHANGE EVERYTHING

Presenter: Renato Zaccheddu, MD

Authors: Sforza M, Andjelkov K, Zaccheddu R

Dola Park Hospital

Introduction: Patients seeking for remarkable changes in their face, usually find in a facelift the procedure of choice. Unfortunately, the modern lifestyle doesn't allow patient to have long abstinence periods from work and more conservative “mini face-lifts” became very popular. However, “mini” procedures often are associated with “mini” results, what utterly is not ideal. Many surgeons largely accept the use of fat as natural filler. The filling effect of fat has the potential to be used as a natural volumizing agent, improving the facial tissue distribution. Moreover, the anti-inflammatory properties of fat-derived stem cells are well recognized subsequent to the cell transfer to other areas, naturally giving the facial skin a better aspect. In this paper authors present retrospective study of 36 patients who had a “mini” face-lift associate with fat injections in order to improve the deformities caused by the residual excess skin and aging and a “thread lift” to maximize the lifting effect.

Method: All thirty-six patients presented a formal indication for a facelift, but decided for a mini face lift. The age ranged between 42 and 67 years. In all patients, the harvested fat was processed using the Puregraft® system and the threads used were the Silhouette Lift®. The fat was usually harvested from the abdominal area and the volume of fat transferred ranged from 10cc to 30cc, with average of 20cc per procedure. The procedures were performed under TIVA sedation anesthesia only.

Results: The results were evaluated by comparing before and after pictures, 3D imaging and a satisfaction rate from the patients. Patient's evaluation of results at 6 months were “excellent” in 100%. After 12 months the results were “excellent” in 80% of cases and “good” in 20%.

Conclusion: The medical team was highly satisfied with the Silhouette® threads and the lifting effect on the midface in all cases. At 12 months, a percentage of the injected fat had been reabsorbed, but the results with the Puregraft® system were very predictable and reproducible. This technique has minimal associated risks and complications and has been shown to be very effective, especially in patients that could not afford an extensive facial rejuvenation procedure.



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ARTERIOGENESIS AND INFLAMMATORY CELL RECRUITMENT IN A MURINE FLAP DELAY MODEL

Presenter: Scott A. Seaman, BS

Authors: Seaman SA, Cao Y, Peirce SM

University of Virginia

Introduction: A commonly used pre-treatment surgical technique, flap delay, involves the ligation of a main vascular pedicle into the fat 10-14 days prior to tissue transfer. It is believed that this initiation of ischemia leads to an increase in vascularity within the flap tissue and leads to better graft survival and long-term volume retention. Previous studies have focused on changes at the protein/single cell level following ligation, but little work has been done at the vascular network level. Here we will characterize microvascular remodeling events at the network level within flaps.

Methods: We used a published murine flap delay model in which we selectively ligated the vascular pedicle supplying the inguinal fat pad¹. Confocal imaging of whole-mounted immunofluorescently labeled tissue allowed us to visualize angiogenesis (growth of new blood vessels), arteriogenesis (expansion of preexisting blood vessels), recruitment of innate immune cells, and proliferation of cells involved with these processes (smooth muscle cells, macrophages).

Results: Immediately after ligation, we observe an 85% decrease in perfusion to ligated tissue and observe nearly complete perfusion recovery 72 hours post-surgery by using intravascular injection of isolectin to visualize patent vessels. No significant difference in angiogenic metrics at the network level (vessel length density, vessel volume fraction, angiogenic sprouting assays) were observed between ligated and control fat; however, significant arteriogenesis was present in the ligated fat. There was a 5-fold increase in the recruitment of circulating monocytes to the ligated tissue, a 3-fold increase in anti-inflammatory macrophages in the ligated tissue, a 40% increase in collateral vessel diameters supplying the ligated fat pad with blood, and an increase in the number of proliferating cells in the ligated tissue.

Conclusions: We provide an in depth look at microvascular adaptations in delayed fat and provide a potential mechanism to target (arteriogenesis and recruitment of anti-inflammatory macrophages) to improve vascularity within flaps. These studies may allow advances in flap delay techniques and better clinical outcomes during fat grafting.

References: 1. Suga et al. *Plast. Reconstr. Surg.* 126:1911, 2010.

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IN SEARCH OF THE IDEAL NERVE CONDUIT, SUPPLEMENTATION OF DECELLULARIZED NERVE ALLOGRAFTS WITH STEM CELLS DERIVED FROM TWO DIFFERENT SOURCES

Presenter: Rezarta Kapaj, MD

Authors: Kapaj R, Alhan D, Uysal C, Akgun H,

Nisanci M, Kurt B, Isik S

National Trauma Center

Question: Regeneration is very difficult in nerve gaps and often scarification of a healthy nerve is necessary.

Hypothesis: 1. Supplementation of acellular nerve allografts (ANA) with stem cells will provide similar results to nerve autografts in the sciatic nerve gap model of the SD rats. 2. The neurogenic regeneration activity of Adipose Derived Stem Cells (ASCs) is similar to Bone Marrow Derived Stem Cells (BSCs) in nerve gaps.

Material And Methods: The sciatic nerves of 12 SD rats were used as donors for the allografts. The Sondell et al. protocol was used for the decellularization. 54 rats were used (9 rats/group). 10 mm defects were created in the right sciatic nerves.

1. The sciatic nerve was exposed in the Sham group
2. The retrieved segment was reconnected in the autograft group,
3. No repair was performed in the defect group, The 12 mm ANA's were connected to the stumps in three groups.
4. 0.05 ml control medium was injected in the 12 mm ANA in one group,
5. 10⁶ DiI stained ASC's were injected in the 12 mm ANA in one group and
6. 10⁶ DiI stained BMSC's were injected in the 12 mm ANA in the last group.

Results: Data was compared using a one way ANOVA test. PostHoc test was applied as appropriate. Statistical significance was accepted at $p < 0.05$. Walking Gait Analysis: (12th week) The highest SFI (Sciatic Function Index) belonged to the Sham group followed by the ANA+ASC's group. There was no difference between the ANA+Medium group, but no difference between the autograft and SC enhanced groups. Electrophysiology, histomorphometry and immunohistochemistry analysis (S100 and GFAP) (12th week). The lowest latency and highest amplitude was recorded on the Sham group, followed by the MSC enhanced groups. The highest myelin count belonged to the Sham group, followed by the autograft group. The MSC enhanced groups showed statistically significant difference when compared to the ANA +medium group and no statistically significant difference from the autograft group. DiI and S100 stained cells were interpreted to be MSC derived Schwann like cells.

Conclusion: Similar results to the autografts have been achieved using the stem cell enhanced ANA's. statistically significant difference between the stem cell enhanced



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METHACRYLATED GELATIN AND MATURE ADIPOCYTES: PROMISING COMPONENTS FOR ADIPOSE TISSUE ENGINEERING

Presenter: Birgit Huber, MD

Authors: Huber B, Hoch E, Tovar G, Borchers K, Kluger PJ

University of Stuttgart

Large fatty tissue constructs are urgently needed as implants after cancer surgery or high-graded burns. To date, no suitable replacement is available. Autologous fatty tissue transfer results in high tissue shrinkage. Also the use of differentiated stem cells has not yet shown notably success. Our strategy is to build up a subcutis equivalent by using mature adipocytes in a crosslinked gelatin matrix, which allows an easy and fast tissue composition.

For the encapsulation of mature adipocytes, methacrylated bovine gelatin was used. The cytocompatibility of the encapsulation procedure was evaluated by testing the GM, the photoinitiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as well as the photo-irradiation time. Mature adipocytes were characterized in a 2D culture by comparison to adipogenic differentiated adipose-derived stem cells (ASCs) regarding cell morphology, size and lipid accumulation. Adipocytes were encapsulated into gels of GM. The elasticity of the constructs was adapted to that of native fatty tissue which was evaluated performing rheological measurements. Viable cells were detected by staining with fluorescein diacetate (FDA). Furthermore, H&E staining and the staining of perilipin A and collagen IV were performed. Cell functionality was determined by glycerol and leptin release.

GM as well as the photoinitiator LAP showed no cytotoxicity in the relevant range of concentrations. Isolated mature adipocytes showed a different morphology, cell size and lipid accumulation pattern compared to differentiated ASCs. A method for the encapsulation of mature adipocytes into macroscopic gels of crosslinked GM was successfully established (Figure 1). The elasticity of the subcutis equivalents was shown to be comparable to that of native fatty tissue. Encapsulated adipocytes were viable and functional for up to 14 days, which was shown by FDA staining, glycerol and leptin release as well as perilipin A and collagen IV stainings.

Human adipocytes are a suitable cell type to build up 3D subcutis equivalents. GM is an excellent matrix due to its biocompatibility and tunable properties. Our long-term goal is the composition of large subcutis constructs supplied by a vascular system and culture in a bioreactor.

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TISSUE ENGINEERED BROWN ADIPOCYTE CONSTRUCTS

Presenter: Francisco Silva, BS

Authors: Silva F, Vargas V, Holt D, Boudina S, Cho S, Atkinson D, Yockman J, Bull D, Patel A

BioRestorative Therapies

WITHDRAWN



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ADIPOSE STROMAL VASCULAR FRACTION; ARE MECHANICAL METHODS OF ISOLATION AS EFFECTIVE AS THE TRADITIONAL ENZYMATIC DIGESTION?

Presenter: Alexandra Conde-Green, MD

Authors: Conde-Green A, Rodriguez RL, Slezak S, Singh DP, Goldberg NH, Holton III L, McLenithan J

Johns Hopkins Bayview Medical Center and University of Maryland Medical Center

Background: Adipose-derived cells are recognized as being an effective regenerative cell population with a wide application in autologous fat grafting and reconstructive procedures in various fields. There is a unanimous agreement on the method of isolation of regenerative cells which is based on the use of enzymatic digestion to obtain the stromal vascular fraction. Since this method involves extensive work, skill, material, cost and time, several researchers are attempting simpler processing and isolation methods to obtain this fraction.

Methods: A population of adipose stromal vascular cells was isolated from subcutaneous adipose tissue harvested from eight healthy female patients aged 21-54 years undergoing body contouring. We used three isolation protocols: high speed centrifugation, vibration then centrifugation, both without using serum or animal-derived reagents, and collagenase digestion. Stromal vascular cells were subjected to cell counting, viability measurements and flow cytometric analysis with expression of surface markers.

Results: Stromal vascular cells' yields differed between enzymatic and mechanical protocols. Collagenase digestion showed a great quantity of mesenchymal and endothelial cells and very few hematopoietic cells (CD45+). Mechanical processed lipoaspirate cells comprised more hematopoietic and inflammatory cells, and significantly less regenerative cells (Mesenchymal CD45- CD 73+, CD 90+, CD 105+) and Endothelial cells (CD45- CD31+).

Conclusion: Stromal vascular cells can be isolated from human lipoaspirate samples by mechanical procedures however the yield of the cell population is different in regards to the method used. The increase hematopoietic and inflammatory cells obtained with mechanical protocols may be hazardous to some grafts. Also enzymatic digestion yielded a significantly greater quantity of mesenchymal stem cells and endothelial cells than mechanical protocols.

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XENOTRANSPLANTATION OF HUMAN ADIPOSE STROMAL CELLS INTO IMMUNOCOMPETENT RATS AS WELL AS MICE, SIGNIFICANTLY REDUCES INFLAMMATORY MARKERS AND M1/M2 MACROPHAGE RATIOS IN CERULEIN-INDUCED ACUTE PANCREATITIS

Presenter: Pamela I. Rogers, RLatg

Authors: Rogers PI, Maxwell T, Serezani H, Feng D, Dey D, Gangaraju R, Murphy M, Zyromski N, Babbey C, March KL

Indiana University

Acute pancreatitis (AP) is an inflammatory disease of the pancreas with high morbidity and mortality, yet no specific medical therapy exists. Blockade of inflammatory cytokines or their receptors decreases severity of pancreatitis and improves survival in animal models; however, these findings have not been translated into effective therapies. The stromal-vascular cell fraction of adipose tissue provides a rich source of adipose stem/stromal cells (ASC), which have multiple anti-inflammatory and immunomodulatory properties. In a pilot study we addressed the hypothesis that modulation of macrophage polarization by human ASC (hASC) could significantly contribute to the resolution of pancreatitis.

Rats and mice of outbred strains were intentionally raised in a husbandry environment exposed to conditions to consequently develop robust immune and inflammatory systems. AP was induced by six hourly IP injections of the secretagogue peptide cerulein (Ce). Cells were delivered by IP injection. Groups included vehicle (V), Ce only, ASC only, V+ASC and Ce+ASC. M1 presence was determined by the qRT-PCR analysis of iNOS and TNF-alpha, and M2 macrophage by RetnLA. Injections of Ce resulted in marked elevation of both pancreatic and pulmonary iNOS and TNF-alpha from baseline, and little change in RetnLA. Human ASC delivery was found to robustly suppress M1 cells as well as TNF-alpha expression, resulting in more than tenfold increase in the M2/M1 ratio vs. Ce alone ($p < 0.001$). Immunohistologic evaluation of CD68, CD206, CD3 and FOXP3 further confirmed the results. Dapi labeled hASC were present throughout the pancreas postmortem. Pancreatic extracts from rats injected with Ce demonstrated significant ($P < 0.05$) increase in mRNA expression for cell adhesion molecules and chemokines in addition to iNOS and TNF-alpha, suggesting severe inflammation. On the other hand, ASC significantly ($p < 0.05$) reduced these transcripts.

IP delivery of hASC provides protective effects in pancreatitis and reduced acinar death and necrosis in both species at two different dosages. There was no evidence of increased inflammatory changes resulting from Xenotransplantation. hASC therapy provides an important approach to further evaluation for treatment of AP.



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INTERACTION OF GELATION KINETICS AND NEEDLE SIZE ON DELIVERY OF SVF AND ADSCS USING RENEVIA: EFFECTS ON CELL SURVIVAL

Presenter: Isaac E. Erickson, PhD

Authors: Matas-Palau A, Dos Anjos Vilaboa S, Onorato MV, Llull R, Zarembinski T, Erickson IE

Stem Center SL

Introduction: Enriching fat graft with cells of the stromal vascular fraction (SVF), or cell-assisted lipotransfer (CAL) is a popular method for improving engraftment. Many report that CAL results in better engraftment or permanence than traditional fat grafting. The intrinsic regenerative potential of SVF cells has been implicated in this effect. Renevia is a hyaluronan and collagen based hydrogel that may replace autologous fat for the delivery of SVF cells within a convenient and consistent matrix. An important factor for consideration is the viability of encapsulated SVF cells after passing through syringe needles at varying stages of the hydrogel gelation process.

Methods: To determine the effect of needle injection, SVF cells were encapsulated within Renevia and expressed (50 μ l) through 4 needle sizes (20G-30G) every 30 minutes for 120 minutes. Calcein AM (live cell label) and propidium iodide (dead cell label) were incorporated in the Renevia-SVF suspensions, the aliquots were imaged with a fluorescence microscope, and the live and dead cells were manually counted to determine percent viability. A similar experiment was performed using human culture expanded adipose derived stem cells (ADSCs) in Renevia. Human adipose tissue lipoaspirates were obtained after informed consent and SVF isolation was carried out using the GID SVF-I device and collagenase. Baseline SVF viability was analyzed by image cytometry (Nucleocounter NC-3000).

Results: The mean viability of freshly isolated SVF cells used in these experiments was 68% (Fig. 1). The viability of SVF cells after simulated injections were similar for the 4 needle sizes early in the liquid to gel transition (0-30min); however, after the transition to a gel is completed (>60min) there was an observed loss of viability that was significantly more pronounced for smaller needle sizes. Further, when repeated with ADSCs in Renevia, the resulting viability was 50% of SVF cells for 30G needles (Fig. 2).

Conclusions: Hydrogel encapsulated cells are best to inject before the liquid-gel transition is complete, especially if fine needles are required for the desired application. SVF cells appear to be more robust than culture expanded ADSCs in their ability to avoid physical damage caused by needle injection.

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INTERACTION OF GELATION KINETICS AND NEEDLE SIZE ON DELIVERY OF SVF AND ADSCS USING RENEVIA: EFFECTS ON CELL SURVIVAL

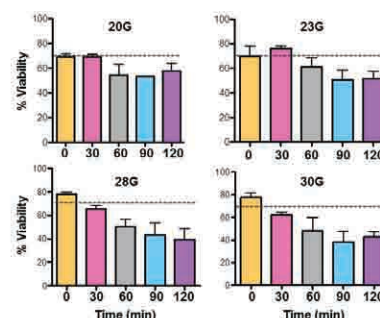


Figure 1 Viability of Renevia encapsulated SVF cells ejected through needles of various gauge every 30 minutes during gelation. Dotted line represents the mean baseline viability of the SVF cells (n=4). Note the decrease in viability with both gelation time and decreasing needle size. Optimum viability may be obtained by injecting Renevia-SVF within 30 minutes of initiating gelation.

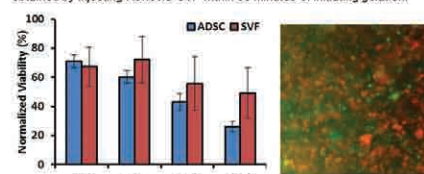


Figure 2 Normalized viability of both SVF and culture expanded ADSCs when ejected through different sized needles after 90 minutes of hydrogel gelation (Left). Note the similar trend in viability for ADSC, decreasing with smaller needles, however, the loss of ADSC viability nearly doubles as the needle size approaches 30G. Representative fluorescence image (right), demonstrating nearly 75% loss from passage through 30G needle after 90 minutes of gelation. Dead cells are labeled red and live cells are labeled green.



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ROLE OF EXOSOMES IN ANGIOGENIC POTENTIAL OF HASC-DERIVED CONDITIONED MEDIUM

Presenter: Yameena T. Jawed, MD

Authors: Jawed YT, Traktuev D, March KL

Indiana University School of Medicine

WITHDRAWN

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PLATELET-RICH PLASMA (PRP) AUGMENTS THE PRO-ANGIOGENIC CAPACITY OF ADIPOSE TISSUE-DERIVED STROMAL CELLS (ADSC)

Presenter: JCN Willemsen, MD

Authors: Willemsen JCN, Van Der Lei B, Harmsen MC
UMCG Groningen

Introduction: Clinically, it is recognized that PRP improves survival of lipograft survival while PRP augments wound healing too. However, only few clinical trials have studied this phenomenon and also reach inconclusive data. Moreover, none of these trials is randomized, double-blind and placebo controlled. Lack of standardization in creating PRP and PRP-fat mixture ratio may be a confounding bias in the published studies due to the potential concentration dependent effect of PRP. In the current in vitro study, the concentration dependent effect of PRP on ADSC (Adipose tissue-Derived Stromal Cells) is explored.

Methods: PRP was generated from whole blood of three healthy volunteers using the Biomet GPS III unit, and mixed with DMEM to a concentration of 15%, 5% and 1.7%, while DMEM with 10% FCS served as a control. Pooled ADSC from three human donors were cultured in these media. After 3 days a proliferation was assessed through immunofluorescent detection of Ki-67 antigen in the nuclei of the ADSC. At the same time point, expression of mesenchymal, angiogenic and reference genes was assessed by qRT-PCR. Alternatively, conditioned medium from ADSC (n=3 donors) was prepared by 3d pre-culture in endothelial culture medium with resp. 15, 5 or 1.7% PRP or 10% FCS as control followed by 16h incubation in the absence of PRP or FCS. The influence of this serum-free conditioned medium was assessed on sprouting of HUVEC on Matrigel for 16h. The number of sprouts as well as their length was calculated by automated software.

Results: A total of 3 cc of PRP was created per donor. There was no significant difference in platelet count among the donors. Total cell count and Ki-67 expression peaked at high PRP concentration ($p < 0.001$ compared to FCS). PRP suppressed smooth muscle cell markers and collagen I and III expression while up-regulating TGF- β 1, β 3, FGF-2 and PDGF-R. This effect was concentration determined. Conditioned medium from ASC exposed to PRP, influences HUVEC sprouting capabilities significantly.

Conclusions: Addition of PRP significantly changes proliferation and expression of several genes compared to control conditions in vitro and influences HUVEC sprouting capabilities. Results from this in vitro study may contribute to a more standardized clinical



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CHARACTERISATION OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS WITH ENHANCED ANGIOGENIC AND ADIPOGENIC PROPERTIES

Presenter: Anne Therese Lauvrud, MD

Authors: Lauvrud AT, Wiberg M, Kingham PJ

Norrland University Hospital UMEAA University

Introduction: Autologous fat grafting is a popular method for soft tissue augmentation and breast reconstruction after cancer. However, the success of such procedures relies on the graft surviving until a sufficient quantity of new blood vessels have revascularised the tissue. Survival can be improved by supplementing the fat graft with a stromal vascular fraction cell mix or isolated adipose tissue derived stem cells (ASC). With a view to identifying an optimal cell type for transplantation we have evaluated the angiogenic and adipogenic properties of CD146+ cells isolated from cultured ASC.

Materials & Methods: Human abdominal fat (n = 8 female patients, mean age = 43 ± 2.2 years) was treated with collagenase type I followed by centrifugation to pellet the ASC, which were then plated onto tissue culture plastic. The adherent cells at early passage were selected for the CD146 surface antigen using immunomagnetic beads.

Results: The mean yield of CD146+ cells was 18.16 ± 3.67%. Both CD146- and CD146+ cells expressed CD90 and alpha smooth muscle actin protein and were negative for CD31 and CD34. The CD146+ cells expressed more NG2 protein, consistent with an overall phenotype characteristic of pericytes. qRT-PCR and ELISA showed that CD146+ cells expressed higher levels of a number of angiogenic molecules including angiopoietin-1, FGF-1 and VEGF-A. Conditioned medium taken from CD146+ cells significantly increased formation of in vitro endothelial cell tube networks whereas CD146- cells did not. Treatment of both CD146- and CD146+ cells with adipogenic differentiating medium resulted in formation of Oil Red O positive adipocytes. However, the number of adipocytes was significant greater in the CD146+ treated cells. Consistent with this, the CD146+ cells showed higher expression of the adipocyte markers, adiponectin and leptin.

Conclusions: These results suggest that CD146+ cells selected from a heterogeneous mix of ASC have more favourable angiogenic and adipogenic properties, which might provide significant benefits for reconstructive and tissue engineering applications.

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HAIR FOLLICLE STIMULATION BY STROMAL VASCULAR FRACTION ENHANCED ADIPOSE TRANSPLANTATION

Presenter: Eric J. Daniels, MD

Authors: Daniels EJ, Sforza M, Ball E, Perez-Meza D, Ziering C, Krishnan G, Daniels E

Kerastem Technologies LLC

NOT PRESENTED



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**HAIR FOLLICLE STIMULATION BY STROMAL VASCULAR
FRACTION ENHANCED ADIPOSE TRANSPLANTATION**

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**AUTOLOGOUS FAT GRAFTING TO TREAT PENILE
LICHEN SCLEROSUS**

Presenter: Aurora Almadori, MD

Authors: Almadori A, D Andrea F
Second University of Naples

NOT PRESENTED



CHANGED PRESENTER

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FAT GRAFTING WITH SILICONE CALF IMPLANTS: NEW RESULTS THAT WE COULD NEVER ACHIEVE BEFORE

Presenter: Marcos Sforza, MD (Katarina Andjelkov, PhD)

Authors: Andjelkov K, Sforza M, Zaccheddu R

Private Clinic

Introduction: Number of calf augmentation with implants is increasing every year. Unfortunately, due to the fact that silicone implants available for surgery have very limited options of pre manufactured sizes and shapes (3 to 5 options only), fine symmetry in volume and contour is often difficult to achieve. Moreover, as implants are foreign bodies and the implanted area is small, a non natural aesthetic is not uncommon. Patients with large asymmetries due to trauma, previous surgeries or sequelae of diseases like poliomyelitis, find it impossible to match their calves with the options available in the market. We present a retrospective study of 24 patients who had fat injections to correct deformities or asymmetries together with silicone calf surgery.

Methods: 24 patients presented either need for aesthetic calf surgery or an asymmetry due to a previous injury/disease. All preferred insertion of calf implants (with possible correction with small amount of fat) rather than fat transfer only. We divided the patients in two groups: aesthetic (n=18) and asymmetries/deformities (difference in shape/volume, n= 6). The age range was between 19 and 35 years. In all patients, the fat was harvested and processed using the Puregraft® system. The fat was usually harvested from the abdominal area and the volume of fat transferred ranged from 60cc to 160cc, with average of 120cc per procedure.

Results: The results were evaluated by comparing before and after pictures and a satisfaction rate from the patients. Patients with satisfaction rate after 6 months were “excellent” in 83.79% of cases, “good” in 3.8%, and “fair” in 2.32%. The medical team evaluation after 6 months rated as “excellent” 87.6% of cases, “good” 10.2% and fair 2.2%. In all cases, a successful correction of the previous problems was achieved without any complications in this series.

Conclusion: Patients were offered fat transfer to improve their results associated with calf implant surgery. At 6 months, a percentage of the injected fat had been reabsorbed, but the high satisfaction rate was sustained. The medical team was highly satisfied with the Puregraft® system as we could process more fat, quicker. This technique has minimal associated risks and complications and has been shown to be very effective.

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ADIPOSE VERSUS FASCIAL SLING FOR ANTERIOR SUBCUTANEOUS TRANSPOSITION OF THE ULNAR NERVE

Presenter: Joseph M. Lombardi, MD

Authors: Lombardi JM, Verveld CJ, Danoff JR, Rosenwasser MP

Columbia University

NOT PRESENTED



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**ADIPOSE VERSUS FASCIAL SLING FOR ANTERIOR
SUBCUTANEOUS TRANSPOSITION OF THE ULNAR
NERVE**

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**A RANDOMIZED PHASE II, DOUBLE-BLIND, DUAL
ARM STUDY TO ASSESS THE EFFICACY OF ADIPOSE
DERIVED STROMAL VASCULAR FRACTION (SVF)-
ENRICHED AUTOLOGOUS FACIAL FAT GRAFTS, &
CONCURRENT UPPER ARM FAT GRAFTS, ISOLATED VIA
THE ANTRIA CELL PREPARATION PROCESS (ACPP)**

Presenter: Shah Rahimian, MD, PhD

Authors: Rahimian S, Maliver L, Bizousky D, Tatarko B,
Johnson T

Antria Inc

Facial lipoatrophy, a resultant of either pathology or aging, has been treated by various methods such as autologous lipografts and artificial dermal fillers. Specifically, autologous fat grafting has become associated with adverse events (i.e. lipograft necrosis, micro-calcification etc.) leading to inconsistent and, at times, ineffective results. These adversities may be overcome by the administration of SVF to autologous fat grafts. Hence, this study aims to evaluate the efficacy of autologous facial fat grafts, which have been supplemented with autologous adipose-derived stromal vascular fraction (SVF).

The retention of autologous facial fat grafts, over a period of 12 months, will be assessed in two groups: 1) SVF-enriched facial fat graft, and 2) non-SVF-enriched facial fat graft. A total of thirty-four (34) subjects will be enrolled in this study, with 17 subjects in each group. The quantified facial locale was defined by specific anatomic location, and referred throughout as the inframalar region.

Quantification of the volume retention within both groups will be facilitated, primarily, by a 3-D imaging camera. Furthermore, efficacy will be assessed by physical assessment, graded on a standardized facial laxity scale. A secondary end-point, referencing safety of the procedure, will be assessed, primarily, through ultrasonography, blood lab work-up, urinalysis, and electrocardiography (ECG).

Volume retention was further analyzed by the placement of a 4 mL fat graft, superficial to the triceps, within the non-dominant upper arm. This fat graft will be deposited into the arm, and volume analysis, via 3-D imaging, will parallel the respective facial fat graft analyses. Amongst the two groups, comparison will further help to evaluate retentive properties of the SVF-enhanced fat graft. At the time of presentation, Antria will have preliminary findings, with initial subjects completing their three month follow-up.



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QUALITATIVE AND QUANTITATIVE DIFFERENCES OF ADIPOSE TISSUE-DERIVED STROMAL CELLS FROM SUPERFICIAL AND DEEP SUBCUTANEOUS LIPOASPIRATES: A MATTER OF FAT

Presenter: Wanda Lattanzi, MD, PhD

Authors: Lattanzi W, Di Taranto G, Cicione C, Visconti G, Barba M, Baranzini M, Bernardini C, Michetti F, Salgarello M, Michetti F, Salgarello M

Universit Cattolica del Sacro Cuore

Introduction: A significant variable to be considered for the clinical outcome of fat grafting is the site for liposuction, which seems to affect stem cell yield and growth kinetics. Both surgeons and biologists, have been focusing on the donor site, though the anatomy of adipose tissue suggests that it should be analyzed also “vertically”: subcutaneous tissue is structured into two layers, a superficial areolar tissue and (SAT) and the deep lamellar layer (DAT), separated by the fascia superficialis. Our study was aimed at comparatively characterize the two tissue layers, focusing on the stem cell compartment.

Methods: We enrolled 13 female patients undergoing elective breast reconstruction; SAT and DAT were collected through manual liposuction, using a Mercedes cannula (1.8 mm in diameter with each hole of 0.9 mm and a bullet-like tip), with a constant pressure of 500 mmHg. The tissue morphology was analyzed histologically, hence adipose-derived stem cells (ASCs) were isolated by plastic-adherence and immunophenotyped (CD45, CD34, CD31, CD105 and CD90). Total RNA isolated from ASCs was used for real time PCR to analyze the expression of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), S100B, RAGE, adiponectin (ADIPOQ), ADIPOQR1, SOX2 NANOG, POUF5S1.

Results: Both SAT and DAT samples displayed an integer morphology: small lobules, intact cell membranes and adipocyte structural integrity. ASCs displayed a spindle shaped, bipolar morphology, upon seven days of culture. Flow cytometry revealed a higher number of CD105+ cells in SAT compared to DAT ($p<0.05$), which has been correlated with higher growth kinetics and differentiation potential, in previous studies. The expression of VEGF, SOX2, NANOG, POUF5S1 was significantly higher in the abdominal SAT ($p<0.05$), while IGF expression was higher in DAT ($P<0.05$), both collected from the abdominal wall.

Conclusion: Overall our data seems to suggest that the areolar fat may represent a naturally enhanced autologous filler; due to increased stem properties. This results originally provide the biological rationale for the clinical evidence indicating that the abdominal SAT is the best donor site for both surgical and regenerative medicine purposes.

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LIPOASPIRATE STORAGE TEMPERATURE AFFECTS YIELD AND VIABILITY OF ISOLATED SVF CELLS

Presenter: Michael A. Zieger, PhD

Authors: Zieger MA, Tholpady SS, Sood R, Gupta MP
Indiana University School of Medicine

Introduction: The successful storage of fat grafts has the potential to make delayed or repeated grafting possible when immediate implantation is not feasible. Although optimized cryopreservation can yield high cell viability and unlimited storage time, it is a procedure that requires adherence to predetermined protocols, specialized equipment and the use of animal sera and cryoprotective agents that can cause adverse reactions. In most clinical settings, simple cold-ischemic storage is more practicable. We hypothesized that the viability of SVF cells isolated from stored lipoaspirates would be inversely proportional to the storage temperature.

Methods: 20 g samples of lipoaspirates were stored in 50 ml centrifuge tubes for up to 4 days at 37°, 30°, 25° or 4°C. Additional aliquots were stored at -20° or -80°C or were mixed with a cryopreservation solution containing 10% DMSO + 10% FBS and dispensed into 1 ml cryovials, which were cooled slowly to -80°C and then directly immersed into liquid nitrogen. Frozen samples were thawed rapidly in a 37°C bath and all lipoaspirates were digested with 0.1% collagenase P for a fixed time. The number of viable cells in the isolated SVF was determined using trypan blue and growth curves for SVF cells were established using a tetrazolium (WST-1) assay.

Results: The yield of viable cells following 37°C/24 h storage was roughly 3-fold higher than cell yields that followed storage at lower temperatures, but this difference diminished with longer storage times. Over the same period, only storage at 25°C resulted in an increase in the number of viable cells recovered over time. Interestingly, the slope of the SVF growth curve was maximal following 25°C/24 h storage and was 5-fold greater than when lipoaspirates were stored at 37°C, which resulted in the lowest rate of cell growth. Cells recovered from 30° or 4°C storage had intermediate growth rates. Cells recovered following cryopreservation had a rate of cell growth that was comparable to cells following 25°C/24 h storage.

Conclusions: The viability of SVF cells was not inversely proportional to the storage temperature as expected but was optimal with short-term storage at 25°C (about room temperature) and was comparable to cryopreservation.



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AUTOLOGOUS PLATELET RICH PLASMA: A BIOLOGICAL SUPPLEMENT TO ENHANCE ADIPOSE-DERIVED MESENCHYMAL STEM CELL EXPANSION

Presenter: Fatemeh Atashi, PhD

Authors: Atashi F, Jaconi M, Pittet-Cunod B, Modarressi A

Geneva University

Introduction: Mesenchymal stem cells derived from adipose tissue (AT-MSCs) are promising candidates for cell therapy and tissue engineering strategies. Currently the use of non-autologous cell culture media (e.g animal-derived or allogenic serum) for clinical applications of mesenchymal stem cells (MSCs) is criticized by regulatory agencies. Autologous platelet-rich plasma (PRP) is proposed as a safer alternative medium supplement for adipose-derived mesenchymal stem cells (AT-MSC) culture.

Materials and Methods: To study its efficiency on cell proliferation, AT-MSCs were cultured for 10 days in media supplemented with different concentrations of autologous non-activated (nPRP) or thrombin-activated PRP (tPRP) (1%-60%). AT-MSC proliferation, cell phenotype and multipotency capacity were assessed and compared to AT-MSCs expanded in a classical medium supplemented with 10% of fetal bovine serum (FBS). Platelet count and viability in the presence or absence of AT-MSCs, was assessed for up to 10 days of culture.

Results: Culture media supplemented with nPRP showed dose-dependent higher AT-MSC proliferation than did FBS or tPRP. 20% nPRP was the most effective concentration to promote cell proliferation. This condition increased 13.9 times greater AT-MSC number in comparison to culture with FBS, without changing the AT-MSC phenotype and differentiation capacity. 57% of platelets were viable up during 10 days of culture.

Conclusion: We concluded that 20% autologous nPRP is a safe, efficient and cost-effective supplement for AT-MSC expansion. It should be considered as an alternative to FBS or other non-autologous blood derivatives. It could serve as a potent substitute for the validation of future clinical protocols as it respects good-manufacturing practices and regulatory agencies standards.

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EFFECT OF PORE SIZE AND POROSITY ON ADIPOSE-DERIVED STEM CELL BEHAVIOUR ON NANOCOMPOSITE POLYMER SCAFFOLDS

Presenter: Naghmeh Naderi, MSc

Authors: Naderi N, Griffin M, Kalaskar D, Butler PE, Mosahebi A, Whitaker IS, Seifalian AM

UCL

Introduction: Biomaterials are useful tools for cell delivery in tissue engineering. We have developed a family of scaffolds for ADSC delivery. Our non-biodegradable nanocomposite scaffold is based on incorporating polyhedral oligomeric silsesquioxane (POSS) into polycarbonate-based urea-urethane (PCU), already in clinical use as the World's first synthetic trachea, bypass graft and lacrimal duct. Its biodegradable counterpart is composed of POSS incorporated into poly-(caprolactone/carbonate)-urethane/urea (PCL). Geometric variables of a scaffold such as porosity, pore size, and pore morphology can influence cellular behaviour. In this study we investigated the effect of pore size and porosity of POSS-PCL and POSS-PCU scaffolds on ADSC behaviour.

Methods: Human adipose tissue was processed to yield ADSC. Characterisation of ADSC was performed using flow cytometry and differentiation down the mesenchymal lineage, assessed by qPCR and immunohistochemistry. 8×10^3 ADSC were seeded on POSS-PCL and POSS-PCU disks fabricated with porogen particles (NaCl) ranging from 25-300 μm in size and 25%-75% in concentration. TCP and nonporous samples served as controls. ADSC proliferation and adhesion on the disks were compared between the different pore sizes and concentrations using scanning electron microscopy (SEM), immunofluorescence, alamarBlue and total DNA analysis.

Results: After expansion the ADSC population was CD34+/CD73+/CD90+/CD105+/CD19-/CD14-/CD45- and able to differentiate into adipocytes, chondrocytes, and osteoblasts. At 14 days: porous POSS-PCU and POSS-PCL samples were associated with significantly better ADSC proliferation than nonporous controls. 75% NaCl content in the 25-100 μm pore size group was associated with significantly better ADSC proliferation compared to 25% and 50%. In 100-200 and 200-300 μm pore size groups 50% NaCl content gave significantly better results compared to 25% and 75%. Comparing different pore sizes, 100-200 μm group was associated with significantly improved ADSC proliferation in the 50% and 75% NaCl groups, but not 25%.

Conclusion: Pore size and porosity are valuable determinants of cellular attachment, proliferation, and organisation and require careful consideration when synthesising the ideal scaffold for ADSC delivery.



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HUMAN ADIPOSE STEM CELLS CULTURED IN TENOGENIC DIFFERENTIATION MEDIUM ON BRAIDED POLYLACTIDE SCAFFOLD IS A POTENTIAL APPROACH FOR TENDON TISSUE ENGINEERING

Presenter: Kaisa Vuornos, MS

Authors: Vuornos K, Bjorninen M, Talvitie E, Kellomaki M, Miettinen S, Haimi S

BioMediTech

Currently, there is a growing demand for an efficient tendon tissue engineering construct due to an increasing amount of tendon injuries in relation to sports activities and degenerative conditions. This *in vitro* study proposes a strategy to differentiate multipotent mesenchymal human adipose stem cells (hASCs) towards tenogenic lineage on braided poly-L-D-lactide (P(L/D)LA) scaffold stimulated with soluble factors.

A suitable tenogenic differentiation medium (TM) containing tenogenesis promoting growth factor combined with ascorbic acid was chosen based on significantly higher cell proliferation and total collagen content together with positive tenogenic marker gene and protein expression profile in phase 1 of study. The braided 8-filament PLA scaffold was chosen based on higher cell proliferation and total collagen content alongside positive gene and protein expression profile in phase 2 of study. The TM for hASCs was combined with the braided 8-filament PLA scaffold for phase 3, where the cell proliferation and total collagen content results were significantly higher compared to control, in addition to which the hASC tenogenic differentiation gene expression profile was gained.

The results demonstrated that growth factor and ascorbic acid supplementation significantly increased hASC proliferation and ECM collagen content. This tissue engineering strategy of pretreating hASCs with TM in braided PLA scaffolds prior to *in vivo* transplantation might be potential for functional musculoskeletal tissue engineering applications. However, more investigations are needed to confirm the optimal TM composition and scaffold architecture required to support further the hASC tenogenic differentiation process and to study the *in vivo* functionality of the engineered tendon construct.

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CYTOKINES AND GROWTH FACTORS, PRESENT AT THE SITE OF IMPLANTATION OF A TISSUE ENGINEERING CONSTRUCT, AFFECT OSTEOGENIC DIFFERENTIATION OF ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS

Presenter: Astrid Bakker, PhD

Author: Bakker A

Academic Center for Dentistry

Introduction: Adult mesenchymal stem cells isolated from adipose tissue (ASCs) that are seeded on scaffolds and used for the healing of large bone defects will be exposed to platelet derived growth factors, inflammatory factors, and growth factors and cytokines produced by bone cells in close proximity to the implant. These parameters likely have a strong effect on the behavior of ASCs, thereby determining the clinical outcome of bone healing strategies.

Aim: To investigate the effect of human platelet lysate, cytokines, and conditioned medium from human primary bone cells on ASC proliferation, trophic factor production and osteogenic differentiation.

Methods: ASCs were cultured in culture medium containing 10^{-8} M 1,25 dihydroxy vitamin D₃ and either fetal calf serum (FCS; 10%) or human platelet lysate (2 or 5%). Alternatively, ASCs were cultured in the presence or absence of 0.1, 1, or 10 ng/mL interleukin-6 (IL-6), interleukin-8 (IL-8), or tumor necrosis factor alpha (TNF- α), or in 25% conditioned medium (CM) from human primary bone cells that had been either or not exposed to mechanical loading by pulsating fluid flow (PFF). RNA was isolated after 4, 7 and 10 days for RT-PCR. Alizarin red staining was performed at days 7 and 10.

Results: Platelet lysate (2%) strongly enhanced the proliferation, and accelerated differentiation, of ASCs. Both IL-6 and IL-8 enhanced the expression (~2-fold) of VEGFA, FGF7, and ALP by day 7. TNF- α had a dose-dependent, stimulatory effect on the expression of Runx2 (2.5-fold), TNF- α (1.7-fold) and IL-8 (80-fold) on day 4, VEGFA (1.9-fold) and Ki67 (4-fold) on day 7, and IL-6 (5.7-fold) on all days. CM from primary bone cells cultured in absence of mechanical stimulation did not affect proliferation, trophic factor gene expression or bone nodule formation by ASCs, while CM from PFF-treated primary bone cells enhanced bone nodule formation by ASCs.

Conclusion: Our results suggest that the environment, particularly with regard to the presence of platelet-derived factors and TNF- α , stimulate ASC proliferation, osteogenic differentiation, and expression of trophic factors. Simulation of such an environment *in vitro* may better predict which parameters (e.g. substrates) will aid bone tissue healing in a clinical setting.



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AUTOLOGOUS ADIPOSE-DERIVED STEM CELLS FOR CRANIOFACIAL RECONSTRUCTION

Presenter: Sophie E. New, PhD

Authors: New SE, Guasti L, Ibrahim A, Bulstrode NW, Seifalian AM, Ferretti P

University College London

Introduction: The regeneration capabilities of cartilage and bone in the craniofacial region are limited, thus congenital deformities, such as ear, nose and throat abnormalities, are typically corrected by reconstructive surgery. Autologous stem cells could prove valuable both therapeutically and in the modelling of disorders. Their use in craniofacial reconstruction could improve surgical practice and bypass the need for repeated surgical interventions. The aim of this study was to evaluate the suitability of pediatric adipose-derived stem cells (ADSC) - readily available source of stem cells - seeded on/in biodegradable nanocomposite polymer (POSS-PCL) for craniofacial defect repair.

Methods: ADSC isolated from a number of pediatric patients as previously described were used in this study (1). The viability, migration, proliferation and differentiation potential of pediatric ADSC seeded either onto monolayer or 3D biodegradable nanocomposite (POSS-PCL) was assessed using imaging, histological, immunocytochemical and RT-qPCR techniques. Grafting to the chick chorioallantoic membrane in ovo (CAM grafting) was used to monitor the response of the host to the differentiated/undifferentiated ADSC-seeded bioscaffolds in vivo.

Results: The pediatric ADSC proliferated and differentiated towards the mesenchymal and epithelial lineages on plastic and monolayer POSS-PCL. The ADSC readily infiltrated 3D POSS-PCL and the appropriate lineage-specific matrix proteins were observed in the pores throughout differentiated samples. The structure of ADSC-seeded 3D POSS-PCL was well preserved under in vitro conditions of the 4-week differentiation protocol. Analysis of CAM-grafted ADSC-nanoscaffold composites suggested that they can be encapsulated and vascularized by host tissue without any apparent negative consequences.

Conclusions: Together, our findings demonstrate that pediatric ADSC readily differentiate on biodegradable synthetic scaffolds along different lineages. This suggests they can provide an ideal autologous cell source for engineering complex structures containing multiple tissues for organ reconstruction in young patients and identify POSS-PCL as a promising scaffold for this purpose.

(1) Guasti et al. (2012) Stem Cells Transl Med. 1:384-395

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BONE TISSUE ENGINEERING TO PROVIDE PERSONALISED IMPLANTS FOR TREATMENT OF CRITICAL BONE DEFECTS IN CHILDREN WITH CRANIOFACIAL SYNDROMES

Presenter: Amel Ibrahim, MBBS, BSc

Authors: Ibrahim A, New S, Bulstrode N, Britto J, Seifalian A, Ferretti P

UCL Institute of Child Health

Introduction: 1 in 1000 babies is born with a craniofacial malformation. Congenital craniofacial bone deformities frequently occur in conditions like Hemifacial Microsomia and Treacher-Collins Syndrome. As well as functional impairment to feeding, speech or vision; deformities can negatively impact the child's social interactions and self-esteem. Reconstruction requires multiple invasive surgeries.

Human adipose-derived stem cells (hADSCs) possess multi-lineage differentiation capabilities and can be derived using minimally invasive surgery. They can provide a permanent autologous bone implant when combined with a suitable scaffold which can give 3-D structural support to cells and mimic the necessary biomechanical environment. As these bone implants will be used in facial reconstruction of paediatric patients, the scaffold needs to be biocompatible, bioabsorbable, mouldable and able to support vascularisation.

Methods: Polyhedral Oligomeric Silsesquioxane-Poly (Caprolactone-urea) urethane (POSS-PCL), Collagen and Fibrin were tested as biodegradable scaffolds alone and in combination. They were assessed for osteogenic, angiogenic and structural properties. Scaffolds were seeded with hADSCs and cultured in osteogenic media for 3 weeks. Chick chorioallantoic membrane (CAM) grafting was employed to assess biocompatibility and vascularisation. Protein and RNA analysis were used to objectively compare the results. Each experiment was repeated three times using different cell lines.

Results: hADSCs were driven to osteogenic differentiation, as verified by histochemical and molecular techniques at three weeks on POSS-PCL, Collagen and Fibrin. Angiogenesis was observed in CAM grafts. Combination of the scaffolds (e.g. coating of POSS-PCL with Fibrin and Collagen with Fibrin) resulted in optimisation of cell seeding, osteogenic differentiation and vascularisation.

Conclusions: Osteogenic differentiation into 3-D bone constructs is achieved on three novel bioabsorbable scaffolds using osteogenic media and human adipose-derived stem cells. Additionally, combining the gels and porous scaffolds resulted in optimisation of the constructs by providing both structural and biological support. These technologies provide for a bio-integrated bespoke reconstructive option.



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**SURGICAL PROCEDURE FOR A MECHANICAL FAR
LATERAL DISC HERNIATION MODEL IN SHEEP
FOR EVALUATION OF REGENERATIVE THERAPY
APPROACHES**

Presenter: Stefan Schwan, MD

Authors: Schwan S, Friedmann A, Meisel HJ
BG Kliniken Bergmannstrost

NOT PRESENTED

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**EFFICIENT DIFFERENTIATION OF HUMAN ADIPOSE
STEM CELLS TOWARDS ANNULUS FIBROSUS TISSUE
IN VITRO IN BIOMIMETIC SCAFFOLDS**

Presenter: Suvi Haimi, PhD

Authors: Gebraad A, Blanquer SB, Miettinen S,
Grijpma DW, Haimi SP

BioMediTech University of Tampere

The origin of chronic back pain is mainly assigned to the degeneration of intervertebral disc tissue, leading to tearing of the outer annulus fibrosus (AF) and extrusion of the inner nucleus pulposus. Current surgical strategies to restore the AF are technically demanding and mechanical repair only is not sufficient for all defects. Tissue engineering based approaches could offer an effective biological repair of the ruptured AF by enabling new extracellular matrix (ECM) synthesis at the defect site.

The use of human adipose stem cells (hASCs) differentiated towards an AF phenotype could evade the problems of limited availability and expansion capacity of autologous AF cells in AF tissue engineering. This study evaluated 3 different in vitro seeding methods for the efficient differentiation of hASCs towards AF phenotype in biomimetic scaffolds.

Using resins based on poly (trimethylene carbonate) (PTMC), a rubber-like surface eroding polymer, biomimetic scaffolds of AF tissue were prepared by stereolithography (SL). The scaffolds were seeded with hASCs using either direct seeding, the micromass technique (Mäenpää et al. 2010; Denker et al. 1995) or the fibrin gel method (Ameer et al. 2002). Scaffolds seeded with primary human AF cells (hAFCs) were used as control. Scaffolds with hAFCs and hASCs were cultured for 21 days in commercial nucleus pulposus culture medium and serum-free chondrogenic differentiation medium supplemented with TGF- β 3, respectively.

Compared to hAFCs, hASCs showed substantially higher production of sulphated glycosaminoglycans and collagen, which are important ECM components of AF tissue. Seeding using fibrin as a cell-carrier, resulted in the best distribution of the cells in the scaffolds and the highest cellular content. Furthermore, when using the fibrin gel method, the differentiated hASCs showed most abundant deposition of an ECM compared to the other seeding techniques. Moreover, the alignment of collagen fibres produced in the biomimetic scaffolds was comparable with the alignment of native AF tissue collagen fibres.

Our results showed that hASCs differentiated towards an AF phenotype seeded into biomimetic PTMC scaffolds using the fibrin gel method could form a potential construct for the effective repair of AF defects in the future.



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IN VITRO MODEL USING BOVINE ARTICULAR CARTILAGE EXPLANT FOR INVESTIGATION OF ADIPOSE-DERIVED STEM CELL KINETICS WITH HYALURONIC ACID

Presenter: Peter Succar, PhD

Authors: Succar P, Medynskyj M, Herbert H
Macquarie University

Hyaluronic acid (HA) is an anionic non-sulfated glycosaminoglycan found in connective tissue extracellular matrix and joint synovial fluid. HA has been shown to have an anti-inflammatory effect by reducing the secretion of inflammatory cytokines, such as IL-1B. This results in reduced expression of matrix metalloproteinases, catabolic enzymes involved in cartilage destruction.

The use of alternative biological therapies, such as adipose-derived stem cells (MSCs), for the treatment of cartilage defects and osteoarthritis is increasing. MSCs have an anti-inflammatory effect by modulating the immune response to injury. In addition, MSCs stimulate cartilage proliferation via paracrine signalling. The beneficial effects of HA and MSCs have led some physicians to combine these treatments for joint injuries and OA.

A key technique for determining the likely therapeutic effect of cells is their secretion profile, which can be quantified in vitro and in vivo by multiplex cytokine assays, such as Bio-Plex. The secretion profile can be an indication of positive or negative effects of media additives in culture or by priming MSCs, i.e. by changes in concentration of inflammatory or anti-inflammatory cytokines. Whilst HA and MSCs have beneficial effects in treating joint disease, it is not clear whether combining them results in a synergistic effect.

In-vitro investigations of MSCs are mostly conducted using plastic culture flasks; however, the use of plastic to model MSC kinetics inside a joint is negated as only the true target tissue can represent in vivo architecture with which MSCs will interact. In this study, we explored MSC-cartilage interactions by the use of a reproducible model of culturing human MSCs onto bovine cartilage explants to assay MSC properties. Using a concentration series of high molecular weight HA, we primed or co-cultured MSCs and then seeded them onto cartilage explants assaying the following; Kinetics (adhesion and proliferation) using a formazan dye colorimetric assay; secretion profile using a 27-plex cytokine assay and morphology with confocal fluorescence microscopy. Initial results indicate HA is a suitable candidate as an adjunct to MSC therapy for the treatment of joint disease.

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PREPARATION OF SVF FROM LIPOASPIRATE INFRANATANT

Presenter: Robert E. Bowen, MD

Author: Bowen RE
The Center for Positive Aging

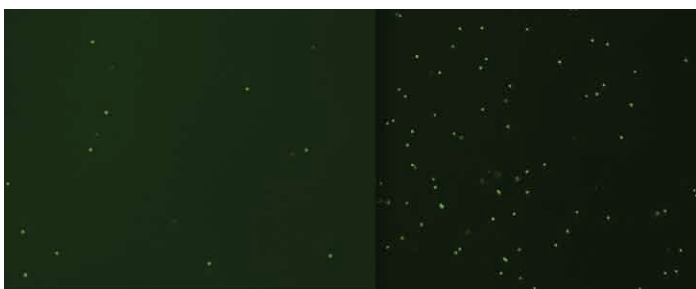
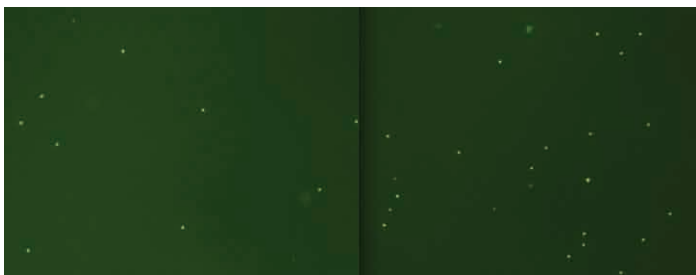
Introduction: Lipoaspirate has shown great promise as a source of progenitor cells for use in regenerative medicine. The stromal vascular fraction (SVF) can be isolated from lipoaspirate using enzyme digestion and centrifugation, but this approach may be limited by the labor intensive nature of the technique as well as ambiguities in current governmental regulations. An alternative approach to obtain SVF from lipoaspirate was studied.

Method: Lipoaspirate obtained from nutational infrasonic liposuction (NIL) was collected and processed by 2 methods: 1.) 50 ml of supernatant adipose tissue was incubated with a collagenase/neutral protease enzyme blend (Vitacyte/Clzyme) according to the manufacturers protocol and under GTP conditions. Time = 80-105 min. 2.) The infranatant from 200ml of adipose tissue (LAF) was centrifuged at 400 g X 5 min. Time = 15-20 min. The respective SVFs cell populations were counted using an optical fluorescent cell counter (Nexcelom A2000) and the fluorescent stains acridine orange (AO) and propidium iodide (PI). The number of live nucleated cells were counted. A separate but related study compared the nucleated cell yield from NIL to SAL infranatants. Paired specimens from 5 patients were acquired by both SAL and NIL and the infranatant cells were counted by the same method as reported above. Live cell numbers, viability %, and cell size were recorded.

Results: N=15. Enzyme: $2.75 \times 10^6 \pm 1.4$ cells/ml, cell size 12.5 ± 0.6 microns. LAF: $2.44 \times 10^6 \pm 0.5$ cells/ml, cell size 12.6 ± 0.49 microns. N=5. SAL: 880,000 live cells, 53% viability, 13.2 microns; NIL: 3,980,000 live cells, 84.5% viability, 12.7 microns.

Conclusions: Infranatant obtained from NIL contains substantial numbers of regenerative cells. Approximately 4 times the quantity of adipose tissue is required to yield the same number of cells as from enzyme digestion. Other methods of obtaining lipoaspirate such as PAL or SAL may also yield regenerative cells in the infranatant. The regenerative effects of minimally manipulated point of care SVF can be obtained by a more cost effective method when using marginally larger aliquots of lipoaspirate. NIL yields a greater quantity of live cells than SAL by both an increased total cell yield and higher viability.

PREPARATION OF SVF FROM LIPOASPIRATE INFRANATANT



RENEVIA(TM) SVF DELIVERY MATRIX: TEMPORAL GELATION CONTROL OF A NOVEL CELL RETENTION SYSTEM

Presenter: Aina Matas Palau, BS

Authors: Matas Palau A, Dos-Anjos Vilaboa S, Erickson IE, Zarembinski T, Llull R

Stem Center

Introduction: Cell based therapies are a recent trend in modern medicine, but many hurdles to acceptance still exist. One way to increase therapeutic efficacy is to increase and retain cells density at implantation site. Current Hydrogels are already cross-linked, so the hydrogel cannot incorporate (retain) cells within. Renevia™ (BioTime, Inc.) is a hyaluronan and collagen hydrogel that can be mixed with any cell fraction before gelation and is being developed as an SVF cell delivery matrix to be used in the place of autologous fat to improve consistency and allow fine gauge needle delivery. Important considerations are the effects of cell dose and reconstitution buffer on gelation kinetics.

Methods: SVF isolation was carried out using the GID SVF-I device. Briefly, the human lipoaspirate was washed using warmed Lactated Ringer's (LR), digested with GIDzyme-2 and centrifuged at 800 g for 10 minutes to obtain the SVF pellet. We analyzed cell viability using method of Calcein AM and Propidium Iodide. Renevia components were prepared by reconstituting in either PBS or LR. 1cc of hydrogel mixture was added to each microcentrifuge tube containing 0, 1, 5, 10 or 20 million SVF cells. The resistance to flow was observed every 5-10 minutes to determine the time of hydrogel formation.

Results: The results demonstrated two important effects: 1) as cell concentration increased, the rate of gelation decreased, and 2) the rate of gelation was significantly increased when using PBS compared to LR (Fig. 1). The time to achieve full gelation for Renevia with LR increased from 75 min to 350 min as the cell number increased, while Renevia with PBS only increased gelation time from 21 to 50 min for 0 to 10 million SVF cells/mL. The slowing of gelation with cell number is likely due to slight Renevia dilution and pH changes from acidic cellular byproducts. PBS has a stronger buffering capacity compared to LR, which helps maintain the pH around 7.4, which is optimal for Renevia gelation.

Conclusion: In order to realize a product for SVF or other cell delivery, quick and consistent gelation is a necessary attribute. The results indicate that a wide concentration range of SVF cells may be optimally delivered with Renevia using PBS between 20-50 min.

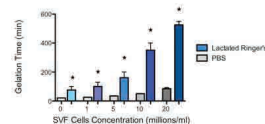


Figure 1. Gelation time with PBS or LR. Each form was used with SVF cells at increasing concentrations. Note that gelation time increases with cell number. For every cell concentration, Renevia with PBS forms a hydrogel significantly faster than LR. *Significant difference between PBS and LR (p < 0.05).



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CULTURE EXPANSION OF ADIPOSE TISSUE-DERIVED STROMAL CELLS: MANUAL FLASK-BASED COMPARED WITH CLOSED, AUTOMATED QUANTUM CELL EXPANSION SYSTEM

Presenter: Sonja K. Brorsen

Authors: Haack-Soerensen M, Juhl M, Brorsen SK, Follin B, Soendergaard RH, Kastrup J, Ekblond A

Rigshospitalet

Background: Adipose tissue-derived stromal cells (ASCs) are a rich and convenient source of cells for regenerative therapeutic approaches. However, applications of ASCs often require cell expansion to reach the needed dose. The current method of manufacturing ASCs utilizes T-flasks in a complex manual process. Movement toward a more standardized cell product requires a paradigm shift to an automated system that can provide a consistent and closed process. In this study, the manual cultivation of ASC from stromal vascular fraction (SVF) over two passages is compared with the automated functionally closed hollow fiber Quantum Cell Expansion System (Terumo BCT).

Methods: SVF was isolated from abdominal fat, suspended in -MEM supplemented with 10% Fetal Bovine Serum (FBS) and seeded onto T75 flasks or a bioreactor coated with cryoprecipitate. The two-passage cultivation of ASCs from SVF was performed via three methods: flask to flask; flask to bioreactor; and bioreactor to bioreactor. In all cases, quality controls were conducted by testing sterility, mycoplasma, and endotoxin, in addition to the assessment of cell counts, viability, and immunophenotype.

Results: The viability of ASCs Po and Pi was above 96% regardless of cultivation in flask and bioreactor. Antigen expression was consistent with ISCT standards for ASC phenotype. Sterility, mycoplasma and endotoxin tests were consistently negative. An average of 53×10^6 SVF loaded into a bioreactor yielded 80×10^6 ASCs Po (1.6 times higher number of ASCs relative to the number of cells seeded), while 4.5×10^6 SVF seeded per T75 flask yielded an average of 1.75×10^6 ASCs (0.4 times the number of ASCs relative to the number of cells seeded). ASCs Pi expanded in the Quantum system demonstrated a population doubling (PD) around 2.1 regardless of whether Po was cultured in flasks or Quantum, while ASCs Pi in flasks only reached a PD of 1.0.

Conclusion: Manufacturing of ASCs in a Quantum Cell Expansion System enhances ASC expansion rate and yield significantly relative to manual processing in T-flasks, while maintaining the purity and quality essential to safe and robust cell production. Notably the use of Quantum includes significant reduced staff and cost.

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THE EXTERNAL INTRAOPERATIVE SOFT-TISSUE EXPANSION SYSTEM (PALMPUMP) – AN INNOVATIVE APPROACH FOR ENHANCED AUTOLOGOUS STRUCTURAL FAT GRAFTING

Presenter: Carlo Oranges, MD

Authors: Oranges C, Tremp M, Largo RD, Schaefer DJ
University Hospital Basel

Introduction: External volume expansion by suction has been proposed to improve fat graft survival. The objective of this study is to present an innovative and simple intraoperative approach to enhance autologous fat grafting and to discuss the background and mechanisms of action.

Methods: Autologous fat grafting preexpansion is performed using a patented PalmPump™ soft-tissue vacuum system (Figure 1). In order to obtain edema, inflammation, cell proliferation and vessel remodeling before grafting, the external volume expansion device was applied in a 69 year-old female patient for soft tissue body contouring. Hereby, the recipient site of the right breast was expanded several times for 30 seconds with a vacuum up to 550mmHg before autologous fat injection. A sequential explanation of components and advantages of use of this device is presented. Patient satisfaction using a Likert scale from 0-10 (0=poor result, 10=very satisfied) and clinical outcome were assessed.

Results: External volume expansion-treated tissues with the PalmPump™ soft-tissue vacuum system are grossly expanded within few minutes of stimulation, developing a macroscopic swelling that regresses slowly over the course of hours following stimulus cessation. This gross swelling is reflective of intense inflammation. The system sets in motion various mechanisms, including mechanical stimulation, edema, ischemia and inflammation providing a conducive environment for cell proliferation and angiogenesis. In our patient, little edema and hemorrhage was noticed for four days postoperatively which resolved without sequelae. Satisfactory results were confirmed after a follow-up of three and six months with a high patient satisfaction (Likert scale 8).

Conclusions: We developed and applied an efficient, time-saving and cheap method for enhanced autologous structural fat grafting by using an external soft-tissue expansion device (PalmPump™). It has a low morbidity, offers intraoperative effective means of harnessing the regenerative capabilities of mechanical forces to increase the vascularity, decres interstitial fluid pressure and finally leads to a high patient satisfaction. However, further clinical and molecular analyses are required in a large scale study to validate our findings.





CHANGED PRESENTER

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ENHANCEMENT OF DECELLULARIZED ADIPOSE TISSUE WITH LIPOASPIRATE AND ADIPOSE-DERIVED STEM CELLS

Presenter: Arta Kelmendi-Doko, MD, MSc
(Kacey Marra, PhD)

Authors: Chnari E, Schilling B, Lannau B, Minteer D,
Huang YC, Kelmendi-Doko A, Marra KG,
Rubin JP

University of Pittsburgh

Introduction: There is a strong clinical need for improved therapies for fat retention after soft tissue loss. The use of autologous fat grafting results in variable and unpredictable outcomes. This study examines the use of a decellularized adipose matrix in combination with human adipose-derived stem cells (ASCs) and/or human lipoaspirate to promote fat retention.

Methods: Human fat was obtained under approval from the Institutional Review Board, and decellularized using both published methods and novel methods. Decellularization was verified by DNA analysis. Key matrix protein retention was also verified through histological staining, including collagens I, III, IV and VI. The decellularized matrix was gelled. Human adipose-derived stem cells were isolated and characterized, and seeded within the gels. Adhesion, proliferation, and adipogenic differentiation of ASCs within the gels were determined. Injection properties of the decellularized matrix in combination with human lipoaspirate were also assessed. Decellularized adipose matrix alone was also injected subcutaneously in mice and the implants were evaluated histologically over time up to 12 weeks.

Results: We have established a reproducible decellularization protocol that results in DNA content of < 50 ng per mg of sample. ASCs were able to attach to the matrices, and adipogenic differentiation was determined. Histological evaluation of the implanted decellularized adipose matrix indicates that the implant promotes host adipocyte infiltration that increases over time. Future studies will include a combination of the adipose matrix with human lipoaspirate to investigate the combined effect in fat retention and cell infiltration.

Conclusion: Preliminary animal studies support adipoinductive properties of the decellularized adipose matrix. The combination of decellularized adipose matrices with adipose-derived stem cells and/or lipoaspirate is promising for enhanced fat graft retention after soft tissue loss.

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HUMAN ADIPOSE SPHERE-DERIVED STEM CELLS INCREASE THE MESENCHYMAL POTENTIAL: THERAPEUTIC IMPLICATIONS

Presenter: Anna Barbara Di Stefano, PhD

Authors: Di Stefano A, Giammona A, Leto Barone AA,
Giunta G, Cordova A, Todaro M, Moschella F,
Stassi G

Cellular and Molecular Pathophysiology Laboratory

Introduction: Adipose tissue represents a plentiful source of these stem cells. Adipose stem cells are a heterogeneous population and have multilineage differential potential. They represent a promising source in cell therapy and tissue engineering. These cells are used accordingly in regenerative medicine, as in "lipofilling" and "rhagades" application. We isolated non-adherent Adipose-derived Stem Cells (ASCs) from adipose tissue, which grow as spheres in suspension and showed multipotency.

Methods: Samples were collected from a heterogeneous population of healthy patients undergoing liposuction for aesthetic reasons. Cells obtained from lipoaspirates were dissociated into a single cell suspension and plated in a serum-free medium containing growth factors that encourage sphere growth. ASCs and adherent ADSCs were subjected to flow cytometry analysis and were incubated with the specific antibodies. Culturing in particular media conditions leads to differentiation of ASCs toward the osteogenic, chondrogenic and adipogenic cell lineages. Animals were anesthetized and a laminectomy was performed at the T8 vertebral level in order to implant the cells into the lesioned region via dermal matrix Integra.

Results: We compared the adherent ADSCs with ASCs and showed the differences between the two cell types investigated. ASCs can grow in suspension contrarily to adherent cells. They express the putative surface stem cell markers such as CD271 and CD90. Furthermore, they display a limitless in vitro capacity to differentiate into distinctive end-stage cell types such as osteocytes, chondrocytes and adipocytes and facilitate bone interbody repair and regeneration after laminectomy in vivo.

Conclusions: ASCs represent an upstream stem cell progenitor lineage isolated from lipoaspirate. They lack adhesion, grow as spheres and are undifferentiated. These divide asymmetrically by clonal expansion, and can differentiate toward mesenchymal cell lineages. ASCs could have an important therapeutic implication in tissue engineering.



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ADIPOSE-DERIVED STROMAL CELLS (ADSC): THE GOOD, THE BAD AND THE UGLY IN MYOCARDIAL REMODELING AND REPAIR

Presenter: Marco Harmsen, PhD

Author: Harmsen M

University Medical Centre Groningen

Cardiovascular disease is a leading cause of death worldwide. The major risk factors such as high caloric intake, lack of exercise, smoking, obesity to mention a few, cause endothelial dysfunction that may culminate in atherosclerosis and plaque rupture. In the heart a ruptured coronary plaque will trigger a myocardial infarction. This leads to irreversible loss of cardiomyocytes and their replacement by scar tissue and cardiac fibrosis on the long run. Cardiac fibrosis also occurs upon chronic pressure overload of the heart. Therapy is possible at all stages of CVD development.

In the past (almost) two decades, the treatment of myocardial pathology by administration of various types of stem cells, precursor cells and other types of anticipated therapeutic cells has met with limited success. However, stroma-derived cells from bone marrow and, nowadays more popular, adipose tissue (ADSC) have proven most powerful. Often referred to as Mesenchymal Stem Cells (MSC), they are better called stromal cells, because they lack self-renewal capacity.

In vitro and in experimental animal models for cardiac disease, the paracrine action of ADSC is most prominent – they secrete a host of soluble factors that support angiogenesis, suppress apoptosis and inflammation and improve remodeling. In addition, ADSC acquire a juxtacrine role in repair by virtue of their differentiation to pericytes and smooth muscle cells (SMC) and engaging close contact with small vessels. It is confusing that pericytes, SMC and myofibroblasts are virtually indistinguishable, yet their role in cardiac repair and fibrosis is like Yin and Yang. Limited studies have shown that ADSC promote may suppress myofibroblast formation. Together with their well-established anti-inflammatory features, the ADSC would be the ideal cell to suppress cardiac fibrosis. However, neither animal studies nor clinical trials have provided solid evidence for this presumption as yet. Nevertheless, ADSC do markedly improve post-aMI myocardial remodeling. Clinical trials with ADSC such as APOLLO show that infarct size is smaller after treatment. The final verdict on the use of ADSC for cardiac therapy is not out yet and requires a better understanding of mechanisms on the one hand and optimized delivery and retention strategies on the other hand.

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ADIPOSE TISSUE-DERIVED STEM CELLS ENHANCE ENDOTHELIAL SPROUTING IN FIBRIN SCAFFOLDS

Presenter: Ester Weijers, PhD

Authors: Wiejers E, Van Den Broek M, Tasev D,

Van Den Broek LJ, Gibbs S,

Van Hinsbergh VW, Koolwijk P

VU University Medical Center

Generation of a perfused, functional vascular network in scaffolds is important for the success of tissue engineering. At this moment, the slow formation of new blood vessels or non-functional nature of the vascular network is limiting the translation of high potential scaffolds towards patients. Since fibrin provides a temporary matrix structure for invading cells during angiogenesis in wound healing and tumor growth, fibrin is an interesting biomatrix to facilitate vessel formation. Previously, we showed that naturally occurring high molecular weight fibrin facilitates in vitro and in vivo an increased vessel sprouting. Nevertheless, biomatrices and/or endothelial cells solely are not sufficient to create functional vascular networks, and vessel stabilization is a necessity. Addition of human adipose mesenchymal stem cells (ASC) appear to be a promising tool to enhance vessel sprouting via paracrine effects, and as perivascular cell source for vessel stabilization. The present study aims to unravel the role of endothelial - ASC interactions during vessel sprouting in 3D fibrin matrices. A dose-dependent stimulation of sprouting was observed when 0.1 and 1% ASC were in direct contact with the endothelial monolayer. This effect appeared to be independent of the VEGF secretion by ASC, since inhibition of VEGF by bevacizumab or SU1498 did not alter the stimulatory effects of ASC. ASC migrated towards endothelial sprouts and located along the stalk cells in a pericytic manner. Strikingly, part of the ASC showed a novel location; adjacent to endothelial tip cells. The effects of ASC location, pericytic phenotype and matrix degradation upon endothelial - ASC interactions are currently investigated. These data will reveal to what extent the positive effects of ASC are additive to the pro-angiogenic effects of fibrin variants. Together, these data provide new perspectives for enhancing scaffold vascularization in the field of regenerative medicine.

This research is funded by NIRM, Netherlands Institute for Regenerative Medicine.



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ACUTE MYOCARDIAL INFARCTION DOES NOT AFFECT FUNCTIONAL CHARACTERISTICS OF ADIPOSE DERIVED STEM CELLS IN RATS, BUT REDUCES THE NUMBER OF STEM CELLS IN ADIPOSE TISSUE

Presenter: Paul A. Krijnen, PhD

Authors: Krijnen PA, Naaijken BA, Meijster E, Vo K, Musters RJ, Kamp O, Niessen HW, Juffermans LJ, Van Dijk A

VU University Medical Center

In most pre-clinical animal studies investigating stem cell therapy in acute myocardial infarction (AMI), the administered stem cells are isolated from healthy donors. In clinical practice, however, patients who suffer from AMI will receive autologous cells, for example using adipose derived stem cells (ASC). During AMI inflammation is induced and we hypothesized that this might affect characteristics of ASC. To investigate this ASC were isolated from rat adipose tissue one (1D group, n=5) or seven days (7D group, n=6) post-AMI, and were compared with ASC from healthy control rats (Control group, n=6). We found that significantly less ASC were present one day post-AMI in the stromal vascular fraction (SVF), determined by a colony-forming-unit-assay ($p < 0.001$ vs Control and 7D). These data were confirmed by flow cytometry, showing less CD90 positive cells in SVF of the 1D group. When cultured, no differences were found in proliferation rate and cell size between the groups in the first three passages. Also no difference in the capacity of ASC to differentiate towards cardiomyocytes was found.

In conclusion, it was shown that significantly less stem cells were present in the SVF one day post-AMI, however, the stem cells that were present showed no functional differences after AMI.

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ADIPOSE STEM CELL DIFFERENTIATION TOWARDS VASCULAR LINEAGES USING NOVEL 3D ELECTRICAL STIMULATION SYSTEM

Presenter: Miina Bjorninen, MSC

Authors: Bjorninen M, Gilmore K, Pelto J, Kellomaki M, Miettinen S, Wallace G, Grijsma D, Haimi S

BioMediTech University of Tampere

Electrical stimulation (ES) has potential for the directed differentiation of human adipose stem cells (hASCs) towards desired lineages in the presence or absence of chemical induction. To date, reusable in vitro electrical stimulation devices that can stimulate several cell constructs simultaneously are unavailable commercially. We therefore developed a system that could electrically stimulate cells reliably and repeatably. Conductive polypyrrole (PPy) coatings on poly (trimethylene carbonate) (PTMC) scaffolds were tested for smooth muscle cell (SMC) and endothelial cell (EC) differentiation of human ASCs with and without ES.

In the first part of the study PTMC scaffolds were chemically coated with PPy in the presence of hyaluronic acid (HA) and their effects on SMC and EC differentiation of hASCs was studied. In the second part of the study, pulsed biphasic electric current (BEC) was applied through the hASC-seeded PPy-coated constructs using a novel in-house ES electrodes composed of titanium nitride (TiN) coated metallic titanium. BEC was applied under SMC differentiation conditions with pulse widths of 0.25 and 1 ms. Cells were characterized for their attachment, viability, cell number as well as EC and SMC marker expression via immunofluorescence staining.

PPy-coated scaffolds provided stronger cell attachment, induced higher proliferation as well as stronger SMC and EC marker expression for hASCs, compared to uncoated PTMC scaffolds under both differentiation conditions. Cells on both scaffold types showed similar viability. Cells under BEC with 1 ms pulse width showed similar viability to controls whereas with 0.25 ms pulse width viability was reduced after 14 days of BEC. Similarly, cells undergoing BEC with 1 ms pulse width showed similar marker expression as that of controls whereas 0.25 ms pulse width showed lower expression levels.

In conclusion, the novel ES electrodes were found to be suitable for delivering BEC for hASCs and can be recommended for in vitro use due to their stability and reliability with repeated use. PPy coating promoted hASC attachment, proliferation and SMC and EC marker expression. The novel electrical stimulation system therefore has potential for vascular tissue engineering applications.



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INFLUENTIAL ROLES OF HMGB1 PATHWAYS IN ACTIVATION OF ASCS AND ADIPOSE TISSUE REPAIR

Presenter: Shinichiro Kuno, MD

Authors: Kuno S, Kanayama K, Kinoshita K, Feng J, Yoshimura K

University of Tokyo

Introduction: We had demonstrated that damaged adipose tissue (AT) released HMGB1, a well-known damage associated molecular pattern molecule (DAMP) and that major receptors for HMGB1, RAGE, TLR2 and TLR4, were expressed on hASCs. We sought to further dissect ASC-activating mechanisms through HMGB1 and its receptors for establishing a therapeutic strategy.

Methods: AT-damage associated factors were collected as AT-soaked buffer (ATSB) by incubation fragmented ATs in buffered saline. We examined hASC response to ATSB under HMGB1 neutralization or blocking of its receptors. In an ischemia-reperfusion model of mice, we evaluated AT repairing process with or without inhibition of HMGB1 or its receptors.

Results: ELISA showed that ATSB contained HMGB1 at a substantial concentration. ATSB-induced proliferation, migration and angiogenesis of ASCs were attenuated by neutralization of HMGB1 or its receptors (RAGE, TLR2 or TLR4) by antibodies. In the ischemia-re-perfusion models, inhibition of HMGB1 pathway resulted in relative inactivation of ASCs and impaired healing of AT. Particularly under inhibition of TLR2, the decreased numbers of CD34-positive cells and infiltrated macrophages in the damaged AT were noted.

Discussion: Many reports suggested that damage-associated factors activate innate immunity systems and tissue-resident stem cells. Our results suggested HMGB1 pathway plays a crucial role in repairing process after AT injury such as ASC activation and macrophage recruitment, suggesting a therapeutic potential of HMGB1.

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IN VITRO ASSESSMENT OF ADIPOSE STROMAL VASCULAR FRACTION CELL DELIVERY UTILIZING A PERFUSION BALLOON CATHETER

Presenter: Jeremy S. Touroo, MS

Authors: Touroo JS, Khana A, Taylor JL, Williams SK

University of Louisville

Introduction: Localized delivery of therapeutic agents to blood vessels is evolving with the continued use of coronary and peripheral vascular interventions. Autologous regenerative cells offer potential for catheter-based treatment of damaged and diseased blood vessels. Adipose stromal vascular fraction (SVF) cell delivery utilizing a perfusion balloon catheter was evaluated through a series of in vitro studies. In vitro experiments included the use of a 3D blood vessel mimic (BVM), a technology that has been developed for preclinical testing of intravascular devices.

Methods: The perfusion balloon catheter was evaluated using both cultured human SVF cells and freshly isolated human SVF cells across a range of test conditions. Cell morphology, viability, and infusion efficiency were assessed. Electrospinning of biological and synthetic materials was utilized to create BVMs for further testing of catheter-based cell infusion in a 3D in vitro system that includes bioreactor fluid flow. Cellular adherence and distribution of cells were assessed in BVMs using scanning electron microscopy (SEM) and fluorescent nuclear staining. Histological staining and SEM were utilized to evaluate tissue formation on BVM surfaces following bioreactor flow.

Results: Balloon infusion had a negligible effect on SVF cell viability. The device delivered SVF cells onto collagen-containing, fiber-based surfaces of 3D BVMs with immediate cellular adherence (Figure 1). Additionally, cellular penetration of the porous BVM wall was accomplished following balloon infusion. Cell deposition in BVMs was highly localized. Human SVF cells delivered using the catheter remained in BVMs following 14 days of luminal flow, and localized areas in which cells appear to be generating a flow-aligned luminal tissue lining were observed.

Conclusions: In vitro testing using a 3D BVM and clinically relevant, point-of-care adipose SVF cells has provided a proof of concept of cell delivery using the perfusion balloon catheter. The perfusion balloon catheter has high potential for the effective delivery of viable regenerative cells to denuded and damaged blood vessels. In vivo testing can provide further insight into the use of the perfusion balloon catheter as a cell delivery device for vascular treatment.

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IN VITRO ASSESSMENT OF ADIPOSE STROMAL VASCULAR FRACTION CELL DELIVERY UTILIZING A PERFUSION BALLOON CATHETER

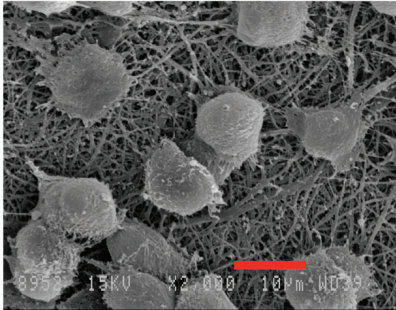


Figure 1. Scanning electron micrograph depicting human adipose stromal vascular fraction cells adhered to the collagen-containing fibers of a 3D blood vessel mimic (BVN). These regenerative cells were delivered to the luminal surface of the BVN utilizing a perfusion balloon catheter. Scale bar = 10µm; 2,000x magnification.

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ENHANCING WOUND HEALING OF FULL-THICKNESS MURINE SKIN DEFECTS: EFFECTS OF TISSUE-ENGINEERED BIOLOGICAL DRESSINGS BASED ON ASCS

Presenter: Julie Fradette, PhD

Authors: Maux A, Morissette Martin P, Moulin VJ, Fradette J

Universite Laval

Introduction: Recent investigations suggest that mesenchymal cells such as adipose-derived stromal/stem cells (ASC) can promote wound healing through their secretome. In this study, tissue-engineered substitutes containing human ASC and its naturally-derived matrix components were produced and applied to murine full-thickness skin defects. We postulated that such living tissues acting as biological dressings would have enhancing effects on skin healing. We particularly aimed to evaluate the specific contribution of reepithelialisation while minimizing wound contraction.

Methods: An animal protocol of splinted full-thickness excisional wound (8 mm diameter) was adapted. The specific monitoring of reepithelialisation was performed non-invasively by using a mouse strain featuring a fluorescent epidermis combined with the Lumina IVIS imagery system. The kinetics of global healing over 18 days were determined by measuring the residual wound areas on macroscopic images. Histology analyses were also performed on healed skin. Untreated wounds were compared to ASC-based dressings placed onto wound beds and changed every 3 days (8 to 18 wounds/group from 2 experiments).

Results: The kinetics of wound reepithelialisation did not differ between the experimental groups. However, measurements of wound areas indicated a faster rate of global healing for the group that received the ASC-based dressings. In addition, histological tissue cross-sections of the regenerated skin revealed a thicker dermal compartment (1.6x, $p=0.0214$) and overall homogenous aspect for the ASC group. This is in accordance with the secretion profiles of bioactive molecules produced by the dressings which can enhance granulation tissue and angiogenesis such as angiopoietin-1, HGF, PAI-1 and VEGF that were quantified in conditioned media before/after being applied to the wounds.

Conclusions: The use of ASC-based biological dressings did not delay nor accelerate reepithelialisation during skin healing but rather seemed to enhance dermal regeneration. Therefore, we provided evidence supporting the in vivo promotion of cutaneous healing by these ASC-based engineered tissues. It will be important to assess their capacity to stimulate regeneration in the context of impaired healing such as diabetic ulcers.



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INDUCTION OF ADIPOGENESIS IN VIVO BY MECHANICAL STIMULATION WITH EXTERNAL VOLUME EXPANSION

Presenter: Luca Lancerotto, MD

Authors: Lancerotto L, Lujan-Hernandez JR, Nabzdyk C, Hassan ZK, Khouri Jr. RK, Giatsidis G, Chin MS, Lalikos JF, Bassetto F, Orgill DP

University of Padova

Introduction: The ability to control adipogenesis may allow improved treatment of pathologic fat accumulation as well as the stimulation of fat deposition for tissue engineering purposes. Fat pathologically deposits systemically in obesity, but also limitedly to localized districts in lymphedema as a consequence of lymph stasis. We previously observed in a mouse model the capacity of external volume expansion (EVE), a clinically available non invasive method of 3D tissue stretching based on suction that has pro-proliferative and pro-angiogenetic effects, to thicken the subcutaneous tissue when applied continuously for 28 days. The model having some similarities with lymphedema, we tested the pro-adipogenic response to its acute application.

Methods: A rubber dome connected to -25mmHg suction was applied to the dorsum of mice for a single 2h stimulation or for 2h/daily for 5 days. Tissues were harvested at 48 hours from the last stimulation and analyzed for edema, inflammation and adipogenesis by staining for H&E, CD45 (pan-leukocytic marker) and Perilipin-A (mature adipocytes marker) respectively. Expression of PPAR-gamma (a pro-adipogenic factor) and PREF-1 (marker of pre-adipocytes) was quantified by WB.

Results: Short single or repeated EVE treatment induced local transient edema and a slowly regressing inflammation. 2h stimulation and cyclical 2h stimulation for 5 days induced 1.5 and 2.0 –fold increase in number of adipocytes/mm respectively, with 2.5 and 1.9 –fold increase in adipocyte surface. PPAR-gamma was significantly increased at the end of stimulation, while PREF-1 had a decreasing trend in the following 2 days, suggesting preadipocytes commitment to maturation.

Discussion: Even short (2h) mechanical stimulation by EVE seems to possess impressive pro-adipogenic effects. Stretch is known to stimulate proliferation, and edema and inflammation are increasingly appreciated pro-adipogenic factors and thought to be key players in lymphedema. While our model appears interesting to investigate adipogenesis and develop strategies for its control, our study suggests mechanical forces may be implemented in adipose tissue regeneration strategies in vivo as a way of maintaining a pro-adipogenic environment.

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XENOGENIC-FREE CULTURE SUPPLEMENTS FOR GMP-COMPLIANT EXPANSION OF MESENCHYMAL STROMAL CELLS

Presenter: Karen Bieback, PhD

Authors: Bieback K, Kinzebach S, Hecker A, Uhlig S, Elvers-Hornung S, Kltter H

Institute of Transfusion Medicine and Immunology

The idea of stem cell therapy sounds simple: obtain sufficient numbers of cells from human tissues, isolate and expand the stem cells and then transplant the cells at the correct location. However, the translation into routine therapies is a complex, multistep process, necessary to comply with regulatory guidelines. Mesenchymal stromal cells (MSC) are interesting examples as these cells have been therapeutically applied in a very early phase of research. Scarcity of MSC often requires ex vivo expansion. Still expansion protocols rely on the use of fetal bovine serum (FBS), albeit critically rated by the regulatory authorities. “Humanized” culture conditions appear highly encouraging to replace FBS for clinical-scale manufacturing.

We compared the effects of pooled human serum (HS) as well as platelet lysate (pHPL) on MSC from bone marrow (MSC) and adipose tissue (ASC). Interestingly whereas expansion of MSC was strongly accelerated by pHPL, ASC proliferated stronger in HS. A differential proteomic approach comparing the human supplements combined with functional tests identified proteins with differential effects on MSC and ASC proliferation. Comparability assays ensured that basic therapeutic features of MSC such as senescence, differentiation potential, immunosuppression and secretion of bioactive factors were maintained. However, differences in cell size and adhesion force correlated to differences in gene and protein expression. These affected adhesion, migration and homing, important therapeutic features of MSC. Albeit the effect of changing the cell culture supplement is not ultimately clarified yet, clinical trials already utilize MSCs expanded in “humanized” supplements indicating feasibility, safety and efficacy. Nevertheless differences in the clinical outcome, e.g. in studies for treatment of graft versus host disease have been observed using either BM-MSCs in FBS or PL. To provide success of MSC-based therapies, the establishment of standardized manufacturing protocols and quality control parameters and assays is of utmost importance.



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ADIPOSE TISSUE-DERIVED STEM CELL SHEETS TO PROMOTE WOUND REPAIR

Presenter: Panithi Sukho, DVM

Authors: Sukho P, Kirpensteijn J, Verseijden F, Bastiaansen-Jenniskens YM

Utrecht University The Netherlands

Introduction: Main reasons for impaired wound healing are restricted blood supply and uncontrolled inflammation. Although human adipose tissue-derived stem cells (ASCs) are known to secrete a myriad of trophic factors that can promote repair of injured tissue, detailed information about the effects of different densities of ASCs on their ability to stimulate angiogenesis and modulate the local inflammatory reaction remains largely unknown. Therefore, we developed ASC-sheets in different densities under 'normal' and 'inflammatory' conditions. We compared the ability of these sheets to express angiogenic and inflammatory factors and evaluated the effect of conditioned medium (CM) on endothelial cell (EC) proliferation and fibroblast migration

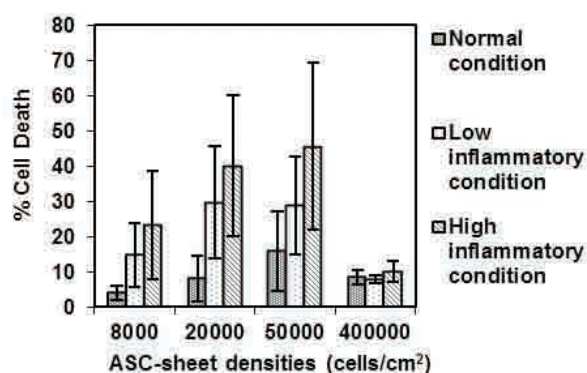
Methods: ASCs from 3 donors were used to develop low-density (8.000 or 20.000 cells/cm²) and high-density (50.000 or 400.000 cells/cm²) cell sheets. After 24 hours pre-culture, these sheets were cultured in 3 different conditions: a normal condition, a low inflammatory condition with 25ng/ml interferon gamma (IFN γ) and 10ng/ml tumor necrosis factor alpha (TNFa) and a high inflammatory condition with 50ng/ml IFN γ and 20ng/ml TNFa. Medium and cell sheets were harvested after 48 hours of culture.

Results: Percentage of cell death, as measured by the level of lactate dehydrogenase in the culture medium, was increased with increasing concentrations of inflammatory factors. Interestingly, less cell death was seen in the highest-density ASC-sheets than in the low-density ASC-sheets (figure 1). Similarly, IDO, PTGS2, TNFa and FGF-2 were increased in the inflammatory conditions and again, the highest-density ASC-sheets were less influenced by pro-inflammatory cytokines and expressed TNFa at much lower levels than low-density ASC-sheets. Gene expression and secretion of VEGF was higher in highest-density ASC-sheets. Additionally, CM from highest-density ASC-sheets enhanced EC proliferation and fibroblast migration compared to low-density ASC-sheets.

Conclusions: In summary, high-density ASC-sheets may have a superior potential to improve wound healing in inflammatory conditions due to enhanced viability, enhanced VEGF and reduced TNFa expression, and the ability to promote EC proliferation and fibroblast migration.

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ADIPOSE TISSUE-DERIVED STEM CELL SHEETS TO PROMOTE WOUND REPAIR





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FREE ADIPOCYTE ISOLATION TECHNIQUES AND HARVEST (FAITH) STUDY: PROSPECTIVE DIRECT COMPARISON OF COLEMAN VS. CYTORI FAT GRAFT HARVESTING TECHNIQUES

Presenter: Edward I. Chang, MD

Authors: Chang EI, Scaglioni MF, Zhang LQ, Iyyanki TS, Butler CE, Beahm EK

MD Anderson Cancer Center

Introduction: Fat grafting is becoming a common adjunct in reconstructive surgery for volume replacement. Consequently, a number of techniques have emerged claiming increased adipocyte counts and stem cell yield; however, the current literature is often biased by conflict of interest and the validity of these claims remain unknown.

Methods: Ten prospective patients undergoing fat grafting underwent harvest using the Coleman and Cytore techniques following institutional review board approval. Each patient served as their own control for each technique. Samples (n=3 per patient) were analyzed for total adipocyte count.

Results: Ten patients (male: 1 and female: 9) with an average age of 53.2 years and mean body mass index of 30.2 kg/m² were included in the study. Comorbidities included smoking (n=5), hypertension (n=3), diabetes (n=1), prior chemotherapy (n=1), prior radiation (n=1), and both chemotherapy and radiation therapy (n=2). None of the patient demographics or comorbidities had a significant impact on overall adipocyte counts. All female patients were undergoing fat grafting for refining results following breast reconstruction. The single male patient underwent fat grafting to augment lip volume following head and neck reconstruction. Two patients were taking statins at the time of fat graft harvest. Average adipocyte count from Coleman samples was nearly two-fold higher than Cytore samples (3.0×10^6 vs. 1.6×10^6). Overall, there was a trend toward higher cell counts with the Coleman technique, but the difference did not reach statistical significance ($p=0.068$). Cost analysis demonstrated an increase in \$525/case with the use of the Cytore system.

Conclusions: The use of the Cytore system did not increase viable adipocyte yield compared to the Coleman technique, but was associated with an increase in cost. Further analysis on stem cell viability and larger studies are needed to justify the use of the Cytore system over the Coleman technique in an era of increasing healthcare costs and dwindling reimbursement.

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DEVELOPING ANIMAL MODEL FOR LOCAL BREAST CANCER RECURRENCE IN AUTOLOGOUS FAT GRAFTING

Presenter: Wakako Tsuji, MD, PhD

Authors: Tsuji W, Valentin J, Marra K, Donnenberg A, Donnenberg V, Rubin JP

University of Pittsburgh

Introduction: Autologous fat grafting for breast reconstruction after breast cancer surgery offers several advantages over prosthetic devices including less immunological problems and a more natural appearance. However, the relationship between cancer cells and fat tissue or adipose-derived stem cells has not yet fully elucidated. The aim of this study was to develop animal model for breast cancer local recurrence in autologous fat grafting.

Materials and Methods: Cancer cell characterization was performed using a flow cytometry. Graded numbers of viable MDA-MB-231 or BT-474 cells were sorted directly into human fat tissue, and injected into the mammary fat pads of female NSG immunodeficient mice. MDA-MB-231 or BT-474 in a Matrigel vehicle were injected as a positive control. After 6 weeks, explants were excised, weighed and graft volume was measured with a gas-pycnometer. Graft density was calculated from weight and volume. Histological evaluations were performed on paraffin sections (H&E, human-specific pan-cytokeratin, and Ki67 as a measure of proliferation).

Results: BT-474 showed epithelial features (Cytokeratin+, CD44-, CD90-, E-cadherin+ and CD73-) and MDA-MB-231 showed mesenchymal features (Cytokeratin+, CD44+, CD90-, E-cadherin- and CD73+). Proliferating mesenchymal-like MDA-MB-231 cells were readily visualized interspersed in the fat grafts (human-specific pan-cytokeratin and Ki67). However, the more epithelial BT-474 were not detected in the fat graft. The addition of both types of tumor cells to the fat grafts increased graft density in a dose-dependent manner, suggesting that fibrosis as well as the growth of tumor cells contribute to increased graft density.

Conclusion: Sorting of measured numbers of viable tumor cells directly into adipose graft preparations is a novel method to establish an animal model for local breast cancer recurrence in the context of fat grafting. Further experimentation is required to determine whether the indication for fat grafting is depended on the breast cancer subtype.



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**CHARACTERIZATION OF ADIPOSE DERIVED CELLS
FROM HUMAN ESCHAR TISSUE**

Presenter: Zeni Alfonso, PhD

Authors: Alfonso Z, Foubert P, Zhao S, Hicok K,
Arm D, Tenenhaus M, Fraser J

Cytori Therapeutics

NOT PRESENTED

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**THE COMBINED USE OF ENHANCED STROMAL
VASCULAR FRACTION AND PLATELET-RICH PLASMA
IMPROVES FAT GRAFTING MAINTENANCE IN BREAST
RECONSTRUCTION: CLINICAL AND INSTRUMENTAL
EVALUATION**

Presenter: Pietro Gentile, MD, PhD

Authors: Gentile P, Cervelli V

University of Rome Tor Vergata

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.



EXHIBITORS



Cook Medical

Sandet 6, DK 4632
Bjaeverskov, Denmark
Tel: 353-61-250674

Email: Henk.Snyman@CookMedical.com

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3590 Grand Forks Boulevard
 Charlottesville, VA 22911
 Tel: 1-434-975-8000
 Fax: 1-434-975-4144
 Email: theresaj@microaire.com
 www.microaire.com



MicroAire Aesthetics, a world leader in aesthetic plastic surgery, produces the PAL® LipoSculptor™ (power-assisted lipoplasty); Endotine® bioabsorbable multi-point fixation devices for cosmetic facial procedures; and LipoFilter™ high-volume fat filtration and harvesting systems. MicroAire Aesthetics also makes EpiCut (epithelium tissue removal), and SurgiWire™ (subcutaneous dissection). For more information, please visit microaire.com.

Musculoskeletal Transplant Foundation - Gold Sponsor

125 May Street
 Edison, NJ 08837
 Tel: 1-732-661-0202
 Fax: 1-732-661-2298
 Email: laura_dey@mtf.org
 www.mtf.org



MTF is a non-profit tissue bank dedicated to providing quality tissue through a commitment to education, research, and care for recipients, donors and their families. FlexHD, our human acellular dermal matrix, is used by general and plastic surgeons to address naturally occurring defects in abdomen, breast and face.

SERVA Electrophoresis GmbH

Carl-Benz-Str. 7
 69115 Heidelberg, Germany
 Tel: 49-4122-712413
 Fax: 49-4122-712286
 Email: collagenase@serva.de
 www.serva.de



SERVA offers various superior Collagenase NB qualities particularly suitable for the isolation and passaging of adipose tissue derived stem cells. For clinical applications our Collagenase NB 6 GMP Grade offers the highest quality and safety. Collagenases NB are produced by the renowned pharmaceutical company Nordmark, the world's largest collagenase manufacturer.



Shippert Medical

6248 S. Troy Circle, Suite A
Centennial, CO 80111

Tel: 1-303-754-0044

Fax: 1-303-754-0318

Email: am@shippertmedical.com

www.shippertmedical.com

Shippert Medical's Tissu-Trans® devices offer a solution for simple, affordable collection of adipose tissue. The devices work with all standard harvesting equipment and aspirators, and do not alter the surgeon's technique. The sterile devices connect inline via tubing between the cannula and the aspirator. Tissue is harvested with minimal air exposure or handling. Multiple collection methods and volumes are available from 1cc to 2000cc.



Tulip Medical Products

4360 Morena Boulevard, Suite 100

San Diego, CA 92117

Tel: 1-858-270-5700

Fax: 1-858-270-5901

Email: sacsy@tulipmedical.com

www.tulipmedical.com

Tulip® is the leader in liposuction and fat transfer instrumentation. As innovators in liposuction cannulas, injector design and manufacturing, Tulip creates instruments and accessories for successful liposuction and fat transfer procedures. With our patent-pending CellFriendly™ Technology and our new premium Gems™ Single-Use fat transfer instruments, Tulip is your source for liposuction, fat-transfer and regenerative medicine cannulas, micro-injectors and accessories.



Coming Soon!
Not approved for use in Europe.

REVOLVE™

NATURAL BEAUTY BY DESIGN™

**In a pre-clinical study^{*,†},
REVOLVE™ System showed more
reliable fat processing results.**

REVOLVE™ System is a high-volume fat processing system that results in high quality adipose tissue.

In a pre-clinical study, REVOLVE™ System:

- Yielded significantly higher fat graft retention (73.2%) than that from decanted samples (37.5%) and similar to that from centrifuged samples (67.7%)
- Produced higher percentage of adipose tissue
- Removed significantly more blood cell debris and a higher percentage of free oil
- Maintained fat in physiological conditions (pH and osmolality)

*Correlation of these results to results in humans has not been established.

Find out more at the LifeCell Booth

Before use, physicians should review all risk information, which can be found in the *Instructions for Use* and *User Manual* for the REVOLVE™ System.

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



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LifeCell
An Acelity Company



IFATS Executive Office

45 Lyme Road - Suite 304 Hanover, NH 03755 USA

Tel: 1-603-643-2325 • Fax: 1-603-643-1444

Email: ifats@conmx.net • Web: www.ifats.org