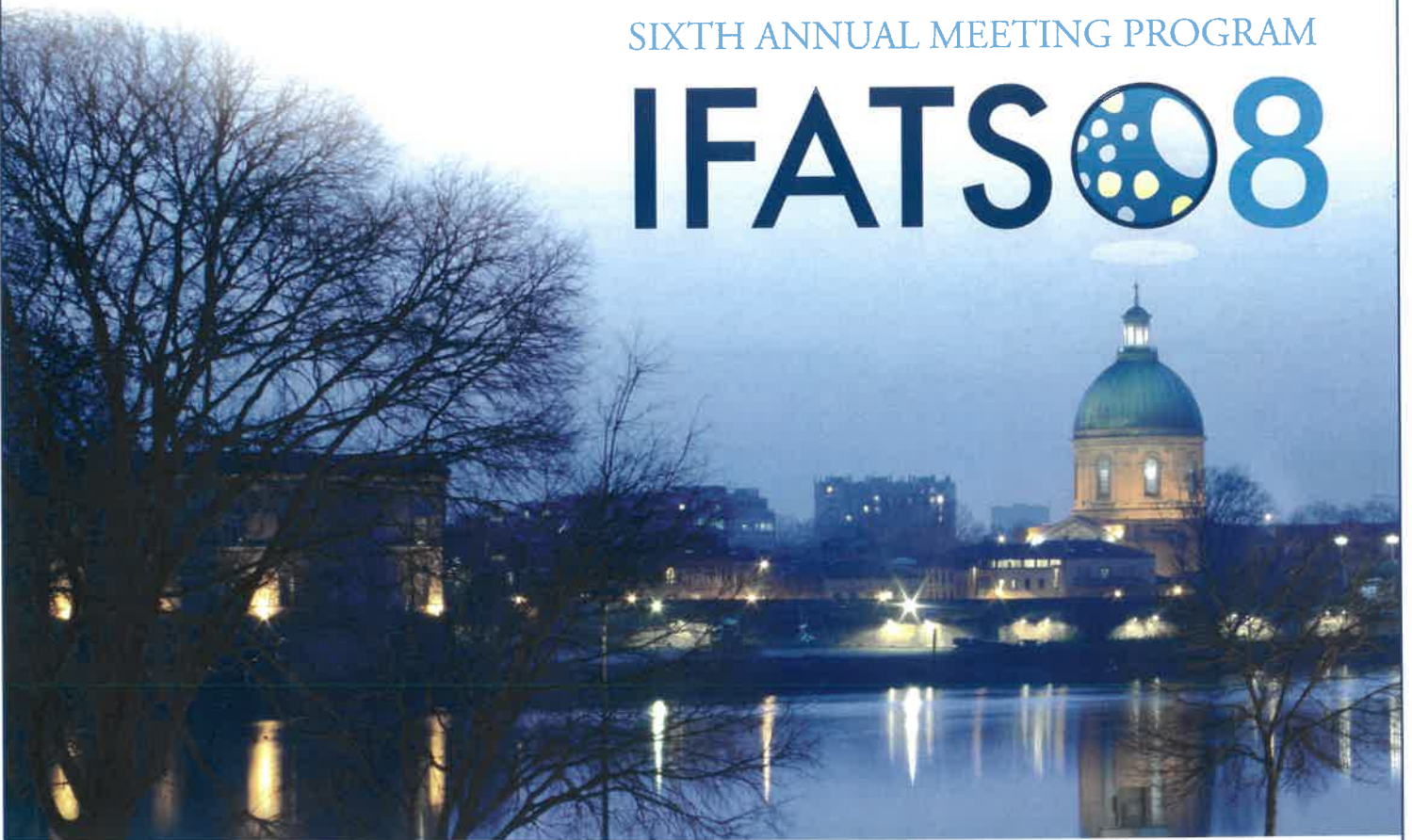


SIXTH ANNUAL MEETING PROGRAM

# IFATS 8



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[www.ifats08.org](http://www.ifats08.org)

**Toulouse, France**  
October 24 – 26, 2008



# Mission

To improve world health through a better understanding of adipose biology.

# Description

The International Federation of Adipose Therapeutics & Science (IFATS), a 501 (c) (3) non-profit organization incorporated in August of 2002, is dedicated to facilitating the development of new technology derived from and directed toward adipose tissue. IFATS is a leading source of information about adipose biology and related technology. The Society identifies new technology and brings together scientists and industry for productive collaborations. Such collaborations will relate to adipose tissue, with far-reaching applications in obesity reduction products and services, fat-derived stromal and cell-based therapies, and fat-applied instrumentation devices which may be directly translated to therapeutic application. IFATS' current scientific areas of interest relate to facilitating the development of treatments for excess body fat, the generation of new fat tissue for reconstruction after cancer or birth-related defects, and the use of adipose tissue as a source of mesenchymal stem cells that have the potential to regenerate and repair many different tissues of the body. IFATS is currently the world's only interdisciplinary fat tissue society. IFATS' hope is to serve as the premier global incubator in the field of fat applied and derived technology.

## Leadership of the Society

Co-Presidents:

**Anne Bouloumie, PhD**

Team Leader AVENIR INSERM U858  
Toulouse, France

**Louis Casteilla, PhD**

Professor, Institut Louis Bugnard  
Toulouse, France

Immediate Past President:

**Keith L. March, MD, PhD**

Professor of Medicine, Cellular & Integrative Physiology, and Biomedical Engineering Indiana University School of Medicine Director, Indiana Center for Vascular Biology and Medicine Cryptic Masons Medical Research Foundation Chair in Vascular Biology and Medicine

## Scientific Program Chairs

**Dr. Jae Ho Jeong**

Professor, Yeungnam University

**Dr. Sang Hong Back**

Director, Cell Therapy Center  
KangNam St Mary's Hospital

# Welcome

*A message from our co-presidents Anne Bouloumie, PhD and Louis Casteilla, PhD.*

Bonjour! We look forward to welcoming you to the Sixth Annual meeting of the International Federation of Adipose Therapeutics and Science (IFATS).

IFATS is the world's only multi-disciplinary society that focuses on the use of stromal/stem cells derived from adipose tissue to regenerate and repair many tissues. This fascinating tissue now appears as one of the best and most powerful sources of regenerating cells.

Investigators in basic and translational science and therapy will share knowledge, ideas, applications and prospective issues in an interactive and collaborative meeting from October 24-26, 2008 in downtown Toulouse. Highlights of the conference will be characterization of the adipose stromal/stem cells, mechanisms and processes involved in the control of their fate, engineering and therapeutic applications in soft tissue reconstruction, bone formation, cardiovascular repair as well as plastic surgery.

We anticipate more than 150 researchers attending from dozens of countries around the world, joining with representatives of the more than 15 companies that are actively working in the area of adipose stem cells as of this writing.

Capital of the Mid-Pyrenees Region, Toulouse is the fourth largest town in France. Easy to reach by frequent plane shuttles from Paris, Toulouse is located between the Mediterranean Sea and the Bordeaux vineyards and the Atlantic coast. It is approximately 100 km from the Pyrenees Mountains and the border of Spain and 730 km from Paris. Dating back to the fourth century, Toulouse displays a long and rich history offering magnificent downtown buildings, churches, monasteries and museums. Its typical culinary traditions and high quality of life have been associated with one of the lowest rates of cardiovascular disease around the world. Today, Toulouse is a very dynamic town and looks to the future with its many research areas and nine thousand researchers including aerospace and aircraft industries, biology and medical science as well as robotics and computer science.

We look forward to welcoming you to Toulouse!!

A. Bouloumie and L. Casteilla, Ph.D. Presidents, IFATS 2008





October 24-26, 2008

Catholic Institute of Toulouse - Toulouse, France

Thursday, October 23, 2008

3:30 – 5:00 PM Exhibitor and Poster Set up (Catholic Institute of Toulouse)

5:30 – 7:30 PM Social Gathering and Welcome (Mercure St. Georges)

Friday, October 24, 2008

8:00 AM – 5:00 PM Exhibitors on site - Exhibitor Hall - Léon XIII

6:00 – 7:15 AM IFATS Founders Meeting Breakfast (Mercure St. Georges)

8:00 AM Opening remarks from organization committee - L. Casteilla and A. Bouloumie (Tolosa Amphitheatre)

8:30 – 9:30 AM Keynote Address I: Regeneration in the Newt – G. Weidenger, Max Planck Institute Dresden, Germany (Tolosa Amphitheatre)

9:30 – 10:30 AM **Symposium One:** Adipose Tissue (Tolosa Amphitheatre)  
Moderators: Drs. A. Katz and J. Gimble

In vivo effects of hypoxia on adipose tissue: Implications for obesity-related dysfunction  
*Hirotaka Suga; Hitomi Eto; Kotaro Yoshimura*

Influence of body mass index, age and adipose tissue location on the CD34+/CD31- stem/progenitor cell number and phenotype  
*Marie Maumus; Coralie Sengenès; Auralie Villaret; Virginie Bourlier; Pauline Decaunes; Alexia Zakaroff-Girard; Jean Galitzky; Anne Bouloumie*

Transforming Growth Factor- $\beta$  Is a Key Factor Responsible For Preventing ASC Adipogenesis and Promoting a Pericytic Phenotype  
*Dmitry Traktuev; Stephanie Merfeld-Clauss; Gangaraju Rajashekhar; Matthias Clauss; Keith March*

Purification of Four Distinct Cell Populations in the Stromal Vascular Fraction of Human Adipose Tissue: Adipogenic Potential and Implications for Soft Tissue Engineering  
*J. Peter Rubin; Han Li; Ludovic Zimmerlin; Kacey Marra; Vera Donnenberg; Albert Donnenberg*

10:30 – 10:45 AM Coffee break - Exhibitor Hall - Léon XIII

10:45 AM – 12:00 PM **Symposium Two:** Adipose Tissue (Tolosa Amphitheatre)  
Moderators: Drs. M. Lafontan and A. Bouloumie

Involvement of adipose-derived stromal cells in remodeling and expansion process of adipose tissue  
*Hirotaka Suga; Hitomi Eto; Harunosuke Kato; Kotaro Yoshimura*

Human Coq2 expression in 3T3-F442A cells reduced adipose differentiation and increased reactive oxygen species production  
*Sandy GalinierBour; Anne Galinier; Sylvie Caspar-Bauguil; Mamen Carmona; Luc Pénicaud; Louis Casteilla*



## ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Friday, October 24, 2008 (continued)

Cytoskeleton reorganization by thymosin beta-4 alters cell fate determination of human mesenchymal stem cells

*Jennifer Ho; Kuang-Ching Tseng; Wei-Hsien Ma; Yeu Su; Oscar Lee*

Direct co-culture of human adipocytes, adipose tissue macrophages and adipose stem/progenitor cells (ASPCs) reveals a mechanism for generation of new preadipocytes and a novel population of CD34 (+) ASPCs

*Saleh Heneidi; Cristina Bertolotto; Gregorio Chazenbalk; Charles Simmons; Ricardo Azziz*

Differential Adipogenesis Related Gene Expression in Mesenchymal Stromal Cells derived from Bone Marrow, Cord Blood and Adipose Tissue.

*Marianna Karagianni; Karen Bieback; Harald Klueter*

12:00 – 1:30 PM

Lunch & Poster Session I Translational

12:00 – 1:30 PM

IFATS Leadership Team Meeting/Working Lunch (Mercure St. Georges)

1:30 – 2:30 PM

Keynote Address II: Fat and Metabolism - M. Lafontan, I2RM, Toulouse, France (Tolosa Amphitheatre)

2:30 – 3:45 PM

*Symposium Three: ASC, Inflammation & Immunity* (Tolosa Amphitheatre)

Moderator: Dr. Jae Ho Jeong

Regulation of lymphocyte proliferation by human adipose-derived stem cells requires IFN-gamma, IDO activity and generates T cells with suppressor activity

*Olga De La Rosa*

Immunogenicity of Allogeneic Adipose-Derived Stem Cells in a Rat Spinal Fusion Model

*Nakia Spencer; Paul Anderson; Jeffrey Gimble; Kevin McIntosh; Mandi Lopez; Jade Borneman*

Toll-like receptor-mediated signaling in human adipose-derived stem cells: Implications on immunogenicity and immunosuppressive potential

*Eleuterio Lombardo*

Therapeutic effect of adipose-derived mesenchymal stem cells on experimental colitis by inhibiting inflammatory and autoimmune responses

*Aitor Beraza*

Anti-adipogenic effect of adipose tissue T lymphocytes on the human CD34+/CD31- cells

*Carine Duffaut; Alexia Girard; Coralie Sengenes; Jean Galitzky; Anne Bouloumié*

3:45 – 4:00 PM

Coffee Break - Exhibitor Hall - Léon XIII

4:00 – 5:00 PM

Keynote Address III: Fat : From ES to Adult Stem Cells – C. Dani, Institut de Biochimie, CNRS, Nice, France (Tolosa Amphitheatre)

## ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

### Friday, October 24, 2008 (continued)

5:00 – 6:15 PM

**Symposium Four:** Translating to Clinic (Tolosa Amphitheatre)

Moderators: Drs. R. Llull and Sang Hong Baek

First-In-Man Experience of Adipose-Derived Stem Cell Transplantation in the Treatment of Patients with an Acute ST-Elevation Myocardial Infarction (APOLLO Trial - Cytori)

*H.J. Duckers; Patrick Serruys*

Safety and efficacy of autologous adipose-derived stromal cells on Type II diabetes patients: 6 month post-procedure results

*Florencio Lucero; Emerita Barrenechea; Letitia Lucero-Palma; Kribah Krishnan; Eduardo Barrenechea; Bill Paspaliaris*

Expanded Adipose-derived Stem Cells (Cx401) for the Treatment of Complex Perianal Fistula. A Phase II Clinical Trial

*Damian Garcia-Olmo; Dirk Büscher*

7:00 PM – 1:00 AM

Museum Dinner at Fondation Bemberg, Hôtel d'Assézat  
Place d'Assézat 31000 TOULOUSE

### Saturday, October 25, 2008

8:00 AM – 5:00 PM

Exhibitors on site - Exhibitor Hall - Léon XIII

8:45 – 9:45 AM

Keynote Address: Heart & Cell Therapy in Humans Up to Now - P. Menasché,  
Hospital Assistance Publique, Paris, France (Tolosa Amphitheatre)

9:45 – 10:00 AM

Coffee Break - Exhibitor Hall - Léon XIII

10:00 – 11:30 AM

**Symposium Five:** ASC Heart & Vasculature (Tolosa Amphitheatre)

Moderators: Drs. K. March and P. Menasché

Evidence of a robust trafficking capability: Adipose-derived stem cells slowly roll on P-selectin and enhance firm adhesion to VCAM-1 following exposure to SDF-1alpha  
*Alexander Bailey; Michael Lawrence; Hulan Shang; Adam Katz; Shayn Peirce*

Endothelial differentiation of adipose-derived stem cells: How close have we really come?  
*Paul DiMuzio; Ping Zhang; Stephen McIlhenny; Lisa Harris; Hamid Abdollahi; Matthew Ferroni; Kristi Wasson; Eric Hager; Neil Moudgil; Nicholas Tarola; Daniel Grabo; Vic Srinivas; Irving Shapiro; Thomas Tulenko*

Adipose Derived Adult Stem cells (ADAS) capacity to differentiate into Endothelial Cells expressing von Willebrand marker and forming capillary-like structures in a Skin Equivalent model.

*Celine Auxenfans; Lauriane Thivillier; Charlotte Lequeux; Nicolas Bechetoille; Stephanie Maillet; Nathalie Tan; Beste Kinikoglu; Valérie André; Odile Damour*

Treatment of chronic myocardial infarction with adipose-derived cells leads to an improvement in cardiac performance

*Manuel Mazo, Beatriz Pelacho, Juan José Gavira, Gloria Abizanda, Felipe Prósper*

## ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Saturday, October 25, 2008 (continued)

Adipose Derived Stromal Cells Express Tissue Factor in a Passage-Dependent Manner: Implication for Intracoronary Cell Therapy and Use of Heparin with ASCs  
*Vineet Kumar; Malgorzata Maria Kamocka; Elliot D Rosen; Clayton A Smith; Brian Johnstone; Keith L March*

Automated isolation of adipose-derived stromal cells and rapid fabrication of enhanced vascular graft  
*H. Joon Paek; Eugene Boland; Erik Vossman; Anthony Yang; Thomas Cannon; Stuart Williams; Paul Kosnik*

12:30 – 2:30 PM

Lunch – Poster Session II Basic

2:30 – 3:45 PM

**Symposium Six:** ASC & Engineering (Tolosa Amphitheatre)  
Moderators: Drs. Yoshimura and J. S. Jung

Towards a human supplement replacing fetal bovine serum for clinical scale MSC manufacturing: Differential Gene and Protein Expression Analysis of Adipose Tissue derived MSC cultivated in different supplements.  
*Viet Anh-Thu Ha; Andrea Hecker; Asli Kocaoemer; Hermann Solz; Harald Klueter; Peter Bugert; Karen Bieback*

The Effect of Hydrostatic Pressure on Three-Dimensional Cartilage Regeneration Using Human Adipose-Derived Stem Cells  
*Rei Ogawa; Shuichi Mizuno; George Murphy; Dennis Orgill*

Osteogenic Potential of Adipose Stem Cells in Electrospun Fibrinogen  
*Michael Francis; Gary Bowlin; Shawn Holt*

Enhanced Fat Protection and Survival in Fat Transplantation via Treatment with Poloxamer 188  
*John Nguyen; Mike McCormack; Mark Randolph; William Austen Jr.*

3:45 – 4 :00 PM

Coffee Break - Exhibitor Hall - Léon XIII

4:00 – 5:00 PM

**Symposium Seven:** ASC & Mesenchymal Tissue (Tolosa Amphitheatre)  
Moderator : Dr. Casteilla

Evaluation of the stamness and of chondrogenic and osteogenic differentiation potential of different human Adipose Stem Cells (hASC) subpopulation.  
*Tommaso Rada; Rui L. Reis; Manuela E. Gomes*

Adipose Derived Stem Cells accelerate Primary Tendon Repair  
*A. Cagri Uysal; Hiroshi Mizuno; Hakan Orbay; Takahisa Okuda; Hiko Hyakusoku*

Adipose derived mesenchymal stem cells in the treatment of osteoarthritis in the veterinary patient. A clinical experience  
*Jerrold Bausman*



## ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

### Saturday, October 25, 2008 (continued)

- 5:30 PM Departure for Gala Awards Dinner  
(transportation provided from each of the three conference hotels)
- 6:00 PM Gala Awards Dinner - Chateau Saint Louis  
Announcement of IFATS 2007 Pre-doctoral Student Award  
Announcement of IFATS 2007 Post-doctoral Fellow Award

### Sunday, October 26, 2008

- 8:30 – 9:30 AM Special lecture: Stem Cell & Plasticity - N. Askenasy, Schneider Children's Medical Center of Israel, Petach Tikva, Israel (Tolosa Amphitheatre)
- 9:30 – 9:45 AM Coffee Break - Exhibitor Hall - Léon XIII
- 9:45 – 11:15 AM **Symposium Eight:** ASC & Non-Mesenchymal Tissues (Tolosa Amphitheatre)  
Moderator: Dr. Rubin
- Cell therapy based on adipose tissue-derived stroma cells promotes physiological and pathological wound healing  
*Tommaso Rada; Rui L. Reis; Manuela E. Gomes*
- Mesenchymal stem cells for liver regeneration  
*A. Cagri Uysal; Hiroshi Mizuno; Hakan Orbay; Takahisa Okuda; Hiko Hyakusoku*
- Microstructural Fat Grafting Improves Radiation Skin Damage in a Murine Model  
*Jerrold Bausman*
- Adipose derived stem cells accelerate primary nerve repair  
*Ebrahimian TG, Squiban C, Pouzoulet F, Buard V, André M, Cousin B, Silvestre JS, Benderitter M, Casteilla L, Tamarat R.*
- Assessing the Risk of Tumorigenesis Through the Interactions of Adipose Derived Stem Cells and Breast Cancer  
*Oscar K. Lee*
- 11:15 AM – Noon Closing Remarks (Tolosa Amphitheatre)
- Noon Adjournment

# Exhibits

Friday Oct. 24, 2008, 8:00am - 5:00pm  
and Saturday Oct. 25, 2008, 8:00am - 5:00pm

## IFATS 08 Exhibitors

**AVISO** is an expert for robotic systems and automation solutions which are mainly employed in the field of life science. We are an industry partner for international research projects focusing on cell biology. By close scientific collaboration with several universities, institutes and research facilities, marketable products with high acceptance are originated.

**BioSpherix Ltd.** provides enabling technology for the development and commercialization of stem cell and other cell therapies. Our advanced incubation systems provide cell scientists with unprecedented new ways to perfect stem cell differentiation and proliferation, and will accelerate your research and development by a large factor. Furthermore, our unique "closed-system incubation" offers the first economical and practical alternative to typical "bricks-and-mortar" clean-rooms for cGMP compliant cell production. This makes clinical trials with stem cells faster and easier to run, at a much lower cost, and reduces your risk of investment considerably. Finally, if your cells prove effective in clinical trials, our technology offers the perfect vehicle for rollout of new cell therapies to clinics worldwide, enabling successful commercialization. Please stop by and see us at the exhibit table and pick up a brochure!

**The Cell Therapy Foundation** has a three-fold mission: to foster healthy public awareness, to increase research funding, and to improve communication and collaboration among leading adult stem cell researchers. The Foundation's lead project is the Web-based Adult Stem Cell Research Network, [www.ascrnetwork.org](http://www.ascrnetwork.org). The site is designed to encourage communication among scientists, to accelerate the rate of progress of active clinical trials and to increase the rate of patients' enrollment. Adult stem cell laboratories are invited to become members of ASCR Network to promote their research, post clinical trial availability, collaborate with other medical researchers and view recent news articles regarding adult stem cell research. Laboratory membership currently includes sites located in Taiwan, South Korea, Sweden, France, Brazil, Netherlands, USA and Singapore.

**Cytori** is dedicated to the science, development and commercialization of adipose-derived cell therapies and the Celution® System tissue processing device. We are commercializing the Celution® System into European and Asian cosmetic and reconstructive surgery markets, conducting cardiovascular disease clinical trials, and collaborating with the global research community to bring new therapeutic applications into clinical development. We look forward to meeting with participants at IFATS 2008 and exploring how, together, we can advance this emerging field.

**Invitrogen Corporation** is a global company that provides products and services to pharmaceutical and biotechnology companies, as well as academic and government research institutions to support disease research, drug discovery and commercial bio-production. Invitrogen's own research and development efforts are focused on breakthrough innovation in all major areas of biological discovery including functional genomics, proteomics, stem cells, cell therapy and cell bio-logy-placing Invitrogen's products in nearly every major laboratory in the world.

# Exhibits

Friday

Oct. 24, 2008

8:00am - 5:00pm  
and

Saturday

Oct. 25, 2008

8:00am - 5:00pm

## IFATS 08 Exhibitors

**Tissue Genesis, Inc.** is a leading authority in regenerative cell therapy and delivery systems. We are a rapidly growing biotechnology company focused on the use of a patient's own therapeutic cells to prevent and repair injury to damaged tissue. Our company brings together pioneers and medical practitioners, including Dr. Stuart Williams, and a consortium of companies targeting the therapeutic utility of regenerative cells. The TGI 1000 advanced cell recovery and delivery system provides point of care solutions for clinical validation efforts worldwide.

**Toucan Capital Corporation** is focused on seed and early-stage life science and advanced technology investments. Toucan Capital Corp. launched its first fund in 1997 and began investing its current fund in August of 2001. Toucan invests nationwide and is one of the largest and most active venture capital funds in seed and early-stage life science. Toucan has also been an early investor in nanotechnology. Our investment professionals have extensive backgrounds in science, engineering, and finance and have significant operational and management experience at various technology-based companies prior to joining Toucan Capital.

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## Adipose Tissue

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## ASC & Engineering

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## In vivo effects of hypoxia on adipose tissue: Implications for obesity-related dysfunction

Hiroataka Suga; Hitomi Eto; Kotaro Yoshimura

### Introduction:

It has been suggested that hypoxia and chronic inflammation of adipose tissue induced by obesity are associated with dysfunction of adipose tissue, leading to insulin resistance and metabolic syndrome. Hypoxia has been known to have various effects on adipocytes and adipose-derived stromal cells (ASCs), but direct effects of hypoxia independent from obesity have been examined only in vitro, presumably because there is no good model for non-obese hypoxic adipose tissue.

### Methods:

We established a new animal model for hypoxic adipose tissue in mice by transecting nutrient vessels for the inguinal fat pad. We prepared three types of hypoxia models; mild, intermediate, and severe hypoxia. Interstitial partial pressure of oxygen (pO<sub>2</sub>) was measured with a needle-type oxygen sensor. Using a real-time RT-PCR, we examined mRNA expression of various genes, which have been reported to change in obesity. Histological changes and cellular events after hypoxia were examined with immunohistology of sectioned and whole mount tissues.

### Results:

Immediately after hypoxia induction, mild-hypoxia, intermediate-hypoxia, and severe-hypoxia models showed pO<sub>2</sub> of 37.3 mmHg, 29.2 mmHg, 11.6 mmHg, respectively, while sham models showed pO<sub>2</sub> of 50.5 mmHg. In every model except sham, HIF1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF $\alpha$ , PAI-1, and GLUT1 were up-regulated as early as at 6 hours, while HGF and MMP2 were up-regulated as late as on day 7. However, the up-regulations of these genes were temporary. On the other hand, leptin, adiponectin, and PPAR gamma were down-regulated as early as at 6 hours, and the down-regulation of these transcripts continued through 1 month and appeared to be irreversible. Only the severe-hypoxia model showed a prolonged hypoxic condition (pO<sub>2</sub> of 27.0 mmHg at 2 weeks) and degenerative changes of adipose tissue frequently seen in obesity, while other models showed a gradual improvement of pO<sub>2</sub> within 7 days and little degenerative changes in histology. The degenerative changes in the severe-hypoxia model resulted in atrophy (a lower weight of the adipose tissue) after 1 month. Under the severe hypoxia, Ki67+ proliferating cells increased on day 3, peaking on day 7. Many ASCs (CD34+/lectin-) were also detected on day 7, suggesting ASCs were involved in the remodeling process of the hypoxic adipose tissue. Aggregation of F4/80-positive macrophages was observed in the later phase, from day 14.

### Conclusions:

Dysfunction of adipose tissue was seen under an experimentally-induced hypoxia. Although mild hypoxia (70% of normal pO<sub>2</sub>) temporarily induced the dysfunction, prolonged and severe (below 50% of normal pO<sub>2</sub>) hypoxia resulted in seemingly-irreversible dysfunction as well as degenerative changes in adipose tissue, which further induced adaptive and remodeling cellular events.

## **Influence of body mass index, age and adipose tissue location on the CD34+/CD31- stem/progenitor cell number and phenotype.**

Marie Maumus; Coralie Sengenes; Auralie Villaret; Virginie Bourlier; Pauline Decaunes; Alexia Zakaroff-Girard; Jean Galitzky; Anne Bouloumie

### **Introduction:**

Adipose tissue (AT) stem/progenitor cells positive for the CD34 marker and negative for the CD31 marker (CD34+/CD31-) exhibit adipogenic and angiogenic capacities. While a number of work have investigated their differentiation abilities, the influence of the body mass index (BMI), the age of patients and the AT location on this cell population was poorly studied. The aim of the present work was to evaluate the effect of the BMI and the age on the proportion, the phenotype and the gene expression of human CD34+/CD31- progenitor cells in different AT depots.

### **Methods:**

Flow cytometry analyses were performed on freshly harvested stroma-vascular fraction (SVF) of human AT originating from different depots (abdominal, gluteal and omental) of lean to obese individuals using a combination of various cell surface markers (CD34, CD31, CD44, CD146, CD36). Additionally, CD34+/CD31- progenitor cells were isolated by an immunoselection/depletion approach with magnetic nanoparticles coupled to specific antibodies. Gene expression analysis regarding stemness (nanog, oct4), adipogenic lineage commitment (Pref-1) and adipogenesis (aP2, FAS, PPARgamma) was evaluated by real-time PCR.

### **Results:**

Flow cytometry analysis of freshly harvested SVF showed that the majority of the CD34+/CD31- progenitor cells express CD44 that  $20.1 \pm 3.6$  % are CD36 positive and that  $1.2 \pm 0.6$  % are CD146 positive. We found no correlation between the number of progenitor cells per gram of AT and the BMI neither with the age of patients, whatever the AT depot studied. Although progenitor cell number in subcutaneous abdominal and gluteal AT was similar, progenitor cell quantity was increased in omental AT as compared to abdominal subcutaneous AT obtained from the same obese patients. Further analysis of transcripts encoding for stemness and adipogenic genes expressed by the freshly harvested progenitor cells revealed the influence of BMI on their level.

### **Conclusions:**

The present study gives new insights regarding the biology of AT progenitor/stem cells, potentially involved in fat mass development and thus in the genesis of the pathologies associated with obesity.

## Transforming Growth Factor- $\beta$ Is a Key Factor Responsible For Preventing ASC Adipogenesis and Promoting a Pericytic Phenotype

Dmitry Traktuev; Stephanie Merfeld-Clauss; Gangaraju Rajashekhar; Matthias Clauss; Keith March

### Introduction:

Introduction: We have previously shown that adipose stromal cells (ASCs) reside in vascular periendothelial layers where they may function as pericytes. Additionally, in vitro and in vivo experiments demonstrated that isolated ASCs readily undergo adipogenesis. The control mechanisms governing the switch among progenitor pericytic and differentiated adipocyte states of ASCs are not well defined. Based on the fact that ASCs in adipose tissue are in direct contact with endothelial cells (EC), we hypothesize that ECs, either through physical interaction or by paracrine signaling, are fundamentally important in controlling ASC transitions between these two states.

### Methods:

Methods: ASC (passage 2-5) alone or in co-culture with ECs (passage 5) at a 1:1 ratio were exposed to (1) control basal medium (EBM-2/5%FBS), (2) adipogenic differentiation medium/5%FBS (ADM), or (3) ADM combined at a ratio of 1:2 with either basal or EC conditioned medium (EC-CM). EC-CM was produced by culturing EC in basal medium for 72h. Protein analysis of EC-CM was performed using an antibody array (RayBiotech). To evaluate the effects of TGF $\beta$  contained in EC-CM on ASC adipogenic potential, media were supplemented with neutralizing antibodies to TGF $\beta$ . To directly evaluate the effect of TGF $\beta$  on adipogenesis and smooth muscle cell differentiation, ASC were grown in ADM or basal medium with and without TGF $\beta$  supplementation. Adipocyte or smooth muscle cell formation was assessed, respectively, by Nile Red staining or immunological staining with smooth muscle actin antibodies.

### Results:

Results: Co-culture of ASCs with ECs in ADM suppressed adipogenesis of ASCs. To determine if this effect was due to factors secreted by ECs, we exposed ASCs to the ADM:EC-CM medium. Culturing ASCs in ADM:EC-CM medium also significantly decreased adipogenesis compared to the cells exposed to ADM/EBM-2/5%FBS. Analysis of the EC-CM revealed that ECs secrete TGF $\beta$  during culture. The presence of TGF $\beta$  in ADM significantly inhibited lipid accumulation in ASCs (up to 63%). Decreased expression of lipase and PPAR $\gamma$  mRNA expression was also observed. Pretreatment of EC-CM with anti-TGF $\beta$  significantly decreased the suppression of adipogenesis by EC-CM. Similar results were observed in co-culture experiments where the presence of anti-TGF $\beta$  in ADM enhanced ASC adipogenesis. Analysis of direct effects of TGF $\beta$  on ASCs revealed upregulation of smooth muscle actin expression and its organization into stress fibers. Conversely pre-treatment of ASCs in ADM with TGF $\beta$  reduced adipogenesis.

### Conclusions:

Conclusion: Based on the results of this study we conclude that the factors secreted by EC are involved in modulating differentiation of ASCs to adipocytes. The major factor involved in regulating this particular switch is TGF $\beta$ .



## Purification of Four Distinct Cell Populations in the Stromal Vascular Fraction of Human Adipose Tissue: Adipogenic Potential and Implications for Soft Tissue Engineering

J. Peter Rubin; Han Li; Ludovic Zimmerlin; Kacey Marra; Vera Donnenberg; Albert Donnenberg

### Introduction:

Adipose stem cells (ASCs) represent a truly heterogeneous population. An understanding of the functional characteristics of subpopulations will be helpful in planning new ASC cell based therapies for soft tissue reconstruction. The aim of this study was to define four distinct populations within the stromal vascular fraction based on surface marker expression, and evaluate the ability of each cell type to differentiate to mature adipocytes.

### Methods:

Subcutaneous whole adipose tissue was obtained by abdominoplasty from human patients. After mechanical and enzymatic dissociation, removal of mature adipocytes by centrifugation and lysis of erythrocytes, the stromal vascular fraction was isolated on a Ficoll/hypaque density gradient and analyzed using a Dako CyAn cytometer and sorted using a Dako MoFlo High Speed Sorter. To distinguish isolated cell populations, we performed an 8-color analysis based on the expression of CD3, CD31, CD34, CD45, CD90, CD117 and CD146. DAPI staining was performed to exclude apoptotic cells and cell clusters. Cell proliferation was determined by DNA quantification (Cyquant), and cells exposed to adipogenic culture media. Adipocyte differentiation was assessed by PCR for PPAR Gamma, fatty acid binding protein 4 (FAB4), and Leptin. Lipid accumulation was confirmed with Oil Red-O staining.

### Results:

Using eight-color multiparameter flow cytometry and prototype high throughput parallel processing analytical software (Venturi, Applied Cytometry Systems) four cell populations were purified and studied. Cells with 2N DNA (DAPI staining, FL6 Log) were selected, and cell clusters (doublet discrimination on FSc), cell debris (FSc x SSc) and cells binding anti-CD3 or CD45 or autofluorescent in the FL1 or FL2 channels were excluded. Candidate perivascular cells (pericytes), defined as CD146+ CD90dim, were characterized as CD31 and CD34 negative. These comprised 1.45 +/- 0.68% of cells studied. Two CD31+ endothelial populations were detected and discriminated by CD34 expression and tentatively designated mature endothelial (CD 31+/CD34-: 0.75 +/- 0.29%), and immature endothelial (CD 31+/CD 31-:6.48 +/- 3.7%). CD90+ cells comprised 66% of mature endothelial cell candidates, and 96% of the CD31+ CD34+ population. Both endothelial populations were heterogeneous with respect to CD146. The CD31-/CD34+ fraction (preadipocyte candidate: 74.26 +/- 11.88%) was also CD90+, but lacked CD146 expression. Proliferation was greatest in the CD31-/CD34+ group and slowest in the CD146+ CD90dim group. PPAR gamma mRNA expression was significantly higher in the CD31-/CD34+ group compared with all other populations after exposure to adipogenic medium. Moreover, the PPAR gamma mRNA expression increased from day 7 to day 14 during culture in adipogenic medium. A disproportionately high expression of FAB4 mRNA, a downstream target of PPAR gamma and an early marker of adipogenesis, was also seen in this group. The highest proportion of positive Oil Red-O staining was noted in this group, as well.

### Conclusions:

We have isolated four distinct stromal populations from human adult adipose tissue. Of these four populations, the CD31-/CD34+ group is the most prevalent and has the greatest potential for adipogenic differentiation. This cell type appears to hold the most promise for engineering of adipose tissue for reconstructive applications.

## Involvement of adipose-derived stromal cells in remodeling and expansion process of adipose tissue

Hiroataka Suga; Hitomi Eto; Harunosuke Kato; Kotaro Yoshimura

### Introduction:

Adipose-derived stromal cells (ASCs) are considered to act as tissue-specific progenitor cells and have multipotency, but actual cellular events in remodeling or expansion process of adipose tissue are not well studied. Unlike conventional histological analysis of adipose tissue, whole mount histology using a confocal microscopy-based method [Nishimura et al. Diabetes, 56: 1517, 2007] enables detailed and 3-dimensional visualization of adipose tissue such as relationships between adipocytes, other cellular components and vessels.

### Methods:

We analyzed remodeling and expansion processes of adipose tissues using original murine models for ischemia-reperfusion and external tissue suspension, respectively. For whole mount staining of living adipose tissue, samples were stained with fluorescent agents (BODIPY for adipocytes, lectin for endothelial cells, and Hoechst 33342 for nuclei), and images (single, multiple-serial, or surface-rendered 3-dimensional images) were captured by a confocal microscopy. Other agents were also combined to further analyze cellular events; propidium iodide (PI) for necrotic cells, acetylated low-density lipoprotein (acLDL) for endothelial progenitor cells, and anti-CD34 antibody for ASCs.

### Results:

After ischemia-reperfusion, adipose tissue underwent a remodeling process (degeneration and regeneration of adipocytes and capillaries), while adipose tissue expansion (adipogenesis and angiogenesis) was seen during a continuous external tissue suspension. After ischemia-reperfusion injury, increase of interstitial space and small-sized adipocytes (smaller than 50  $\mu\text{m}$  in diameter), an increased number of nucleated cells including lectin-positive round cells, and capillaries especially around small-sized adipocytes were observed. Combination with PI enabled detection of necrotic adipocytes, which appeared 3 and 7 days after injury. We also detected migration of endothelial progenitor cells (acLDL+/lectin+), which were most frequently observed 7 days after injury. With an antibody against CD34, pericytic localization of ASCs and their proliferation and increase in number after injury were confirmed. During a continuous external tissue suspension, a rapid thickening of adipose tissue layer, increase in small-sized adipocytes and ASCs (CD34+/lectin-), and an increased density of capillaries and small vessels arranged in the direction of external suspension were observed. Most of Ki67+ proliferating cells were lectin-negative and suggested to be ASCs, and a substantial number of CD34+/lectin+ cells, which may be ASCs differentiating into endothelial cells, were seen especially in the marginal area of expanding adipose tissue on day 14.

### Conclusions:

Three-dimensional visualization of adipose tissue with a confocal microscopy and fluorescent agents provided us with detailed images of adipose tissue, which helped us precisely examine cellular events in adipose tissue in various situations. ASCs were highly involved in adipose tissue remodeling and expansion as a main dividing cell population and contributed to adipogenesis and angiogenesis.

## Human Coq2 expression in 3T3-F442A cells reduced adipose differentiation and increased reactive oxygen species production

Sandy GalinierBour; Anne Galinier; Sylvie Caspar-Bauguil; Mamen Carmona; Luc Pénicaud; Louis Casteilla

### Introduction:

Coenzyme Q (CoQ) is the only lipophilic antioxidant molecule synthesised in mammals. Besides its antioxidant function, it plays a role in electron transport between complexes I/II and III of mitochondrial respiratory chain. In mice as in humans, we have previously shown that coenzyme Q level in adipose tissue inversely correlates with body or adipose tissue weight. Therefore, we wanted to determine if coenzyme Q level directly controls adipocyte differentiation.

### Methods:

In order to study long term effects of coenzyme Q content on adipocytes, 3T3-F442A murine preadipocytes were transfected with a vector containing cDNA for human Coq2 gene (polyprenyl-4OH-benzoate transferase, enzyme involved in CoQ synthesis) under the control of CMV promotor. Clones expressing human Coq2 mRNA (hCoq2) were screened and compared to untransfected 3T3-F442A (3T3) or clones transfected with a vector containing a scramble DNA (Neg).

### Results:

Coq2 expression induced a strong regulation of genes involved in coenzyme Q synthesis. Indeed, amounts of mRNA encoding Coq6, Coq7 and TPTE, three enzymes of coenzyme Q synthesis pathway, were reduced. However, human Coq2 expression enhanced significantly coenzyme Q content without modification of its redox status. Adipose differentiation was strongly reduced in hCoq2 clones compared to 3T3 or Neg clones, as evidenced by a decrease in triglycerides accumulation and aP2 or PPAR $\gamma$ 2 mRNA abundance. In addition, hCoq2 clones produced more mitochondrial reactive oxygen species (ROS) than 3T3 or Neg cells.

### Conclusions:

This study demonstrate for the first time the crucial role of coenzyme Q biosynthetic pathway in adipose differentiation regulation. The increase in intracellular CoQ level in hCoq2 clones induces mitochondrial ROS production, probably due to a modification of respiratory chain activity, and reduces adipocyte differentiation. These results were in accordance with a previous study wherein we demonstrated that ROS negatively control adipose differentiation. In conclusion, this work suggest a strong regulation of adipocyte differentiation by both CoQ and ROS production.

## Cytoskeleton reorganization by thymosin beta-4 alters cell fate determination of human mesenchymal stem cells

Jennifer Ho; Kuang-Ching Tseng; Wei-Hsien Ma; Yeu Su; Oscar Lee

### Introduction:

Change of actin filament organization at the early stage of differentiation of human mesenchymal stem cells (MSCs) directs cell commitment. Thymosin beta-4 (Tb4), a major G-actin sequestering peptide, possesses cytoskeleton regulation ability. The purpose of this study is to investigate whether Tb4 modulates cell fate determination of human bone marrow-derived MSCs by changing the organization of cytoskeleton.

### Methods:

MSCs were derived from human bone marrow and Tb4 (1 ug/ml) were added to induction medium once every 3 days with each medium change. Osteogenic, chondrogenic and adipogenic differentiation of MSCs were evaluated by histologic, cytochemical, and immunocytochemical analysis. TRITC-labeled phalloidin was used for detection the F-actin rearrangement and F-actin/G-actin ratio was measured by Western blotting while MSCs differentiation. Gene expression of adipocyte-specific adhesion molecule (ASAM) was measured by real-time RT-PCR and membrane protein expression of ASAM was detected by immunofluorescence staining while adipogenic induction of MSCs.

### Results:

We found Tb4 decreased F-actin formation as well as the ratio of F-actin/G-actin during osteogenic differentiation of MSCs and resulted in the inhibition of osteogenic maturation evidenced by decreased caspase-3 activity, alkaline phosphatase activity, expression of osteogenic marker genes and mineralization matrix formation. On the contrary, Tb4 reciprocally facilitated adipogenic differentiation evidenced by increased expression of adipogenic marker genes as well as deposition of intracellular fat drop. From the macroarray analysis, it was further found that Tb4 highly up-regulated the expression of adipocyte-specific adhesion molecule (ASAM) and which was not due to associated with differential expression of peroxisome proliferator activated receptor gamma (PPAR $\gamma$ ). Moreover, Tb4-induced membrane expression of ASAM in early adipogenic differentiation was accompanied with early phenotypic maturation.

### Conclusions:

Sequestration of G-actin by Tb4 resulted in the inhibition of osteogenic differentiation, and up-regulation of ASAM, the cell-cell adhesion molecule of adipocyte, facilitated adipogenic differentiation of MSCs. Biophysical effects of Tb4 on early stage of MSC differentiation are not associated with regulation of key transcription factors, Runt-related transcription factor 2 (Runx2) and PPAR $\gamma$ . Our finding implicated that Tb4, the ubiquitous peptide, may involved in obesity or osteoporosis when its intracellular concentration was increased.



## **Direct co-culture of human adipocytes, adipose tissue macrophages and adipose stem/progenitor cells (ASPCs) reveals a mechanism for generation of new preadipocytes and a novel population of CD34 (+) ASPCs**

Saleh Heneidi; Cristina Bertolotto; Gregorio Chazenbalk; Charles Simmons; Ricardo Azziz

### **Introduction:**

Increased obesity is associated with accumulation of macrophages in adipose tissue. In addition to regular phagocytotic function, adipose tissue macrophages may also influence the adipocyte growth as well as its metabolism and secretory activity through the production of cytokines and chemokines. We have recently demonstrated that CD14(+) adipose tissue macrophages represent a potential progenitor pool for preadipocytes through direct co-culture between adipocytes, adipose tissue macrophages and adipose stem/progenitor cells (ASPCs). Understanding the range of interactions between adipocytes, tissue adipose macrophages and ASPCs is central to unlocking many of the cellular and molecular biological mechanisms of wound healing and adipose stem cell plasticity. Hypothesis: Direct cell to cell contact of adipocytes, adipose tissue macrophages and ASPCs generate (i) new preadipocytes in part through adipose tissue macrophages differentiation

### **Methods:**

Human adipose tissue obtained from obese patients was treated with collagenase and adipocytes were isolated by centrifugation. Adipose tissue macrophages and ASPCs were isolated from the stromal vascular fraction using a Ficoll gradient. Adipocytes and the macrophage adipose tissue/ASPC fraction were cultured separately for 24 hours, co-cultured for a further 24 hours, and then separated and cultured alone for a further 48 hours. In some experiments the adipose tissue macrophage/ASPC fraction was incubated with fluorescent anti-human CD14 nanobeads. After separation of unbound or non-internalized nanobeads, the macrophage/ASPC fraction containing nanobeads was plated for 24 hours and co-cultured was performed as described above.

### **Results:**

Approximately 90% of adipose tissue macrophage/ASPC fraction incorporated fluorescent anti-human CD14 nanobeads after internalization of these nanobeads. Co-culture between the CD14 nanobead-internalized macrophage/ASPCs fraction and the untreated adipocytes, majority of preadipocytes generated contained internalized CD14(+) nanobeads. The resulting CD14 nanobead labeled preadipocytes were also positive for S-100 and DLK confirming that most of the new preadipocytes were of CD14 precursor cell origin. These new preadipocytes were also CD34(+), CD105(+), and CD146(+). Preadipocyte proliferation required direct cell to cell contact, since proliferation was greatly reduced if co-culture was performed using a transwell system. Interestingly, we also observed after co-culture a population of tiny CD34 (+) ASPCs. These cells were also positive for S-100.

### **Conclusions:**

Through co-culture of adipocytes, and adipose tissue macrophage and ASPCs fraction it is possible (i) to generate new preadipocytes derived from adipose tissue macrophages (ii) to generate a novel population of CD34 (+) ASPCs, that were also positive for markers commonly found in preadipocytes/adipocytes. These findings could have far reaching implications with adipocyte growth, wound healing and adipose stem cell plasticity modulated, at least in part, by this novel cellular differentiation pathway.

## Differential Adipogenesis Related Gene Expression in Mesenchymal Stromal Cells derived from Bone Marrow, Cord Blood and Adipose Tissue.

Marianna Karagianni; Karen Bieback; Harald Klueter

### Introduction:

Introduction: Mesenchymal Stromal Cells (MSCs) from Bone Marrow (BM- MSCs) and Adipose Tissue (AT- MSCs) undergo in vitro differentiation into mesodermal derivatives such as adipose-, bone- and cartilage tissue. Cord Blood MSCs behave differently in response to adipogenic stimuli. They fail to develop the mature adipocyte phenotype with perinuclear lipid droplets and inversion of the nuclear-cytoplasmic relation. We tried to track specific protein markers and transcriptional factors during adipogenic induction of BM-, AT- and CB-derived MSCs to identify a potential inhibitory pathway in CB-MSCs.

### Methods:

Methods: AT-, BM- and CB-MSCs were cultivated in MSC medium as undifferentiated condition. Differentiation towards the adipogenic lineage was induced by adipogenic stimuli (insulin, dexamethasone, isobutylmethylxanthine, indomethacin) after reaching postconfluency. The expression of lipid droplets associated proteins (LDAP) like adipophilin, and perilipin was followed by immunofluorescence (IF). Quantitative RT-PCR gave relative quantification comparisons on the time-dependent expression of transcriptional factors and genes such as PPAR $\gamma$ , CEBP $\alpha$ , adiponectin and DLK/Pref-1. Knock-down of DLK1/Pref-1 was performed via si-RNA in CB-MSCs. qRT-PCR was performed subsequently for PPAR-g and adiponectin.

### Results:

Results: Following culture of BM-, AT- and CB-MSCs under adipogenic conditions, we found induction of the adipogenic phenotype and positive IF signals for perilipin, a specific LDAP for adipocytes, in BM- and AT-MSCs. This was associated with increased levels in mRNA expression of perilipin, c-EBP-a, PPAR-g and adiponectin. In contrast the phenotype of the CB-MSCs remained unaltered, the IF-detection of perilipin persisted negative. The m-RNA expression of perilipin, c-EBP-a, PPAR $\gamma$  and adiponectin in CB-MSCs was decreased in relation to AT- and BM-MSCs. DLK/Pref-1, a transcriptional factor, associated with an undifferentiated state of preadipocytes, was downregulated in induced BM- and AT-MSCs. In treated CB-MSCs it remained unsuppressed, supporting the notion that DLK/Pref-1 behaves as a growth factor, maintaining the proliferative state of undifferentiated cells. Inhibition of DLK/Pref-1 via si-RNA in CB-MSCs resulted in an upregulation of PPAR-g and adiponectin mRNA. A mature adipogenic phenotype however was not achieved.

### Conclusions:

Conclusions: CB-MSCs fail to undergo adipogenic differentiation in contrast to BM- and AT-MSCs. Our data indicate a correlation with the expression of DLK/Pref-1, which was shown to be unsuppressed in CB-MSCs in contrast to its downregulation in BM and AT-MSCs under adipogenic induction. mRNA interference with DLK/Pref-1-si-RNA in CB-MSCs corroborated our thesis, as it resulted an upregulation of PPAR-g and adiponectin -but still no adipogenic phenotype- in treated CB-MSCs. We suggest CB-MSC as a potent model for studying modulatory pathways of the adipogenic cascade, especially influenced by aging, with a focus on the inverse correlation between adipogenesis and osteogenesis, which are amongst others controlled by Pref-1 expression.

## Regulation of lymphocyte proliferation by human adipose-derived stem cells requires IFN-gamma, IDO activity and generates T cells with suppressor activity

Olga De La Rosa

### Introduction:

Human adipose-derived stem cells (hASCs) are mesenchymal stem cells with reduced immunogenicity and the capability to modulate immune responses. These properties make hASCs of special interest as therapeutic agents in the settings of both chronic inflammatory and autoimmune diseases. Although similar to bone-marrow mesenchymal stem cells (BM-MSC), hASCs may differ from them in the mechanism of immunosuppression. We have studied the mechanism responsible for the hASC-mediated immune regulatory effect.

### Methods:

hASCs isolation and expansion. Lymphocyte proliferation assays. CD3+, CD4+, CD8+ isolation. Cytometric bead arrays. Flow cytometry. Neutralization assays. HPLC.

### Results:

We report that hASCs inhibit PBMC, CD4+ and CD8+ T cell proliferation in both cell-cell contact and transwell conditions. This inhibition is accompanied by a reduction of proinflammatory cytokines. We demonstrate that hASCs do not constitutively express immunomodulatory factors. Conditioned supernatants from hASC stimulated by IFN, PBMCs or activated PBMCs highly inhibited PBMC proliferation, indicating that inhibitory factors are released upon hASC activation. hASC-mediated immunosuppression is highly mediated by IFN and IDO activity. Furthermore, interaction between hASCs and PBMCs generates CD3+ T cells with a regulatory phenotype.

### Conclusions:

hASCs prevent lymphocyte proliferation by releasing soluble factors upon activation through inflammatory mediators and contribute to the generation of regulatory T cells. Collectively, these data provide immunological support for the safety and efficacy of hASCs in the treatment of inflammatory diseases

## Immunogenicity of Allogeneic Adipose-Derived Stem Cells in a Rat Spinal Fusion Model

Nakia Spencer; Paul Anderson; Jeffrey Gimble; Kevin McIntosh; Mandi Lopez; Jade Borneman

### Introduction:

Adipose-derived stem cells (ASCs) express a non-immunogenic profile as shown by in vitro studies which demonstrate a lack of T cell proliferation to allogeneic ASCs as well as ASC mediated suppression of mixed lymphocyte reactions (MLR). To determine whether these observations translate in vivo, immune monitoring studies were carried out in rats transplanted with allogeneic ASCs as part of a scaffold-based spinal fusion study.

### Methods:

A total of 56 Fischer strain rats were randomly assigned to four different treatment cohorts after bilateral decortication of the L4 and L5 transverse processes (n=14/cohort). ASCs, derived from Fischer or ACI rats, were loaded onto scaffolds and implanted in Fischer recipients according to the following treatments: 1) No treatment; 2) Scaffold only; 3) Syngeneic ASCs + Scaffold; or 4) Allogeneic ASCs + Scaffold. Half of each group was sacrificed at 4 weeks post implantation and the remaining animals were sacrificed at 8 weeks. Cellular and humoral immune responses were evaluated at both timepoints to determine whether donor ACI strain ASCs induced an immune response in recipient Fischer rats. Recipient Fischer lymph node cells were assessed for T cell proliferation to ACI spleen cells by one-way MLR assays and serum was collected to assess antibody binding to donor ASCs by flow cytometry.

### Results:

No T cell priming to donor ACI alloantigens was observed in recipients at 4 or 8 weeks after implantation of ASCs as determined by the kinetics and magnitude of MLR responses. A significant antibody response was detected in Fischer recipients implanted with either Fischer or ACI strain ASCs when compared to the "No Treatment" group. Antibodies were of the IgG isotype and non-cytotoxic in the presence of complement. Antibodies specific for fetal bovine serum proteins were detected in the "Scaffold Only", "Syngeneic ASCs + Scaffold" and "Allogeneic ASCs + Scaffold" groups.

### Conclusions:

These results support the use of allogeneic ASCs for spinal fusion and suggest that ASCs be propagated in syngeneic serum to mitigate the production of antibodies.



## Toll-like receptor-mediated signaling in human adipose-derived stem cells: Implications on immunogenicity and immunosuppressive potential

Eleuterio Lombardo

### Introduction:

Human adipose-derived stem cells (hASCs) are mesenchymal stem cells with reduced immunogenicity and the capability to modulate immune responses. These properties make hASCs of special interest as therapeutic agents in the settings of both chronic inflammatory and autoimmune diseases. Toll-like receptors (TLR) ligands, both exogenous and endogenous, have been linked with the perpetuation of inflammation in a number of chronic inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis, due to the permanent exposure of the immune system to TLR-specific stimuli. Therefore, hASCs employed in therapy are potentially exposed to TLR ligands which may result in the modulation of hASC activity and therapeutic potency.

### Methods:

We investigated the role of TLR signalling in hASCs function in vitro. hASCs differentiation assays as well as lymphocyte proliferation assays were carried out. Expression of inflammatory cytokines upon stimulation was determined by cytometric bead arrays and ELISAS.

### Results:

In this study we demonstrate that hASCs possess active TLR2, TLR3 and TLR4 as the activation with specific ligands resulted in induction of the NF- $\kappa$ B-dependent gene manganese superoxide dismutase (MnSOD) and the release of IL-6 and IL-8. Whereas osteogenic differentiation was increased by TLR3 and TLR4 ligands, no effect on adipogenic differentiation or proliferation was observed. Moreover, we show that TLR activation does not impair the immunogenic and immunosuppressive properties of hASCs.

### Conclusions:

hASCs are activated by TLR ligands. However, this activation does not affect the immunogenic and immunosuppressive abilities of hASCs. Therefore, these results may have important implications with respect to safety and efficacy of hASC-based cell therapies.

## Therapeutic effect of adipose-derived mesenchymal stem cells on experimental colitis by inhibiting inflammatory and autoimmune responses

Aitor Beraza

### Introduction:

Crohn's disease is a chronic debilitating disease characterized by severe T helper cell (Th)1-driven inflammation of the colon partially caused by a loss of immune tolerance against mucosal antigens. Mesenchymal stem cells (MSCs) of allogeneic origin have been reported to suppress effector T-cell responses in vitro, to have therapeutic effects in some immune disorders and certain capacity to restore immune tolerance. The aim of this work is to investigate the potential anti-inflammatory and therapeutic effect of human adipose-derived MSCs (hASCs) in two well-established murine models of inflammatory bowel diseases.

### Methods:

We examined the therapeutic action of hASCs in the colitis-induced by administration of trinitrobenzene sulfonic acid or dextran sodium sulfate, evaluating diverse clinical signs of the disease. We also investigated the mechanisms involved in the potential therapeutic effect of hASCs, such as inflammatory cytokines and chemokines, Th1-type response, and the generation of regulatory T (Tr) cells.

### Results:

Systemic infusion of hASCs significantly ameliorated the clinical and histopathologic severity of colitis, abrogating body weight loss, diarrhea, and inflammation, and increasing survival. The therapeutic effect was associated with down-regulation of both inflammatory and Th1-driven autoimmune response, by regulating a wide spectrum of inflammatory mediators directly through activated macrophages, and by generating interleukin-10-secreting Tr cells with suppressive capacity on autoreactive T cells. In addition, hASCs protected from mortality caused by sepsis by downregulating the exacerbated inflammatory response characteristic of this disease.

### Conclusions:

Therefore, these adult MSCs emerge as key regulators of immune tolerance in physiological conditions by inducing the generation/activation of Tr clones in the periphery and as attractive candidates for a cell-based therapy for the treatment of inflammatory and autoimmune disorders.

## Anti-adipogenic effect of adipose tissue T lymphocytes on the human CD34+/CD31- cells

Carine Duffaut; Alexia Girard; Coralie Sengenès; Jean Galitzky; Anne Bouloumié

### Introduction:

Obesity is characterized by a low grade inflammatory state. The white adipose tissue appears as a primary player in the settlement of such a condition as both source and site of inflammation. Accumulation of macrophages within the fat mass in obese conditions has been well described but the potential role of their major cell partners, i.e. T-lymphocytes, has been less studied. The aim of the present study was to describe the population of human adipose tissue lymphocyte (ATLs) and to study their effects on adipogenesis.

### Methods:

Flow cytometry analyses were performed on the stroma-vascular fraction (SVF) of human subcutaneous adipose tissue of lean to obese patients (n=130) using cell surface markers of lymphocyte subtypes (CD3, CD19, CD4, CD8, CD56, CD45RO, CD45RA). Human adipose tissue progenitor cells (CD34+/CD31-) and T-lymphocytes (CD3+) were isolated through immunoselection/depletion protocols from the human SVF. CD3+ transcript expression was analysed by real time PCR analysis. Conditioned media from native human ATLs, obtained after 24h culture period of CD3+ cells, were used to treat CD34+/CD31- cells maintained under adipogenic culture conditions.

### Results:

Flow cytometry analyses demonstrated the presence of T-lymphocytes (CD3+) in human subcutaneous adipose tissue that are increased with the body mass index of the patients. ATLs were mainly composed by memory (CD45RO+/CD45RA-) and effector (CD45RO-/CD45RA-) helper (CD4+) and cytotoxic (CD8+) T cells. Analysis of the ATLs transcript expression showed high amount of RANTES, interferon gamma as well as TNFalpha. Treatment of the native CD34+/CD31- cells cultured under adipogenic culture conditions with ATL-derived conditioned media led to a strong inhibition of cell number associated with decreased adipogenesis assessed by triglyceride accumulation and expression of adipocyte specific markers lipoprotein lipase and fatty acid synthase.

### Conclusions:

The present study demonstrates that obesity in human is associated with accumulation of T-lymphocytes, i.e. helper as well as cytotoxic T cells, within the adipose tissue. Such a cell population might play a major role through their anti-adipogenic effect on the extension capacity of the fat mass and thus indirectly to the obesity-associated pathologies.

## First-In-Man Experience of Adipose-Derived Stem Cell Transplantation in the Treatment of Patients with an Acute ST-Elevation Myocardial Infarction (APOLLO Trial - Cytori).

H.J. Duckers; Patrick Serruys

### Introduction:

A number of pilot trials as well as two larger randomized trials have shown a significant benefit of treatment with bone marrow mononuclear cells in acute myocardial infarction (AMI). Autologous adipose-derived regenerative cells (ADRCs) can be obtained from subcutaneous adipose tissue in sufficient amount for therapy and require no cell culture step before use. While similar to bone marrow derived adult stem cells in differentiation potential, the usual abundance of adipose tissue in human patients and the higher frequency of adult stem cells per unit mass (including mesenchymal stem cells and EPCs) allows fast isolation of an efficacious number of cells without having to culture expand them. Preparation of therapeutic doses of ADRCs involves no cell culture and can be achieved in less than two hours from the time of donor tissue acquisition. This allows for treatment during the same procedure as the harvest of the adipose tissue and can thus be appended to the primary percutaneous coronary interventional treatment (PCI) of an acute myocardial infarction.

### Methods:

The APOLLO is a prospective, double-blind, randomized, placebo-controlled, dose escalation cell therapy study that will enroll up to 48 patients to determine the safety and feasibility of different doses of adipose-derived stem cells delivered via the intracoronary route in the treatment of patients with ST-elevation myocardial infarction.

### Results:

The study includes up to four consecutively enrolled dose cohorts of 12 patients each. Within each dose cohort, patients are randomly assigned 3:1 to receive cell therapy or placebo. Eligible patients undergo liposuction and intracoronary infusion of the adipose-derived regenerative cells isolated from the lipoaspirate within 24 hours after the primary PCI. Patients are evaluated for 36 months, whereas 3D echocardiography, SPECT scintigraphy, invasive hemodynamic analysis (P/V loop analysis), cardiac MRI and BNP blood analysis at 6 and 18 months are used to evaluate global and regional cardiac function and perfusion. Currently, 7 patients have been enrolled in the first dose cohort, whereas the therapy has been deemed safe in these patients.

### Conclusions:

The APOLLO study is a prospective, dose-escalating cell therapy study to evaluate the safety and feasibility of adipose-derived regenerative cells in the treatment of patients with a myocardial infarction.



## Safety and efficacy of autologous adipose-derived stromal cells on Type II diabetes patients: 6 month post-procedure results

Florencio Lucero; Emerita Barrenechea; Letitia Lucero-Palma; Kribah Krishnan; Eduardo Barrenechea; Bill Paspaliaris

### Introduction:

Stem cell therapies hold great promise for anti-aging benefits as they are regenerative in nature. Autologous adipose-derived stem cell transplants hold even more potential as they have no ethical barriers and require no out-of-surgery culture requirements.

### Methods:

We have devised a procedure that entails the isolation of stromal cells from adipose-tissue derived from a mini-liposuction procedure, their activation from a quiescent stage to an active stage, and their reintroduction back into the patient via intravenous mode. This single procedure has now been performed on 176 subjects over a two and a half year period in four countries with no adverse effect. Because these were isolated case studies, a formal clinical trial was then initiated to assess the safety and efficacy of the procedure on a controlled group of 34 patients with non-insulin and insulin-dependent type II diabetes mellitus with no cardiovascular or nephrological complications.

### Results:

After three months post-operation the patients showed a significant and sustained reduction in fasting glucose levels (from  $9.64 \pm 3.88$  mmol/l to  $7.01 \pm 1.64$  mmol/l;  $p=0.005$  at 2 weeks to  $7.71 \pm 2.29$  mmol/l;  $p=0.01$  at 12 weeks), glycosylated haemoglobin (from  $9.11 \pm 2.06\%$  to  $7.73 \pm 1.19\%$ ;  $p=0.00001$ ), C-peptide (from  $2.75 \pm 1.02$  to  $2.27 \pm 1.45$ ;  $p=0.045$ ) and triglycerides (from  $2.31 \pm 1.53$  to  $1.91 \pm 1.63$ ;  $p=0.03$ ). At six months post procedure nearly half the patients reverted back to pre-op conditions while the other half continues to see sustained decreases in diabetic parameters as compared to pre-op levels. Six month statistics showed Fasting blood sugar went from  $9.64 \pm 3.88$  mmol/l at pre-op to  $8.50 \pm 2.86$  mmol/l;  $p=ns$  at 24 weeks), glycosylated haemoglobin (from  $9.11 \pm 2.06\%$  to  $8.10 \pm 1.82\%$ ;  $p=0.001$ ), C-peptide (from  $2.75 \pm 1.02$  to  $2.83 \pm 1.37$ ;  $p=ns$ ) and triglycerides (from  $2.31 \pm 1.53$  to  $2.01 \pm 1.35$ ;  $p=ns$ ). Most patients have noticed an increase in well-being parameters post-op. There was no significant change detected post-op in total cholesterol and other CBC, LFT and KFT values and no obvious adverse reaction has been noted.

### Conclusions:

The results of the trial to date suggest that the autologous adipose derived stromal cell therapy appears to be safe and beneficial to type II diabetes patients by decreasing their resistance to insulin and decreasing diabetic cardiovascular risk factors. We believe that the stromal cell transplant is probably acting by increasing adiponectin levels in these subjects, an adipocytokine that is produced by adipose stromal cells and known to regulate insulin-resistance. Lifestyle and hypoglycemic medication changes also plays an important role in sustaining the effects observed.

## Expanded Adipose-derived Stem Cells (Cx401) for the Treatment of Complex Perianal Fistula. A Phase II Clinical Trial

Damian Garcia-Olmo; Dirk Büscher

### Introduction:

Management of complex fistulas is a challenge due to the limitations of current treatments. Expanded adipose derived mesenchymal Stem Cells (Cx401) is a novel cell therapy based on immunoregulation and cell proliferation which helps repair damaged tissue.

### Methods:

A multicenter, randomized, controlled trial is being conducted to evaluate the efficacy and safety of Cx401 in 49 adult patients with complex perianal fistula from Cryptoglandular (35) or Crohn (14) diseases. Patients received fibrin glue or 20 million cells plus fibrin glue intralesionally. Fistula healing was evaluated at 8 wks. If not healed, a second dose of fibrin glue or 40 million cells plus fibrin glue was administered, with healing evaluated 8 wks later. Healing was defined as absence of drainage (spontaneous or by gentle compression) and complete re-epithelization of the external openings.

### Results:

The proportion of patients whose fistulas were healed was significantly higher with Cx401 than with fibrin glue [17(70.83%) vs 4(16.00%) respectively,  $RR=4.43$ (CI 1.74, 11.27); $p$ -value=0.0001]. Efficacy was observed in Crohn and non-Crohn. 45% of Cx401 patients received a cumulative dose of 60 million cells. At 6 months follow-up, 2 SAEs were observed in each group. Only one SAE was related to fibrin glue, no SAE was related to Cx401

### Conclusions:

A dose of 20–60 million Expanded Adipose-derived Stem Cells (Cx401) in combination with fibrin glue is an effective and safe treatment for complex perianal fistula

## Phase II/III Clinical Trial of Autologous Differentiated Adipocytes

Sa-ik Bang; Mihyung Kim; In-Ok Kim; Songyi Han

### Introduction:

Autologous fat transplantation has become a popular method for correcting soft tissue defects since its introduction in the late of 1800s. However, the limitations of fat transplantation are well known, particularly the long-term unpredictability of volume maintenance. Although adipose tissue derived stem cells(ASCs) are an alternative cell source for adipose tissue generation, the enhancement of the in vivo adipogenic conversion of them remains a major task. Previously, we demonstrated that adipogenic differentiation of ASCs before transplantation could successfully enhance adipose tissue regeneration.

### Methods:

Autologous differentiated adipocytes are produced by well-established techniques including cell harvesting from lipoaspirates, expansion of preadipocytes, and differentiation into pure and immature adipocytes. To determine optimal dose of autologous differentiated adipocytes(Adipocell) for depressed scar and to evaluate the safety and efficacy of Adipocell after injection into patients phase II/III clinical trial was conducted. 3D scanning system and software for 3D scan data processing were introduced to evaluate the clinical efficacy objectively.

### Results:

A total 31 of patients were injected with Adipocell. Patients were followed for 12 weeks after injection and additional follow-up ranged from 9 to 15 months. When  $3.8 \times 10^7$  cells/ml of Adipocell was injected into depressed scars subcutaneously average volume recovery rate was 74.6% at 12 weeks. The long-term follow-up revealed that volume correction at 12 weeks was well maintained up to 1 year. There was no any significant adverse effect.

### Conclusions:

Autologous differentiated adipocytes therapy is a safe and effective way for treatment of soft tissue defects with relatively long-term volume maintenance.

## Evidence of a robust trafficking capability: Adipose-derived stem cells slowly roll on P-selectin and enhance firm adhesion to VCAM-1 following exposure to SDF-1alpha

Alexander Bailey; Michael Lawrence; Hulan Shang; Adam Katz; Shayn Peirce

### Introduction:

Intravenous delivery of adipose-derived stem cells (ASCs) is a promising option for the treatment of ischemia. Following delivery, ASCs that reside and persist in the injured extra-vascular space have been shown to aid recovery of tissue perfusion and function, although low rates of incorporation currently limits the safety and efficacy of these therapies. We submit that a better understanding of the trafficking of therapeutic ASCs through the microcirculation is needed to address this and that selective control over their homing (organ- and injury-specific) may be possible by targeting critical adhesion proteins. Towards this end, we investigated cellular- and molecular-level interactions between ASCs and endothelial cells under flow conditions, *in vivo* and *in vitro*, to identify methods to increase the number of cells that successfully traffic to and incorporate into injured/ischemic tissues.

### Methods:

Early passage human hASCs (P=0-3) were perfused at a wall shear stress equal to 0.5 dynes/cm<sup>2</sup> over immobilized human VCAM-1 or P-selectin in a parallel plate flow chamber. Firm adhesion and slow rolling interactions with these endothelial cell adhesion molecules were recorded and quantified. To identify which hASC adhesion molecules may be mediating these interactions, hASCs were incubated with competitive antibodies to PSGL-1 or alpha4 integrins for 30 minutes prior to assay, and flow cytometry was preformed separately. For *in vivo* studies, DiI-labeled hASCs (P=3; 750,000 cells/ml) were injected into the mesenteric artery of male rats, and the subsequent adhesive interactions between injected cells and exposed mesenteric microvascular endothelium were observed in real time using intravital microscopy. hASCs were also chemically pre-conditioned to increase adhesive interactions by incubating with SDF-1alpha (0.1 microg/ml) for 30 minutes prior to both the *in vitro* and *in vivo* assays.

### Results:

Under flow conditions, hASCs slowly rolled on VCAM-1 and P-selectin at speeds as low as 2 microns/sec and exhibited the characteristic stop-and-go behavior typical of rolling leukocytes. Furthermore, hASCs firmly adhered to VCAM-1 (8-cells/mm<sup>2</sup> min), which was enhanced following SDF-1alpha pre-conditioning (23-cells/mm<sup>2</sup> min). Incubation of hASCs with competitive antibodies to the alpha4 integrins eliminated all VCAM-1 interactions, while blocking PSGL-1 activity had no effect on hASC ability to roll on P-selectin. These data were complemented by *in vivo* observations that hASCs slowly rolled and firmly adhered to injured endothelium in patent arterioles and venules in rat mesenteric microvasculature. Similar to *in vitro* studies, a greater number of SDF-1alpha pre-conditioned hASCs (versus non-conditioned hASCs) persisted in the microvasculature (8.0% versus 1.3%) at 1 hour following injection.

### Conclusions:

Our study suggests that hASCs possess robust trafficking capabilities and interact directly with injured endothelium by capitalizing on well-known molecular adhesion interactions. Moreover, we present a viable method for enhancing these interactions using a biochemical pre-conditioning scheme, with the long term goal of improving hASC trafficking efficiencies.

## Endothelial differentiation of adipose-derived stem cells: How close have we really come?

Paul DiMuzio; Ping Zhang; Stephen McIlhenny; Lisa Harris; Hamid Abdollahi; Matthew Ferroni; Kristi Wasson; Eric Hager; Neil Moudgil; Nicholas Tarola; Daniel Grabo; Vic Srinivas; Irving Shapiro; Thomas Tulenko

### Introduction:

We have demonstrated that adipose-derived stem cells (ASC) are abundant in the peri-umbilical fat of patients with cardiovascular disease, suggesting these cells may be practical for use in tissue engineering/regenerative medicine. In particular, we have focused on their use in creating a tissue engineered vascular graft produced by seeding ASC differentiated into endothelial cells (EC) onto a natural vascular tissue scaffold. The current studies evaluate the efficacy of growth factors, shear stress, and hypoxia in stimulating ASC to acquire EC characteristics.

### Methods:

ASC (CD13+29+90+31-45-) isolated from the peri-umbilical fat of patients undergoing vascular surgery were cultured in either Endothelial Cell Growth Supplement (ECGS) or EGM2 media (up to 4wk), under normoxic vs. hypoxic (21% vs. 2% O<sub>2</sub>, up to 2wk) conditions, with or without the application of shear stress (12dyne x 72h) in vitro. After differentiation, we assessed: 1) expression of eNOS, vWF, CD31, veCadherin, VEGF; 2) cord formation in Matrigel, 3) re-alignment in the direction of shear force, and 4) production of nitric oxide (NO). The effect of hypoxia was determined by expression of HIF1 $\alpha$ . In a canine model, differentiated ASC were seeded onto vascular grafts and implanted within the carotid arterial circulation for two weeks. Explanted grafts underwent histological evaluation to assess endothelial function of the stem cells in vivo.

### Results:

Culture in either ECGS or EGM2 for 1 wk resulted in ability to form cords and re-align in the direction of shear. EGM2 stimulated more vWF and CD31 expression than ECGS at 2wk, but significantly less than in EC controls. In cultures differentiated for 2wk, the addition of shear stress increased both vWF and CD31 expression. The addition of hypoxia (and resultant HIF1 $\alpha$  upregulation), however, decreased CD31 expression, despite significant increases in VEGF expression. None of the stimuli resulted in expression of eNOS or veCadherin; only the addition of VEGF (50ng/ml) to EGM2 medium resulted in minimally detectable levels of eNOS at 3wk. NO was detected in the media of cells cultured in either ECGS and EGM2 for 2wk, but at levels inferior to control EC; the differentiated stem cells expressed nNOS, but not eNOS or iNOS. Finally, at physiological levels of shear, only ECGS-cultured cells remained attached to vascular grafts. After 2wks in vivo, 6 of 7 ECGS-cultured stem cell grafts remained patent; however, the luminal surfaces of the patent grafts were uniformly coated with fibrin.

### Conclusions:

These results suggest that adipose-derived stem cells acquire several characteristics associated with endothelial cells when stimulated by various growth factors and shear stress. Conversely, hypoxic conditions appear to promote their "stemness." Despite these manipulations of the microenvironment in vitro, the stem cells do not express significant amounts of eNOS and appear mildly thrombogenic in vivo, two essential characteristics of differentiated endothelial cells. We therefore conclude that the ability of these tissue-specific stem cells to commit fully to an endothelial lineage is limited, and that genetic manipulation is likely required to bring about full differentiation.



## **Adipose Derived Adult Stem cells' (ADAS) capacity to differentiate into Endothelial Cells expressing von Willebrand marker and forming capillary-like structures in a Skin Equivalent model.**

Celine Auxenfans; Lauriane Thivillier; Charlotte Lequeux; Nicolas Bechetoille; Stephanie Maillet; Nathalie Tan; Beste Kinikoglu; Valérie André; Odile Damour

### **Introduction:**

Adipose tissue is now recognized as a source of autologous stem cells with multilineage capacity, able to differentiate into various cell types, notably into endothelial cells [1, 2]. In cell therapy, its abundance and accessibility make it an alternative to bone marrow and peripheral blood, which are the only current sources of autologous endothelial progenitor cells (EPCs). Thus, this route broadens therapeutic perspectives on regenerative medicine, such as revascularization of ischemic tissues or repair of damaged vessel walls and vascularized tissue engineering (vascular prostheses and skin equivalents for the treatment of large burns). After optimizing culture conditions for the proliferation of the SVF cells and their differentiation into EPCs, the aim of this study was to demonstrate their functionality by the expression of von Willebrand Factor and their capacity to form tubular structures in a skin equivalent (SE) model. Indeed, our SE model was prepared from cocultured fibroblasts and keratinocytes in a chitosan cross-linked collagen-glycosaminoglycan (CGC) scaffold. Endothelialized skin equivalent was previously obtained by seeding both HUVECs and fibroblasts into the dermal part of this model. Under these conditions, capillary-like structures were formed in a coculture environment containing newly synthesized extracellular matrix by fibroblasts and keratinocytes [3]

### **Methods:**

ADAS cells, differentiated along endothelial lineage by treatment with VEGF, were seeded together with fibroblasts into the CGC scaffold at the same density, and then cultured for 3 weeks in fibroblast medium supplemented with VEGF before seeding the surface with keratinocytes. After keratinocyte seeding, the model was cultured under submerged conditions for 1 week, then lifted to an air/liquid interface and cultured for 2 more weeks. The samples were then fixed in formalin and Tissue Tek for histology and immunohistology

### **Results:**

The average percentages of CD 133+ and CD31+ cells, measured by flow cytometry on freshly isolated cell suspension, are 29% and 11%, respectively. At passage 1, before predifferentiation, they were 36% and 11%, respectively. Immunostaining shows that ADAS are able to form capillary-like structures in which cells express both von Willebrand marker and basal membrane components such as Laminin 5. No fluorescence was detected in the non-endothelialized control SE

### **Conclusions:**

In the endothelialized skin equivalent (ESE), cell-cell and cell-matrix interactions, VEGF supplementation and long-term culture induce differentiation of preamplified ADAS cells into ECs that are able to aggregate into capillary like structures and express von Willebrand marker. These results suggest that adipose tissue may be considered as an accessible source of endothelial cells for cell therapy and ESE reconstruction. 1. Cao, Y., et al., Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. *Biochem Biophys Res Commun*, 2005. 332(2): p. 370-9. 2. Planat-Benard, V., et al., Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation*, 2004. 109(5): p. 656-63. 3. Black, A.F., et al., A novel approach for studying angiogenesis: a human skin equivalent with a capillary-like network. *Cell Biol Toxicol*, 1999. 15(2): p. 81-90.

## Treatment of chronic myocardial infarction with adipose-derived cells leads to an improvement in cardiac performance

Manuel Mazo, Beatriz Pelacho, Juan José Gavira, Gloria Abizanda, Felipe Prósper

### Introduction:

Cardiovascular diseases are a leading health concern in Western Countries with myocardial ischemia as the main mechanism for loss of cardiomyocyte (CM) mass and contractile force. Actually, regeneration of the damaged tissue has not been successfully achieved and new approaches and therapies like the transplantation of stem cells are being tested. In our study, we have assessed in a rat model of chronic myocardial infarction, the putative positive effect of a particular stem cell population, the stromal vascular fraction (SVF) derived from the white adipose tissue (WAT).

### Methods:

Cardiac ischemia was provoked by permanent ligation of the left descending coronary artery, and 1 month later, WAT-SVF cells (10 millions) isolated from transgenic GFP-rats or media as a control, were injected in the peri-infarct zone of the heart. One and three months after transplantation, cardiac function was assessed by echocardiography and the degree of tissue metabolism by PET technique. Also, histological studies were performed at the sacrifice point, in order to analyze the levels of cell engraftment and differentiation, angio- and vasculogenesis, the tissue inflammation and fibrosis degree and the infarct size.

### Results:

Cell-injected animals presented a significant improvement in the cardiac contractility (Left Ventricular Ejection Fraction: Pre-implant:  $26.46 \pm 2.26\%$ ; 3 months:  $38.25 \pm 4\%$ ;  $p < 0.05$ ) and in the tissue metabolism of the damaged zone (PET values: Pre-implant:  $50.55 \pm 4.41\%$ ; 3 months:  $60.64 \pm 5.26\%$ ;  $p < 0.01$ ) whereas medium-treated ones did not (LVEF: Pre-implant:  $29.87 \pm 2.84\%$ ; 3 months:  $28.1 \pm 1.59\%$ ; PET: Pre-implant:  $45.3 \pm 2.54$ ; 3 months:  $42.7 \pm 1.91$ ). At the histological level, cell-treatment was able to induce a significant increase in small and large caliber vessels density, and also to diminish infarct size and fibrosis. Furthermore, injected cells were found in the peri-infarct zone of treated animals 3 months after their injection ( $1.29 \pm 0.46\%$  of injected cells) demonstrating a wide differentiation capability, including endothelial, smooth muscle, myofibroblast, adipose, mesothelial and even cardiomyocytic phenotypes.

### Conclusions:

WAT-derived SVF injection induced a significant and long-lasting improvement in the cardiac function, not only by paracrine mechanisms involved in vascular and remodeling processes but also, although in low percentage, by directly contributing to cardiac and vascular new tissue.

## Adipose Derived Stromal Cells Express Tissue Factor in a Passage-Dependent Manner: Implication for Intracoronary Cell Therapy and Use of Heparin with ASCs

Vineet Kumar; Malgorzata Maria Kamocka; Elliot D Rosen; Clayton A Smith; Brain Johnstone; Keith L March

### Introduction:

Adipose stromal cells (ASCs) are readily available autologous cells with potential for treatment of cardiac disease. Several trials of ASC involve intravascular delivery, including intracoronary delivery, and accordingly we wished to determine whether ASC exhibit procoagulant (PC) properties

### Methods:

Human ASC either before passage (P0) or after 1 or 2 passages in EGM2-MV, or else human endothelial cells (EC) at passages 2-3, as a control, were evaluated for PC activity in normal human plasma, using standard protocols for testing prothrombin time (PT) in the absence of exogenously added thromboplastin (purified tissue factor). Time to coagulation in standard 150  $\mu$ l volumes was measured using change in viscosity by a Start 4 coagulometer (Diagnostica Stago). From 0 to  $5 \times 10^5$  ASC, obtained from 3 different subjects and at the different passages, or EC were added to test vials. Assays were performed with cells alone; or in the presence of increasing concentrations of heparin, the factor X inhibitor fondaparinux, or with a neutralizing anti-human tissue factor antibody. Cell surface-exposed activated tissue factor (TF) was quantitated by comparing activity to that of known TF quantities. The expression of coagulation factors, such as TF, was also evaluated by Affymetrix gene chip analysis.

### Results:

The time to coagulation increased with ASC concentrations and was inversely proportional to passage number. The PC activity of  $5 \times 10^5$  P0 ASC was equivalent to 0.53% of the concentration of thromboplastin normally employed in the PT assay, and resulted in coagulation at 39.2 seconds. The same number of ASC at P1 and P2 exhibited, respectively, 0.29% and 0.05% of the standard thromboplastin activity. In the absence of thromboplastin or ASC, as well as in the presence of equivalent numbers of EC, there was no coagulation. The PC activity of ASC was inhibited by neutralizing tissue factor antibody and also in a dose-dependent fashion by heparin, with full inhibition at 1 U heparin/106 ASC. Indirect quantification of tissue factor expressed by ASC at P0, P1 and P2 indicated that 598, 328 and 56 picogram TF, respectively, was present on cell surface. The ASC at P2 consistently expressed tissue factor transcript at levels in the upper quartile of all transcripts.

### Conclusions:

ASC express TF in a passage dependent manner and may potentially increase thrombogenesis in vivo by activating the extrinsic coagulation pathway. The potent blockade of the activity by heparin suggests that admixture of heparin with ASC would be of potential utility for optimizing clinical safety, especially with of intracoronary delivery.

## Automated isolation of adipose-derived stromal cells and rapid fabrication of enhanced vascular graft

H. Joon Paek; Eugene Boland; Erik Vossman; Anthony Yang; Thomas Cannon; Stuart Williams; Paul Kosnik

### Introduction:

In recent years, tissue engineering and regenerative medicine have provided valuable foresight in future medical treatments for a number of ailments and injuries. Despite the enormous promise that regenerative medicine offers, the most significant obstacle is a cell source due to a severe shortage of donor organs. Adipose-derived stromal cells (ASC) can be obtained more readily than bone marrow-derived stem cells, and donor site morbidity is low. They also do not pose ethical and political issues as embryonic stem cells do. Although many studies have reported the therapeutic benefits of ASC, to date results have varied greatly. One of the major contributing factors to the discrepancies among different groups is the isolation method. It is also likely that operator techniques during isolation further increased variability in results. In addition, ASC isolation from adipose tissue is an extremely labor-intensive process. Tissue Genesis (TGI) has been developing an automated ASC isolator for point-of-care regenerative medicine. This instrument eliminates operator variability and reduces the required labor for cell isolation. TGI is currently using this technology to evaluate a number of point-of-care applications, including vascular grafts.

### Methods:

Lipoaspirated human adipose tissue was washed in saline and digested in the automated isolator with Adipase™ Solution. Following the isolation process, cell yield and viability were assessed, and ASC were subjected to a flow cytometric assay. Canine adipose tissue was excised from falciform ligament and minced into small pieces. Minced tissue was rinsed and digested to collect ASC. The lumen of ePTFE conduits were rapidly coated with ASC by a single-stage method called "pressure sodding", using a low sustained transluminal pressure for 5-10 minutes. Vascular grafts were sodded at 0.2 million cells/cm<sup>2</sup> of the luminal surface area. A total of 8 sodded ePTFE vascular grafts were implanted into the carotid arteries of 8 adult dogs and compared to unsodded control ePTFE grafts implanted in the contralateral vessels. Patency of the grafts was evaluated using angiography at implant and termination at Day 140.

### Results:

The automated instrument could isolate 0.4-0.9 million cells per gram of tissue or up to 54 million therapeutic cells per single run with 60 g of lipoaspirated tissue in approximately one hour. ASC population is consistent in composition, containing 10-20% endothelial-like and 20-50% stem-like cells. Cell viability ranges from 75-95%. All animal survived the 140-day study period. Occlusion of control grafts began on Day 28 and increased throughout the duration of the study. At the time of study termination, 50% of the control grafts were occluded, and all sodded grafts were patent.

### Conclusions:

TGI's automated instrument can isolate therapeutically viable ASC from adipose tissue in one hour. The utility of ASC in point-of-care treatment was demonstrated in vascular grafts in contrast to conventional tissue-engineered vascular grafts, which often require weeks, if not months, of in-vitro culture. We demonstrated the safety and patency of ASC-sodded ePTFE vascular graft for up to 140 days in a canine carotid model, with a 100% improvement in patency compared to the "off-the-shelf" ePTFE graft.

## **Towards a human supplement replacing fetal bovine serum for clinical scale MSC manufacturing: Differential Gene and Protein Expression Analysis of Adipose Tissue derived MSC cultivated in different supplements.**

Viet Anh-Thu Ha; Andrea Hecker; Asli Kocaoemer; Hermann Solz; Harald Klueter; Peter Bugert; Karen Bieback

### **Introduction:**

Mesenchymal stromal cells (MSC) are promising candidates for various different cell based therapies. For clinical scale production, most MSC expansion culture protocols focus on supplementing medium containing fetal bovine serum (FBS). FBS has repeatedly been shown to cause adverse immunological reactions in humans and additionally poses a considerable risk of transmitting xenogenic agents. Previous studies have shown that pooled human AB-Serum (AB-HS) and thrombin-activated Platelet-Release-Plasma (tPRP) are suitable alternatives for FBS; however, differences in growth pattern and adhesion were evident (Kocaoemer et al. Stem Cells, 2007). To investigate the effect of AB-HS and tPRP in comparison to FBS supplement on MSC regarding their growth pattern and adhesion properties at the molecular level we performed a differential gene and protein expression analysis of adipose-tissue derived MSC (AT-MSC).

### **Methods:**

MSC from adipose-tissue of 6 donors were isolated and expanded using: (a) 10% FBS, (b) 10% AB-HS (pool of 5 AB-donors) and (c) 10% tPRP (buffy coat derived platelet concentrate representing 8 donors). Differentiation capacity toward the adipogenic and osteogenic lineages were assessed and cell surface markers analyzed with flow cytometry at passage 3. A whole genome gene expression analysis was performed at passage 2 using microarray hybridization. For verification of microarray data, quantitative real-time PCR, immunofluorescence and flow cytometry were applied.

### **Results:**

AT-MSC cultivated in both HS-AB and tPRP maintained differentiation capacity and cell surface marker expression but showed an altered morphology, modified plastic adhesion and significantly accelerated expansion. Analysing the microarray data, gene-expression was considered as significantly changed, if exceeding the multiple testing cut-off, computed in this case with  $-\log_{10}(\text{p-value})$  of 4.18 according to the Bonferroni criterion. Based on this, we identified 91 genes significantly differentially expressed between the groups FBS and AB or tPRP. Differences between cells cultivated in AB-HS and tPRP were hardly evident, but 78 out of 91 identified genes showed a higher expression in FBS compared to AB or tPRP (fold change 2). In contrast, only 13 of the 91 genes showed a lower expression in FBS compared to AB or tPRP (fold change 0.5). Proving the observations of reduced adhesion of cells cultivated in the human alternatives we detected a number of adhesion molecules expressed at lower levels in AT-MSC cultivated in these supplements. Likewise a number of bone and cartilage associated proteins were higher expressed in cells grown in FCS. Confirmative assays using quantitative RT-PCR as well as immunofluorescence/FACS analysis with selected genes supported these results.

### **Conclusions:**

Here we demonstrate that AB-HS and tPRP, both suitable alternatives for expanding MSC, have an effect on the gene expression profile of AT-MSC. In contrary xenogenic conditions as provided by FBS induce a modified gene expression compared to the human environment. This profile is consistent with existing observations regarding growth pattern and adhesion. However, the clinical relevance of these findings still remains unknown and therefore further functional studies need to exclude any potential impact for a clinical use, for example bone and cartilage regeneration.



## The Effect of Hydrostatic Pressure on Three-Dimensional Cartilage Regeneration Using Human Adipose-Derived Stem Cells

Rei Ogawa; Shuichi Mizuno; George Murphy; Dennis Orgill

### Introduction:

Establishment of an in vitro three-dimensional (3-D) tissue regeneration technique from an easy to obtain donor cell source may facilitate production of large cartilage constructs for clinical use. To build 3-D cell constructs in vitro, both inductive factors and optimal culture conditions (e.g., hydrostatic pressure, gas concentration, and nutrient supply) are necessary to promote cell viability and to maintain phenotype. In this study, we focused on 3-D optimizing culture conditions for induction of cartilage using adipose derived stem cells (ASCs) and collagen scaffolds.

### Methods:

Under an approved IRB protocol, discarded normal human adipose tissues (5.0 g) obtained from plastic surgical procedures were finely minced and digested with 0.075% type I collagenase. After centrifugation, the pellet was resuspended with DMEM and maintained in growth medium (DMEM + 10% FBS + 1% antibiotic-antimycotic solution) at 37 °C and 5% CO<sub>2</sub>. After the primary culture in and expansion to three passages, the cells were harvested and re-suspended with neutralized collagen solution, and subsequently  $1 \times 10^6$  cells / 50 $\mu$ l were suspended in neutralized type I collagen solution. The cell suspension was absorbed with collagen sponge scaffold and incubated at 37°C to achieve gelation. After a day of incubation in growth medium at 37°C and 5% CO<sub>2</sub>, cell constructs were incubated for one week using cyclic hydrostatic pressure (HP) at 0-0.5 MPa, 0.5 Hz, with a medium flow rate of 0.1 ml/min, at 37°C, 3% O<sub>2</sub>, and 5% CO<sub>2</sub> using an automated pressure/perfusion bioprocessor. The atmospheric pressure (AP) controls were incubated in the same bioprocessor. In both groups (HP and AP), the medium subsequently was switched to chondrogenic differentiation medium (DMEM with 1% FBS and 1% antibiotic-antimycotic solution, 10 ng/ml transforming growth factor-beta 1,  $1 \times 10^{-7}$  mol dexamethasone, 50  $\mu$ g/ml ascorbic acid 2-phosphate, 1 mM sodium pyruvate, and 40  $\mu$ g/ml l-proline). One, two, three and four weeks after incubation, the cell constructs were harvested for histological, immunohistochemical, and genomic evaluation. Chondrogenic differentiation and cell proliferation were assessed by Toluidine blue staining, immunohistochemistry using antibodies of type II and keratin sulfate, real time RT-PCR for type II and X collagen, aggrecan and sox9.

### Results:

Accumulation of pericellular and extracellular metachromatic matrix that stained with Toluidine blue within collagen scaffolds was observed in both groups and increased over 4 weeks. Accumulation of the matrix in the HP group was much greater than AP groups especially after 2 weeks. Immunohistochemical evaluation revealed that expression of type II and keratin sulfate of both groups increased with time, and HP groups showed greater expression than AP groups at each time point. Chondrogenic specific gene expression of type II and X collagen, aggrecan and sox9 was increased in the HP group especially after 2 weeks.

### Conclusions:

These results show that ASCs differentiate into chondrocytes in a 3-D collagen scaffolds with treatment of a cyclic HP. Cyclic HP was effective in enhancing chondrogenic differentiation of ASCs and in inducing increased accumulation of extracellular matrix and genes indicative of chondroid differentiation. This approach establishes a basis whereby discarded ASCs may potentially be employed for in vitro production of histocompatible cartilage constructs for potential use in clinical applications such as joint reconstruction and tissue stabilization and remodeling.

## Osteogenic Potential of Adipose Stem Cells in Electrospun Fibrinogen

Michael Francis; Gary Bowlin; Shawn Holt

### Introduction:

In the U.S. alone over 500,000 surgical procedures are performed annually requiring new bone tissue or bone analogues, generating a pressing need for new bone and bone substitutes clinically. Here we explore the engineering of bone using adipose-derived stem cells (ASCs) as a source of osteoblast precursors, along with electrospun fibrinogen as an ideal, potentially entirely patient-derived, bone template scaffold.

### Methods:

Electrospun sheets were formed of pure fibrinogen, pure PDO and PDO:fibrinogen dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol in a 25kV electrostatic field, grounded onto a round rotating and translating mandrel. Around  $2 \times 10^8$  early passage ASCs were electrosprayed into concurrently electrospun fibrinogen:pdo and formed uniformly pink sheets of tissue. Sheets of spun matrix were then punched into 10mm discs, seeded on the surface with around 50,000 ASCs, then placed in 48 well plates or in NASA designed rotating vessel bioreactors. Regular growth media was then replaced with either osteogenic media, or regular growth media or adipogenic media as controls. Media was changed every 3 days throughout the experiment. Constructs were harvested weekly and fixed. Histological analysis was via H&E, Mason's Trichrome, Alizarin Red and Oil Red O staining. Confocal analysis was performed using DAPI, Osteocalcin, Nile Red and phalloidin probes. SEM analysis was used to assess changes in tissue topography. Additionally, ASC seeded discs were kept in culture on spun fibrinogen discs for up to 10 weeks in culture in growth media to assess proliferative and migratory potential of ASCs.

### Results:

As early as 14 days and up to 28 days, the appearance of regular, porous, natural bone-like structures were seen in osteogenic induced scaffolds alone, as seen under SEM, and only observed on scaffolds containing fibrinogen. Alizarin red staining indicated pronounced mineralization on only the bone-induced scaffolds beyond 14 days in culture, as seen with fibrinogen containing scaffolds, with only trace staining seen on pure PDO scaffolds. Control and adipogenic scaffolds negatively stained for alizarin red, yet all scaffolds showed good cellularity via H&E and DAPI at least on the perimeter of the construct, with rich cellularity seen throughout the co-electrosprayed constructs. After 21 days of bioreactor culture, scaffolds with cells and with osteogenic media became hard and brittle. Confocal revealed dense patches of cells staining strongly for DAPI and phalloidin in all scaffolds. Scaffolds histology showed robust new collagen synthesis and matrix remodeling throughout on all fibrinogen-containing scaffolds, with new collagen production increasing with time and with bioreactor cultivation. In static culture ASC only poorly penetrated and migrated through any of the electrospun scaffolds, requiring 2 months to fully migrate throughout the scaffold in pure fibrinogen, while never migrating into spun PDO.

### Conclusions:

Electrospun fibrinogen appears to be an excellent material for ASC growth, proliferation and osteogenic differentiation. Fibrinogen scaffolds can be blended with a synthetic filler, PDO, and still retain good cellular and osteogenic differentiation characteristics. Remarkably, simultaneous electrospraying ASC within the spinning matrix produces in 20 minutes the cellularity seen with over 2 months of culture with conventional seeding of cells atop the scaffold.

## Enhanced Fat Protection and Survival in Fat Transplantation via Treatment with Poloxamer 188

John Nguyen; Mike McCormack; Mark Randolph; William Austen Jr.

### Introduction:

Autologous fat transplantation is an essential tool in soft tissue reconstruction. Damaged and apoptotic cells, however, are eventually resorbed by the body and provide inconsistent and undesirable results for soft tissue restoration. Poloxamer 188 (P188) is a nonionic surfactant that interacts with damaged cell membranes and inserts into lipid monolayers. P188 effectively seals the membrane of damaged cells and has been shown to protect against injury and apoptosis. The ability of poloxamers to interact with lipid membranes has led us to hypothesize that by sealing portions of fat cells damaged during fat harvesting, we can restore and protect the structural integrity of damaged cells and thus improve cell survival. This study was designed to investigate the ability of P188 to effectively restore and protect damaged tissues, improve cell survival, and improve transplantation results.

### Methods:

Fat was obtained from liposuction aspirate and transplanted into nude mice within an hour of harvest from the OR. A volume of 0.6cc of fat was placed subcutaneously in the right dorsum of each mouse. Our experimental study has three groups: 1) Fat treated with P188 at a concentration of 10mg/ml 2) Fat treated with dextran at a concentration of 10mg/ml 3) Fat treated with normal saline. After a period of 6 weeks, mice were euthanized and fat implants were harvested. Fat implants were evaluated prior to implantation and after harvesting using weight, volume, live/dead assay, mitochondrial ATP levels, and real time PCR for leptin and PPAR $\gamma$ 2 levels.

### Results:

Transplanted fat developed into a well circumscribed, demarcated nodule in each animal after a six week period. The saline controls exhibited up to 30% resorption based on weight and volume. Dextran treated fat grafts exhibited a similar resorption rate. Fat grafts treated with P188 demonstrated a 67% decrease in resorption. This is a statistically significant reduction ( $p < 0.001$ ). Differences were also seen at the molecular level and histologically, with notable increases in proportion of fat cells, and decreased vacuolar areas and fibrosis formation.

### Conclusions:

In this study, we examined the efficacy and survival of human fat grafts using a nude mouse model of transplantation. Poloxamer 188 a non ionic surfactant significantly reduced resorption of our fat grafts and improved cell survival. In contrast, treatment with dextran, a comparable molecular weight control, gave results similar to saline. We conclude that the use of P188 effectively restores and protects fat grafts, improves cell survival, and decreases fat graft resorption. This therapy has potential benefits for future clinical applications.

## Evaluation of the stemness and of chondrogenic and osteogenic differentiation potential of different human Adipose Stem Cells (hASC) subpopulation.

Tommaso Rada; Rui L. Reis; Manuela E. Gomes

### Introduction:

Adipose tissue is becoming an elected source of cells for tissue engineering (TE) applications because ASCs have been easily isolated and have shown good differentiation potential for several different lineages. The ASCs have several common characteristics with the bone marrow MSC and both the stem cells population have a mesenchima origin. Studies on BMSC have shown the presence of several subpopulations that show distinct differentiation potential, but up to date there are no explicit published studies that show the existence of several subpopulations of ASCs. Most of the isolation methods used until now are based on an enzymatic digestion followed by a cell selection simply based on the capacity of ASCs to adhere to the surface of the plastic culture flasks. However, this method has several disadvantages, which we expect to overcome using a newly developed isolation method based on the use of immunomagnetic beads (patent submitted), that has been optimized to increase the number of ASCs in the cell culture and to allow the isolation of different subpopulation. This study describes the use of this method to obtain subpopulation isolated from human ASCs and analyses the stemness of the isolated subpopulation. Additionally, it was assessed the chondrogenic and osteogenic differentiation potential of the obtained subpopulations.

### Methods:

The hASC were isolated using an immunomagnetic beads coated with different antibodies, namely, CD29, CD44, CD49d, CD73, CD105, STRO-1 and NGFr (p75). Once isolated, cells were cultured with Basal Medium until confluence and then trypsinized and divided into 3 groups: the first one was used to characterize the stem cells by RT Real-Time PCR for CD44, CD105; CD73, CD90 and STRO-1; the second group was cultured with Osteogenic Medium for 3 weeks, to analyse the osteogenic potential; the third one was used to set up a pellet culture with chondrogenic medium and cultured for 3 weeks, to assess the chondrogenic differentiation ability. For characterization of the differentiation potential, cells were retrieved after 3 weeks of culture with osteogenic medium or chondrogenic medium for Alizarin Red Staining and RT Real-Time PCR for Osteocalcin and Osteopontin and for Alcian Blue, Toluidine Blue and Safranin O staining and RT Real-Time PCR for Agrecan, Collagen I, Collagen II, Collagen X and Sox 9, respectively.

### Results:

All the cell subpopulations isolated express the characteristic marker genes for stem cells considered and all the cell subpopulations are able to differentiate in chondrocyte and osteoblast like cells. However, there are some differences (in some cases, significant) among the several cells subpopulation isolated, regarding the expression of the stem cell marker and also regarding their ability to differentiate into the osteoblastic and/or chondrogenic lineage. The results obtained show that the hASCs isolated with the immunomagnetic beads coated with CD29 Ab have the highest gene expression of the genes considered as stem cells markers. Furthermore, the subpopulation isolated with CD29 and CD105 show to be the ones with highest chondrogenic differentiation potential while the subpopulations isolated with CD29 and STRO-1 antibodies show the highest osteogenic differentiation potential.

### Conclusions:

With the described method it was possible to isolate distinct subpopulations which present different genes expression relative to the stem cell markers studied. The osteogenic and chondrogenic markers expression showed that these subpopulations exhibit differentiation potentials significantly different. The results obtained show a complex picture where the ASCs population is formed of several subpopulations each one with distinct ability to differentiate to the chondrogenic or osteogenic lineage.

Acknowledgements: Marie Curie Actions Alea Jacta Est, Project HIPPOCRATES, NoE EXPERTISSUES.

## Adipose Derived Stem Cells accelerate Primary Tendon Repair

A. Cagri Uysal; Hiroshi Mizuno; Hakan Orbay; Takahisa Okuda; Hiko Hyakusoku

### Introduction:

Adipose derived stem cells(ASCs) were proven to have a positive effect on healing processes. In in vitro experimental models, ASCs were indicated to differentiate into different cell lineages including tenocytes. We have performed an experimental study to find out the effect of ASCs on primary tendon repair model.

### Methods:

The ASCs were gathered from inguinal fat pads of Japanese Rabbits. For autologous transplantation, fat that was harvested from each rabbit was cultured and then utilized in the same animal. After three passage in control medium (DMEM,10% FBS), the cells were labeled with DiI and Hoecht 33342 for tracing.  $1 \times 10^7$  cells were used in every application. 6 rabbits were used in the study. The right Achilles tendon was used as the experimental and the left one was designated as the control group. The tendons were exposed and sharp incisions were accomplished. The tendons were then repaired with Modified Kessler method. 7cc of blood was taken from each rabbit and centrifuged at 800rpm for 15 to yield Platelet Rich Plasma(PRP). In the experimental group, the ASCs were mixed with the PRP and 2% Calcium chloride was added to have a gel form. Then this gel is applied over the repaired tendon. In the control group, same procedure was done except ASCs mixture. The legs of the rabbit were immobilized with plaster of Paris for 2 weeks. The animals were euthanized at the 4th week. Then biomechanical testing, histology and immunohistochemical staining were performed.

### Results:

The tensile strength was found to be  $29.464 \pm 3.657$ ,  $43.058 \pm 3.798$  in the control and experimental group respectively. There was a statistical significance between the groups. The immunohistochemical staining revealed the differentiation of the injected ASCs to tenocytes and endothelial cells around the tendon. TGF – beta1, beta2, beta3, FGF and VEGF immunohistochemical stainings revealed difference between the experimental and control groups.

### Conclusions:

ASCs might help in the primary healing process of the tendon directly and indirectly. Direct effect is mainly the differentiation to tenocytes and endothelial cells. Indirectly, the differentiation to fibroblasts could increase the growth hormones and cytokines and this indirect effect is the main controller of the healing process.

## Adipose derived mesenchymal stem cells in the treatment of osteoarthritis in the veterinary patient. A clinical experience

Jerrold Bausman

### Introduction:

Osteoarthritis is reported to be the most common cause of chronic pain in canines in the U.S. and it is estimated that approximately one in five adult dogs in the U.S. is arthritic. In published reports of hospitalized felines 33.9% were found to have arthritis and in those over 12 years of age approximately 90% had arthritis. Similarities in human and veterinary non-surgical management of arthritis includes joint supplements such as glucosamine chondroitin sulfate, weight management, controlled exercise (physical therapy), acupuncture and a heavy reliance on non-steroidal anti-inflammatories (NSAID's). Complications associated with non-surgical management are primarily associated with NSAID's in both human and veterinary medicine and our patients share similar pathologies – GI upset/ulceration, liver and kidney damage. Surgical management such as joint replacement surgery in veterinary medicine is primarily isolated to the coxofemoral joint however newly developed elbow replacements show promise. A safe minimally invasive effective treatment of arthritis could dramatically improve the quality of life for both human and veterinary patients that suffer this painful debilitating disease and the potential complications associated with current therapy.

### Methods:

10 veterinary patients comprising 18 arthritic joints treated with intra-articular injection of autologous adipose derived mesenchymal stem cells (AD-MSC's) are presented. One of these ten patients was treated additionally with both intravascular and inter-fragmentary AD-MSC's for peripheral neuropathy and chronic non-union fracture with secondary pseudo-arthritis. Adipose tissue in all patients was collected via surgical lipectomy from either an external thoracic fat pad caudal to the scapula (9) or the falciform ligament (1). AD-MSC's were isolated via enzymatic digestion and centrifugation. All patients were treated with intra-articular injection of 5 million (MM) viable autologous AD-MSC's in 0.6 ml's of phosphate buffered saline (PBS). One patient additionally received 5MM cells in 10ml's of PBS intravenously as well as 5MM AD-MSC's in 0.6 mls PBS interfragmentary. All patients were evaluated approximately 14 days post intra-articular injection. Patients were scheduled for re-evaluation every 30 days post AD-MSC therapy for 120 days. Patients were all evaluated by a single veterinary surgeon blinded as to previous evaluation results. Veterinary evaluation consisted of gait analysis, range of motion of treated joint(s), pain level, muscle mass estimation. Both owners and veterinarian also evaluated the pets condition using a numerical scale 1-5 (1 = normal, 5 = so severe they would consider euthanasia) at each recheck. Parameters evaluated using the numerical scale were lameness, range of motion and pain. To avoid bias previous evaluations were not accessible to the owner or veterinarian.

### Results:

Number of days post AD-MSC therapy to last re-evaluation ranged from 14 to 268 days with an average of 116 days. No adverse events associated with injection of the AD-MSC's in PBS were reported or identified. All patients recovered from surgery within 14 days with minimal morbidity and complications. All patients showed moderate to dramatic improvement in all parameters measured by both owner and veterinarian. Due to continued lameness and pain isolated to the hip joint, a total hip arthroplasty was performed in one patient 174 days after the initial injection (99 days after a second stem cell injection). All owners were pleased with the rapidity and low morbidity associated with the procedure. 9 of 10 owners were pleased with the outcome and would have the procedure done again. All owners would recommend the procedure to family or friends with pets with arthritis.

### Conclusions:

This series of cases demonstrates the safe and effective use of AD-MSC therapy for debilitating arthritis. No patients in this case series suffered any adverse events associated with the injection of AD-MSC's. All but one patient was successfully treated using AD-MSC's, with success defined as acceptable improvement in the patient's condition as determined by a veterinary surgeon and the patients owners. In many of the cases a profound improvement was observed by both owner and veterinarian. The procedure was well tolerated by both patient and owner and carried with it a low morbidity. Liposuction is a well established and acceptable procedure in human plastic and reconstructive surgery that has been reported in one veterinary patient. The development of this technique in veterinary surgery has the potential to decrease the already low morbidity associated with AD-MSC therapy in our animal companions. Current veterinary clinical trials using AD-MDSC's include liver and kidney diseases, gingival stomatitis in cats and canine atopy as well as recurrent obstructive airway disease (Heaves or COPD) in horses. Areas of interest for future exploration may include diabetes, degenerative myelopathy, and other neurologic diseases.



## Cell therapy based on adipose tissue-derived stroma cells promotes physiological and pathological wound healing.

Ebrahimian TG, Squiban C, Pouzoulet F, Buard V, André M, Cousin B, Silvestre JS, Benderitter M, Casteilla L, Tamarat R.

### Introduction

Regenerative properties of adipose derived stromal cells (ADSC) are now well-established in ischemic and osteoarticular diseases. Few studies investigated the putative therapeutic interest in the context of wound healing and skin regeneration. We aimed in this study to investigate these points in normal and irradiated situation.

### Methods

GFP Adipose tissue-derived stroma cells (ADSC) were administrated to skin punched wounds of both non-irradiated and irradiated mice (20 Gy, locally).

### Results:

At Day 14, ADSC enhanced wound closure, viscoelasticity and collagen tissue secretion by 5-, 1.12- and 1.10-fold respectively in non irradiated mice and by 4-, 1.3- and 1.29-fold, respectively in irradiated mice when compared to PBS injected animals ( $p < 0.05$ ).

GFP-positive ADSC incorporated in dermal tissue and expressed epidermal markers K5 and K14. At Day 7, ADSC also improved skin blood perfusion assessed by laser Doppler Imaging, capillary density and VEGF plasma levels by 1.46-, 1.66- and 4.75-fold respectively in non irradiated mice and by 2.9, 1.85- and 6-fold, respectively in irradiated mice when compared to PBS injected animals ( $p < 0.01$ ). GFP-ADSC isolated from female mice were transplanted to male mice. We observed GFP-positive cells stained with 2 X chromosome probe and 1 Y chromosome probe suggesting that ADSC can fuse spontaneously in our experimental conditions.

### Conclusions:

This study demonstrates, for the first time that ADSC participate to dermal wound healing by both cell fusion, differentiation in keratinocyte cells and KGF secretion. ADSC also trigger angiogenesis. Adipose lineage cells represent a new cell source for therapeutic dermal wound healing.

## Mesenchymal stem cells for liver regeneration

Oscar K. Lee

We have demonstrated that extra-hepatic human mesenchymal stem cells (MSCs) differentiate into hepatocyte-like cells with functions *sine qua non* of normal hepatocytes, and that in utero transplantation (IUT) of MSCs can contribute to numerous organs including the liver. However, their therapeutic potential is questioned by the low frequencies of engraftment in IUT models as well as animal models of non-lethal liver injury. Here, we investigate the feasibility of rescuing lethal fulminant hepatic failure (FHF) with MSCs. The significance of a lethal model is that it reflects more closely the clinical demand for alternative or bridging therapies while waiting for available donor organs. FHF in non-obese diabetic severe combined immunodeficient mice was established by carbon tetrachloride (CCl<sub>4</sub>) administration, and MSCs were intrasplenically or intravenously transplanted. While placebo groups died from CCl<sub>4</sub>-injury, liver regeneration and long-term engraftment of donor-derived cells was demonstrated in MSC-recipients. Engraftment frequencies as well as comparison of MSCs with pre-differentiated hepatocytes-like cells indicate that rescue of FHF was not mediated by functional differentiation but, rather, by paracrine effects. We further found that undifferentiated MSCs express higher basal levels of anti-oxidative enzymes than pre-differentiated cells and, up-regulate anti-apoptotic genes under oxidative stress. It was also demonstrated that intravenous transplantations were more effective than intrasplenic transplantations. We propose that MSCs rescue FHF by scavenging and clearance of reactive oxygen species, and promote endogenous liver regeneration through the release of trophic factors. Our pre-clinical results here demonstrate that mesenchymal stem cells can offer a novel therapy for lethal fulminant hepatic failure.

## Microstructural Fat Grafting Improves Radiation Skin Damage in a Murine Model

Robert Allen, Jr.; Phuong Nguyen; Jeffrey Schachar; Vishal Thanik; Pierre Saadeh; Stephen Warren; Sydney Coleman; Alexes Hazen

### Introduction:

Recent clinical reports have shown fat grafting to be a useful tool in the treatment of radiation fibrosis. The mechanism responsible for this clinical improvement, however, remains unknown. Since radiation fibrosis is known to be mediated by upregulation of the TGF- $\beta$ /Smad3 pathway, we hypothesize that microstructural fat grafting improves radiation fibrosis by downregulating this fibrotic response and increasing the vascularization of these tissues.

### Methods:

Lipoaspirate was harvested from healthy human donors. Dorsal skin of wild-type FVB mice was isolated and irradiated with 45Gy using a Varian 2300 Linear Accelerator. Four weeks following radiation, mice were either grafted with 1.5-cc of lipoaspirate or sham-grafted into the dorsal subcutaneous tissue. Hair growth, skin color, and degree of ulceration were analyzed photometrically. Fat graft survival was measured by volume displacement. Collagen production, fibrosis, and vascular density (% positive staining/hpf) of irradiated tissue was assessed via gomori trichrome, Smad3, and CD31 staining at 4 weeks post-grafting, respectively. Fat graft vascularization was analyzed by en bloc  $\beta$ -galactosidase staining of the fat graft and dorsal skin of tie2/lacZ transgenic mice at 4 weeks post-grafting.

### Results:

Irradiated mice began to exhibit radiation damage at  $13 \pm 2$  days. Chronic ulceration and fibrotic skin thickening became stable 4 weeks post-irradiation. Fat grafts harvested at 4 weeks following injection had  $82.7 \pm 5.3\%$  survival. Hair regrowth, skin color/texture, and degree of ulceration were improved in fat-grafted mice compared to sham-treated controls when analyzed photometrically. Smad3 production was significantly decreased in treated animals ( $18.16 \pm 0.5\%$  vs  $29.34 \pm 0.7\%$ ,  $p < 0.03$ ). Collagen production subsequently had a 2.3-fold decrease in fat grafted mice compared to controls. Vascular density of irradiated skin was also increased in fat grafted mice ( $7.3 \pm 0.04\%$  vs  $5.2 \pm 0.09\%$ ,  $p < 0.01$ ). Examination of the fat-grafts on tie2/lacZ mice demonstrate neovascularization within the grafted fat that does not stain positive for  $\beta$ -galactosidase, signifying that these vessels do not have murine endothelial cells. There is also a clear point of inosculation between human and murine vasculature at the perimeter of the graft, with obvious perfusion of these vessels.

### Conclusions:

Microstructural fat grafting improves radiation fibrosis. Four weeks following grafting, treatment animals have a decreased fibrotic response, with decreased expression of Smad3. This downregulation of the TGF- $\beta$ /Smad3 pathway correlates with a decrease in collagen production. There is also a concomitant increase in the skin vascularity of these previously irradiated tissues. Furthermore, we have demonstrated for the first time that de novo vessels are forming from human elements within the fat grafts. There is direct inosculation of vessels originating from the grafted tissue (human) to the host vasculature (murine). It is likely that this neovascularization establishes blood flow to the graft and improves perfusion to the surrounding tissues. It is probable that the combination of these mechanisms result in the improvement in radiation fibrosis following microstructural fat grafting.

## Adipose derived stem cells accelerate primary nerve repair

Hiroshi Mizuno; Uysal A. Cagri; Hakan Orbay; Takahisa Okuda; Hiko Hyakusoku

### Introduction:

Adipose derived stem cells(ASCs) were proven to have a positive effect on healing processes. In in vitro experimental models, ASCs were indicated to differentiate into different cell lineages including neurons. We have performed an in vivo experimental study to find out the effect of ASCs on primary nerve repair model.

### Methods:

The ASCs were gathered from inguinal fat pads of fisher rats. After three passage in control medium (DMEM,10% FBS), the cells were labeled with DiI and Hoecht 33342 for tracing.  $1 \times 10^7$  cells were used in every application. The sciatic nerves of 6 rat were explored and sharp incision was performed before the branching into peroneal and tibial nerves. The nerve was coapted under operating microscope. The right side was covered with fibrin glue and ASCs and the left sciatic nerve was applied fibrin glue only. Walking gate analysis was performed on 3rd and 6th months. Electro neurography(ENG) was performed on the 6th month. Histological and immunohistochemical staining with anti S100 protein antibody and VEGF were done. SEM was performed.

### Results:

Walking gate analysis at the 3rd month revealed that the sciatic function indexes (SFI) were  $-108.677 \pm 7.436$ ,  $-83.061 \pm 8.183$  in the control and experimental groups respectively. At the 6th month SFI was  $-83.246 \pm 7.352$ ,  $-62.256 \pm 7.349$  in the control and experimental groups respectively. The nerve conduction velocity (m/sec) was  $34.88 \pm 5.15$  in the control and  $48.01 \pm 6.83$  in the experimental group. The conduction percentage of the given voltage was  $5.37\% \pm 1.89$  in control and  $9.33\% \pm 2.01$  in the experimental group. There was statistical significance in all of the evaluation methods. ( $p < 0.05$ ) The immunohistochemical staining revealed the differentiation of the stem cells to endothelial and schwann cells. VEGF was increased in the experimental group. TGF beta1, TGF beta2, TGF beta 3 and FGF was significantly different between the groups.

### Conclusions:

ASCs might help in the primary healing process of the nerve directly and indirectly. Direct effect is mainly the differentiation to endothelial cells and neural cells, mainly schwann cells, supported by the ENG and histologically. Indirectly, the differentiation to fibroblasts could increase the growth hormones and cytokines mainly and this indirect effect is the main controller of the healing process.

## Assessing the Risk of Tumorigenesis Through the Interactions of Adipose Derived Stem Cells and Breast Cancer

John Nguyen; William Austen Jr.; Mark Randolph; Raymond Wadlow; Mike McCormack

### Introduction:

Fat grafts supplemented with adipose derived stem cells (ADSC) have been shown to have improved graft blood supply as well as improved longevity and graft volume. The clinical feasibility of using adipose derived stem-cell supplemented fat for breast augmentation or reconstruction raises the issues of tumor initiation, promotion and progression. There is compelling evidence that stromal cells, including fibroblasts can influence epithelial transformation and are prominent modifiers of cancer progression. Little is known about the dynamics of interaction between tumors and therapeutic stem cells. The purpose of this study is to perform an examination of the cell-cell interaction and assess potential tumorigenesis between ADSC and breast cancer cells.

### Methods:

To examine the behavior between immortalized tumors and mesenchymal stem cells we developed a uniform monolayer co-culture system. ADSC were isolated from over 20 patients and grown until confluent on the base of a 96 well plate. This layer of stem cells acts as a feeder layer and is seeded with various green fluorescence protein (GFP)-labeled breast cancer cell lines. Breast cancer cell lines included ER, PR, and HER2 positive and negative receptors. Analysis of GFP expression was performed daily using a fluorescence plate reader to assess important characteristics of GFP-expressing cancer cells such as viability, cell growth, and proliferation. ADSC and breast cancer cells showing increased GFP expression are FACs sorted, examined for expression of relevant surface markers, and further co-cultured with a semi-permeable membrane using minicell co-culture inserts.

### Results:

We have enabled the stable transduction of the GFP gene into a large series of human breast tumor cell lines and the tumor cell lines were able to stably express GFP at high levels. The present study demonstrated that the presence of ADSC in co-culture with a benign mammary epithelial cell line remained unchanged in growth and proliferation in vitro as compared to fibroblast controls and benign mammary epithelial cells alone. Additionally, co-culture of various human breast cancer cell lines remained unchanged in the presence of all but one ADSC of the patients examined. We have found in repeated experimentation that co-culture of a single patient's ADSC (1/20) had significantly increased the growth and proliferation of one cancer cell line (1/5). There was an exponential increase in growth and proliferation as compared to fibroblast controls and breast cancer cell growth alone. Furthermore, 1 out of 5 of our cancer cell lines exhibited an increase in growth and proliferation when co-cultured with our fibroblast control.

### Conclusions:

The purpose of this study was to perform an examination of the cell-cell interaction and assess potential tumorigenesis between ADSC and breast cancer cells. We have developed a uniform monolayer co-culturing system that effectively allows us the ability to assess important characteristics of cancer cell growth and proliferation using GFP expression. Thus far, we have found a population of ADSC that when co-cultured with an ER positive breast cancer cell line, seems to stimulate an increase in growth and proliferation. This finding does not indicate that ADSC stimulate cancer but does suggest that there are patient phenotypes that may have a factor in tumorigenesis. The mechanism in which these ADSC enhances proliferation and growth in these breast cancer lines is unclear; however, examination of cells for expression of relevant surface markers, and further examination using this and other models is warranted.

## Transplantation of human adipose-derived stem cells promotes axonal regeneration and functional recovery in sciatic nerve defects of nude rats

Euk-Sik Yoon; Han-Woong Ko; Yi-Hwa Ji; Eun-Sang Dhong; Seung-Ha Park

### Introduction:

Tissue engineering approaches for promoting the repair of peripheral nerve injuries have focused on cell-based therapies involving multipotent stromal cells, including human adipose derived stem cells (hASCs). The aim of this study is to evaluate whether transplantation of hASCs within silicone tubes bridging peripheral nerve defects promotes axonal regeneration and functional recovery in nude rats.

### Methods:

Transplantation of ASCs and neuronal-differentiated cells was carried out on the sciatic nerves of 30 nude rats. A 10mm defect was created and a silicone tube was used as a nerve conduit. The tubes were then filled with one of the following: 1) fibrin glue only, 2) cultured undifferentiated cells and 3) differentiated neurogenic cells suspended in fibrin glue. Nerve and chamber contents were harvested after 4 and 8 weeks. The specimens were harvested from the midpoint of the silicon tubes for hematoxylin-eosin, toluidine and immunohistological staining and electron microscopy to observe the effect of hASCs on nerve regeneration.

### Results:

ASCs differentiated into neuronal cell in vitro after 10 days of being cultured in the neuronal-differentiation inducing medium. Western blotting showed expression of glial fibrillary acidic protein (GFAP), a structural element of fibrillary astrocytes. No regenerated nerve fibers were present within the silicone tubes of the control group at 4 weeks. Regenerated nerve fibers were confirmed in all groups at 8 weeks. The number and diameter of the myelinated fibers from the experimental groups with both undifferentiated and neuronal differentiated hASCs were significantly higher and larger than those from the control group without hASCs. In the experimental group with undifferentiated hASCs, the diameters of the myelinated nerve fibers and the thickness of the myelin sheaths had no obvious difference from those of the group with neuronal differentiated hASCs, but the number of regenerated myelinated sheath fibers was lower than that of the group with neuronal differentiated ASCs. It appears that hASCs contribute to the promotion of axonal regeneration.

### Conclusions:

hASCs maintained in neurogenic medium or undifferentiated control medium have neurogenic potential both in vitro and in vivo. hASCs may be an ideal material for further experiments on stem cell biology and regenerative medicine



## Human adipose-derived stromal cells maintain hematopoietic progenitors in vitro

Fabienne DE TONI; Béatrice Cousin; Philippe Bourin; Louis Casteilla; Patrick Laharrague

### Introduction:

Human adipose-derived stem cells (ADSC) and human bone marrow (BM) mesenchymal stem cells (MSC) share many properties, such as their mesodermal origin, and their potential of adipogenic, osteogenic and chondrogenic differentiation. Simultaneous transplantation of MSC and hematopoietic stem cells (HSC) improves engraftment of HSC and reduces the aplastic period after allogeneic bone marrow transplantation. However, MSC could be abnormal in hematologic malignancies and their accessibility is limited. This study aims to determine if ADSC, which are never in contact with malignant cells, could support the survival of HSC, like their bone marrow counterpart. For this purpose, we compared the capacity of human MSC and ADSC feeder-layers to maintain the survival and self-renewal of HSC in a long-term culture system.

### Methods:

Adipose tissue samples were obtained from abdominal lipectomies (or lipoaspiration) and BM during total hip prosthesis procedures, from consenting healthy patients (25-80 years). CD34+ hematopoietic progenitor cells isolated from BM by positive selection were seeded in parallel onto ADSC and MSC layers at confluence. These long-term co-cultures (LTC) supported the growth and development of HSC during at least five weeks. Clonogenic assays in methylcellulose were performed at two and five weeks of culture. The frequency of long-term culture-initiating cells (LTC-IC), and the presence of cobblestone areas were also determined.

### Results:

The numbers of colony forming units (CFC) in methylcellulose, at two and five weeks, obtained from hematopoietic cells cultured either on ADSC or MSC layers were very similar. We observed also the presence of cobblestone area in both co-culture systems. However, LTC-IC frequency (limit dilution experiment) was lower on ADSC than on MSC. Altogether, these results demonstrated that human ADSC are able to maintain HSC survival at long-term.

### Conclusions:

These results indicate that human adipose-derived stem cells may be used for co-transplantation with CD34+ progenitors to sustain long-term hematopoietic reconstitution after chemo- or radiotherapy. This will be soon tested in vivo, using co-transplantation experiments in a NOD-SCID model. Because it is abundant and accessible, adipose tissue could be therefore a convenient source of cells for the reconstitution of hematopoiesis in man.

## Liability Protection for Stem Cell Therapies

James M Wood

### Introduction:

Stem cell research presents as many scientific, legal and, when involving fetal tissue, ethical issues as it does potential therapies. Once the increasingly volatile debate over these issues is resolved, the promises of stem cell research can be brought to clinical therapies. However, they can be just as readily undone without a formal legislative scheme that provides compensation for the patient harmed while simultaneously protecting the industry from unbridled litigation. This program proposes such a liability model for both patients receiving care or those participating in a clinical trial. To do so, this presentation summarizes the history of the undoing of the vaccine industry by product liability cases, identifies past efforts to minimize liability in related endeavors (with mixed results), and summarizes the elements of a national compensation program.

### Methods:

A survey of stem cell therapies, a review of liability for vaccines, and creating a proposed model limiting liability for stem cell research

### Results:

I. INTRODUCTION II. STEM CELL RESEARCH A PRIMER III. PUBLIC POLICY SUPPORTS STEM CELL RESEARCH AND LIABILITY PROTECTION A. Why Stem Cell Research And Treatments Are Good For The Public 13 B. Why The Public Good Associated With Stem Cell Research Is Like That Associated With Vaccines 14 C. The Market For Stem Cell Research And Development Is Fragile 16 IV. THE UNDOING OF THE VACCINE INDUSTRY BY PRODUCT LIABILITY 17 V. ALTERNATIVES TO PRODUCT LIABILITY FOR PROVIDING COMPENSATION TO THOSE HARMED BY PRESCRIPTION PRODUCTS 20 A. The Swine Flu Model 20 B. National Vaccine Injury Compensation Program 22 1. Overview 22 2. The Vaccine Injury Table 23 3. Filing A Claim 23 4. Effects Of The VICP 25 5. Criticisms And Limitations 25 C. Safety Act and Project BioShield 27 1. Overview 27 2. Effects 29 3. Criticisms And Limitations 30 D. Blood Shield Laws 33 E. California AIDS Vaccine Victims Compensation Fund 34 F. The Federal Tort Claims Act And Stem Cell Liability Protection 38 1. Overview 38 2. Application Of FTCA To Stem Cell Research 40 VI. ELEMENTS OF A SUCCESSFUL COMPENSATION SCHEME 41 VII. CONCLUSION 43

### Conclusions:

ELEMENTS OF A SUCCESSFUL COMPENSATION SCHEME If stem cell researchers and manufacturers cannot be brought under the Federal Tort Claims Act (similar to what was done for Swine Flu) , a compensation scheme that should be enacted by Congress in creating a national compensation program for harm caused by stem cell therapies would have most or all of these elements: 1. Create a national standard of liability and compensation that preempts the product liability laws of the states. 2. The national fund would be created from a combined tax on stem cell therapy manufacturers, purchasers and attorney fees from awards. 3. The national fund would be the sole source for compensation of injuries related to stem cell research. 4. As part of a national stem cell compensation program for risks that are known, a special master would be created to determine the amount of compensation (based upon an existing table of injuries) that would include past unreimbursed as well as future medical expenses, lost earnings, damages for pain and suffering with a cap of \$250,000 and reasonable attorneys' fees and costs. If the patient has died, there is a cap of \$250,000. 5. The injuries entitled to compensation must be clearly defined and a mechanism for adding newly discovered injuries should be recognized. The California AIDS Vaccine Victims Compensation Fund provides a model for the addition of injuries as they become associated with a stem cell therapy. 6. If there is proof of the administration of a therapy as well as proof of a compensable injury there is a presumption that the therapy was the cause of the injury. 7. If proof of either fails, the patient bears the burden of proving that the stem cell therapy was the cause of the specific harm. 8. If state tort claims are permitted in any way at all, there would be a presumption in the state court proceeding that a warning on a therapy is adequate and the claim for a failure to warn is preempted if the FDA has approved the warning; such a presumption can be overcome only with proof by clear and convincing evidence of fraud or intentional misrepresentation. 9. If state tort claims are permitted, there should be preemption in the absence of fraud or intentional misrepresentation that must be established by clear and convincing evidence including evidence that there was reliance upon the misrepresentation. 10. If state tort claims are permitted, the FDA regulatory compliance defense should be adopted. 11. If state tort claims are permitted, and if a manufacturer petitions to have a warning to a therapy added and the petition rejected by the FDA, there will be no liability in a state court if the adverse event resulted from failure to warn. 12. If state tort claims are permitted, punitive damages cannot be recovered.

## Assessing the Risk of Tumorigenesis Through the Interactions of Adipose Derived Stem Cells and Breast Cancer

John Nguyen; William Austen Jr.; Mark Randolph; Raymond Wadlow; Mike McCormack;

### Introduction:

Fat grafts supplemented with adipose derived stem cells (ADSC) have been shown to have improved graft blood supply as well as improved longevity and graft volume. The clinical feasibility of using ADSC supplemented fat for breast augmentation or reconstruction raises the issues of tumor initiation, promotion and progression. There is compelling evidence that stromal cells, including fibroblasts can influence epithelial transformation and are prominent modifiers of cancer progression. Little is known about the dynamics of interaction between tumors and therapeutic stem cells. The purpose of this study is to perform an examination of the cell-cell interaction and assess potential tumorigenesis between ADSC and breast cancer cells.

### Methods:

To examine the behavior between immortalized tumors and mesenchymal stem cells we developed a uniform monolayer co-culture system. ADSC were isolated from over 20 patients and grown until confluent on the base of a 96 well plate. This layer of stem cells acts as a feeder layer and is seeded with various green fluorescence protein (GFP)-labeled breast cancer cell lines. Breast cancer cell lines included ER, PR, and HER2 positive and negative receptors. Analysis of GFP-expression was performed daily using a fluorescence plate reader to assess viability, cell growth, and proliferation. ADSC and breast cancer cells showing increased GFP-expression are FACs sorted, examined for expression of relevant surface markers, and further co-cultured with semi-permeable membranes.

### Results:

We have enabled the stable transduction of the GFP gene into a large series of human breast tumor cell lines and the tumor cell lines were able to stably express GFP at high levels. The present study demonstrated that the presence of ADSC in co-culture with a benign mammary epithelial cell line remained unchanged in growth and proliferation as compared to fibroblasts controls and benign mammary epithelial cells alone. Additionally, co-culture of various human breast cancer cell lines remained unchanged in the presence of all but one ADSC of the patients examined. We have found in repeated experimentation that co-culture of a single patient's ADSC had significantly increased the growth and proliferation of one cancer cell line. There an exponential increase in growth and proliferation as compared to fibroblast controls and breast cancer cell growth alone. Furthermore, 1 out of 5 of our cancer cell lines exhibited an increase in growth and proliferation when co-cultured with our fibroblast control.

### Conclusions:

In this study, we have developed a uniform monolayer co-culturing system that effectively allows us the ability to assess important characteristics of cancer cell growth and proliferation using GFP-expression. Thus far, we have found a population of ADSC that when co-cultured with a specific breast cancer cell line, seems to stimulate an increase in growth and proliferation. This finding does not indicate that ADSC stimulate cancer but does suggest that there are patient phenotypes that may have a factor in tumorigenesis. The mechanism in which these ADSC enhances proliferation and growth in these breast cancer lines is unclear; however, examination of cells for expression of relevant surface markers, and further examination using this and other models is warranted.

## Vascular Endothelial Growth Factor and Hepatocyte Growth Factor Play Dominant Roles in ASC Stimulation of Endothelial Migration and Proliferation

Stephanie Merfeld-Clauss; Brian Johnstone; Keith March

### Introduction:

Introduction: It is becoming increasingly apparent that paracrine support is the predominant mechanism by which many stem cell types promote recovery and repair of injured tissues. However, it is not known which of the many factors secreted by these cells are important for the positive effects seen in animal models of various human diseases and injuries. We are addressing this question in adipose stem cells (ASC) by systematically inhibiting the expression and activity of growth factors secreted by ASCs. In this study we addressed the consequences of specific factor depletion on the ability of ASC conditioned medium (ASC-CM) to promote mature and progenitor endothelial cell (EC) recruitment and proliferation

### Methods:

Methods: Cell-free ASC-CM was collected after growth for 72 hours in basal medium. Neutralizing antibodies were added to ASC-CM to inactivate either VEGF, hepatocyte growth factor (HGF), HGF receptor (c-Met), monocyte chemoattractant protein-1 (MCP-1), and insulin-like growth factor-1 (IGF-1). Untreated or treated ASC-CM were then assayed for the ability to stimulate chemoattraction and proliferation of human endothelial progenitor cells (EPC) and mature microvascular EC (HMVEC). For proliferation, growth of EPC over 4 days was assessed in basal medium by itself or with a 1:1 mixture of ASC-CM that had been pretreated with antibodies to the specific factors. Migration assays were performed using a transwell system, placing cells in basal media in the upper chamber and 1:1 mixture of ASC-CM/basal medium in the lower chamber.

### Results:

Results: Untreated or control antibody-treated ASC-CM stimulated EPC migration by  $73.5 \pm 2.8\%$  over basal medium alone ( $p < 0.00015$ ). Neutralization of VEGF in ASC-CM reduced EPC migration by  $37.4 \pm 3.9\%$  ( $p < 0.0015$ ), whereas pre-incubation with neutralizing HGF antibodies completely blocked migration. This effect was confirmed by performing the migration after inactivating c-Met on EPC. Simultaneous neutralization of both HGF and VEGF resulted in migration below baseline. Although it has been reported that EPC express the CCR2 receptor for MCP-1, no migration towards purified MCP-1 was observed. Only minor effects were observed with IGF-1 neutralization. In EPC proliferation assays, neutralizing either VEGF or HGF in ASC-CM blocked EPC proliferation by  $26.99 \pm 0.03\%$  and  $32.19 \pm 0.05\%$ , respectively ( $p < 0.00012$  and  $p < 0.00015$ ). Pre-treatment simultaneously with both antibodies reduced proliferation to levels observed with basal medium only. Similarly, neutralizing VEGF or HGF in ASC-CM reduced EC proliferation. These effects were most pronounced with neutralizing HGF  $49.7 \pm 0.03\%$  ( $p < 3.3 \times 10^{-12}$ ).

### Conclusions:

Conclusion: ASC secrete many chemoattractant and mitogenic factors; remarkably, these activities in ASC-CM require predominantly HGF and VEGF. Both of these factors are known to have potent activities in this regard. It may be, however, that the therapeutic potential of ASC could be enhanced by inducing (either genetically or otherwise) secretion of additional complementary factors or, conversely, by attenuating factors which are inhibitory toward angiogenic processes.

## **Mammospheres generated from human mammary tissue are derived from the adipose fraction of the organ and do not contain luminal epithelial mammary progenitors.**

Elodie Bachelard-Cascales; Marion Chapellier; Emmanuel Delay; Thibault Voeltzel; Veronique Maguer-Satta

### **Introduction:**

The human mammary gland is a tubulo-alveolar gland composed of a series of branched ducts that drain sac-like alveoli. These ducts and alveoli are composed of two general lineages of epithelial cells: the luminal cells and the underlying smooth muscle-like myoepithelial cells. In normal human breast, the functional structure is organized as an inner layer of luminal epithelial cells and an outer layer of myoepithelial cells, delimited by a basement membrane. These structures are surrounded by a microenvironment mainly constituted by collagenous matrix, stroma and adipose tissue. The environment composition evolves through time as for example the proportion of adipose tissue in breast is highly variable but becomes predominant with age.

### **Methods:**

In mice breast, previous observations have demonstrated the presence of phenotypically distinct progenitor and non-progenitor epithelial cell subpopulations. Subsets of progenitors include the bipotent progenitor (EpCAM+MUC1-CD49f+CD10+) which can generate both luminal and myoepithelial cells, and the luminal-restricted progenitor (EpCAM+MUC1+CD49f+CD10-). These distinct subpopulations can be isolated from normal mammary tissue or from mammary epithelial cell cultures and their growth and differentiation potential can be monitored by their ability to form colonies when plated at low density in vitro. We adopted the same strategy to isolate bipotent- and luminal-restricted progenitors from human normal mammary samples.

### **Results:**

We then observed the presence of different subpopulation that expresses very high CD10 along very low EpCAM and Vice-versa. In order to identify the biological properties of these human CD10 or EpCAM expressing subpopulations, we sorted them separately by flow cytometry and analyzed their biological properties according to phenotype, ability to form mammary colonies and to generate "mammospheres", previously described as an indicator of their "stem cell" like status. Only CD10+ subpopulation (very low EpCAM+) was able to form "mammospheres" but did not contain luminal progenitors, whereas the EpCAM+ (CD10-) fraction contained mammary colonies but were unable to form spheres. Studies have demonstrated that adipose tissue from various body sites contains a Stroma Vascular Fraction (SVF) constituted by multipotent Mesenchymal Stem Cells (MSCs) which can form "spheres" in presence of EGF (Epithelial Growth Factor). More over a common feature between mammary primary cells and SVF fraction of the adipose tissue is the presence of a large fraction of cells expressing the CD10 antigen which is considered in the SVF as a "stem cell marker". We then wondered if "mammosphere" generated from human mammary tissue might derive from the adipose fraction of the organ. To address this question we compared the phenotype of cells isolated from abdominal or mammary (at the border of the gland) lipo-aspirates versus human breast. We sorted CD10+ from the three different tissues and compared their capacity to form "spheres" and E-CFUs (Epithelial Colony forming unit). A transcriptional analysis was also performed on SVF primary cells or derived "spheres" and compared to results obtained with mammary cells.

### **Conclusions:**

For the first time, our results argue for the fact that mammary adipose microenvironment could constitute a reservoir of a wider variety of stem cells than expected, beyond of maintenance of tissues integrity and cell growth regulation. [maguer@lyon.fnclcc.fr](mailto:maguer@lyon.fnclcc.fr)

## Adipose Tissue Derived Cells and Articular Chondrocytes Combined with Injectable Gellan Gum Hydrogels in the Treatment of Full Thickness Articular Cartilage Defects in Rabbits

João Oliveira

### Introduction:

João T Oliveira<sup>1,2</sup>, Leandro Gardel<sup>3</sup>, Luís Martins<sup>4</sup>, Tommaso Rada<sup>1,2</sup>, Manuela E Gomes<sup>1,2</sup>, Rui L Reis<sup>1,2</sup> 1 3B's Research Group - Biomaterials, Biodegradables, and Biomimetics, Department of Polymer Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal 2 IBB- Institute for Biotechnology and Bioengineering, PT Associated Laboratory 3 Department of Clinical Veterinary, Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal 4 Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal Introduction The field of cartilage tissue engineering is one of the most prominent in the regenerative medicine area. Although the cartilage tissue is somehow simple in its structural organization, the generation of a fully functional substitute is a difficult task and until now no efficient system or technology has been able to completely deal with this problem. Our group has originally proposed the use of gellan gum hydrogels for the regeneration of damaged cartilage tissue. Gellan gum is an extracellular microbial polysaccharide produced by *Sphingomonas paucimobilis* which forms stable hydrogels in the presence of cations. It is not cytotoxic and can be injected into the body in a minimally invasive manner, delivering cells and/or bioactive agents to a defect site. In this work, we tested the in vivo performance of injectable gellan gum hydrogels combined with adipose tissue derived cells (with and without chondrogenic pre-differentiation) in comparison to injectable gellan gum hydrogels combined with articular chondrocytes. These systems were used in the treatment of full thickness articular cartilage defects in New Zealand white rabbits.

### Methods:

Gellan gum was dispersed in a water solution and heated at 90°C. The temperature was lowered to 42°C and kept stable under constant stirring. Full thickness cartilage knee defects (Ø 4 mm x 2-3 mm thickness) with subchondral drill (1 mm thickness) were created in the femoral condyles of New Zealand white rabbits. The gellan gum solution was mixed with the following groups and injected in the defects: a) rabbit adipose tissue derived cells pre-differentiated; b) rabbit adipose tissue derived cells; c) rabbit articular chondrocytes; d) gellan gum alone (no cells); e) empty defect. These cells were isolated from the same animals where they would be implanted, being this study conducted in an autologous approach. The implants were kept for periods of up to 8 weeks. At the defined time points, explants were retrieved from the defects and characterised. Histology was performed using hematoxylin-eosin (H&E) for general cells observation, and alcian blue staining for proteoglycans identification. Pineda scoring system was conducted by three independent individuals to evaluate the quality of the new formed tissue. Realtime PCR analyses quantified the up and downregulation of Sox9, collagen type I, collagen type II, and aggrecan during the course of the experiments.

### Results:

The histological analysis performed with H&E staining showed homogeneous distribution and normal cell morphology, as well as good lateral integration in the native cartilage in most cases. The Pineda scoring system retrieved the best results for adipose tissue derived cells with chondrogenic pre-differentiation with a continuous increase in the quality of the formed tissue up to 8 weeks. Both adipose tissue derived cells without chondrogenic pre-differentiation and articular chondrocytes presented the same increasing tendencies in newly formed cartilage tissue quality, although in a more moderate fashion. Alcian blue staining revealed metachromatic staining in adipose tissue derived cells groups and articular chondrocytes mostly in the last time points. Realtime PCR analyses were in accordance with these observations evidencing collagen type II and aggrecan upregulation in both subsets of adipose tissue progenitor cells. Articular chondrocytes exhibited a downregulation on both markers by the end of the experiments, although the absolute values were higher compared to those of adipose derived cells.

### Conclusions:

Adipose tissue derived cells appear to perform as well as articular chondrocytes in the regeneration of cartilage tissues when combined with injectable gellan gum hydrogels. Cell distribution and morphology was comparable to that observed for articular chondrocytes and the Pineda histological score retrieved the best results for adipose tissue derived cells with chondrogenic pre-differentiation. Proteoglycans identification using alcian blue staining was similar between the studied systems. The quantification of cartilage associated markers, mainly collagen type II and aggrecan reinforced these facts. In conclusion, it can be stated that these gellan gum-adipose tissue derived cell systems may constitute a potential candidate for future tissue engineering clinical applications in the field.

## DIFFERENTIATION OF ADIPOSE DERIVED STEM CELLS INTO SCHWANN-LIKE CELLS IS NOT MEDIATED BY THE NOTCH SIGNALLING PATHWAY

Paul Kingham; Giorgio Terenghi

### Introduction:

Adipose derived stem cells (ASC) offer great potential for clinical applications due to their easy accessibility and multi-lineage differentiation properties. Recently we have shown that these cells can be differentiated into functional Schwann-like cells which could potentially be used to repair injured peripheral nerves. Notch signalling in the neural crest has been suggested to play a role in the development of Schwann cells. In this study, we have therefore investigated the expression of components of the notch signalling pathway in ASC and determined whether it is important for differentiation of the cells into a Schwann-like phenotype.

### Methods:

ASC were isolated from rat visceral fat and differentiated with a mixture of glial growth factors (GGF-2, bFGF, PDGF and forskolin) for a period of two weeks. Using RT-PCR, notch-1, jagged-1 and the notch responsive hes-1 gene expressions were determined at key stages of differentiation. ASC were treated with N-[N-(3,5-Difluorophenacetyl-L-alanyl)-S-phenylglycine t-butyl ester (DAPT), a gamma-secretase inhibitor which blocks notch signalling. The effect of DAPT on ASC differentiation was examined by using immunocytochemistry and western blot analysis of glial cell markers, S100 and GFAP, and the ability of the differentiated Schwann-like cells to promote neurite outgrowth.

### Results:

ASC express notch-1 throughout the differentiation process but at markedly lower levels than found in Schwann cells isolated from adult rat sciatic nerve. Jagged-1 was expressed at high levels in control ASC but was down-regulated progressively through the differentiation process. Hes-1 was expressed in both control and differentiated ASC. DAPT treatment of differentiating ASC did not affect the expression levels of either S100 or GFAP proteins. When co-cultured with a motor neuron cell line (NG108-15), differentiated ASC cultures treated in the absence or presence of DAPT both enhanced neurite outgrowth. Compared with control NG108-15 neurons the average neurite length ( $n = 3$ ) was increased from  $41.04 \pm 7.60\mu\text{m}$  to  $147.7 \pm 9.44\mu\text{m}$  (differentiated ASC;  $p < 0.001$  by one-way ANOVA) or  $133.5 \pm 1.26\mu\text{m}$  (differentiated ASC plus DAPT;  $p < 0.001$ ).

### Conclusions:

ASC express components of the notch signalling pathway but there is no evidence to suggest its involvement in the differentiation of ASC into Schwann-like cells.



## EFFECT OF ADIPOSE DERIVED STEM CELLS ON PERIPHERAL NERVE REGENERATION

Daniel Kalbermatten; Pietro di Summa; Daniel Espinoza; Daniel Egloff; Wassim Raffoul; Mikael Wiberg; Giorgio Terenghi; Paul Kingham

### Introduction:

Traumatic injuries resulting in peripheral nerve lesions often require a graft to bridge the gap. Although autologous nerve autograft is still the first choice strategy in reconstructions, it has the severe disadvantage of the sacrifice of a functional nerve. Cell transplantation in a bio-artificial conduit is an alternative strategy to create a favourable environment for nerve regeneration. With this aim we analyzed effects of various regenerative cells in two different nerve conduits.

### Methods:

We decided to test fibrin as a new conduit material compared with collagen, which is currently used in the clinical practice in case of nerve lesions. The nerve conduits were constructed to treat a 1cm sciatic nerve gap injury in rats. The two materials were tested both empty and in combination with different types of regenerative cells including Schwann cells and both bone marrow derived and adipose derived stem cells (ASC). The stem cells were differentiated into a Schwann cell phenotype in vitro using a mixture of glial growth factors (neuregulin, bFGF, PDGF and forskolin). Two weeks after implantation, conduits were harvested and examined by immunohistochemistry using the axonal marker PGP 9.5 and Schwann cell marker S100.

### Results:

Preliminary data from in vivo analysis at two weeks post-operatively showed a positive tendency in the fibrin conduit group seeded with differentiated ASC compared with the fibrin alone. Axonal regeneration distance was  $4.74 \pm 0.24$  mm vs.  $4.16 \pm 0.06$  mm and Schwann cell intrusion  $4.79 \pm 0.18$ mm vs.  $4.17 \pm 0.06$ mm respectively. Bone marrow derived stem cells gave similar values to ASC. The greatest effect was from Schwann cells which significantly ( $p < 0.01$  by one way ANOVA) enhanced regeneration ( $5.75 \pm 0.15$ mm (PGP9.5) and  $5.85 \pm 0.15$ mm (S100)). In contrast to the fibrin conduits, cells seeded in collagen conduits did not enhance regeneration.

### Conclusions:

These initial findings show a positive role of differentiated ASC in fibrin conduits. Differences between fibrin and collagen's structure may be responsible of the variation of results when seeding with cells. It will be of great importance to evaluate the target organ function in longer term studies.

## Potential of adipose tissue-derived stem cells compared to bone marrow-derived stem cells for cell transplantation therapies against cerebral ischemia

Yuka Ikegame; Kentaro Yamashita; Shinichiro Hayashi; Fukka Yo; Shigeru Nakashima; Hiroshi Mizuno; Shinichi Yoshimura; Toru Iwama

### Introduction:

Transplantation of bone marrow-derived or adipose tissues-derived Mesencymal stem cells (MSC) has been shown their ability to attenuate the functional deficits after brain ischemia. Among them, adipose tissue-derived stem cells (ADSC) and bone marrow-derived stem cells (BMSC) are now increasingly expected as a promised stem cell source. Thus, we compared therapeutic potential of these MSC for preservation and recovery from tissue damages under cerebral ischemia.

### Methods:

ADSC and BMSC were prepared from C57BL/6J mice. We analyzed proliferative activity of these cells, and also measured concentrations of angiogenic growth factors in the cell culture supernatants by ELISA. The left middle cerebral artery (MCA) occlusion was made in age-matched 8weeks C57BL/6J male mice, and the ADSC or BMSC were injected via tail vein just after the occlusion. Assessment of ischemic damage was performed after 90 min occlusion followed by 24 h reperfusion.

### Results:

ADSC showed higher proliferative activity than BMSC in vitro. In addition, ADSC could produce higher concentration of vascular endothelial growth factor (VEGF) than BMSC, while concentration of Angiopoietin1 (ANGPT1) in ADSC was slightly lower than that in BMSC. Interestingly, ADSC transplanted group resulted in better physiological and pathological recovery than BMSC transplanted group. It is also note worthy that a few cases of hemorrhagic infarction occurred in ADSC group, suggesting that balanced expression of VEGF and ANGPT1 need for ADSC transplantation against cerebral infarction.

### Conclusions:

ADSC preparation is easier than BMSC. ADSC also exhibit superior efficiency for preserving brain tissue integrity and function after cerebral ischemia than BMSC. These results indicate that ADSC can be a more useful source in cell transplantation than BMSC. Alternatively, our study suggest the possibility that modulation of VEGF or ANGPT1 expression in ADSC will be necessary for a safer cell therapy against ischemic brain diseases.

## Estrogens exert a site-specific influence on lymphocyte distribution in adipose tissues

Sylvie Caspar-Bauguil

### Introduction:

Obesity is characterized by a chronic low-grade inflammation resulting, in a large part, from the infiltration of adipose tissues by inflammatory cells. In addition to macrophages, different lymphocyte subpopulations have been recently identified in adipose tissue. As described in mouse models, most of them are characterized by an ancestral phenotype, namely natural killer cells (NK), natural killer T (NKT) and gamma/delta T (gdT) lymphocytes, and their number in adipose tissue is highly altered in obesity state. Interestingly, beside their pivotal role in sexual development and reproduction, the sexual hormones estrogens influence numerous physiological and pathophysiological processes, including body fat distribution and immune responses. Since estrogens have been demonstrated to influence lymphocyte biology, and especially the number and functions of NKT, the present study aimed to analyze the effect of 17 $\beta$ -estradiol (E2) administration on the distribution of lymphocyte subpopulations in adipose tissues.

### Methods:

To determine the effect of *in vivo* chronic estrogens administration, 4 week-old C57Bl/6 female mice were subcutaneously implanted with time-release pellets delivering either E2 (0.25 mg/60 days) or a placebo, and maintained on a normal chow diet. After a 6 weeks treatment period, mice were sacrificed, and white adipose pads (subcutaneous inguinal and deep perigonadic) were dissected and digested with collagenase. Lymphocyte content was analyzed by flow cytometry in non adipocytic fraction, called stroma-vascular fraction, using specific surface markers.

### Results:

Although the total body weight was not influenced, E2 administration led to a significant reduction in both subcutaneous inguinal and perigonadic adipose pad weights (-41% and -33% respectively), as compared to control mice. Analyses of lymphocyte subpopulations in the stroma-vascular fractions revealed a site-specific influence of E2 administration. Indeed, no significant change was observed in subcutaneous inguinal adipose tissues. In contrast, in perigonadic adipose tissue, percentages of NK and NKT cells were significantly decreased, whereas percentages of gdT lymphocytes were significantly increased in E2-treated mice as compared to control mice. Finally, the percentages of alpha/beta T and B lymphocytes were not altered by E2 treatment.

### Conclusions:

Extending the current knowledge on the influence of estrogens on the immune system, the present study indicates that chronic exposure to E2 *in vivo* alters the distribution of ancestral lymphocyte subpopulations in deep adipose tissues. Whether this specific effect contributes to the beneficial effects of estrogens on body fat distribution and insulin sensitivity remains to be determined in future studies.

## Adipose Skin Equivalent Model: advantage of using Preadipocytes instead of mature adipocytes

Charlotte Lequeux; Ali Mojallal; Celine Auxenfans; Lauriane Thivillier; Valérie André; Odile Damour

### Introduction:

Since the first monolayer cultivation technique of keratinocytes and its clinical applications, tissue engineering has evolved [1, 2]. In recent years, the three-dimensional reconstruction of adipose tissue from preadipocytes (only cell of the adipose tissue able to differentiate) seeded in different types of scaffold has become an attractive axis of research in plastic surgery [3-5]. In view of these two areas of tissue reconstruction, it seemed attractive to combine these two types of culture. That would spread the clinical applications and could be used for pharmacotoxicological tests. In this paper, we presented a new adipose skin equivalent (ASE) model based on collagen-glycosaminoglycan (CGC) scaffold [6] and we compared results obtained with ASE prepared either with mature adipocytes or with preamplified preadipocytes.

### Methods:

ASE model is prepared as followed: collagen-glycosaminoglycan (CGC) scaffold were seeded with both fibroblasts and either freshly extracted mature adipocytes or preadipocytes (from 3 donors in triplicate). After 3 weeks culture in a fibroblast medium supplemented with bFGF, keratinocytes were seeded at the top of the model and cultured one week in submerged condition before elevation at air liquid interface and cultured for 2 more weeks. ASE Characterization used i) histology: Oil Red O staining, and ii) radio immune assay: adiponectin and leptin secretion was quantified.

### Results:

Our ASE model allows the differentiation of preadipocytes, into mature adipocytes stained by Oil Red O and secreting adiponectin and leptin (figures 1a, b). Culture conditions allow the development of a nice ASE model with both a dermal part where extracellular matrix fills the porous structure of the scaffold and a pluristratified and differentiated epidermis. Freshly extracted mature adipocytes survived the 42 days culture in our SE model, but the neosynthesised extracellular matrix (ECM) appears very poor leading the keratinocytes penetration into the dermis (figures 1c and d) but kept their capacity to synthesise leptin and adiponectin.

### Conclusions:

We proof that it is possible to prepare ASE with preamplified preadipocytes. Mature adipocytes can survive in this model during 41 days but they inhibit the development of fibroblasts and their ECM component synthesis as if they were cytotoxic. So we can recommend the use of preadipocytes for cell-cell or cell-matrix interactions studies. Moreover, the possibility of doing preadipocytes amplification and freezing them, are more suitable and could allow a better reproducibility. Their capacity to differentiate into mature adipocytes was proved by Oil red O staining both in vitro in our ASE model and in vivo on nude mice 2 month after grafting ( in press). Preadipocytes amplification able to differentiate in mature and functional adipocytes open perspective in adipose tissue deficiencies. ASE bring a new model cells for sliming active substance testing. References 1. Braye, F., et al., [Cultured epidermis for the treatment of severe burns. A 2-year study (18 patients)]. *Ann Chir Plast Esthet*, 2001. 46(6): p. 599-606. 2. Rheinwald, J.G. and H. Green, Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell*, 1975. 6(3): p. 331-43. 3. Flynn, L., et al., Adipose tissue engineering with naturally derived scaffolds and adipose-derived stem cells. *Biomaterials*, 2007. 28(26): p. 3834-42. 4. Hemmrich, K., et al., Implantation of preadipocyte-loaded hyaluronic acid-based scaffolds into nude mice to evaluate potential for soft tissue engineering. *Biomaterials*, 2005. 26(34): p. 7025-37. 5. Torio-Padron, N., et al., Engineering of adipose tissue by injection of human preadipocytes in fibrin. *Aesthetic Plast Surg*, 2007. 31(3): p. 285-93. 6. Auxenfans, C., et al., [Porous matrix and primary-cell culture: A shared concept for skin and cornea tissue engineering.]. *Pathol Biol (Paris)*, 2008.

## Transplantation Of Human Adipose Tissue-Derived Mesenchymal Stem Cells Is Associated With Functional Improvement In Mouse Acute Myocardial Infarction Model.

Sang Hong Baek; Seung Hyun Choi; Dongchoon Ahn; Jichang Yoo; Sung Cil Lim

### Introduction:

Introduction: Cell transplantation is a new developing treatment modality to improve cardiac function in ischemic damaged heart. We have investigated to determine the effect of transplantation of human adipose tissue-derived mesenchymal stem cells (hAdMSC) in mouse acute myocardial infarction model.

### Methods:

Methods: Thirty-three nude mice underwent left coronary artery ligation and received by either intravenous injection or direct intramyocardial injection into the peri-infarct region 30 minutes after permanent myocardial infarction with hAdMSC. The control group was treated with saline. Heart function was assessed by echocardiography. Cell engraftment, differentiation were evaluated with lentivirus-eGFP transfected hAdMSC. Angiogenesis and fibrosis in the scar tissue were also evaluated by immunohistochemistry and immunofluorescence. Measurements of LV pressure, dP/dt will be measured with Millar catheter.

### Results:

Results: Three weeks after cell transplantation, hAdMSC induced a significant improvement in heart function; LVEF(46.7 +/- 6.3 % v.s. 43.6 +/- 7.2 % v.s. 30.0 +/- 8.0 %; im v.s. iv v.s. control) and LVFS ( 20.8 +/- 3.0 % v.s. 18.8 +/- 3.8 % v.s. 11.9 +/- 3.4 % %; im v.s. iv v.s. control). An increase in the degree of angiogenesis and a decrease in fibrosis were also detected. Transplanted cell engraftment and differentiation to endothelial cells were detected in peri-infarcted area.

### Conclusions:

Conclusion: These results indicate that direct intramyocardial injection of hAdMSC in acute infarct provides a superior benefit for functional improvement.

## Enhanced Adipogenic Differentiation of Adipose Stem Cells Cultured as 3-Dimensional Multicellular Aggregates

Hulan Shang; Adam Katz

### Introduction:

ASCs have a clear capacity for adipogenic differentiation, and therefore have potential to provide insights and solutions to both soft tissue reconstruction and obesity-related challenges. Since 3-dimensional (3-D) cultures more likely reflect *in vivo* conditions than “traditional” monolayer cultures, we hypothesized that ASCs cultured as 3-D multicellular aggregates (MAs) would demonstrate more robust adipogenic differentiation than ASCs in monolayer. Additionally, we hypothesized that ASC MAs would permit adipogenic differentiation under defined, serum-free conditions.

### Methods:

Human ASCs were isolated using established techniques and initially plated in growth factor enriched medium with 1% human serum (LADPM-1%). Cells were subsequently expanded as monolayers in serum free medium (LADPM). 3-D multicellular aggregates (MAs) were fabricated and placed into suspension culture. Parallel groups of cells were plated in similar numbers into adherent monolayer (ML) culture. Both MA and ML ASCs were placed in either serum free medium (LADPM) or “traditional” DMEM/F12+10%FBS culture medium (D10). Differentiation was initiated by adding adipogenic supplements (Dexamethasone, Insulin, Isobutyl-methylxanthine (IBMX) and Indomethacin) to each of the base medias (LADPM, D10). After two weeks in adipogenic conditions, cells were evaluated by Oil Red-O staining for intracellular lipid and by quantitative RT-PCR for lineage-related genes (LPL, PPAR $\alpha$ , and FABP).

### Results:

After two weeks of culture in D10 medium with adipogenic additives, abundant Oil Red-O staining was identified in both monolayer and 3D cultures. However, ASCs in 3-D (MA) culture demonstrate twice the levels of adipogenic gene expression than parallel cells in 2-D (monolayer) culture. After two weeks of culture in completely defined serum free adipogenic medium, ASCs MAs demonstrate 5 times the levels of adipogenic gene expression than ASCs in 2-D culture. However, gene expression levels are notably lower in LADPM medium relative to D10 culture conditions. Oil Red-O staining reflects these gene expression findings, being minimal after 2 weeks in serum free LADPM, but becomes readily apparent after 6 weeks in inductive medium.

### Conclusions:

ASCs cultured as 3-D MAs display more robust adipogenic potential than ASCs in monolayer culture, whether in serum-containing or serum-free conditions. Furthermore, 3-D MA culture permits adipogenic differentiation in completely defined, serum-free conditions, and without the addition of synthetic or exogenous matrix factors/scaffolds. These findings have implications for the mechanistic study of adipogenic differentiation, as well as for translational therapeutic objectives.

## Genomic and Proteomic Characterization of Human ASCs Grown in 3-D Suspension Culture

Sahil Kapur; Hulan Shang; Stefan Bekiranov; Adam Katz

### Introduction:

Emerging evidence supports the therapeutic potential of Adipose-Derived Stem Cells (ASCs) in the healing of cutaneous wounds. Using a murine model of delayed diabetic wound healing, our team has demonstrated enhanced in vivo potency of ASCs formulated as 3-dimensional multicellular aggregates (MAs), as compared to ASCs grown as adherent monolayers and delivered as single cell suspensions. The purpose of this study was to elucidate transcriptional and translational differences between the two cell formulation strategies that may provide mechanistic insights into the basis for our in vivo findings.

### Methods:

Human ASCs were isolated and plated using established techniques. Cells were expanded in adherent monolayer culture in growth factor enriched medium with 1% human serum (AR8-1%). After sufficient expansion, half of the cells were formed into 3-D MAs and maintained in suspension culture, and half were maintained in adherent monolayer culture. On day 6, cells or culture medium from each group were harvested and analyzed by one of three techniques depending on the specific experiment: 1) microarray analysis (3 donors); 2) ELISA analysis (1 donor); and 3) mass spectrometry (1 donor).

### Results:

Microarray analysis revealed the statistically significant upregulation of at least 85 genes by a factor of 2 fold or greater ( $p < 0.05$ ). Upregulated genes included IGF-1 (57x), BGN (21x), IGFBP (31x), PDGF (16x) VCAM-1(14x) MMP-1(14x), TNC(12x), and HGF (11x) among others. On categorizing these genes, we found gene expression patterns demonstrating a profile reflective of tissue repair, ECM remodeling, wound healing, and angiogenesis. We also observed an upregulation in skin/hair follicle stem cell markers that include Sox9 (8x), TCF3 (2x), and NFATc (1.5x). ELISA and mass spectrometry analysis confirmed the translational upregulation of most of these genes.

### Conclusions:

ASCs prepared as 3-D MAs statistically up-regulate the expression of many important factors involved in wound healing and tissue repair, including soluble growth factors and extracellular matrix proteins. Given the emerging evidence for the synergistic bio-activity of ECM-growth factor complexes, these findings may help explain the enhanced potency of 3-D MAs that we have observed in related in vivo studies.



## Stromal Vascular Fraction derived from adipose tissue contains hematopoietic potential in vivo

Jinah Han; Gou Young Koh

### Introduction:

Adipose tissue consists of lipid-filled adipocytes and stromal cells called stromal vascular fraction (SVF). SVF is composed of heterogeneous cell populations including preadipocytes, pericytes, endothelial cells, immune cells and multipotent stem cells. However, little is known about existence of hematopoietic stem and progenitor cells (HSPC) in SVF. Recent reports reveal that HSPC is found not only in bone marrow (BM) but other non-lymphoid organs such as muscle and thoracic duct. In terms of cellular characteristics, SVF shares similarities with BM stromal cells. Based on this idea, we have sought whether adipose tissue contains HSPC (SVF-HSPC) and SVF-HSPC is capable of maintaining hematopoietic homeostasis in vitro and in vivo.

### Methods:

SVF was obtained from the epididymal adipose tissues by collagenase digestion. They were analyzed by FACS for expression of lineage-, Sca-1+, c-Kit+ (LSK) which are general markers for HSC. In vitro hematopoietic activity was evaluated by culturing SVF in methylcellulose based hematopoietic differentiating medium. In vivo activity of SVF-HSPC was determined by GFP+SVF (5x10<sup>6</sup> cells) transplantation into irradiated recipient mice. GFP+BM transplanted mice were analyzed to ensure origin of SVF-HSPC.

### Results:

FACS analysis revealed that SVF contained ~0.004% LSK cells versus BM contained ~0.05%. When they were cultured, SVF gave rise to various types of hematopoietic colonies, including BFU-E, CFU-E, CFU-GM, CFU-GEMM, versus BM developed ~150 times more colonies than SVF. In vivo hematopoietic activity was confirmed by the transplantation experiments with GFP+ mice. At 24 hours after transplantation, SVF successfully homed to BM of recipient ~1% versus BM ~10% homing frequency. The difference of homing ability between SVF and BM was matched to that of LSK frequency. Transplanted SVF was able to repopulate the circulating system of the irradiated mice up to 40% at 20 weeks, albeit BM achieved 90%. SVF-derived cells were capable of differentiating into each lineage of hematopoietic cells including T, B lymphocytes and myeloid cells in vivo. Additionally, SVF-HSPC was originated from BM.

### Conclusions:

This study indicates that SVF possesses BM-derived HSPC. Hence, SVF could be other alternative source of HSPC for therapeutic applications in patient with hematologic diseases.

## Comparative characterization of adipose-derived stromal cells isolated with the TGI 1000 automated method versus a standardized manual method

Michelle Stein; Joon Paek; Anthony Yang; Shannon Iwami; Jennifer Cannon; Renia Sylvester; Elyse Ryan; Erik Vossman; Eugene Boland; Stuart Williams; Paul Kosnik

### Introduction:

A growing body of literature indicates the importance and utility of stem and progenitor cells for regenerative medicine and tissue engineering applications. Adipose tissue provides an abundant, readily-accessible source of stromal cells without donor site morbidity or the need to expand or culture the cells. Fully realizing the potential of stem cell-based therapies will partly hinge on integrating the process of isolating and concentrating such cells in a safe, efficient, reliable manner that is clinically convenient. Tissue Genesis has developed an automated cell isolation system (TGI 1000) that digests lipoaspirated tissue and delivers concentrated adipose-derived stromal cells (ASCs) in a syringe for autologous point-of-care use. The purpose of this study was to characterize and compare the cells delivered by the automated system with the same pellet of cells obtained using a manual laboratory method.

### Methods:

Each donated human lipoaspirate sample was divided into two groups and either digested, centrifuged, and washed using the TGI 1000 automated system or using a standardized manual method. Cell yield and viability of the subsequent cell suspensions were determined. Flow cytometry analysis with CD34, CD31, and CD45 allowed the characterization of each cell suspension. Finally, each final cell suspension was assayed for endotoxin concentration, residual collagenase activity and sterility.

### Results:

Our results demonstrated no statistically significant differences between the means of the automated and manual methods with regard to cell viability ( $81.1 \pm 3.16\%$ ), endotoxin level ( $0.195 \pm 0.05$  EU/ml), collagenase activity ( $2.1 \pm 0.751$  U/ml) and sterility (3-day and 14-day sterility maintained). Cell yield was lower using the automated system ( $0.5 \times 10^6$  cells/g fat) as compared to the manual method ( $1.16 \times 10^6$  cells/g fat) due to residual fluid losses in the automated system. The heterogeneous population of cells from both manual and automated processing is composed of stem-like cells and endothelial-like cells.

### Conclusions:

The TGI 1000 automated cell isolation platform is an effective device for isolating ASCs from human lipoaspirate. Digestion of lipoaspirate using this device resulted in a similar cell population as tissue digested using a standardized manual laboratory method. The results indicated that cell viability, endotoxin level, residual collagenase activity, sterility and marker presence were identical among the isolation methods. ASCs, isolated and concentrated in a rapid, safe, reliable manner in the operating-room compatible TGI 1000 automated device may serve to advance the fields of regenerative medicine and cell-based therapies.

## Adipose-Derived Stem Cells Self-Aggregate Through Surface Divalent Cation and Protein-Mediated Adhesion

Clayton Smith; Brian Johnstone; Keith March

### Introduction:

**Introduction** We recently determined that administration of autologous porcine adipose stromal vascular fraction (SVF) cells directly into normal hearts via intracoronary artery delivery resulted in significant elevation of cardiac damage markers, increased incidence of arrhythmia, and histological evidence of regional ischemia. Visual inspection before administration indicated the occurrence of time-dependent aggregation of cells within SVF, which may have created micro-emboli within the coronary vasculature. Empirical tests with readily available agents suggested that aggregation and subsequent microinfarctions could be substantially diminished by pre-treating SVF with heparin before infusion. This study aimed to identify factors mediating aggregation as well as to define agents suitable for use in humans which may block this phenomenon.

### Methods:

**Methods** SVF was isolated from either excised dorsal hump (porcine) or lipoaspirated subcutaneous (human) adipose tissues using reagents and protocols supplied by Tissue Genesis Inc. (TGI). Stromal cells, containing adipose-derived stem cells (ASC), were selected by attachment to and growth on uncoated tissue culture plastic. Low passage (P1-P4) cultured ASCs were detached from plastic by 2 mM EDTA treatment. Fresh SVF or cultured ASCs were incubated for increasing times in TGI Suspension Medium under varying conditions: (1) 2 mM EDTA, (2) 2 mM EDTA followed by 7 mM calcium and magnesium, (3) 25, 100 or 400 g/ml heparin sulfate, (4) the factor X inhibitor fondaparinux (4 g/ml), and (5) a neutralizing antibody to tissue factor. In some experiments ASCs were first subjected to brief (3 minute) treatment with trypsin. Cell suspensions were observed macroscopically, noting aggregate size and number, as well as microscopically, to quantitate aggregates and individual cells.

### Results:

**Results** Both human- and porcine-derived fresh SVF and cultured ASCs aggregated within 30 minutes after being placed in suspension. Pre-treatment of ASCs with trypsin prevented aggregation of both SVF and ASCs. Aggregation of both SVF and ASCs was reversibly inhibited in the presence of EDTA. Heparin also inhibited aggregate formation of both SVF and ASCs in a concentration-dependent manner; while, fondaparinux, a non-ionic heparin analog that is specific for factor X, and a blocking antibody to tissue factor both had no effect.

### Conclusions:

**Conclusions** This study demonstrates that ASCs possess an aggregative property that may limit their clinical safety and utility if not controlled properly. Heparin is a promising candidate to inhibit aggregation in this context. Aggregation is mediated by proteinaceous cell surface factors and requires divalent cations, but which are distinct from classic coagulation pathways. The identity of the surface factor(s) responsible for promoting aggregation is currently under investigation. Candidate proteins could be proteoglycans or other proteins, which mediate adhesion amongst cells as well as between cells and extracellular matrices.

## **Proliferative effects of combined use of PRP and FGF-2 on human adipose-derived stem cells and fibroblasts**

Satoshi Kushida; Natsuko Kakudo; Kenji Suzuki; Kenji Kusumoto

### **Introduction:**

It has been shown that platelet-rich plasma (PRP) and FGF-2 singly promote cell growth, thereby greatly contributing to wound healing. In recent years, they have been actively applied clinically. In this study, we examined whether their combined use synergistically promotes wound healing by human adipose-derived stem cells (ASC) and fibroblasts (Fb).

### **Methods:**

PRP was prepared from human blood using the double-spin method, and activated PRP (aPRP) was prepared by activating PRP with self thrombin and CaCl<sub>2</sub>. The concentrations of growth factors (TGF- $\beta$ , PDGF, and FGF-2) in PRP were measured by ELISA. aPRP and FGF-2, singly or in combination, were added to serum-free medium to culture ASC or Fb, which had been cultured and isolated from human adipose tissue and dermis, respectively. The cells were evaluated for growth, collagen production, and collagen gel contraction.

### **Results:**

The addition of aPRP and FGF-2 singly at optimal concentrations promoted maximal cell growth, and that of higher concentrations gradually reduced cell growth promotion. The combined addition of aPRP and FGF-2 promoted maximal cell growth, which exceeded the level of growth that occurred after the addition of aPRP or FGF-2 alone. Similarly, the production of collagen was maximal after the combined addition of PRP and FGF-2.

### **Conclusions:**

Although aPRP and FGF-2 were useful for cell growth, their addition at higher than optimal concentrations was found to gradually reduce cell growth promotion, suggesting that they need to be used at appropriate concentrations. The use of PRP and FGF-2 in combination exhibited a stronger effect than that of PRP and FGF-2 singly, suggesting that their combined use may be a new, effective treatment for wound healing by ASC and Fb.

## EFFECT OF HYPOXIA ON MESENCHYMAL STEM CELL ADIPOGENIC DIFFERENTIATION.

Maria Giuditta Valorani; A. Germani; W.R. Otto; C.P. Khoo; M.I. Hawa; M.R. Alison; P. Pozzilli; P. Patrizi

### Introduction:

Previous studies indicate that murine mesenchymal stem cells (MSCs) have specific morphological and antigenic characteristics and have the potential to contribute to regeneration of different cell types in vivo. In this study we aimed to find the best source of MSCs. We investigated bone marrow (BM) cells and adipose tissue (AT) from NOD mice, a model of T1D, as a source of pure MSCs and assessed the expansion and purification of these cells at low and normal oxygen levels. The ability and capacity of cultured BM- and AT-MSCs to differentiate in vitro into mature cells of different lineages was also analyzed.

### Methods:

MSC were isolated from both BM and AT of 8-12 week-old NOD mice. Briefly, BM cells were collected by flushing femurs, tibias and iliac crests while AT cells were obtained from the epiploon which was excised, cut into small pieces, collagenase digested and filtered. The cells were grown under atmospheric (21%) and low oxygen levels (<2%). Isolated cells were phenotyped by Flow Cytometry for surface antigen expression of CD44 and Sca-1 (as evidence of MSCs).

### Results:

To confirm isolated cells were true MSC populations, in vitro differentiation into adipocytic, osteocytic and chondrocytic phenotypes was carried out. The BM- and AT-MSCs were capable of trilineage differentiation when grown in specific media. FACS analysis of cultured BM-MSCs and AT-MSCs showed that when grown in hypoxic conditions, Sca-1+ cells were increased in both populations and after 90 days, 98% of BM-MSCs were Sca-1+/CD44+, whereas from normoxic culture only 22% were Sca-1+/CD44+. Moreover, after only 10 days in hypoxic culture 81% of AT-MSCs were Sca-1+/CD44+, whereas only 35% were Sca-1+/CD44+ following growth in normoxic culture. Therefore, if CD44 and Sca-1 identify pure AT-MSCs, we expected high differentiation potential from hypoxic-cultured cells which are enriched in Sca1+CD44+. Then, we assessed the ability of normoxic and hypoxic cells to differentiate into adipogenic cells. Although hypoxia inhibits AT-MSC differentiation into adipocytes, hypoxic cultured AT-MSCs, displayed higher adipogenic differentiation potential when transferred to normoxic conditions, compared to normoxic-cultured MSCs.

### Conclusions:

Our data suggest that prior exposure to hypoxia culture condition enhances MSC purification and subsequent differentiation potential when transferred to normoxic conditions.

## **miR-196a regulates proliferation and osteogenic differentiation in mesenchymal stem cells derived from human adipose tissue**

Jin Sup Jung; Yeon Jeong Kim; Sang Woo Bae; Young Chan Bae

### **Introduction:**

Elucidating the molecular mechanisms that govern human adipose tissue-derived mesenchymal stem cell (hASC) differentiation and proliferation could improve hASC-based cell therapy. In this study, we examined the roles of microRNA (miRNA) 196a on hASC proliferation and osteogenic differentiation.

### **Methods:**

hASC was isolated from fat tissue by liposuction and culture expanded. We used lentiviral vectors encode miR-196a and facilitate isolation of transduced cells based on lentivirus-mediated EGFP expression.

### **Results:**

Lentiviral overexpression of miR-196a decreased hASC proliferation and enhanced osteogenic differentiation. Overexpression of miR-196a decreased protein and mRNA levels of HOXC8, a predicted target of miR-196a. Expression of HOXC8 was decreased during osteogenic differentiation of hASC in concordance with increase in miR-196a level. In contrast, inhibiting miR-196a with 2'-O-methyl-antisense RNA increased protein levels of HOXC8 in treated hASC, accompanied by increased proliferation and decreased osteogenic differentiation. Activity of the luciferase construct in which the miR-196a target site from the HOXC8 3'UTR was lower in LV-miR196a-infected hASC than in LV-miLacZ infected cells.

### **Conclusions:**

RNA interference-mediated downregulation of HOXC8 in hASC increased their proliferation and decreased their differentiation into osteogenic cells.

## Multi drug resistance protein expression on adipose tissue derived stem cells

Annemieke van Dijk; wouter Jurgens; Ruud Oerlemans; George Scheffer; Frans Visser; Gerrit Schuurhuis; Florine van Milligen; Hans Niessen

### Introduction:

The efflux of Hoechst dye, which is caused by the presence of Multi drug resistance (MDR) proteins, is an widely accepted characteristic of stem cells. Several MDR proteins have been identified on different types of stem cells. However, only little research has been done to investigate the expression of MDR proteins in adipose tissue derived stem cells (ASC). In this study, we therefore investigated the expression and activity of MDR proteins in ASC. Since expression of these proteins might protect the cells in harmful conditions, we further studied whether these proteins protected ASCs during ischemia, and whether ischemia affected MDR protein expression.

### Methods:

BCRP, MRP-1, MRP-4 en PGP protein expression was investigated over time (passage 2-6) using both western blot analysis and immunohistochemical staining of cytopsin slides. Activity of the proteins was investigated by FACS analysis of MDR protein specific substrate extrusion. Ischemia was mimicked using metabolic inhibition.

### Results:

Immunohistochemical staining and western blot analysis showed protein expression of BCRP, MRP-1 and MRP-4, but not of PGP, on ASC. This expression was found to be most prominent in early passage (p2), and decreased during culture. Furthermore 5 hours of ischemia induced protein expression of BCRP, MRP-1 and MRP-4. It was also shown using a substrate extrusion assay that the MDR proteins indeed were functionally active. Finally, it was shown that BCRP protects ASC for ischemia, namely when the BCRP protein is blocked, more stem cells die after 1 hour of ischemia.

### Conclusions:

ASCs express BCRP, MRP-1 and MRP-4 protein, however expression decreases during culture. MDR proteins protect stem cells in harmful environments like ischemia. Therefore, transplantation of ASCs in ischemic environments like myocardial infarctions, is optimal when expression of these proteins are high, thus in early passage.

## Effect of in vivo long-term inhibition of CXCR4 activity with AMD3100 on adipose tissue cellular dynamics

Coralie Sengenès; Marie Maumus; Genevive Tavernier; Jean Galitzky; Anne Bouloumie

### Introduction:

The chemokine CXCL12 (Stromal derived factor 1 or SDF-1) and its receptor CXCR4 are known to play a central role in the regulation of bone marrow stem cell trafficking. We have already shown that capillary endothelial cells from human adipose tissue (AT) induce a strong chemotaxis of human AT stem cells via the production of CXCL12. Interestingly it was recently reported that the couple CXCL12/CXCR4 could play a pivotal role in the immune cell (macrophages and T-lymphocytes) infiltration that is associated with the development of AT. The present work examines the role of long term AMD3100 administration, a CXCR4 antagonist, on the cellular composition of AT.

### Methods:

AMD3100 was administered subcutaneously, 3 times a week, for 6 weeks at a dose of 2.5 mg/kg to C57BL/6J male mice. In order to study AT cellular dynamics, the expression of stem cell markers CD34 and stem cell antigen-1 (Sca-1) and of the common leukocyte marker CD45 or of lymphocyte markers (CD4 and CD8) was studied. The analyses were performed by using multi-colour flow cytometry on different AT depot-derived stroma-vascular fractions and on bone marrow, at the beginning and at the end of the protocol. Furthermore, the weights of the animals, their fat mass content, their energy expenditure as well as their physical activity were measured.

### Results:

Flow cytometry experiments allowed the identification of the various cell populations present within AT that is CD45+ cells (leukocytes including lymphocytes) and also populations of Sca-1+/CD34- and Sca-1+/CD34+ cells which both do not express CD45. The administration of AMD3100 led to changes in cell population proportions within the bone marrow as well as within AT, but in a depot specific manner. Furthermore, AMD3100 treatment was associated with fat mass content changes that were not explained by decrease in energy expenditure and/or physical activity.

### Conclusions:

The present study suggests that the couple CXCL12/CXCR4 may regulate in vivo the cellular dynamics occurring within AT, thus shedding new light on bone marrow and AT compartment interactions.



## Use of ASCs and BM-MSCs for Adipose Tissue Engineering

Maud Vallée; Guillaume Marceau-Fortier; Lucie St-Pierre; Julie Fradette

### Introduction:

Mesenchymal stem cells are multipotent somatic stem cells that hold promise for numerous applications in tissue engineering. They were originally isolated from bone marrow (BM-MSCs), but similar populations have been reported in other tissues such as adipose tissue (ASCs). Since both cell sources have been successfully used in tissue engineering, the main objective of this study was to compare their potential to generate entirely natural human three-dimensional adipose tissue substitutes using the self-assembly approach.

### Methods:

ASCs were isolated from adipose tissue (lipoaspirate) of healthy patients, which underwent cosmetic surgery procedures. Commercially available human BM-MSCs were used. A characterization of transcripts and surface proteins was performed by RT-PCR and flow cytometry respectively, on cultures of thawed primary cells. Adipose tissue substitutes were generated using the self-assembly approach. This consisted of stimulating cells with ascorbic acid and serum to produce and organize an endogenous extracellular matrix (ECM), and to concomitantly induce adipogenic differentiation. The adipocyte-containing manipulatable cellular sheets obtained were then superposed. Adipogenic differentiation was analyzed through lipid accumulation by Oil Red O staining and thickness of the reconstructed tissues was measured. Expression levels of genes related to adipogenic differentiation and ECM was quantified by QRT-PCR.

### Results:

RT-PCR performed on primary cell cultures showed that genes normally associated with stem cells such as Kit, Nanog, and Gnl3 were expressed in both cell populations. Flow cytometry analyses revealed that they express CD73 and CD105; surface markers previously associated with mesenchymal stem cells, and are negative for CD34 and CD45 markers. Both cell sources were able to generate adipose tissue substitutes using the self-assembly approach. However, lipid accumulation was greater when BM-MSCs were used whereas tissue thickness and extracellular matrix formation were enhanced when ASCs were used. Quantitative RT-PCR results showed differences in gene expression levels for genes related to adipocytes (Lep, Lpl, and Pparg2) and extracellular matrix formation (Col1a1, Col5a2, and Sparc) between the two cell sources.

### Conclusions:

In conclusion, both cell sources can be used to generate entirely natural human adipose tissue substitutes using the self-assembly approach. Tissues engineered from BM-MSCs show a greater adipogenic potential with a limited extracellular matrix formation, whereas adipose tissues engineered from ASCs produce more extracellular matrix while maintaining an adequate adipogenic differentiation. The results presented here suggest that ASCs, which are abundant and represent an easily accessible cell source, offer a greater potential for the reconstruction of manipulatable autologous adipose tissues using the self-assembly approach. Presenting author's email address: [maud.vallee.1@ulaval.ca](mailto:maud.vallee.1@ulaval.ca)

## Human adipose-tissue derived stromal cells in clinical trials : evolution of the phenotype and definition of quality control standards

Julie-Anne Peyrafitte; Marilyn Gomez; Elodie Labat; Valérie Planat-Benard; Béatrice Cousin; Louis Casteilla; Philippe Bourin

### Introduction:

Human adipose tissue-derived stromal cells (ADSC) are currently used in various clinical trials. As quality controls are required, we tried to establish relevant standards for their evaluation. This study focuses on the phenotypic characterization of freshly isolated cells, as well as its evolution during in vitro expansion.

### Methods:

The stromal-vascular fraction (SVF) was isolated from lipo-aspirates by collagenase digestion and centrifugation. The adherent fraction was expanded in vitro in a culture medium developed for cell therapy protocols. After 8 days, ADSC at passage 0 were harvested with trypsin EDTA and then cultured for 6 additional days (passage 1). Immediately after harvesting (SVF) or after in vitro expansion (ADSC P0 and P1), cells were phenotyped by triple-labelling flow cytometry analysis using 61 directly coupled monoclonal antibodies and their matched controls.

### Results:

About 75% of the SVF cells were non-hematopoietic, as identified by the lack of CD45 expression. This subpopulation, known for containing the multipotent ADSC, was therefore further characterized. Three groups of markers were distinguished according to their percentage of expression : absent or weak (less than 10%), intermediate (between 10 and 50%) and high (more than 50%). The first group comprised CD3, CD6, CD11c, CD14, CD15, CD16, CD18, CD38, CD41, CD49b/c/d, CD50, CD56, CD61, CD64, CD71, CD95, CD103, CD106, CD115, CD117, CD130, CD133/2, CD144, CD150, CD166, CD184, NGF-R, ABCG2, LAP, HLA-DR, KDR, EGF-R, PDGF-Ra, IGF-R1, SSEA-1 and 4. The second group consisted of CD9, CD10, CD13, CD26, CD31, CD44, CD47, CD49a/e/f, CD54, CD55, CD73, CD105, CD146, CD147, CD164, HLA-ABC and PDGFR-b. Finally, CD29, CD34, CD90 and CD157 constituted in the third group. Moreover we found that the markers CD13, CD49e, CD73, CD90 and CD157 were predominant (more than 70% of expression) in the CD34pos/CD45neg fraction of the SVF. A marked homogenization of the phenotype was observed in cultured cells. Hematopoietic cells represent less than 1% of the adherent population, and some markers were expressed by more than 95% of the ADSC (CD9, CD10, CD13, CD26, CD29, CD44, CD47, CD49b/e, CD55, CD73, CD90, CD105, CD130, CD147, CD157, CD166, LAP, EGF-R, PDGFR-b). Surprisingly, the expression of several markers that were weakly expressed in the SVF (CD49b/d, CD61, CD71, CD130, CD166, LAP and EGF-R) was dramatically increased after in vitro expansion. Culture also resulted in a progressive loss of CD34 expression. We did not observe any marked phenotype evolution between P0 and P1.

### Conclusions:

Taking these data into account, we propose a triple-labelling procedure using at least CD45 and CD34 in addition to other surface antigens to identify cells of interest among the subpopulations of the human SVF. Furthermore, regarding the decrease of CD34 expression during in vitro expansion, CD13, CD73, CD90 and CD157 seem to be the most relevant markers for clinical grade-cultured ADSC and could be used as quality controls in a cell therapy context.

## Seeding and Incorporation of Human ASCs into an Acellular Dermal Scaffold: Impact of Cell Formulation and Culture Medium

Yihwa Yang; Hulan Shang; Shayn Peirce; Adam Katz

### Introduction:

Dermal defects can be associated with severe scarring as well as secondary aesthetic and functional sequelae. At present, there is no autologous cell-based dermal replacement available to the clinician. Given the translational potential of adipose-derived stem cells (ASCs), we hypothesized that an autologous dermal construct could be fabricated using ASCs and existing, commercially available acellular dermal scaffolds. The purpose of this study was to evaluate the ability of human ASCs to proliferate and incorporate after seeding onto acellular dermal scaffolds (ADS), and to define the influence of culture medium and cell formulation on this process.

### Methods:

Human ASCs were isolated and culture expanded in monolayer adherent culture using 10% FBS (D10) or 1% human serum (AR8 1% HuS) culture media. After sufficient expansion, half of the cells were formulated into 3-dimensional multicellular aggregates (MAs) and maintained in suspension culture for 3 days in D10 or AR8 1% HuS, the other half maintained in adherent monolayer culture. Equal numbers of ASCs were then seeded onto acellular dermal scaffolds as either monolayer-derived single cell suspensions or 3-D suspension-cultured MAs. The cell-scaffold constructs were maintained in D10 or AR8 1% HuS and harvested after 6 and 19 days. Cell proliferation and incorporation were evaluated by MTT proliferation assay and histological analysis.

### Results:

MTT analysis revealed that AR8 1% HuS media induced 3-fold greater proliferation of ASCs than control medium (D10) whether seeded as MAs or single cell suspensions on both AlloDerm and tissue culture plastic. When seeded onto ADS specifically, there was no difference in ASC proliferation in AR8-1% HuS by day 19 whether seeded as MAs or single cell suspensions. Histological analyses demonstrated that ASCs in AR8-1% HuS incorporated (i.e. penetrated) on average ~50% more into ADS when seeded as MAs compared to single cell suspensions at both harvest time points (Day 6: 48% vs. 0%; Day 19: 79% vs. 25%).

### Conclusions:

This pilot study confirms that hASCs can be successfully seeded onto, remain viable, proliferate and incorporate into acellular dermal scaffolds. Our results demonstrate that cell proliferation and incorporation into ADS are significantly influenced by cell preparation methods and culture media. Further testing is planned to confirm these initial findings, as well as to evaluate the impact of these variables on in vivo engraftment of ASC-ADS constructs.

## Adipose-derived Stem Cells Improve Liver Regeneration after Partial Hepatectomy in Rats

Il-Hwa Hong; Ok-Kyung Hwang; Jin-Kyu Park; Kyung-Sook Hong; Jung-Youn Han; Ae-Ri Ji; Mi-Ran Ki; Young-Mi Moon; Se-Il Park; Jae-Ho Jeong; Kyu-Shik Jeong

### Introduction:

The replacement of diseased hepatocytes by stem cells or the stimulation of endogenous as well as regeneration by exogenous stem cells is the main aims of liver-directed cell therapy. Adipose-derived stem cells (ASCs) have been demonstrated to be capable of differentiation towards hepatic lineage in vitro and in vivo, however, there are still lack of theses demonstration in vivo. Moreover, there are a few reports of differentiation of bone marrow stem cells into the liver cells after partial hepatectomy, but theses demonstration is still unclear in ASCs.

### Methods:

ASCs were collected from the abdominal fat of rats and labeled with Feridex and DiI dye. 70% partial hepatectomy (PH) was performed onto 8 week-old Sprague-Dawley rats and  $1 \times 10^6$  of ASCs injected into tail vein. ASCs were detected by prussian blue staining and fluorescence in the liver and ASCs in the liver were examined by the immunolabelling with lineage markers such as albumin, alpha-fetoprotein, laminin, Senescence marker protein-30 (SMP30), etc.

### Results:

ASCs were detected by both prussian blue staining and fluorescence in the liver. ASCs in the liver were found in the hepatic sinusoids expressing laminin. SMP30, which is highly expressed in regenerated hepatocytes, was more expressed in hepatocytes of ASCs-injected liver compare to non-injected ASCs group, indicating improving of liver regeneration.

### Conclusions:

Liver regeneration after PH has been reported that the remaining liver expands in mass to compensate for lost tissue. The laminin is one of the expressing extracelullar matrix after PH and contributes to the capillarization of the sinusoids, moreover, increased expression of SMP30 in the ASCs-injected liver than non-injected ASCs group suggest that ASCs participate and improve to the liver regeneration after PH.

## Effect of Adipose Derived Stem Cell on photo-aged Human Dermal Fibroblast : in vitro model

Lew Dae Hyun; Song Seung Yong ; Jung Ji Eun ; Tark Kwan Chul

### Introduction:

The causes of skin aging can be classified into intrinsic and extrinsic factors. Intrinsic factors are chronologic one and they are similar in all other organs, but extrinsic factor, photoaging, is developed by extrinsic factors especially ultraviolet radiation . In many reports, UV can cause not only senescence but also carcinogenesis. And these are closely associated with apoptosis and cellular senescence. Recently, there has been reports of evidence that ADSC has effects on restoration and regeneration of injured tissues. These effects thought to have influence on injured fibroblasts due to skin aging. Therefore, by studying the functions of ADSC in photo aging induced fibroblasts, the basis of anti-aging treatment using ADSC can be planned. We investigate ADSC can reverse aging process of human dermal fibroblast by detecting changes of several biochemical markers in in vitro experiments. Also, we tried to find out whether this effect is due to direct influence between the cells or secondary owing to secretory agents.

### Methods:

Fibroblast harvested from young age human were irradiated with subcytotoxic dose of UVB(Sankyo Denki, Japan). Then this fibroblast was cultured with ADSC from same person. Experimental model consisted of 3 groups. group1 : UVB irradiated fibroblast with no ADSC (control). The group 2: UVB irradiated fibroblast with adipose derived stem cell(ADSC) by transwell culture plate. Group3 : UVBirradiated fibroblast and cultured with ADSC conditioned medium(without cell). changes of protein markers associated with senescence, fibroblast function, apoptosis and carcinogenesis(SA- $\beta$ -galactosidase, collagen type I, cell proliferation assay, matrix metalloproteinase-1(MMP-1), TUNEL assay for apoptosis, p53) were investigated.

### Results:

After irradiated with UVB, SA- $\beta$ -galactosidase level of fibroblast was increased and we could confirm photoaging of fibroblast. Cell proliferation assay revealed proliferation decrease in control group (the first group) but increase in the experiment group(the second and the third), especially in transwell culture group. Collagen type I production decreased after UVB irradiation. But after culture with ADSC, it was restored. Other markers, including p53, MMP-1, apoptosis were increased by UVB irradiation but they were also decreased by culture with ADSC.

### Conclusions:

These results suggest ADSC can reverse photoaging of human dermal fibroblast and can modify carcinogenesis and apoptosis process caused by UVB irradiation. Comparing transwell culture and cell medium culture, this study suggest that ADSC effect is enforced by two-way communication between ADSC and fibroblast than one-way communication. Additional study will be needed for mechanism of these results in detail.

## Antiaging effect of Adipose-derived stem cells on photoaged hairless mouse model

Jung Ji Eun ; Tark Kwan Chul ; Lew Dae Hyun

### Introduction:

Antiaging has gained much interest these days. Skin aging is due to two biologically discriminative processes both chronological aging and extrinsic aging. The former, so called intrinsic aging, is occurred by natural aging process but the latter is resulted by environmental factors. The extrinsic aging is mainly caused by Ultraviolet B(UVB), so it is called "Photoaging". It also brings on histological alterations such as epidermal thickening, destruction of dermal collagen fiber bundles (DCFBs) and accumulation of amorphous elastotic materials("Elastosis") etc. And it also shows visible alterations of skin such as coarse wrinkles and furrowing that most people do not want. Many reports showed that Adipose-derived stem cells(ADSCs) accelerate dermal wound healing via direct and indirect effects. This concept can be applied to photo-damaged dermis. This experiment was designed under the hypothesis that ADSC can improve the photoaged skin and dermis, and to provide the fundamentals for anti aging improvement using autogenous ADSC. Objective :We investigated that ADSCs can promote recovery of histological alteration on photoaged skin caused by UVB irradiation in hairless mouse model. The goal of this study is clarify the antiaging effect of ADSC and eventually confirm that local injection of ADSC can improve the skin aging and wrinkles.

### Methods:

Hairless mice were exposed to the UVB(peak wavelength : 312nm) irradiation at a dose of 50mJ/cm<sup>2</sup> per irradiation, 3-12 times a week for 6weeks, resulting a total dose of 3900mJ/cm<sup>2</sup>. The day after final irradiation, human ADSCs with Dulbecco's phosphate buffered saline(DPBS) were injected to dorsal neck area of mice intradermally. Control group was injected only DPBS . Before and 5weeks after injection, skin surface replicas of injection sites were taken by silicon rubber and 8 kinds of wrinkle texture parameters were obtained by replica analysis with Optical Profilometry (BioNET Inc.). Pre and post treatment skin biopsy were done and evaluated various histologic alteration by specific stain.

### Results:

8 kinds of wrinkle texture parameters acquired by Replica analysis show that wrinkles are less significant in ADSC group compared to Control group. Quantatively, epidermal and dermal thickness were found to be significantly smaller in ADSC group. Epidermal thickness was 23.23 $\mu$ m in ADSC group compared to 57.42 $\mu$ m in control group( $p < 0.00001$ ). And dermal thickness was 471.4 $\mu$ m in ADSC group compared to 846.8 $\mu$ m in control group( $p < 0.00001$ ). And microscopically, destruction of DCFBs and dermal elastosis were lighter in ADSC group. Collectively, our findings suggest that intradermal injection of ADSCs may promote recovery of photoaging in Hairless mouse model.

### Conclusions:

ADSCs support recovery of histologic alteration caused by photoaging and reduce visible symptoms such as skin wrinkles. It is suggested that ADSCs can be used for treatment of photoaging. We think local injection of autologous ADSC can improve aged appearance and it is very fascinating option for antiaging treatment.

## **In vivo evaluation of uncultured freshly isolated lipoaspirate cells combined with demineralized bone matrix for bone regeneration in a rat critical-size calvarial defect model.**

Eul-Sik Yoon; Seung Chul Rhee; Yi-hwa Ji; Eun Sang Dhong; Seung Ha Park

### **Introduction:**

Adipose tissue is plentiful and can be easily obtained using current liposuction techniques. However, because adipose-derived stem cell and progenitor cells, have not been approved for the use by the FDA, stem cell therapies have much limitation for universal applicability. Although the property of differentiation may be an important aspect of stem cell therapeutic potential, other mechanism such as vasculogenesis, arteriogenesis, anti-apoptosis, anti-inflammation are also important. Although there are many studies suggest that human adipose tissue contains pluripotent stem cells which are similar to bone marrow-derived stem cells, there are a few reports on SVF (stromal vascular fraction) or the pellet of the stem cell. In the present study, we evaluated the bone formation capacity of pellet using the adipose-derived stem cell and P(L/DL)LA [Poly(70L-lactide-co-30DL-lactide) Co Polymer P(L/DL)LA](GmbH Synthes, Oberdorf, Switzerland)(orbital floor plate 1.5, Resorbable, 0.5 mm). We used the demineralized bone matrix [DBXÂ° (Putty, Synthes, Paoli, PA, USA)], and evaluated the ability of implanted pellet to new born formation in rat adipose derived stem cells in vivo.

### **Methods:**

Fifty white rats were randomized into 5 different groups (n=10): 1) Group A: No treatment, 2) Group B: DBM 0.2g + fibrin glue, 3) Group C: Group B + SVF, 4) Group D: Group B + P(L/DL)LA, 5) Group E: Group B + P(L/DL)LA + SVF. After acquiring SVF pellet, an 8-mm critical size circular defect was made in each rat. Specimens were harvested at 8 weeks post-implantation, and evaluated radiographically as well as histologically. New bone formation was qualified through H&E staining and Anti-osteocalcin antibody [Osteocalcin antibody (OC4-30), Abcam, Cambridge, UK] immunostaining of calvarial sections. Mineralization was quantified through 3D CT analysis.

### **Results:**

In gross appearance, group C and E, comprising adipose-derived stem cells, showed more abundant bone formation than group A, B and D. The densitometric evaluation for simple radiologic examination revealed that each group show different bone formation (Group A: 13.48%, B: 39.94% C: 57.69%, D: 24.86%, E: 42.75%) There are statistically significant inter-group differences according to Kruskal-Wallis (Rank) test ( $p = 0.030 < 0.05$ ) and Mann-Whitney T-test [Group AD ( $p = 0.063 < 0.1$ )]. Histogram analysis of 3D CT scan showed similar result. (Group A: 15.77%, B: 31.30%, C: 37.49%, D: 25.73%, E: 26.84%) Group C and E, which comprising SVF, showed statistically significant higher bone formation. Histological evaluation revealed that group A had no new bone formation and defected areas were filled up with fibrous materials. In terms of group B and D, authors found that irregular new bone was regenerated around from the borderline area of defected margin. Island immature new bone and mature new bone were found in the periphery of defected margin of rat calvarias.

### **Conclusions:**

Authors tried to evaluate the osteogenic potential of the uncultured freshly isolated lipoaspirate cells combined with demineralized bone matrix for bone regeneration in a rat critical-size calvarial defect model. We found that undifferentiated adipose-derived stem cell, stromal vascular fraction pellet, induced new bone formation in rat calvarial defect, which was coincident finding as in the case of bone marrow-derived stem cell. Accordingly, stromal vascular fraction pellet, which is relatively free in the ethical issues, might a practical, promising, and potent candidate for tissue engineering or cell-based therapies in the regeneration medicine.

## Specific Alterations of Wnt Pathway Member Expression in ASC Derived from Obese and Lean Donors.

Gangaraju Rajashekhar; Robert V Considine; Dimitry Trakteuv; Brian H Johnstone; Stephanie Merfeld-Clauss; Elliot D Rosen; Keith L March; Matthias Clauss

### Introduction:

Introduction There is a progressive increase in the frequency of obesity in all age groups. The epidemic of obesity in children is expected to result in a significant increase in cardiovascular mortality as this population ages. Recently, we have shown that endothelial cells (EC) can suppress adipogenic differentiation of ASC in vitro, in part via upregulation of Wnt molecules in both ASC and EC, and activation of Wnt signaling pathways in ASC, upon contact of ASC and EC. These findings are consistent with the previous description of Wnt factors as suppressors of adipogenesis, and the identification of genetic susceptibility to obesity due to alterations in the Wnt10b gene. Accordingly, we hypothesized that a selection of Wnt proteins, receptors, or other Wnt signal-mediating molecules would be downregulated in ASC derived from obese donors, linking Wnt signaling and adipogenic differentiation potential with susceptibility to obesity.

### Methods:

Methods We evaluated gene expression differences of Wnt signaling associated genes in ASC isolated from lean (BMI<30, av. BMI 26.1 $\pm$ 2.8), and obese (BMI>40, av. BMI 50.7 $\pm$ 4.0) human donors (N=6 each). These ASC were tested after expansion over 2 passages, with specific focus on Wnt signaling molecules including agonists/antagonists, receptors, and related transcription factors. Expression levels were assessed by real-time RT-PCR, normalized to eF1alpha, and expressed using comparative Ct method.

### Results:

Results ASC from obese subjects manifested a significant 2.1 fold decrease ( $p < 0.05$ ) in expression of the Wnt receptors Fz3 and Fz4 but not Fz1 and Fz2. In addition, Wnt ligand 10b (2.7 fold decrease,  $p < 0.01$ ) but not Wnt's 1, 4, 8a and Wnt 10a were different. On the other hand, the transcription factors involved in Wnt signaling namely, TCF7, Wisp1, Sox17 and Axin1 were unchanged ( $p > 0.05$ ).

### Conclusions:

Conclusions The data from this study provide evidence for our hypothesis that in obese individuals specific Wnt receptors and ligands are reduced in ASC, thus potentially increasing their susceptibility to adipogenic differentiation. Identification of these differences between lean and obese individuals as caused by genetic or epigenetic alterations will be important to determine potential approaches to modulation of these systems in obese-prone individuals.



## Adipose-derived mesenchymal stem cells differentiate into functional endothelial cells cultured in serum free differentiation medium

Masamitsu Konno; Tatsuo S. Hamazaki; Satsuki Fukuda; Makoto Tokuhara; Hideho Uchiyama; Makoto Asashima; Hitoshi Okochi

### Introduction:

Recently, mesenchymal stem cells are considered to be useful for the cell therapy of various tissues in regenerative medicine. Among them, adipose tissue-derived mesenchymal stem cells (ASCs) have been reported to differentiate into endothelial cell lineage when they were differentiated in serum containing medium. Without serum in culture condition, it had been difficult to induce such mesodermal lineage. In this study, we developed a new method that the functional endothelial cells were differentiated from ASCs and it would serve to an approach using the endothelial cell derived from ASCs to cell therapy.

### Methods:

ASCs were isolated from inguinal adipose tissue of adult female and GFP-transgenic B57/CL6J mice. After 5 or 6 passage of ASC were used for the differentiation. They were cultured in serum-free DMEM/F12 culture medium supplemented with some growth factors on collagen type IV coated dish. After 2 weeks, the expression of endothelial cell markers were examined by real time RT-PCR and immunostaining. To examine their function in vivo, these cells were transplanted into mice femoral muscle. One or 2 weeks after cell transplantation, we carried out immunostaining to examine whether the neoangiogenesis occurred.

### Results:

The ASCs have changed their morphology which were similar to that of normal endothelial cells 2 weeks after the culture in the serum-free differentiation medium. Real time RT-PCR and immunostaining showed that the addition of VEGF, EGF, IGF-1, ascorbic acid and heparin into the medium elevated the expression of endothelial major markers such as Flk-1, Tie-2 and vWF of differentiated ASCs. Moreover, the mice which were transplanted of those differentiated ASCs showed the neoangiogenesis and ASCs derived cells formed the endothelial cells. These results indicated that ASCs differentiated into functional endothelial cells in vivo.

### Conclusions:

In this study, we showed mouse ASCs differentiated into endothelial cells in serum-free medium in vitro and they contributed to neoangiogenesis in vivo. We are considering that this differentiation system has an advantage to be used for cell therapy.

## Neural Differentiation of Adipose Stromal Cells is Induced by Brain Derived Neurotrophic Factor and Retinoic Acid and Further Promoted by DNA Demethylation

Tatiana Lopatina; Natalia Kalinina; Galina Pavlova; Yelena Parfyonova; Vsevolod Tkachuk

### Introduction:

Adipose stromal cells (ASCs) are progenitor cells capable to differentiate into a large variety of cell types including neuronal cells. Many active ingredients were suggested for the induction of neural differentiation of stromal progenitor cells. However the combination of pharmaceutically approved agents allowing stable induction of neural differentiation of ASCs is not established. Here, we tested the ability of brain derived neurotrophic factor (BDNF) and retinoic acid (RA) alone as well as together with DNA demethylating agent 5-azacytidine to induce stable neural differentiation of ASCs

### Methods:

ASCs were isolated from human or mouse (Bl6 strain) subcutis as described by Zuk et al. (2001). At passages 2–5 the cells were induced with NIM (DMEM/F12, 3% FBS and 1 mM azacytidine supplemented with 1 mM RA or 20 ng/ml BDNF) for 3 days. The efficiency of neural differentiation was estimated by the change of expression of neuronal markers, including nestin, tubulin-beta3, neuron-specific enolase 2 and microtubule-associated protein 2, at 3 and 7 days after induction using Real Time PCR. The expression of neural markers was also examined by immunocytochemistry.

### Results:

The expression of marker genes increased 6-10 times after incubation in the NIM, supplemented with RA, and up to 4 times in the medium containing BDNF 3 days after induction. The expression of neural markers remained high 7 days following induction. This data was confirmed by immunocytochemistry. Furthermore, ASCs primed to neural differentiation demonstrate significantly better surviving and incorporation in the brain tissue after transplantation into the mouse brain.

### Conclusions:

Taken together, our data suggest that the combination of BDNF or RA with 5-azacytidine could be suggested for the induction of stable neural transdifferentiation. This method could be used in further studies to develop cell therapies for the treatment of neural disorders.

## The Effects of Adipose Derived Stem Cells with a Tubular Scaffold on Neurogenic Differentiation and Induction of Peripheral Nerve Regeneration

Young-Joon Jun; Deuk-Young Oh; Sung-Eun Kim; Sang-Cheon Lee; Byung-Chul Seo; Young-Jin Kim; Jong-Won Rhie

### Introduction:

Autologous nerve grafting has been applied as the best method of treating peripheral nerve defects but it has problems such as donor site morbidity. Thus, this research was planned in order to see if it is possible to culture adipose derived stem cells(ASCs) and differentiate and multiply them to neuronal cells and to graft the neuronal progenitor cells and ASCs into nerve defects bridged with polycaprolactone(PCL) nano-tube in vivo and examine the effects of the grafting on nerve regeneration.

### Methods:

Using ASCs, neurogenic differentiation was induced in a mono layered culture medium containing neuronal induction agents. In addition, we made a 15mm long defect in the sciatic nerve of 30 rats and connected a PCL nano-tube to the defect. Then, we grafted neuronal progenitor cells differentiated from ASCs to a group of rats(the experimental group 1), ASCs to another group of rats(the experimental group 2), and no cells to the other group (the control group). After 10 weeks from the grafting, nerve conduction velocity(NCV) and histological observations were made.

### Results:

ASCs differentiated to the neuronal cells were observed in a monolayered culture. The NCV was improved more in the experimental groups after 10 weeks from grafting (exp 1 :  $18.0000 \pm 7.4864$  m/s, exp 2 :  $17.4750 \pm 6.9259$  m/s) than in the control group ( $4.3250 \pm 2.6174$  m/s) ( $p < 0.05$ ). But, there was no statistical difference between experimental groups( $p < 0.05$ ). Histologic(H&E, Toluidine blue staining) and immunohistochemistry(Nestin, MAP-2, GFAP) staining showed more regenerated nerve findings.

### Conclusions:

This research proved that ASCs could multiply and differentiate into neuronal cells. When they were grafted into nerve defects, the grafted cells were differentiated into Schwann like-supportive cells and contributed to peripheral nerve regeneration by an unknown mechanism, for example trophic factors and cytokines in vivo that might act on surviving host cells. Further evaluation is needed to analyze the facts of grafted cells and clarify the mechanism of nerve regeneration. However, any functional improvement of this research gives much hope for the use of adipose tissue as an alternative source of neuronal cells for treating peripheral nerve defects.

## **Hypoxia-Treated Adipose Stromal Cells Stimulate Angiogenesis via Promoting Progenitor Cell Differentiation, Secretion of Angiogenic Factors and Enhancing Vessel Maturation**

Kseniya Rubina; Natalia Kalinina; Tatiana Lopatina; Zoya Tsokolaeva; Anastassia Efimenko; Veronika Sysoeva; Yelena Parfyonova; Vsevolod Tkachuk

### **Introduction:**

Adipose-derived stromal cells (ASCs) are suggested to be potent candidates for cell therapy of ischemic conditions due to their ability to stimulate blood vessel growth. In this study we analyzed mechanisms of angiogenesis stimulation by ASCs

### **Methods:**

ASCs treated by hypoxia were injected in Matrigel plugs into Balbc mice (n=7). Vascularization of plugs was assessed using immunofluorescent staining of frozen sections. Growth factor production by ASCs was analysed using real-time PCR and ELISA. Mechanisms of ASCs angiogenic action were analyzed in co-culture model of ASCs with cardiomyocyte fraction (CMF) isolated from early postnatal hearts of Wistar rat pups. CMF was depleted from vascular cells using immuno-magnetic sorting.

### **Results:**

ASCs treated by hypoxia stimulated blood vessels growth and maturation in Matrigel plugs. ASCs produced many angiogenic and anti-apoptotic growth factors, and their secretion was significantly enhanced by hypoxia. On the 2nd day in culture CMF cells formed spontaneously beating colonies with CD31-positive capillary-like structures. However, these vessel-like structures disassembled within the next 5 days. Co-culturing of CMF with ASCs resulted in the formation of stable and branched vessel-like structures. We showed that ASCs directly interacted with capillaries as well as promoted the endothelial differentiation of cardiac progenitor cells.

### **Conclusions:**

Taken together these data indicate that ASCs induce angiogenesis via secretion of paracrine-acting factors, direct interaction with capillaries as well as promotion of endothelial differentiation. All these mechanisms of actions could be beneficial for the stimulation of angiogenesis in ischemic tissues upon ASCs administration.

## Adipose-derived Stem Cells Injection Ameliorate Doxorubicin-induced Cardiomyopathy in Mice

Il-Hwa Hong; Ok-Kyung Hwang; Jin-Kyu Park; Kyung-Sook Hong; Jung-Youn Han; Ae-Ri Ji; Mi-Ran Ki; Young-Mi Moon; Se-Il Park; Jae-Ho Jeong; Kyu-Shik Jeong

### Introduction:

Cardiovascular disease highly ranks as the major cause of death in human beings. Cellular cardioplasty is a rapidly progressing field and a large body of work now exists pertaining to its therapeutic potential. However, there are a few reports exist relating to stem cell differentiation into the cardiomyocyte lineage. This study estimate therapeutic potential of adipose-derived stem cells in cardiomyopathy and other skeletal related myopathy.

### Methods:

Twelve week-old, male C57BL/6 mice were treated with 3mg/kg of DOX once a week for 6 weeks or saline by intra-peritoneal injection. Human adipose-derived stem cells (ASCs) labeled with CM-DiI dye were injected intraperitoneally with sodium nitroprusside (vasodilator). Cardiotoxicity was assessed using mortality, weight changes, blood chemistry and immunolabelling.

### Results:

Although DiI-labeled stem cells were not detected in mice hearts, mice injected with ASCs were shown low mortality and low decrease of body weight compare to only DOX-treated group. In blood chemistry, creatine kinase (CK), aminotransferase (AST) and lactate dehydrogenase (LDH) were all significantly decreased in ASCs-injected group. On the immunolabelling study, the expression of caspase-3 in the heart also decreased in ASCs injected group compare to only DOX-treated group.

### Conclusions:

DOX was used to treat a variety of cancers, however, it is associated with increase risk of cardiomyopathy or congestive heart failure and bone marrow depression, because DOX induced generation of H<sub>2</sub>O<sub>2</sub> which is responsible for drug's toxicity and apoptosis. In this study, DOX induced cardiomyopathy was successfully established in the mice and it showed that ASCs therapy ameliorate DOX-induced cardiac injury. Therefore, this study showed that DOX-induced cardiomyopathy model can be applied to the study of ASCs therapy, and suggested that ASCs might be have therapeutic potential in cardiomyopathy.

## Human ASCs Support the Growth and Morphology of Human Keratinocytes through Direct Cell Interactions

Yihwa Yang; Hulan Shang; Shayn Peirce; Adam Katz

### Introduction:

Recent studies support a therapeutic potential for ASCs in cutaneous wound healing. Although ASCs share many similarities to, and offer several harvest-related advantages over dermal fibroblasts (dFB), their ability to support and/or maintain keratinocyte (KC) viability and growth deserves further delineation. The objective of this study was to evaluate direct and indirect (i.e. paracrine) effects of hASCs on the behavior of human KCs in vitro, and to determine if culture medium or cell formulation method impacts such interactions.

### Methods:

Direct cell interactions were characterized by culturing keratinocytes on top of a confluent layer of hASCs (in keratinocyte and non-keratinocyte media) and subsequently evaluating keratinocyte proliferation and morphology. Indirect effects were characterized by culturing keratinocytes in one of two different types of media (containing either 10% FBS (D10) or 1% human serum (AR8-1%)) and conditioned by hASCs grown as either adherent monolayers or suspension multicellular aggregates (MAs). For all studies, human dermal fibroblasts (hDFBs) served as a control/comparison cell population and KC medium (EpiLife) served as control/comparison medium. Outcome analysis was performed using FACS, MTT cell proliferation assay, confocal and/or bright field microscopy.

### Results:

For direct studies, both hASC and dFB feeder layers were effective in maintaining normal keratinocyte morphology in both AR8 1% and D10 media. FACS quantification of KC proliferation was indeterminate due to dilution of fluorescent signal with increased replication. For indirect studies, non-keratinocyte media conditioned by either hASCs or dFBs, grown as either adherent monolayers or multicellular aggregates failed to maintain characteristic keratinocyte morphology. Furthermore, there was no significant difference in keratinocyte proliferation noted with conditioned medium from hASCs vs. dFBs, or when derived from monolayer vs. multicellular aggregate cells. However, conditioned D10 medium may better enhance paracrine-mediated growth of KCs than conditioned AR8 1%.

### Conclusions:

These studies demonstrate that hASCs are able to support the viability, morphology and growth of human keratinocytes, and also strongly suggest that these interactions are primarily mediated by direct interactions between the two cell types. ASCs may prove useful for wound healing and skin repair challenges via direct interactions with keratinocytes, but further in vitro and in vivo studies are required.

## Human adipose stromal cells expressing vascular endothelial growth factor following recombinant adeno-associated viral vector-mediated gene transfer stimulate angiogenesis in a murine model.

Eugeniy Shevchenko; Pavel Makarevich; Zoya Tsokolaeva; Yelena Parfyonova

### Introduction:

Due to sufficient adipose stromal cell (ASC) numbers, their multipotency and the ability to secrete angiogenic growth factors autologous ASC transplantation may become an alternative tool to treat cardiovascular diseases. The ability to efficiently transfer genes of interest into such cells would create a number of therapeutic opportunities.

### Methods:

Low passaged human ASC obtained from different donors were transduced using gene delivery system based on a recombinant adeno-associated virus (rAAV) encoding vascular endothelial growth factor (VEGF) and green fluorescent protein (GFP). Transduction efficiencies and transgene expression level in ASCs were analyzed by quantitative flow cytometry, quantitative real-time PCR and ELISA. The effect of rAAV expression of VEGF in ASC on vessel growth was determined in the model of skin matrigel implants transplanted in nude mice.

### Results:

Firstly, human ASC population was analysed for heparin sulfate proteoglycan expression, the main cellular AAV binding receptor. It was found that 55-65% of human ASC population express this receptor. The efficiency of ASC transduction using AAV delivery system was found to be 60+/-7% although there was significant inter-donor variation. GFP expression was seen a month after transduction, though the number of transduced cells reduced 2-fold. Relative to control, cells transduced by VEGF rAAV vector increased VEGF secretion level by at least 10-fold as compared to unmanipulated ASC. Moreover VEGF-transduced cells significantly increased vascularisation of subcutaneous matrigel implants in immunodeficient mice when compared to controls where either unmanipulated ASC or ASC cultured in hypoxic condition were used.

### Conclusions:

Recombinant adeno-associated virus provide efficient tools for ex vivo modification of human ASCs. AAV-mediated gene-modified ASC expressing a gene of angiogenic potential can stimulate vessel growth in vivo and could become a potential tool for therapeutic angiogenic factor delivery in ischemic tissues.

## Association of hASCs with Murine Retinal Vasculature after Intravitreal Delivery

Alyssa Taylor; Peter Amos; Lara Seltz; Shayn Peirce-Cottler; Paul Yates

### Introduction:

Diabetic retinopathy results from structural abnormalities of retinal vasculature. Loss of vascular integrity results in macular edema and exudation, and retinal and vitreous hemorrhage. An ideal strategy for intervention would be to restore structural integrity of damaged vessels, as well as mature the compensatory neovascularization that occurs in this disease. Bone marrow-derived stem cells injected intravitreally have been shown to incorporate into damaged retinal vessels in murine models of diabetic retinopathy through reendothelialization of acellular vessels. The initial insult in diabetic retinopathy is a loss of pericyte support cells on existing vessels and a subsequent lack of pericytes on immature neovascularization. Recently published work from several labs has demonstrated hASCs can assume a pericyte-like role, associating abluminally with vessels while also expressing pericyte molecular markers such as NG2. The use of human adipose-derived stem cells (ASCs) for treating retinal disease has not yet been investigated. Specifically, the ability of hASCs injected intravitreally to cross the internal limiting membrane (ILM) of the retina and subsequently associate with retinal vasculature is unknown. In this study, we examine the ability of intravitreally-injected hASCs to penetrate the ILM and physically associate with blood vessels of a murine retinal vascular network.

### Methods:

CD-1 (n = 6) and NIH-III (n = 2) immunocompromised 8-week-old female mice received intravitreal injections of DiI-labeled hASCs using a 30 gauge Hamilton syringe through the pars plana. Passage 3 cells were injected in 2  $\mu$ l sterile PBS, with each eye receiving a different concentration of hASCs (25 million cells or 12.5 million cells/mL). Two or ten days post-injection, retinas were harvested, whole-mounted, and immunostained to label blood vessels. Retinal vascular networks were stained with isolectin and TOTO nuclear stain and imaged using confocal microscopy.

### Results:

Two days after hASC intravitreal injection, individual hASCs as well as aggregated masses of hASCs were present above the superficial vascular network, but penetration of the ILM was not detected, as there were no apparent instances of coplanarity between hASCs and retinal vessels. After ten days, hASCs were observed in the vascular bed both in aggregated masses and discrete cellular units. hASCs in the vascular bed did not directly associate with blood vessels, and no quantifiable differences in vascular length density were observed in the retina as compared to sham-injected controls. Many hASCs stained positive for isolectin, a stain that also labels endothelial cells. When hASCs were present in an aggregated mass, lectin staining was almost exclusively limited to cells on the surface periphery of the mass.

### Conclusions:

We have shown that hASCs, when injected into the murine ocular vitreous, have the ability to cross the ILM and reach the vascular bed of the retina by ten days post-injection. As a result, it may be possible to utilize hASCs as a therapeutic strategy to target damaged retinal vessels. Our observations may indicate that in the nondiabetic retinal compartment, hASCs will assume an extravascular endothelial phenotype instead of a perivascular phenotype. The phenotype of injected hASCs in a murine diabetic model remains to be determined.



## Adipose-derived mesenchymal stem cells in biosutures increase resistance of experimental colonic anastomoses

Isabel Pascual; Gemma Fernandez de Miguel; Fernando de Miguel; Mariano Garcia-Arranz; Damian Garcia-Olmo

### Introduction:

Mesenchymal stem cells (MSC) from different sources are pluripotent, have immunomodulatory properties and are involved in wound healing. After digestive surgery, a correct restoration of tissue continuity is critical to diminish anastomotic dehiscence to prevent leakage of colonic contents, with the subsequent risk of peritonitis and new surgeries. Adhesions to the anastomotic line have been considered beneficial due to higher resistance in the short term but also can be associated to abdominal pain and intestinal obstructions in the long term. Moreover, surgery in other regions of the digestive tract, such as oesophagus or lower rectum, is considered critical due to the lack of adhesions to support these anastomoses. The weakest point where dehiscence occurs is the tract of the needle used for suturing the tissue. Recently, we developed what we call biosutures, i.e. sutures coated with MSC derived from adipose tissue, with the intention to supply stem cells at the exact point where they are needed the most. We tested these biosutures in an experimental model of critical anastomoses.

### Methods:

Adipose tissue-derived MSC were isolated from BDIX rats as described and characterized by flow cytometry by standard positive and negative membrane markers. ASC were allowed to adhere to resorbable multifilament 4/0 polyglactin 910 sutures for 24 h in ultralow attachment plates. Syngeneic BDIX rats were used for surgeries. Colon was completely sectioned 5cm from ileocaecal valve, and a colonic end-to-end anastomosis was constructed with six single interrupted stitches using either control sutures or biosutures. To prevent adhesions formation to the anastomosis, 40 ml of 4% icodextrin solution was injected i.p. before skin closure. Adhesion index was calculated after macroscopic analysis of the anastomoses four days after surgery. Bursting at the site of surgery was evaluated ex vivo immediately afterwards.

### Results:

Intraperitoneal icodextrin did not affect the frequency of dehiscences, colonic dilatation or intestinal obstruction compared to control group. Colonic adhesions in the presence of icodextrin were more easily detachable and adhesion index was lower than that from control group. The pool of ASC provided with the biosutures did not improve significantly these parameters except for bursting pressure, which was three times higher than that of control sutures ( $62.96 \pm 14.83$  mm Hg vs.  $21.03 \pm 21.76$  mm Hg,  $p=0.006$ ).

### Conclusions:

Sutures seem to be a good vehicle to deliver ASC at a specific site in surgical procedures. Biosutures with ASC produced colonic anastomosis with higher resistance even in the case of lower adhesion index, four days after surgery, at the time point where clinical dehiscence is more frequent. Biosutures could be useful in digestive surgery with high risk of dehiscence.

## Neuroleptic drugs increase adipose stem cell differentiation - an explanation for the massive weight gain under neuroleptic pharmacotherapy

Ben Kappel; Nora Paul; Daniela Goy; Norbert Pallua

### Introduction:

Antipsychotic-induced weight gain is a key issue in the treatment of psychotic illnesses and affects 80% of individuals treated with antipsychotic drugs. The atypical neuroleptics clozapine and olanzapine show the greatest weight-increasing potential while risperidone and the typical neuroleptic haloperidol induce moderate weight gain during therapy. In contrast, the newer drug aripiprazole causes weight decreases in clinical studies. The weight increase is significantly correlated with clinical improvements, but the causes of treatment-emergent weight gain in schizophrenic patients have not been discovered so far. It has been assumed that neuroleptics impair the perception of satiety, since patients experience a lower suppression of appetite after meals when consuming neuroleptics. However, strict food reduction unfortunately shows little success. This study therefore addressed the question of whether neuroleptic-induced weight gain might be due to neo-formation of adipose tissue from preadipocytes.

### Methods:

Preadipocytes were isolated from human abdominal subcutaneous adipose tissue samples and cultured until confluence. Differentiation was induced by adding insulin, isobuthylmethylxanthine, pioglitazone, dexamethasone, and transferrin in the absence of FCS. Clozapine (5  $\mu$ M, 20 $\mu$ M), olanzapine (5  $\mu$ M, 20 $\mu$ M), risperidone (5  $\mu$ M, 20 $\mu$ M), haloperidol (5  $\mu$ M, 20 $\mu$ M), and aripiprazole (0.5 and 1  $\mu$ M) were added for the first 5 days during differentiation. To evaluate preadipocyte differentiation on the molecular level, the activity of glycerol 3-phosphate dehydrogenase (GPDH), a molecular key marker of adipogenic conversion, was determined after 14 days by measuring enzyme activity. Intracellular superoxide formation was measured for clozapine and confirmed microscopically by ethidium bromide staining (Hydroethidin conversion, 2 $\mu$ M).

### Results:

Our findings show that GPDH-activity was increased by clozapine (+75%  $\pm$  40% ( $p < 0.01$ )), haloperidol (+62%  $\pm$  37% ( $p < 0.05$ )), risperidone (+50%  $\pm$  35%) and olanzapine (+43%  $\pm$  20%), while treatment with aripiprazole decreased GPDH-activity by 40%  $\pm$  20%. All these findings were supported by microscopical results of the extend of differentiation. Superoxide anion formation was highly induced under clozapin treatment.

### Conclusions:

We here present a new explanation for how neuroleptic drugs cause massive weight changes in patients, i.e. weight gain due to enhanced differentiation and weight loss due to reduced differentiation of adipose tissue precursor cells to mature fat cells, respectively. The molecular mechanisms how the neuroleptic drugs induce preadipocytes differentiation are still widely unknown, however, our preliminary findings on oxidative stress analyses with clozapine strongly suggest that neuroleptic drugs fundamentally change the intracellular redox system balance. Such a mechanism has also been proposed for other stem cells and might have important implication for the differentiation of various stem cell types.

## **Pilot Study of Autologous Adipose-Derived Regenerative Cell Therapy in Chronic Liver Disease in the Canine**

Robert Harman, DVM, MPVM; Linda Black, DVM, PhD; David Bruyette, DVM

### **Introduction:**

Liver disease is common amongst many mammals. Chronic hepatitis in the canine has a prevalence of approximately 0.7% in the United States and has features both similar and dissimilar to the human. Many in-vitro and preclinical studies have shown that adipose-derived stem and regenerative cells can both improve the clinical condition and become hepatocytes. This is the first report of the clinical use of adipose-derived stem and regenerative cell therapy in chronic hepatitis in the dog.

### **Methods:**

This study included nine dogs with chronic hepatitis. All dogs had pre-treatment liver biopsy samples collected and complete clinical chemistry and hematology profiling as well as pre and post prandial bile acids. All dogs were treated with a single initial dose of intravenous autologous adipose-derived stromal cells.

### **Results:**

All dogs have improved clinically, but the clinical pathology data did not consistently improve. Two dogs with chronically severe elevation of bile acids died at days 51 and 133. Follow-up biopsy samples will be collected at day 365 and all remaining dogs are being retreated with higher doses of cells and followed through day 365 post-treatment.

### **Conclusions:**

Preliminary data indicate that a single intravenous dose of autologous, adipose-derived stromal cells can improve the clinical condition of dogs with chronic hepatitis. This presentation will present the clinical, laboratory, and dosing data along with a discussion of predictors of successful outcomes along with preliminary data after secondary dosing with higher doses of cells.

## Effect of Murine Adipose Derived Stem Cells (ADSC) on Bone Induction of Demineralized on Bone Matrix (DBM) in a Rat Calvarial Defect Model

Chanyeong Heo, Hak Chang, Kyungwon Minn

### Introduction:

Adipose tissue-derived stem cells (ADSC) has an osteoconductive potential and demineralized bone matrix (DBM) is an osteoinductive material. A combination of DBM and ADSC would probably create osteoinductive properties. The purpose of this study was to determine the effect of the combination of DBM and ADSC mixture on healing of rat calvarial defect.

### Methods:

Thirty adult male Sprague-Dawley rats were randomized 3 groups(n=10): 1) Control, 2) DBM alone, 3) DBM with ADSC mixture. DBM with ADSC mixture group has had a 3-day preculture of ADSC from groin fat pad. An 6 mm critical size circular calvarial defect was made in each rats. Defect was implanted with DBM alone or DBM with ADSC mixture. Control defect was left unfilled. 6 and 12 weeks after implantation, the rats were sacrificed and the defects were evaluated by histomorphometric and radiographical studies.

### Results:

Histomorphometric analysis revealed that DBM with ADSC mixture group showed significantly higher bone formation than DBM alone ( $p < 0.05$ ). Although radiographs from DBM alone group and DBM with ADSC group revealed similar diffuse radiopaque spots dispersed throughout the defect. Densitometric analysis of calvarial defect revealed DBM with ADSC mixture group significantly higher bone formation than DBM alone ( $p < 0.05$ ). There was correlation of densitometry with new bone formation (Spearman's correlation of coefficient = 0.804, 6 weeks, 0.802, 12 weeks)

### Conclusions:

The DBM with ADSC mixture group showed the best healing response and the osteoinductive properties of DBM were accelerated with ADSC mixture. It will be clinically applicable that DBM and ADSC mixture in plastic and reconstructive surgery, such as alveolar cleft and congenital facial deformities that bone graft should be required. The DBM with ADSC mixture group showed the best healing response and the osteoinductive properties of DBM were accelerated with ADSC mixture. It will be clinically applicable that DBM and ADSC mixture in plastic and reconstructive surgery, such as alveolar cleft and congenital facial deformities that bone graft should be required.

## Antiwrinkle effect of adipose-derived stem cell: Activation of dermal fibroblast by secretory factors

JONG-HYUK SUNG; SO-HYUN PARK; BYUNG-SOON PARK

### Introduction:

Adipose-derived stem cells (ADSC) have wound-healing and antioxidant effects on human skin via secretion of growth factors and activation of dermal fibroblasts. Therefore, paracrine mechanism reducing UVB-induced wrinkles by ADSC is investigated in this study.

### Methods:

Wrinkles were induced by an eight-week UVB irradiation in hairless mice and ADSCs were injected subcutaneously. To characterize the paracrine mechanism involving the antiwrinkle effect of ADSC, a conditioned medium of ADSC (ADSC-CM) was directly incubated in human dermal fibroblasts (HDF).

### Results:

Wrinkles were significantly improved by the subcutaneous injection of ADSC in hairless mice. In a replica analysis, parameters involving wrinkles were improved with mid-level and high doses of ADSC ( $1 \times 10^4$  and  $1 \times 10^5$  cells). Dermal thickness and collagen contents in the dermis also were increased in the ADSC-injected groups. UVB irradiation reduced the proliferation of HDF, but this was reversed by the pretreatment of ADSC-CM in a dose-dependent manner. In a cell-cycle analysis, ADSC-CM decreased the UVB-induced apoptotic cell death, which was demonstrated by the reduced sub-G1 phase of HDF. In addition, the ADSC-CM increased the protein expression of collagen type I and decreased the protein level of matrix metalloproteinase 1 in HDF, which may account for the increased collagen contents in the dermis.

### Conclusions:

Collectively, these results indicate that the ADSC and its secretory factors are effective for UVB-induced wrinkles, and the anti-wrinkle effect is mainly mediated by reducing UVB-induced apoptosis and stimulating collagen synthesis of HDF.

## Co-transplantation of Endothelial Progenitor Cells with Adipose stromal Cells Inhibits Adipocyte Maturation In Vivo

Zhong Liang; Katherine Bisordi; Amy Zollman; Shekhar Gangaraju; Dmitry Traktuev; Matthias Clauss; Keith March; Elliot Rosen

### Introduction:

Hemophilia A and B are serious bleeding disorders resulting from insufficiency of coagulation factors FVIII and FIX, respectively. The severity of the bleeding pathology correlates with the level of FVIII or FIX activity, where individuals with less than 2% often suffer severe disease involving spontaneous bleeding events while those expressing 2-5% of wt levels generally suffer prolonged bleeding mainly after trauma. Current treatments involve replacement therapies in which exogenous FVIII or FIX is supplied intravenously. While prophylactic treatment with exogenous proteins provides protection, the expense is often prohibitive and most patients are treated after bleeding episodes. Since relatively low levels of FVIII activity are necessary to reduce the potentially lethal symptoms of hemophilia and provide prophylaxis (5% of wt levels), cell/gene therapy provides an attractive therapeutic strategy to provide sufficient long-term expression of the therapeutic proteins. The goal of this project is to implant genetically modified ASCs for long term FVIII expression by ASC derived grafts. Since fat transplantation has been used successfully for constructive surgery, we propose to transplant ASCs to form adipose tissue. Since the development of adipose tissue from engrafted stem progenitor cells requires differentiation into adipose cells and the establishment of blood supply we tried implantations of combinations of human ASCs, human ACS pre-differentiated into pre-adipocytes and human endothelial progenitor cells (EPC) to determine the combination of transplanted cells best capable of forming an adipose tissue graft.

### Methods:

Various combinations of low passage undifferentiated human ASCs, pre-adipocytes derived from ASCs and EPCs, were mixed with hydrogel (Puramatrix) and HGF and injected subcutaneously into nude mice. At 7-8 weeks animals were sacrificed and the implants were excised and examined histochemically for the presence of blood vessels and lipid loaded cells.

### Results:

ASCs were injected with or without pre-adipocytes derived from the ASCs in the presence or absence of EPCs. Only 2 of 6 injections of ASCs produced detectable grafts 8 weeks after transplantation and only 1 of those contained adipose tissue. However, 70% (7 of 10) implants generated by the co-implantation of ASCs and pre-adipocytes yielded implants containing adipose tissue. Interestingly, the incorporation of EPCs in the transplantation inoculum inhibited adipocyte maturation. Of 5 transplants containing ASCs, pre-adipocytes and EPCs, 4 generated grafts at 8 weeks and only 1 contained adipocytes. In the absence of pre-adipocytes, transplantation of ASCs and EPCs generated only 4 grafts following 9 injections and although the grafts were well vascularized, none of the grafts included adipocytes.

### Conclusions:

These results are consistent with other reports that cross talk between ASCs and EPCs, possibly involving wnt signaling pathways, suppresses adipocyte differentiation. However, the co-injection of undifferentiated ASCs and pre-differentiated ASCs in the presence of HGF leads to efficient engraftment of adipose tissue. Preliminary results suggest these grafts can persist long term (>5 months). The long term engraftment of genetically modified ASCs producing adipose tissue capable of expressing recombinant proteins may provide a cell therapy strategy for pathologies associated with a plasma protein deficiency. We propose to engraft genetically modified ASCs expressing FVIII as a strategy for the treatment of Hemophilia A.

## Regeneration of adipose tissue by transplantation of human adipose-derived stem cell

Natsuko Kakudo; Satoshi Kushida; Kenji Suzuki; Kenji Kusumoto

### Introduction:

Human adipose-derived stem cells (ASC) are adult pluripotent stem cells, which have the potential to be applied in tissue engineering. Vascular development and adipose tissue differentiation are thought to play an important role during the formation of adipose tissue. However, the process and mechanism of the differentiation of transplanted ASC have not been elucidated. In this study, to clarify the process of adipose tissue formation by transplanted ASC, we investigated the influence of the cell condition at the time of transplantation on subsequent tissue formation. We also evaluated the status of angiogenesis in tissue formation.

### Methods:

ASC were prepared from surplus abdominal adipose tissue obtained during plastic surgery. ASC at confluence (C stage) or those after differentiation into adipocytes (A stage) were transplanted into the back of SCID mice, and tissue samples were collected sequentially. Paraffin sections were prepared, and stained with HE, or immunostained for human vimentin and  $\alpha$ -SMA. In addition, frozen sections were stained with Oil Red O. The stained sections were examined under a microscope. VEGF, FGF-2 in the culture supernatants of ASCs during differentiation were determined by ELISA.

### Results:

Fat pads were formed at the transplantation sites of all mice, and engrafted there. However, their morphology varied according to the condition of transplanted cells. The transplantation of ASC at confluence resulted in the formation of clusters of fibroblast-like cells alone, and no fat droplets or blood vessels were observed. In contrast, when ASC were transplanted after their differentiation into adipocytes, the development of fat droplets and blood vessels was observed. High levels of VEGF, FGF-2 were detected in the culture supernatants of ASCs.

### Conclusions:

The conclusion is that the transplantation of ASC after differentiation into adipocytes facilitates the efficient formation of adipose tissue.

## Extending the replicative life span of human adipose-derived stem cells

Anja Peterbauer; Simone Hennerbichler; Guido Stadler; Johanna Aberl; Michael Karbiener; Johannes Grillari; Regina Grillari; Christian Gabriel; Heinz Redl; Martijn van Griensven; Susanne Wolbank

### Introduction:

Cell banking of mesenchymal stem cells from various human tissues has significantly increased the feasibility of stem cell based therapies. Adipose tissue is a very attractive stem cell sources as it is abundantly available and as adipose-derived stem cells (ASC) offer outstanding possibilities displaying high differentiation potential and the ability to modulate immune reaction. Limitations, however, concern the reduced replicative potential which hampers scaleable production and long-term analysis of these cells. Hence, we investigated two approaches to overcome these limitations. Firstly, we optimized expansion media with special regard to culture supplements of human origin including platelet lysate (PL). Secondly, we tried to circumvent growth arrest after a limited number of population doublings due to telomere shortening by transfecting ASC with hTERT, the catalytic subunit of human telomerase. Subsequently, we monitored growth characteristics, surface marker profile, differentiation potential, immunogenic and immunomodulatory properties.

### Methods:

ASC were isolated from liposuction material. PL was produced from pooled thrombocyte concentrates by a freeze-thaw cycle and selected growth factors were determined by ELISA. PL was then used in comparison to fetal calf serum (FCS) as supplement for expanding and differentiating human ASC. hTERT was introduced by retroviral transfection and telomerase activity was determined by real-time telomeric repeat amplification protocol (TRAP) assay. Surface marker profile was characterized by flow cytometry, growth characteristics were demonstrated by calculating population doubling levels, and immunogenic and immunomodulatory properties were investigated by co-culture with peripheral blood mononuclear cells. Adipogenic and osteogenic differentiation was carried out according to standard protocols and subsequently demonstrated by Oilred O, von Kossa and Alizarin Red staining, alkaline phosphatase activity and calcium quantification.

### Results:

ASC cultured in presence of PL displayed a higher proliferation potential and in vitro life span compared to cells cultured under FCS conditions. PL preserved the differentiation capacity of the cells and did neither alter the surface marker expression profile nor the immunomodulatory properties of ASC. Likewise, hTERT overexpression left stem cell characteristics largely unaltered while extending the replicative life span and improving growth characteristics of ASC.

### Conclusions:

Our data suggest that both, PL in expansion medium as well as hTERT transduction substantially extend life span and improve growth characteristics of ASC while maintaining the differentiation potential and immunomodulatory properties. Hence, the two proposed approaches are useful tools in large-scale production of ASC for long-term studies while the PL approach might even be considered for future cell banking strategies. Acknowledgements: Austrian Cluster for Tissue Regeneration, HIPPOCRATES (NMP3-CT-2003-505758), EXPERTISSUES (NMP3-CT-2004-500283), Lorenz Böhler Fonds



## DEVELOPMENT OF MICROPOROUS SCAFFOLDS BIOCOMPATIBLE WITH ADIPOSE DERIVED STEM CELLS FOR NERVE REPAIR

Kai Hei Tse; Mingzhu Sun; Sandra Downes; Giorgio Terenghi; Paul Kingham

### Introduction:

Peripheral nerve injury is a common problem worldwide causing huge economic impact on the working populations. Nerve conduits could be a promising alternative to nerve autografts for repairing peripheral nerve injury. The present study aimed to develop novel microporous scaffolds for the production of an optimised nerve conduit suitable for transplantation of regenerative cells such as adipose derived stem cells (ASC) and Schwann cells.

### Methods:

Polymer pellets of poly-D, L.-lactic acid, (PLA; Mw ~55,000) and polycaprolactone (PCL; Mw ~65,000) were dissolved in 3% (w/v) dichloromethane. Different mass fractions of PLA and PCL were used to cast the binary blends by the "breath figuring" mechanism. The polymer solutions were cast on glass cover slips and the films were left overnight for complete solvent evaporation. Pore size was determined by scanning electron microscopy and wettability of the scaffolds was measured using a contact angle analyser. Rat ASC were differentiated to a Schwann cell phenotype using a mixture of glial growth factors (GGF-2, bFGF, PDGF and forskolin) for a period of two weeks. Differentiated ASC (S100/GFAP positive cells) or Schwann cells were seeded onto the films and biocompatibility determined by measurement of attachment and proliferation (PCNA immunocytochemistry).

### Results:

The atmospheric-surface of the scaffolds in all polymer compositions was dominated by porous morphology. The average pore sizes among PCL and the blends (from  $3.30 \pm 0.16 \mu\text{m}$  to  $4.00 \pm 0.12 \mu\text{m}$ ) were significantly larger than PLA alone ( $1.64 \pm 0.08 \mu\text{m}$ ). All scaffolds showed a relatively hydrophobic surface with a range of contact angles from  $68.6 \pm 2.13^\circ$  (PCL) to  $84.12 \pm 1.75^\circ$  (PLA). Schwann cells showed minimal cell attachment (10-15%) to the scaffolds of all polymer compositions over a six hour period. However, almost all differentiated ASC attached to the scaffolds. Numerous PCNA positive ASC were observed on the scaffolds after 4 days in vitro but Schwann cells rarely expressed PCNA. Behavioural similarities, including formation of cytoplasmic extensions, end-to-end cellular interactions and cellular alignments, were observed between Schwann cells and differentiated ASC when grown on tissue culture plastic. These properties were retained in the ASC on scaffolds with scanning electron microscopy showing strong points of adhesions. In contrast Schwann cells lost these feature and showed a rounded morphology.

### Conclusions:

The observations of this study suggest microporous polyester-based scaffolds produced by the "breath figuring" technique together with ASC differentiated to a Schwann cell like phenotype could be a promising combination for peripheral nerve repair.

## Adipose tissue-derived stem cells (ASCs) migrate into damaged liver: potential cell-based therapy for liver cirrhosis

Makoto Tokuharo

### Introduction:

Liver cirrhosis is the end stage liver disease and difficult to treat. Liver transplantation is most effective treatment for liver cirrhosis. However, this treatment has still many problems. Cell-based therapy is minimally invasive procedure with less potential complications and might be the alternative for the liver transplantation. We have reported that ASCs were able to transdifferentiate into hepatocytes and would be a good source for hepatocytes cell therapy. We examined the capability of undifferentiated ASCs for the therapy of liver cirrhosis, using CCl<sub>4</sub> induced liver fibrosis mouse model.

### Methods:

ASCs were isolated from subcutaneous adipose tissues of gastric cancer patients. Six-weeks olds female Balb/c nude mice were treated with 0.25 ml/kg carbon tetrachloride (CCl<sub>4</sub>) dissolved in olive oil twice a week for 4 weeks. Twenty-four hours after eighth injection of CCl<sub>4</sub>, 1x10<sup>6</sup> human ASCs or same volume of Hanks' balanced salt solutions (HBSS) as a control were injected into the tail vein. After cell injection, mice continued to be treated with CCl<sub>4</sub> until sixteenth injection. Then the mice were anesthetized and serum and liver tissue were harvested. Serum concentration of total bilirubin and albumin were measured to assess liver function. The liver tissue were also examined by histology and immunohistochemistry.

### Results:

Liver fibrosis was observed in CCl<sub>4</sub> treated mice liver. Immunohistochemical analysis revealed that HLA-1 positive cells were found in transplant mice liver tissue. However, azan stain did not show amelioration of liver fibrosis. Unfortunately, serum level of total bilirubin and albumin were not significantly different between transplant group and control group.

### Conclusions:

This present results indicated that transplanted human ASC's could migrate and engraft into damaged liver. Although the improvement of liver function was detected at present, ASC's transplantation might be applicable in future cell-based therapy for liver cirrhosis.

## Intravenous injection of the uncultured stromal vascular fraction of adipose tissue reduces myocardial infarction size in vivo in a rat model

Annemieke van Dijk; Kireejan Nalliah; Frans Visser; Florine van Milligen; Hans Niessen

### Introduction:

Stem cells form a promising therapy for regeneration of injured tissue after myocardial infarction (MI). Bone marrow derived mesenchymal stem cells (MSC) are the most commonly used stem cells. However, to obtain a sufficient number of MSCs for therapy, cells need to be cultured. This is time consuming, expensive, and culturing may also affect the functional characteristics and the size of the stem cells. Recent studies show that the stromal vascular fraction of adipose tissue (SVF) contains a population of cells with properties that are similar to MSC (in vitro and in vivo). Furthermore, it has been shown that cultured adipose derived stem cells (ASC) can differentiate towards cardiomyocytes, and improve cardiac function after MI. In contrast to MSCs, these SVF cells can be obtained in a minimally invasive method. And more important, adipose tissue provides more stem cells than bone marrow, which provides a possibility to use uncultured SVF cells for transplantation, in stead of cultured ASC or MSC, which will be advantageous for clinical practice. We therefore investigated whether transplantation of the SVF improved MI outcome, and compared this to cultured ASCs.

### Methods:

SVF cells were obtained from male wistar rats, and labelled using DiI staining. MI was induced in female wistar rats by 40 minutes of coronary occlusion. Seven days after MI, rats were intravenously injected with either vehicle (n=11),  $5 \times 10^6$  rat SVF cells (n=12) or  $1 \times 10^6$  cultured adipose derived stem cells (n=8).

### Results:

We found that SVF treatment significantly reduced infarctsize the same way as cultured ASCs did, namely SVF reduced infarction with  $51 \pm 10$  % and cultured ASCs reduced infarction with  $58 \pm 12$  % when compared with the vehicle treated rats. Further no adverse effects were found after transplantation of SVF. Using cultured ASCs, in contrast, 3 animals suffered from respiratory problems during transplantation, and one of these animals died due to acute lung emboli.

### Conclusions:

Taken together, we now show for the first time that treatment with SVF cells significantly improves MI outcome in a rat model. SVF transplantation is therefore a promising therapy for MI, with high clinical potential, since cells can be harvested easily, and transplanted the same day, with minimal manipulations.

## Phenotypic Characterization of the Stromal Vascular Fraction of Human Lipoaspirate

Anthony Donofrio; Delara Motlagh; Amy Cohen; David Amrani

### Introduction:

The stromal vascular fraction (SVF) is obtained through collagenase digestion of human lipoaspirate tissue. The SVF is a diverse population and has been reported to contain cells such as but not limited to: adipose stromal stem cells (ASC), hematopoietic stem cells (HSC), mesenchymal stem cells (MSC), preadipocytes, endothelial cells, fibroblasts, macrophages, and mastocytes. Of the cells in the SVF, it has been reported that approximately 50% are positive for the stem cell marker CD34. Preclinical literature has shown that cells in the SVF, specifically CD34+ cells, contain the potential for angiogenesis and tissue repair in the rat left anterior descending artery ligation as well as the mouse hind limb ischemia models. The CD34+ population from the SVF may be enriched using a method of selection. We set out to characterize the phenotype expression profile of the various populations of cells from the SVF.

### Methods:

Lipoaspirate tissues are digested with a collagenase solution. A proprietary bench-top selection method was utilized where the resulting cells are then incubated with anti CD34 antibody. The cells are then rosetted by paramagnetic beads displaying a secondary antibody directed at the CD34 antibody. The CD34+ cells are released from the antibody-bead complex in a release step. Flow cytometry is utilized to examine the surface markers on the pre selection cells (Unselected) as well as the post selection cells (Selected).

### Results:

The stromal vascular fraction contains approximately 48 $\pm$ 12% (n=12) CD34 positive cells. The process captures 92 $\pm$ 9% (n=6) of the CD34 cells and, after release the cell product purity is approximately 88  $\pm$  18% (n=5) CD34 positive cells. Within the population of CD34+ cells in the unselected, at least three distinct sub populations exist. Population A comprises 20  $\pm$ 6% of the total and has an expression profile of 34bright/45-/90+/140b+/31-/73+/44+/105-/146-. Population B comprises 8  $\pm$ 4% of the total and has an expression profile of 34bright/45-/90+/140b-/105+/146+/144+/31+/44+. Population C comprises 31 $\pm$ 16% of the total and has an expression profile of 34dim/45-/90+/105+/146+/31+/44dim. Modifications to the selection method resulted in the retention or elimination of these cell populations.

### Conclusions:

Population A appears to be adipose stromal cells based on the phenotypic profile. These cells display the stem cell marker CD34 and the profile 45-/105+/31-, which implies that cells have stromal properties. The stromal properties of this population may allow these cells to have tissue engineering qualities due to their multipotent potential. These cells also express the PDGFR2 antigen, which is reflective of mesenchymal type cells. The combination of these markers implies that these cells may differentiate into osteocyte, chondrocyte, adipocyte, myocyte, epithelial cell, neuron or others. Population B has an endothelial profile (31+/144+/146+) but also displays the stem cell marker and the endoglin marker CD105, part of the TGFB receptor. Cells displaying these markers have been reported to facilitate angiogenesis and vasculogenesis as well as contribute to tissue repair, and decrease apoptosis. Population C displays the profile 34+/31+/146+/105dim/90+/45-, which has been published as a profile of hematopoietic progenitors, capable of forming myeloid as well as lymphoid cells. Other markers (CD31/CD146) suggest there may be endothelial properties of these cells as well. The SVF is a cell fraction rich in therapeutic potential. The phenotypic profile of each population is consistent with specific characteristics attributed to ASCs, MSCs, HSCs, and endothelial cells. It may be beneficial to separate these populations to better understand their potential. These cells hold much therapeutic promise and could be utilized to address multiple areas of therapeutic need.

## Stem Cell or Mature Fat Cell Survival: Which is necessary for long term graft survival?

Ali Mojallal; Charlotte Lequeux; Fabienne Braye; Michel Saint-Cyr; Rod Rohrich; Spencer Brown; Odile Damour

### Introduction:

The long term aesthetic results of fat grafting may be highly variable among individuals. Overall survival of fat grafts is a function of viability of either triglyceride-filled adipocytes or the associated stromal stem cells or both. The aim of this study was to determine what the relative contribution of each cell type in the presence of a natural or collagen scaffold on long term fat grafts in an animal model.

### Methods:

Human adipose tissue specimens were excised from which the following conditions were prepared: 1) fat grafts; 2) freshly isolated stem cells plated on collagen scaffold; 3,4) cultured preadipocytes (passage 2) plated on a collagen scaffold with or without growth factors; and 5) isolated mature adipocytes. Specimens of each cell isolate or cell scaffolding construct were implanted into SCID mice (n=10) for 2 months. Upon sacrifice, specimens were explanted, weighted and histological assessments (HSP, Oil-red-O, and Vimentin) were performed.

### Results:

Complete adipose tissue grafts had a 31% weight increase after 2 months while implanted mature adipocytes alone varied in response as 50% of the sample were reabsorbed and had weight losses and a decrease in lipid droplet size and number. The control, no cells scaffold was partially reabsorbed. Cultured preadipocytes plated on collagen scaffolds without growth factors were superior to the respective condition in the presence of growth factors (25% vs 16% weight increase). Cultured preadipocytes conditions were associated with the presence of small, lipid-filled adipocytes. In contrast, freshly prepared preadipocytes plated immediately on scaffold and implanted had a 25% reduction on weight.

### Conclusions:

Reproducibility for long term survival of fat grafts is dependent upon the survival of both mature, lipid-filled adipocytes and the presumptive stem cells. In the animal model results, the 3D stabilization of both cells types by a natural or engineered scaffolding provides an environment that allows long term survival. The control of differentiation of preadipocytes to mature adipocytes appears to be under local control and does not require the addition of exogenous growth factors.

## Assessment of the Beneficial Effects of ASCs on the Photoaging Process in Human Skin

Eunsang Dhong; Keith March; Matthias A Clauss; Brian Johnstone; Rajashekhar Gangaraju; Dan F Spandau

### Introduction:

Recent clinical reports indicate that subdermal injections of adipose tissues into the facial region may have beneficial effects on skin tone or texture. It is possible that some of these effects may be due to the presence of stem cells in adipose in addition to mature adipocytes and oils. The present study examined the changes induced in UV damaged skin after administration of autologous adipose-derived stem cells (ASCs).

### Methods:

Ex-vivo skin culture: Abdominal skin samples obtained from 7 caucasian females who underwent abdominoplasties with liposuction. After removal of the fat layer, the skin specimens were cultured in serum-free DMEM plus antibiotics. Skin photoaging model development: The maximum sub-cytotoxic doses of UVB (1600 J/m<sup>2</sup>) and UVA (250 J/m<sup>2</sup>) were determined by preliminary studies using a Philips F20T12/UV B source, IL-1700 radiometer and SED240 UV B detector to deliver a range of different dosages. ASC preparation : Lipoaspirates were digested with collagenase and centrifuged to isolate ASCs. Fresh ASCs were used at 40000 – 80000 cells/skin mm<sup>2</sup> and were used in a feeder layer by first attaching or by injecting intradermally. Treatment groups: Groups were divided into 6 according to preparations (group I: skin ex-vivo culture, control, II: skin + ASCs co-culture, III: skin + irradiation, IV: skin + irradiation+ASCs co-culture, V:skin +ASCs intradermal injection, VI: skin +irradiation + ASCs intradermal injection) and biopsies were done on 3, 5, 7 and 10th days after administration to compare therapeutic effects. The biopsies were analyzed by histology (H&E and Verhoeff's staining) to assess overall morphology, apoptosis by TUNEL assay, and replicative senescence with  $\beta$ -Gal at pH 6.0. In addition, immunohistochemical analysis of epidermal proliferation as well as ASC differentiation and cell integration was performed.

### Results:

Epidermal necrosis was significantly reduced in UV-irradiated skin when ASCs were added to the culture (Day 3: groupIII 52.7  $\pm$  5.2 %, group IV 20.4  $\pm$  7.3 %, group VI 4.2  $\pm$  1.9 %, Day5: groupIII 54.4  $\pm$  24.7 %, group IV 18.2  $\pm$  9 %, group VI 0 %, Day 7: groupIII 66.8  $\pm$  24.2 %, group IV 15.1  $\pm$  10 %, group VI 3.1 $\pm$ 2.3 %, Day 10: groupIII 0 %, group IV 9.2  $\pm$  10.2 %, group VI 2.8 $\pm$  0.4 % ). An increase of parakeratosis was observed at early timepoints in ex-vivo cultures containing ASCs( Day 3: groupIII 11.8  $\pm$  7 %, group IV 10.7  $\pm$  7.9 %, group VI 11.2  $\pm$  8.7 %, Day5: groupIII 22.3  $\pm$  15.7 %, group IV 42.8  $\pm$  24.3 %, group VI 15.2  $\pm$  7.8 %, Day 7: groupIII 5.5  $\pm$  4.8%, group IV 15.2  $\pm$  10.9 %, group VI 13.8 $\pm$ 11.8 %). Apoptosis and senescence in the UV-irradiated epidermal layer were both markedly decreased in the ASC groups. Although groups with ASCs displayed a trend of accelerated epidermal differentiation, there was not a similar consistent result in the basal cell proliferation.

### Conclusions:

Although ASCs administrated promoted decreased epidermal necrosis, apoptosis and aging in the skin photoaging model, further studies will be necessary to determine whether and exactly how these phenomena effect basal cell proliferation or epidermal differentiation.

## Operating Room Compatible Systems to Create Blood Vessels from Adipose Derived Stromal Cells

Stuart Williams

### Introduction:

The stromal cell fraction derived from adipose tissue following enzymatic digestion can be used to create blood vessels for use in a variety of vascular bypass procedures. Studies have been performed to evaluate the technical requirements for transition of this technology from the laboratory setting to the specialized environment of the human operating room. The ability to automate all aspects of adipose tissue processing have been considered, resulting in the design and construction of individual prototype systems and ultimately a complete functional system defined as the TGI 1000™. Component systems have been evaluated and indicate the ability to produce a tissue engineered graft from adipose derived stromal cells that provides long term vascular support when used as a bypass graft. These bypass conduits can be produced in a variety of configurations with an inner diameter ranging from 12 mm to less than 50 microns. Results of these studies indicate the compatibility of these systems to the human operating room.

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## Osteogenic differentiated human Adipose derived Stem Cells maintained on chemically modified titanium supports

Laura de Girolamo; Elena Arrigoni; Silvia Lopa; Roberto Chiesa; Anna T. Brini

### Introduction:

Bone tissue regeneration often requires the combination of cells and scaffolds which should provide adequate mechanical properties and osteointegration ability. Loading multipotent cells on scaffolds in vitro may lead to a faster bone formation and more stable osteointegration in vivo. hASCs may be considered a suitable future source in musculo-skeletal regenerative medicine. Titanium is the more widely used material in joint prosthesis, due to its biocompatibility and its good mechanical properties, however its surface modifications could ameliorate cell adhesion and thus improve the integration with the surrounding bone. Here we show the influence of three chemically modified titanium supports on the in vitro osteogenic differentiation of hASCs.

### Methods:

Undifferentiated and osteogenic pre-differentiated hASCs were seeded both on plastic and on three different discs of titanium (1.13 cm<sup>2</sup>): TIT (standard titanium), TAA modified by two consecutive Anodic Spark Deposition processes, and TAAK consisted in KOH alkali etching process on TAA specimens. hASCs were cultured in static condition both in control and in osteogenic media. At different time points cells seeded on scaffolds have been assayed for Alkaline phosphatase activity and calcium deposition and compared to the ones cultured on plastic. The adhesion of hASCs to the three supports was also evaluated by Scanning Electron Microscope (SEM, JSM-840A, Jeol Ltd.)

### Results:

Both the titanium modifications did not alter cell viability if compared to standard titanium. Indeed, despite the different surface of the three materials, cells well adhere to the discs and actively proliferate, as observed by SEM. As also previously shown, hASCs were able to efficiently differentiate towards cells of the osteogenic lineage. The high levels of alkaline phosphatase and calcium deposition, produced by hASCs in vitro, were differently affected by the presence of the three titanium supports here presented. On hASCs directly differentiated on scaffolds, the chemical modification influence on the differentiation process was more evident than on predifferentiated hASCs. In all the tested condition both TIT and TAA are osteoinductive; indeed ALP levels and calcium deposition of hASCs cultured on these types of titanium were significantly increased compared to the ones cultured without supports. In particular the osteoinductive feature of TAA is shown by an average increase of 116% and 93% for ALP and calcium, respectively, in undifferentiated hASCs. In contrast, TAAK seems to never support per se the osteogenic differentiation of hASCs. TAA modification was able to ameliorate the osteogenic differentiation of undifferentiated hASC in comparison to those seeded on standard titanium (average increase of 30% and 18% for ALP activity and calcium deposition, respectively).

### Conclusions:

Due to their availability and their well-known osteogenic capacity, hASCs are a very good tool for screening biomaterials in vitro. hASCs induced to directly differentiate on scaffolds show rapidly and efficiently the influence of the support, so we claim that to predifferentiate hASCs it is not required. TAA material shows to osteoinduce hASCs better than the standard titanium, whereas the KOH alkali etching process used for the TAAK modification seems to produce a significant decrease of the tested parameters at all time points. Further studies aimed at repairing critical size bone defects in an in vivo rabbit model, employing autologous ASCs in association to different scaffolds, are currently in progress.



## ADIPOSE TISSUE SENSITIVITY TO RADIATION EXPOSURE

Sandrine Poglio; Sylvain Galvani; Mireille Andre; Bénédicte Prunet-Marcassus; Luc Pénicaud; Louis Casteilla; Béatrice Cousin

### Introduction:

A large proportion of the plastic and reconstructive surgical procedures are dedicated to repair soft tissue defects that result from traumatic injury, tumor resection or congenital defects. Autologous fat transplantation has been frequently used to repair damaged soft tissues although its limitations are well known. Since a few years, adipose tissue has regain interest with the identification within fat depots of stem/stromal cells able to contribute to tissue regeneration. For example, therapeutic approach for resolving the late side effects of radiotherapy using these cells has been proposed. However, the acute effects of ionizing radiation on adipose tissue had never been investigated. Therefore the aim of this study was to characterize the alterations induced in adipose tissue by total body irradiation.

### Methods:

Subcutaneous adipose tissue from control, or irradiated mice, was analyzed by immunohistochemistry to determine both proliferation (Ki67 immunostaining) and cell death (TUNEL assay). Evaluation of markers of irradiation damage was performed by qRT-PCR in fat pads from control or irradiated animals. At a cellular level, phenotypic and functional analyses were performed by flow cytometry and culture respectively.

### Results:

Radiation exposure induces an acute response in subcutaneous adipose tissue. Indeed, irradiation was associated with morphological changes reducing both number and size of mature adipocytes. In addition, cell dynamics was profoundly altered by ionizing radiations as well as both proliferation and differentiation potentials of precursor cells present in SVF.

### Conclusions:

In conclusion these data demonstrated for the first time that subcutaneous adipose tissue is very sensitive to irradiation. The profound alteration of developemental potential of subcutaneous adipose tissue following irradiation may be an issue for patients that undergo total body irradiation as radiotherapy. Indeed, in terms of therapeutics, these acute affects may modify the reconstructive capacity of adipose tissue and therefore its use in autologous fat tissue transfer after irradiation. They may also contribute to metabolic dysfunction which could be deleterious to patients presenting with malignant diseases.

## Both expansion and CD34 expression of human adipose-tissue derived stromal cells depend on cell plating density

Julie-Anne Peyrafitte; Valérie Planat-Benard; Béatrice Cousin; Louis Casteilla; Philippe Bourin

### Introduction:

A number of studies have shown that the rate of expansion of human bone-marrow mesenchymal stem cells (MSC) is inversely related to the initial plating density. On the other hand, culture of human adipose tissue-derived stromal cells (ADSC), which have multi-lineage potential similar to MSC, has attracted interest for cell therapy purpose because of the easier and higher yield of multipotential progenitors. Moreover, there is a debate in the literature about CD34 expression by ADSC. Therefore, the goal of this study was to investigate the relation between plating density and growth of ADSC, together with the loss of CD34 expression during in vitro expansion.

### Methods:

The stromal-vascular fraction (SVF) was isolated from lipoaspirates by collagenase digestion and centrifugation. For plating density assays, the adherent fraction was expanded in vitro in MEM alpha medium supplemented with 2 or 10% foetal calf serum (FCS). Cells were initially seeded at densities ranging from 250 to 4000 cells/cm<sup>2</sup>. After 8 days, ADSC were harvested with trypsin EDTA. The number of viable cells was determined using trypan blue exclusion and CD34 expression was evaluated by flow cytometry analysis. For cell sorting experiments, CD34<sup>pos</sup> cells of the SVF were isolated by immunoselection using MACSTM technology. Total SVF and the sorted cells were then cultured in MEM alpha medium supplemented with 2 or 10% FCS. The initial plating density was 4000 cells/cm<sup>2</sup>. Cells were harvested everyday during 8 days, counted and phenotyped for CD34 expression.

### Results:

We first compared the influence of FCS concentration on proliferation of the ADSC. Growth was clearly lower when culture medium was supplemented with 2% FCS instead of 10%. Nevertheless, a strong correlation was found between plating density and the number of harvested cells in both cases. At the same time, decreasing the plating density also resulted in a lower percentage of CD34 expression at day 8. On the other hand, expansion was inversely correlated with plating density between 1000 and 4000 cells/cm<sup>2</sup>, and seemed to stagnate or decrease at lowest plating densities. Furthermore, for those ranging from 1000 to 4000 cells/cm<sup>2</sup>, a negative correlation was found between expansion and the percentage of CD34 expressing cells at day 8, suggesting a relation between proliferation and loss of CD34 expression. We consequently sought to determine this relation. After CD34<sup>pos</sup> cell sorting of the SVF and subsequent culture, growth and phenotype kinetics were analysed. The total SVF and the CD34<sup>pos</sup> sorted fraction displayed similar behaviour during in vitro expansion, while we observed sharp differences according to culture media. Cell proliferation was low and quite stable in presence of 2% FCS, as expansion rapidly increased in the medium supplemented with 10% FCS. At the same time, CD34 expression remained very high in presence of 2% FCS (62% and 82% in total SVF and in the sorted fraction at day 8 respectively). In 10% FCS-containing medium, the increase of proliferation starting at day 3 matched the beginning of the decrease of CD34 expression. Only 14% and 25% were CD34<sup>pos</sup> at day 8 in total SVF and in the sorted fraction respectively.

### Conclusions:

Like MSC, ADSC expansion increases while the cell plating density is decreased from 4000 to 1000 cells/cm<sup>2</sup>. However, unlike MSC, ADSC expansion could not be further increased by plating cells below these densities. This constitutes another argument that MSC and ADSC are distinct cell populations. Secondly, in particular for the medium supplemented with 10% FCS, we observed a negative relation between proliferation and CD34 expression, which could explain why other studies describe ADSC as CD34 negative cells, and particularly if those experiments have been carried out on serially passaged cultures.

## Clinical Manipulation of Adipose Tissue: Use of Stem Cells to Measure Cell Lysis

Diane Duncan

### Introduction:

Introduction. Clinical manipulation of subcutaneous fat is currently possible only at the gross level; liposuction or cavitation with ultrasound are currently the most used methods of fat reduction. Nonsurgical fat reduction is a rapidly growing field, but the results of any treatment are difficult to measure. An adipose tissue protocol utilizing stem cells as the test substrate is shown in this study as a new method of reliably quantifying the efficacy of adipose cell lysis by a particular new drug. Phosphatidylcholine and sodium deoxycholate is a popular fat reducing injectable compound. Although there has been conjecture that the deoxycholate is the active ingredient, causing lipolysis as a detergent, the role of phosphatidylcholine has been speculative as it has been difficult to isolate and test independently. In this study, eight lipolytic compounds and four constituent ingredients, along with a saline control, were tested.

### Methods:

Methods. Twelve compounds were tested for efficacy in causing oncosis and subsequent cell lysis of human subcutaneous adipose tissue. The stem cell substrate was prepared by harvesting subcutaneous fat using a Byron liposuction machine at 7 mm Hg, in order to preserve the intact cell structure and promote viability. Upon receipt of the adipocytes, collagenase was then added and the cells were fractionated to yield preadipocytes. The preadipocytes were then differentiated into mature adipocytes *in vitro*. The rationale for using stem cells was that harvested adipocytes are fragile and are not a reliable substrate for measuring lipolysis. Stem cell derived mature adipocytes provide a sturdy and more accurate substrate for measuring the cytotoxicity of a drug specifically designed to induce adipocyte lysis. Following differentiation of the cells into mature adipocytes, equal parts of test solution and cell food were added to the substrate. The solutions were left in place for 12 hours, and the solutions were carefully rinsed. Four assays were performed; two to assess cytotoxicity and two to assess specificity for inducing lipolysis. Lactate dehydrogenase and Oil Red O assays were performed to determine cytotoxicity, while a triglyceride and a glycerol assay were performed to test for the products of hydrolysis. Oil Red O stain of HAD adipocytes

### Results:

Results. The solutions tested showed varying degrees of cytotoxicity and lipolytic activity. Five compounds showed significant lipolytic activity. As the concentration of phosphatidylcholine increased, the degree of lipolysis and cytotoxicity declined. The level of lipolysis also decreased as the concentration of deoxycholate decreased. Not surprisingly, isolated phosphatidylcholine showed no cytotoxicity nor lipolytic activity. Since phosphatidylcholine is a major component of most cell membranes, it is counterintuitive to imagine it might cause lipolysis—yet many medical journal articles have been written speculating that this is so. Because benzyl alcohol has also been cited as an inducer of lipolysis, this constituent ingredient, often used in injectable drugs as a preservative, was tested as well, and was found to have the same degree of cytotoxic and lipolytic activity as the saline control. Key to diagram below: 1. PC/DC solution #1 (proprietary) 2. PC/DC solution #2 (proprietary) 3. PC 50mg/ml/ DC 42 mg/ml 4. Deoxycholate 10 mg/ml 5. PC/ DC solution #3 (proprietary) 6. PC53/ DC 42 7. PC 45/ DC42 8. Benzyl alcohol 18 mg/ml 9. Deoxycholate 24 mg/ml 10. Phosphatidylcholine 50 mg/ml (isolated, solvent: inert mineral oil) 11. Local anesthetic 12. Mineral oil solvent 13. Saline control

### Conclusions:

Conclusion. The cytotoxic and lipolytic activity induced by various injectable drug compounds can be accurately measured using a stem cell derived substrate. Until now, cultured keratinocytes have been used to measure cytotoxicity and lipolytic indices of drugs for fat reduction. Clearly, the use of skin cells to measure lipolysis is not tissue specific and will be inaccurate. By establishing a new adipose tissue protocol, an industry standard can be set to accurately measure the efficacy of lipolytic compounds.

## Autologous fat transfer in breast reconstruction secondary to implant failure

Alireza Sadeghi; Kamran Khoobehi

### **Introduction:**

Fat grafting to the breast is an infrequently used modality in breast reconstructive surgery. However, it produces exceptional outcomes in patients experiencing implant failure secondary to rupture, capsular contracture, asymmetry and contour deformities.

### **Methods:**

This is a retrospective review of 33 autologous fat transfers to the breast in 17 patients from October 2006 to August 2008. The main indication for fat transfer was breast reconstruction following implant removal secondary to implant related complications. In one patient fat was transferred to adjust reconstruction volume following autologous flap reconstruction. Pre- and postoperatively photography and postoperative mammography were undertaken to monitor the viability of the fat grafts and detect mammographic abnormalities.

### **Results:**

The mean follow-up period of patients was 9.1 months (1-22 months). In patients with postoperative mammograms, mammograms identified changes expected after any breast procedure. Patient and surgeon satisfaction with the outcomes of the procedure and photographic evidence of viability were used to gauge final outcomes. All women had a significant improvement in their breast size and shape postoperatively and all had breasts that were soft and natural in feel and appearance.

### **Conclusions:**

Autologous fat grafting to the breast can produce natural long lasting and permanent improvement in breast shape and size in patients who have undergone multiple procedures for implant related complications.

## DEVELOPMENT OF A SERUM-FREE, XENO-FREE CULTURE MEDIUM FOR HUMAN ADIPOSE-DERIVED STEM CELLS

John P Daley, Lucas G Chase, Shayne E Boucher, Mahendra S Rao and Mohan C Vemuri

### Introduction:

Recent pre-clinical and clinical advancements have shown that human mesenchymal stem cells (MSCs) may hold great promise in regards to therapeutic applications. While numerous studies have been conducted using human bone marrow-derived MSCs (BM-MSCs), current efforts have demonstrated that human adipose-derived stem cells (ADSCs) represent a readily available and alternative source of human MSCs or MSC-like cells. The below study describes the development of STEMPRO MSC SFM XenoFree and LipoMAX, two novel stem cell media products that collectively provide a xeno-free, serum-free medium system that is capable of robust expansion of multipotent human ADSCs.

### Methods:

Passage 3 Human ADSCs, isolated and banked in MesenPRO RS Growth Medium, were expanded for five passages in complete serum-free medium (STEMPRO MSC SFM XenoFree) with or without additional LipoMAX supplementation. To test tri-lineage mesoderm differentiation potential, STEMPRO Differentiation Kits were used to induce commitment of ADSCs to adipogenic, osteogenic and chondrogenic lineages. Oil Red O, alkaline phosphatase, and Alcian Blue staining methods were used to detect the presence of adipocytes, osteoblasts and chondrocytes, respectively.

### Results:

Expansion of human ADSCs under xeno-free conditions in the presence of LipoMAX supplementation revealed robust expansion (5 passages under serum-free conditions). Human ADSCs expanded under serum-free conditions revealed both a primitive spindle-shaped morphology as well as the ability to support high density cultures without compromising multipotentiality.

### Conclusions:

STEMPRO MSC SFM XenoFree supplemented with LipoMAX represents a viable alternative for human ADSC expansion. This development provides a reliable and robust media system and sets the stage for future investigations into the clinical utility of human ADSCs.

## MULTILINEAGE DIFFERENTIATION OF EQUINE ADIPOSE TISSUE DERIVED PROGENITOR CELLS AFTER CRYOPRESERVATION.

Enrico Santos; Lisley Mambelli; Paulo Frazio; Mariana Chaparro; Irina Kerkis; André Zoppa; Alexandre Kerkis

### Introduction:

In horses, stem cell therapies are a promising tool to the treatment of many injuries, which are common consequences of athletic endeavor, resulting in high morbidity and often compromising the performance. The aim of this study was to isolate progenitor cell from equine adipose tissue (eAT-PC), to evaluate their proliferative potential and to evaluate their capacity towards osteogenic, chondrogenic, adipogenic, neurogenic and miogenic differentiation before and after cryopreservation

### Methods:

Proliferation rate of progenitor cells, isolated from horse adipose tissue, was evaluated during 23 passages before and after cryopreservation. Differentiation was induced according to standard protocols previously described by Zuk et al., 2002. For chondrogenic differentiation pellet culture was obtained and stained with Toluidine blue. Immunohistochemical analyses of lineage specific proteins were also used in order to characterize different types of differentiation.

### Results:

The eAT-PC can be easily isolated and expanded in vitro. They present fibroblast-like morphology, constant growth rate during 12 passages before freezing and after thawing. All assays have been done before and after cryopreservation demonstrating similar results. Osteogenic differentiation was evidenced by the mineralization of extracellular matrix at day 11, which became stronger at day 21 and by positive von Kossa staining. Differentiated eAT-PC also presented positive immunostaining for osteocalcin and bone sialoprotein antibodies. After induction of adipogenic differentiation, the cell morphology changed within 24 hours from elongated fibroblastic cells to oval-shaped cells. After 4 days, vacuoles within cell cytoplasm were observed and at the day 7 an increased number of these cells, which presented vacuoles, showed positive Oil Red O staining. Chondrogenic differentiation was observed 21 days after induction by the staining of extracellular cartilage matrix proteoglycans. Both, neurogenic and miogenic differentiation have been presented by morphological typical changes and cell were positive to  $\beta$ -tubulin III and myosin antibodies immunostaining, respectively.

### Conclusions:

Our data suggest that the cells isolated from equine adipose tissue can be successfully isolated and expanded in vitro. We also demonstrated that eAT-PC showed high proliferative rate and capacity to differentiate into derivatives of mesoderm and ectoderm. These characteristics of eAT-PC can be maintained even after cryopreservation

## Quantification of Collagen Synthesis by Human Adipose Stem Cells Cultured as 3-Dimensional Multicellular Aggregates (MAs)

Samuel Yun; Hulan Shang; Adam Katz

### Introduction:

Our lab has developed techniques to suspension culture human ASCs as 3-dimensional multicellular aggregates (MAs). Our previous studies have demonstrated the fabrication of a self-generated extracellular matrix (ECM) by ASCs formulated as MAs, such that a defined, manipulatable structure (≈ "organoid") is generated. In contrast to cells grown as adherent monolayers, MAs enable the easy transfer/transplant of cells and ECM without disruption of cell-cell and cell-matrix interactions. The goal of this study was to confirm the presence of collagen in the extracellular matrix of ASC MAs and to quantify the relative amount of collagen production under varying culture conditions.

### Methods:

Cryopreserved human ASCs were thawed and plated using established protocols. Cells were cultured as adherent monolayers in LADP medium with 1% human serum until confluency. ASC MAs of 105 cells were then fabricated and maintained in suspension culture in one of three different medias (LADPM with 1% human serum (LADPM-1%), LADPM with no serum (LADPM-SF), and DMEM/F12 with antibiotics only (D0)). Each of these study arms was further divided into parallel cultures with or without Ascorbic Acid-phosphate. Media was changed on culture day 3, and MAs were harvested and analyzed on culture day 5 using two methods: Sirius Red F3B dye binding assay (detects all/most types of collagen) and picro-sirius red staining of cryosections.

### Results:

ASC MAs generated detectable collagen under all culture conditions, but more collagen was generated in LADP medium than DMEM medium. Under serum free conditions, the addition of vitamin C did not significantly increase collagen levels. In the presence of serum, however, vitamin C increased collagen production by ~20% (Table). Visualization of picro-sirius red stained MA sections under crossed-polarized bright field microscope showed the presence of Type I and III collagen fibers.

### Conclusions:

This study demonstrates that ASCs produce self-generated collagen when formulated as defined multicellular aggregates in suspension. Even more, ASC MAs support collagen production in defined, serum-free conditions. Based on these findings, ASC MAs may prove useful for therapeutic applications that would benefit from collagen supplementation/replacement.

## A subpopulation of Human Mesenchymal Precursor Cells expressing NANOG in vitro

Enrico Pierantozzi; Barbara Gava; Ivana Manini

### Introduction:

Human Mesenchymal Precursor Cells (h-MPCs) can be isolated from many adult tissues. These cells have been widely characterised and can differentiate into several mesenchymal lineages. Despite the large number of studies on h-MPCs, little is known about the molecular mechanisms underlying their biological properties. Recently, OCT4 and NANOG, two transcription factors responsible of maintaining stemness properties in embryonic stem cells, have also been described in h-MPCs, although these data are still debated.

### Methods:

Biopsies from human tissues were obtained with informed consent of patients undergoing surgical intervention, in accordance with the guidelines approved by the ethical committee. Cells were isolated and cultured according to standard procedure for culturing human MSC.

### Results:

In h-MPCs isolated from different adult tissues, including human adipose tissue, we did not detect expression of OCT4, but confirmed the expression of NANOG by both PCR and immunofluorescence assays. The number of NANOG+ cells varied between 10 to 50 % depending on the tissue of origin. We did not observe a direct correlation between NANOG expression and h-MPCs differentiative potential. However, the expression of NANOG decreased with differentiation and in late passages of h-MPCs. We are currently investigating the properties of this subpopulation of NANOG+ cells aiming to understand their potential role in h-MPCs biology.

### Conclusions:

Our studies identify a subset of cells expressing Nanog, but not of OCT4 in human MSC from adipose tissue. The properties of this subset of cells are being further characterised in order to identify their role within the population of MPC.



## Adipose-derived mesenchymal stem cells in reconstructive breast surgery - pitfalls

Susan Lim; Foong Lian Lam; Kerrie Lim

### Introduction:

The use of autologous fat and its cellular derivatives including the stromal vascular fraction and mesenchymal stem cells as a basis for tissue engineering in breast reconstruction has been extensively investigated. Important considerations in the success of the technique, including the cell type or cell-mix used, the method of processing if any, and surgical techniques of implantation are analysed in this case study.

### Methods:

We report the case of a 30-year old married female who underwent breast augmentation using autologous fat-derived stromal vascular fraction. The procedure was performed elsewhere, but the patient was followed up in our practice over a period of 6 months, using clinical palpation, ultrasonography, digital mammography, MRI and histology as methods of evaluation.

### Results:

At one month post-implantation, the patient appeared satisfied and did not report any nodules. At 3 months post-implantation, the patient reported palpable breast nodules, not previously felt. At 5 months post-implantation, these nodules were noted by both the patient and her husband, to have increased in size. Clinical examination confirmed the presence of multiple fatty nodules of varying sizes, palpable in both breasts. Digital mammography, high resolution ultrasonography and MRI confirmed the presence of multiple fatty nodules throughout the breast tissue. Histological examination confirmed the same.

### Conclusions:

The use of autologous fat and its cellular derivatives is an attractive option for women undergoing breast augmentation as well as for women requiring reconstruction of lumpectomy defects post cancer surgery. This study highlights the importance of surgical techniques in implantation. The necessity to characterise the cell types within the stromal vascular fraction, and the proportion of mesenchymal stem cells implanted, may be relevant in determining the final outcome and potential for growth of these implanted cellular derivatives of fat.

## One-step surgical procedure with stromal stem cells from the infrapatellar fat pad is feasible for regeneration of cartilage tissue

Wouter Jurgens; Zufu Lu; Annemieke van Dijk; Florine van Milligen; Marco Helder; Marco Ritt

### Introduction:

Current therapies for osteoarthritis lack regenerative capacity, and stem cell therapies are evaluated as a promising alternative. Since adipose tissue provides a rich source of mesenchymal stem cells (ASCs) which can be harvested in clinically relevant quantities within a short time frame, surgical procedures for osteoarthritic treatment in which ASC procurement and subsequent cellular therapy are performed within one single surgery, are within reach. To minimize discomfort for the patient and to possibly avoid a second surgical incision, we investigated whether the infrapatellar ("Hoffa") fat pad, readily available when accessing the osteoarthritic cartilage defect, harbors sufficient ASC quantity and quality to comply with this one-step surgical concept for cartilage regeneration.

### Methods:

Infrapatellar fat pads were harvested from 20 patients undergoing knee arthroplasty, with approval of the VU University medical center. The frequency of Colony-Forming Units (CFUs) in the stromal vascular fraction (SVF) of these fat pads was determined, and the percentage of these colonies able to differentiate into the osteogenic and adipogenic lineage. Growth kinetics of cultured ASCs were calculated and both fresh and cultured ASCs were characterized by FACS analysis of surface marker expression profile. Both these fresh and cultured ACSs were then differentiated into the chondrogenic lineage to check the feasibility for a one-step surgical procedure to regenerate cartilage, as analyzed using RT-PCR and (immuno) histochemistry.

### Results:

The frequency of Colony-Forming Units (CFUs) in the stromal vascular fraction (SVF) of these fat pads was  $2.6 \pm 0.6\%$ . These CFUs showed differentiation towards the osteogenic and adipogenic lineage. When cultured up till passage 3, a homogeneous cell population was obtained with a population doubling time of approximately two days and a surface marker expression profile matching that of ASCs. When cultured in a poly (D,L-lactide-co-caprolactone) scaffold, both cultured ASCs and freshly isolated stromal cells showed chondrogenic differentiation potential in vitro, as demonstrated using RT-PCR analysis and (immuno) histochemistry

### Conclusions:

Due to the high ASC quantities in the stroma of the infrapatellar fat pad, the favorable proliferation rate and the potent chondrogenic differentiation potential, this stroma is a suitable candidate for a one-step surgical procedure to regenerate cartilage tissue.

## In vitro simulation of injury activates adipose-derived stromal cells

Hitomi Eto; Hirotaka Suga; Kotaro Yoshimura

### Introduction:

After injury to adipose tissue, adipogenesis accompanied by angiogenesis is seen in the tissue repair process. By characterizing wound exudates within one day after liposuction, bFGF, PDGF, EGF, and TGF-beta were determined as injury-associated soluble factors in the earliest stage, and a mixture of the four factors was called injury cocktail in this study. To simulate adipose injury in vitro, we used the injury cocktail and its biological effects on adipose-derived stromal cells (ASCs) were examined.

### Methods:

To characterize ASCs stimulated by the injury cocktail, surface marker expression using flow cytometry and gene expression using quantitative real-time PCR were examined. In addition, cell proliferation assay, migration assay and three-dimensional capillary formation assay on Matrigel were performed; effects of the injury cocktail were compared with those of VEGF. Human umbilical vein endothelial cells (HUVECs) were also used for comparison.

### Results:

The injury cocktail significantly promoted proliferation and chemotactic activity of ASCs. Migration assay revealed that ASCs migrated into a chamber with injury cocktail, but HUVEC did not. On the other hand, HUVEC migrated into a chamber with VEGF, but ASCs did not. Real-time PCR showed that remarkable upregulation of Flk-1 mRNA was induced by stimulation of injury cocktail as well as an increase in CD31 gene expression, while CD34 mRNA was downregulated. FACS analyses revealed that ASCs cultured with injury cocktail showed a higher expression of Flk-1, Tie-2, and CD117 as well as a greater intake of acLDL than control, suggesting that ASCs were activated as endothelial progenitor cells and maybe partly differentiated into endothelial cells, though CD34 expression was markedly suppressed. CD140b was maintained at a high percentage, while SMA expression was increased compared to control. In Matrigel assay, ASCs cultured in injury cocktail formed lectin-positive capillary networks, suggesting that ASCs efficiently differentiated into endothelial cells in vitro. On the other hand, capillary network formation by HUVECs was not affected by injury cocktail. When ASCs were co-cultured with HUVECs on Matrigel, both types of cells were combined in newly-formed capillary networks.

### Conclusions:

In vitro simulation of injury activated ASCs, inducing proliferation and migration of ASCs. It was demonstrated that ASCs had a great potential for angiogenesis and could be activated as endothelial progenitor cells by injury cocktail.

## Isolation and identification of mesenchymal stem cells from various human adipose tissues

Seok-Whan MOON; Hyeong Sun KWON; Yong Woon CHOI; Young-Sun SOHN; Yeong In KIM; Il Hoan OH; Wan-Shik SHIN; Sang Hong BAEK

### Introduction:

Human mesenchymal stem cells originally discovered from bone marrow and umbilical cord blood harbor differentiation capacities into osteogenic, adipogenic, chondrogenic cells and even further neural cells under appropriate culture conditions. Recently, it has been reported that human adipose tissue presents an alternative source of mesenchymal stem cells mainly because it is relatively easy to obtain a large number of cells in a very convenient way and has the same differentiation abilities into multiple lineages as those isolated from bone marrow and umbilical cord blood. In this study, we have tried to isolate and characterize human mesenchymal stem cells from several different fat sources such as mediastinal fat, thymic fat, adult thymus and omental fat.

### Methods:

Tissue sources were obtained from patients with written consents. Cells were isolated by the treatment of collagenase and RBC lysis buffer. DMEM-based medium was used for cell expansion and induction media were used for osteogenesis or adipogenesis. Flow cytometry was performed to determine phenotypes. In vitro differentiation assays of osteogenesis and adipogenesis were carried out by alizarin red staining and Oil red O staining respectively.

### Results:

During the culture, cells, which were isolated from mediastinal, thymic, and adult thymus showed typical fibroblast-like shape, but cell morphology from omentum was more swelling and spherical. Isolated cells from all the tissues commonly expressed surface markers such as CD73, CD90, CD105 and CD166, but negative for CD34, CD45 and HLA-DR with variable rate of expression. Among four tissues, isolated cells from mediastinal fat and adult thymus had multiple differentiation capability to both osteogenesis and adipogenesis at the rate of 80% and 88% respectively, but 30% for thymic fat and 0% for omental fat.

### Conclusions:

Human mesenchymal stem cells featuring morphology, surface markers and differentiation capability were successfully isolated from mediastinal fat, adult thymus and thymic fat. Mediastinal fat and adult thymus showed higher rate of isolation of human mesenchymal stem cells. These results indicate that cells from mediastinal fat and adult thymus have comparable characteristics to those of bone marrow and umbilical cord blood-derived mesenchymal stem cells and have a potential as an alternative source for mesenchymal stem cells.

## Adipocytes release factors that accelerate keratinocyte proliferation in culture

Chris Campbell; C. Scott Hultman; Bruce Cairns; Anthony Meyer

### Introduction:

Keratinocytes grown directly on adipose tissue have greater proliferation rates than keratinocytes grown alone. It is unknown if factors released by adipose tissue into culture media could increase keratinocyte proliferation without requiring incorporation of adipose tissue into skin graft models, or serve as a substitute for the fibroblast feeder layer.

### Methods:

Human keratinocytes were grown with and without NIH 3T3 fibroblast feeder layer in the following conditions (12 cultures per group): adipose tissue co-culture (AT), cultures supplemented with medium from whole adipose tissue referred to as adipose-conditioned medium (ACM), and control. Proliferation was measured with a colorimetric proliferation assay, and digital calculations of percent confluence over time. Culture morphology was assessed by light microscopy.

### Results:

ACM cultures without 3T3s, AT cultures with and without 3T3s, and 3T3 control cultures demonstrated a similarly significant proliferation increase over non-3T3 control ( $p < 0.05$ ) corresponding with a two-fold increase in percent confluence by Day 7. ACM cultures with 3T3s proliferated significantly faster than all other treatment groups ( $p < 0.05$ ) resulting in complete confluence by Day 5. ACM cultures with and without 3T3s produced a thick keratinized layer by Day 7 whereas all other cultures including AT cultures did not.

### Conclusions:

Engineered tissue replacement can be accelerated and simplified by ACM without requiring the addition of adipose tissue or a fibroblast feeder layer to keratinocyte culture systems. ACM supplementation provides an additive proliferation benefit when combined with a feeder layer producing mature grafts in approximately half the time as keratinocytes alone by accelerating proliferation and increasing keratinization.

## Appropriate application of cultured human adipose-derived stem cells within lipoinjection for soft tissue augmentation

YunYoung Kim; SuHee Lee; SeonHwa Park

### Introduction:

With injective transfer of autologous aspirated fat, it is now possible to use to repair defects for the elimination of facial wrinkles, skin contour defects and soft tissue augmentation. But several issues require attention, including unpredictability and a low survival rate due to partial necrosis.

### Methods:

This study was under taken to evaluate the longer-lasting effects of aspirated fat injected into the back of nude mouse subcutaneously, combined with human adipose-derived stem cells(ASCs) cultured from aspirated fat, at the range of 0(control), 2 and 10 x 10<sup>4</sup>/ml. Aspirated fat was homogenized with mixer homogenizer for blending, suspension and evenly mix with ASCs.

### Results:

Cultured human ASCs were confirmed in their differentiation capacity into the adipogenic, osteogenic, and chondrogenic lineages and also were characterized negative for the hematopoietic stem cell marker CD34 and the endothelial marker CD31. At 6 weeks after the lipoinjection, the weight was measured and histological comparisons were also performed. The mean weight of lipoinjection combined with 1 x 10<sup>5</sup>/ml cultured human ASCs was 152% of the weight of lipoinjection without ASCs. The group of lipoinjection without homogenization makes a lump more than with homogenized lipoinjection group. The capillary vessel was detected more in the histological sections of the group of lipoinjection with ASCs than those of the group of lipoinjection without ASCs.

### Conclusions:

Adipose tissue is known to be rich in microvasculature, and cultured ASCs showed to induce more vasculature around the fat injected and some of them differentiated into vascular endothelial cells. This study demonstrated that the cultured human ASCs have a great advantage for soft tissue augmentation when combined with lipoinjection.

## OSTEOGENIC AND ADIPOGENIC DIFFERENTIATION OF MOUSE ADIPOSE STEM CELLS: EFFECT OF EPIGENETIC MODULATION ON DIFFERENTIATION MARKERS

Björg Roberg; Philippe Collas; Jan Øivind Moskaug

### Introduction:

Adipose stem cells can easily be isolated from mouse gonadal fat deposits. Fractions of such cells can be induced in vitro to differentiate towards the osteogenic, adipogenic, myogenic and neurogenic lineages. Studies of human adipose stem cells have shown that differentiation potentials are partly determined by epigenetically regulated chromatin structure. The current study was undertaken to assess the effect of epigenetic modulators affecting DNA methylation and histone acetylation.

### Methods:

Cells from the stromal vascular fraction were cultured while still retaining their ability to support adipose tissue, as shown by in vivo imaging of luciferase positive cells. Cells in culture were treated with 5-aza-cytidine and/or trichostatin A to inhibit DNA methyl transferases and histone deacetylases, respectively, and expression of osteogenic and adipogenic differentiation markers were measured by quantitative RT-PCR.

### Results:

We found that osteopontin is down regulated by both osteogenic and adipogenic differentiation. Osteogenic induced reduction can be reversed by treatment of cells with either 5-aza-cytidine or trichostatin A. Combination of both gave an additive effect, suggesting that osteopontin expression during osteogenic differentiation is at least partly epigenetically regulated. Adipogenic induced reduction of osteopontin expression was not influenced by epigenetic modulators. Also another osteogenic marker, osteoprotegerin, responded to chromatin modulators by increased expression during osteogenic differentiation.

### Conclusions:

The preliminary conclusion from these studies is that epigenetic modulation of chromatin regulates expression of osteogenic marker genes during differentiation of mouse adipose stromal vascular fraction cells.

## Effects of Adipose-derived Stem Cells in Partial Removal of Skeletal Muscle in Rats

Ok-Kyung Hwang; Il-Hwa Hong; Mi-Ran Ki; Jin-Kyu Park; Moon-Jung Goo; Jung-Youn Han; Kyung-Sook Hong; Ae-Ri Ji; Young-Mi Moon; Se-Il Park; Jae-Ho Jeong; Kyu-Shik Jeong

### Introduction:

Differentiation of mesenchymal stem cells into skeletal muscle and restoration of dystrophin expression in mdx/- mouse by stem cell transplantation have been reported previously. In this study we evaluated the effect of culture-expanded stem cells derived from adipose tissue on the muscle regeneration in partial removal of skeletal muscle in rats.

### Methods:

Stem cells were isolated from rat abdominal adipose tissue by collagenase digestion and cultured in low glucose DMEM. Muscle injury was created by partial removal of quadriceps femoris muscle of rats. We have used undifferentiated adipose derived stem cells (ASCs) labeled with superparamagnetic iron oxide (Feridex). The labeled cells were directly injected into the muscle injured area. Histological analysis was performed using H&E staining and prussian blue staining. The immunolabelling of MyoD, Myogenin, and Pax7 were examined.

### Results:

ASCs were detected by Prussian blue staining in injured muscle area. Microscopical muscle regeneration and neovascularization were more increased in ASCs-injected group compared to ASCs non-injected control group. The expression level of MyoD, Myogenin, Pax7 were more significantly increased in ASCs-injected group compared with ASCs non-injected group.

### Conclusions:

This study suggests that ASCs directly involved in the regeneration process of injured skeletal muscle, therefore ASCs need to be further characterization for the cell therapy.



## Effect of Mechanical Forces on the Differentiation of Human Adipose Derived Stem Cells

Kate O'Brien; Sanjit Nirmalanandhan; Alicia Duren; Sitta Sittampalam

### Introduction:

Human Adipose Derived Stem cells (hADSCs) have been extensively studied for their ability to differentiate into multi-lineage cell types such as adipocytes, osteoblasts, and chondrocytes. These differentiated cells have potential clinical applications in regenerative medicine and in the field of tissue engineering. For example, hADSCs of an arthritis patient can be differentiated into chondrocytes to regenerate and repair cartilage damage. It is known that these cells experience forces in the body as they differentiate and repair damaged tissues, and we propose to simulate such forces and establish better conditions for preparing cells for clinical applications. The purpose of this study is to examine the effect of mechanical forces on the differentiation of hADSCs into adipocytes and chondrocytes using appropriate differentiation media.

### Methods:

The hADSCs (Invitrogen Inc.) were expanded to passage 2 or 3 using MensenPRO RS medium (Invitrogen Inc) supplemented with a proprietary Growth Supplement (Invitrogen Inc.) 1% of L-glutamine, and 1% antibiotics. The hADSCs were then cultured in STEMPRO Adipogenesis and STEMPRO Chondrogenesis differentiation Basal Media (Invitrogen Inc.) supplemented with 10 mL STEMPRO Adipogenesis and Chondrogenesis supplements, 50 uL of gentamicin, and 1% antibiotics in the presence of mechanical forces for 4-7 days. Forces are applied to these cultures using FlexCell Tissue Train system (FlexCell International Inc.) LipidTox neutral green and Alician Blue or Safranin O stain for differentiated lineages of fat and chondrocytes respectively. The ADSCs and differentiated cells were lysed with RIPA buffer and Western blots were performed to measure the protein expression using the selected surface protein markers for hADSCs, CD13, CD44, CD105, CD29, and CD34 (BD pharmingen).

### Results:

Preliminary LipidTox staining data show hADAS differentiate into fat cells. CD13 was negative in both ADSCs and differentiated adipocytes. CD44 was positive in hADSC, but it was negative in the differentiated cells. CD29 and CD105 were positive in both hADSCs and differentiated cells. CD34 was positive in differentiated adipocytes, but it was negative in hADSCs. Work is underway to examine the effect of chondrocyte differentiation and the effect of forces on the differentiation of these cells into adipocytes and chondrocytes.

### Conclusions:

Our results indicate that hADSCs can be differentiated into adipocytes by using the appropriate differentiation medium (Stem-PRO adipogenesis basal medium). The western blots of CD29 and CD44 are consistent with the literature (Mitchell, JB et al, Stem Cells, 2006). CD105, CD34, and CD13 are inconsistent with the literature. These discrepancies could be attributed to differences in cell source, isolation procedures, antibody detection techniques used in the lab. Currently our group is examining the effect the above sources of variation, and in addition to the effect of mechanical forces on the differentiation hADSCs into these lineages.

## Isolation, Culture and Plasticity of Mesenchymal Stem Cells from Horse and Swine Peripheral Blood and Adipose Tissue.

Ricardo GARCIA; Maria MASRI; Cristina VELASQUILLO; Clemente IBARRA

### Introduction:

Studies have shown that harvested cells from peripheral blood have the plasticity potential that bone marrow stem cells have shown in vitro. There are surface markers known to characterize a specific stem cell phenotype that has the ability to produce precursor cells committed to a specific functional tissue, with the potential to regenerate damaged tissue from different origin, hence the functional tissue to heal the lesion. We isolated mononuclear cells from peripheral blood and adipose tissue from adult individuals (6 horses 4-6 years and 6 pigs 6-8 months), to characterize the phenotype looking for specific stem cell surface antigens with flow cytometry analyses; CD34, CD45, CD90, CD14, CD117, CD73 monoclonal antibodies were used. Ever after we made plasticity assays to evaluate if our harvested cells had the ability to produce different cellular phenotypes with culture stimulation. From the horse blood we got a CD34+, CD90+, CD 73+, CD45-, CD14-, CD117- phenotype and with the fat cells CD90+, CD117+, CD14-, CD47-, this surface markers were lost at the end of the cloning phase. From the pig blood we harvested a CD34+ population; and from fat cells we got a CD34+, CD117 y CD90+ phenotype. The mesenchymal stem cells (MSC) have an specific phenotype, this goes to surface markers produced by undifferentiated cells that make easier his recognition and isolation from a wide cell population in different tissues. Between the different markers associated with this undifferentiated cell population we have: CD117, CD73, CD90, CD34, CD22, CD13, CD71. The list of known sources are long from the bone marrow to the liver; and this undifferentiated cells have the potential to originate full functional tissues, because this harvested cells in vitro can give rise to committed cells that leads to functional tissues that could be used to repair different tissues, like cartilage, tendons, bone, nerves, etc. Bone marrow stem cells can generate different functional tissues, in humans 1 of each 10 000 cells is a MSC; the harvest technique is painful leaving the patient with prolonged pain consequences. Besides the blood harvest of MSC ( $4 \times 10^6$ /L human adult) is an easier way to get precursors with the potential of MSC leaving the patient without any pain besides a needle puncture on his arm. Although the precursor cells obtained from peripheral blood had demonstrate plasticity as the MSC isolated from bone marrow. The main goal of this study is to demonstrate that is possible to harvest precursors from peripheral blood and fat tissue with specific MSC surface markers and give rise to other kind of tissue, thinking in future application to repair damaged tissue like articular cartilage or nerves. Hypotesis It is possible to isolate and characterize mesenchymal stem cells from peripheral blood and fat tissue to generate in vitro cultures and give rise to other cell phenotypes (cartilage, fat, neurons and muscle).

### Methods:

Peripheral blood: peripheral blood samples (50 mL) from 6 swine and 6 equine were obtained in EDTA blood collection tubes we use approximately per sample, then the buffy coat were separated carefully without RBC contamination using Ficoll-paque (gradient concentration separation). Centrifugation was performed at a relative centrifugal force of  $400 \times g$  for 30 minutes at  $10^\circ\text{C}$ . The interface layer was placed into a new 50-ml tube, washed twice in phosphate buffered saline (PBS), and counted afterwards (GibcoBRL Trypan blue 0.4%). After an additional wash with PBS, cells were centrifuged at  $300 \times g$  for 5 minutes to remove the supernatant. Flow cytometry analyses was performed and plated for culture half of the cells. Fat tissue: fat tissue cells were harvested from 2 g of tissue resected from swine and equine individuals, the fat tissue of the pigs was taken from the cheek. The fat tissue of the horses was taken from 4 inches lateral and 4 inches anterior of tail insertion (Nixon et al 2006). Adipose tissue was digested with type I collagenase at  $37^\circ\text{C}$  shake for 60 min (Shaker). We use 1mg of type I collagenase for each 0.5 g of adipose tissue (Miyazaki et al 2005). Then the samples were centrifuged at  $200 \times g$  over 10 min. The pellet were analyzed by flow cytometry and plated using 24 well culture plates. From the whole cell samples we designated different cell groups to perform plasticity essays. For chondrogenic committed cells showed sensitivity to the media change, leading us to evaluate different concentrations of transforming growth factors from the original protocol (Pittenger, et al 1999); the adipogenic cells were stimulated with a protocol (Miyaki, et al 2005) using isobutylmethylxanthine, dexamethasone, insulin, and indomethacin. Neurogenic designated cultures were changed in phenotype adding to the media forskolin and dexamethasone (Sanchez-Ramos, et al 2000); finally the muscle generating cell culture were stimulated with a 5-azacytidine protocol (Wenrong, et al 2004). All cells change in shape from the original fibroblast cell like phenotype.

**Results:**

Chondral lineage. Peripheral blood (PB): cells showed chondrocytic appearance by increasing transforming growth factor concentration and the recombinant human insulin. A difference from the published protocol. Adipose tissue (AT): showed no change from the initial fibroblast shape like; but adding the recombinant human insulin swine adipose tissue showed a chondrocytic like phenotype. Adipose Linage. Peripheral blood doesn't show any change with the stimulating media. Adipose tissue from both species shows lipid accumulation evidenced by a Nile red stain. Neural lineage. Peripheral blood (PB) showed immediate change of shape from the initial fibroblast spindle shape, changed for a neural phenotype with cytoplasm prolongations. Stain pending. Adipose tissue show no change in shape. Muscular Linage. Peripheral blood (PB): cells showed sensitivity to the stimulation by 5-azacytidine Adipose tissue showed resistance to different concentrations of 5-azacytidine.

**Conclusions:**

Mononuclear cells were isolated from peripheral blood and stromal cells were harvested from adipose tissue, both from equine and swine individuals. The presence of specific stem cell precursor surface markers were proved using human synthesized monoclonal antibodies; this indicates cross antigenicity and let us presume proteic sequence homology between the species receptors; nonetheless the swine cells showed no positive readings to some of the antibodies tested; this goes with no human proteic sequence recognized by the antibodies in the swine cells.. When the stimulation media were added a change in cell shape was seen, this change of phenotype suggests that the culture cells change metabolism induced by the growth factors and other drugs added to the culture media. We attempt to study this changes of metabolism with immuno-histochemistry tests, looking for specific tissue markers and cellular products founded in the mature tissues of the live organism. In the cartilage like culture we look for collagen II and aggrecan; in the nervous like cultures, nestin a specific neural precursor protein; and with the muscular tissue we will look for alpha-cardiac actin. At this time we are standardizing the assays to create our positive controls in those immuno-histochemistry tests using mature tissue cells isolated from the specialized tissue. *Literatura citada.* Dennis J.E., Merriam A., Awadallah A., Yoo J.U., Johnstone B., Caplan A.I., (1999) A quadripotential mesenchymal progenitor cell isolated from the marrow of an adult mouse, *J. Bone Miner. Res.*;14: 700-709. Kirschstein R. and Skirboll L.R., (2001) Stem Cells: Scientific Progress and Future Research Directions. Report prepared by the National Institutes of Health June Miyazaki Takamichi, Yasuo Kitagawa . Kazuhiro Toriyama . Masato Kobori . Shuhei Torii, (2005) Isolation of two human fibroblastic cell populations with multiple but distinct potential of mesenchymal differentiation by ceiling culture of mature fat cells from subcutaneous adipose tissue, *Differentiation* 73:69-78 Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM., (2006) Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. *Stem Cells*. Feb;24(2):376-85. Epub 2005 Dec 1. Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., Moorman, M. A., Simonetti, D. W., Craig, S., & Marshak, D. R., (1999) Multilineage potential of adult human mesenchymal stem cells. *Science*; 284, 143-147. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR., (2000) Adult bone marrow stromal cells differentiate into neural cells in vitro. *Exp Neurol*; 164:247-256. Wenrong XU, Xiran Zhang,\* Hui Qian, Wei Zhu, Xiaochun Sun, Jiabo Hu, Hong Zhou, and Yongchang Chen., (2004) Mesenchymal Stem Cells from Adult Human Bone Marrow Differentiate into a Cardiomyocyte Phenotype In Vitro, *Exp Biol Med* (Maywood) Jul;229(7) 623-31.

## Prefabrication of Engineered bone using adipose-derived stem cells

Takahisa Okuda; Cagri Uysal; Yurie Itoi; Rei Ogawa; Hiko Hyakusoku; Hiroshi Mizuno

### Introduction:

The purpose of this study is to investigate whether engineered tissue by adipose-derived stem cells (ASCs) and scaffold could be transferred through the flap prefabrication technique.

### Methods:

ASCs were isolated from inguinal fat pads of Fisher rats. The cells were expanded to two passages. They were seeded onto porous beta-TCP. Scaffolds were placed into osteogenic medium for 2 weeks. Then, scaffolds were wrapped with epigastric flaps in rats in order to prefabricate osteocutaneous engineered flaps. They were monitored for 8 weeks. After that, prefabricated osteocutaneous flaps were elevated and their vascular pedicles were clamped for 4 hours. Finally, tissue specimens were harvested after an additional 2 weeks. There are 3 main and 2 subgroups. In Group A, ASCs that are induced osteogenically, In Group B, ASCs without any induction and in Group C, no cells were used. "x" stands for prefabrication and "y" is for the non-prefabricated group. We performed histological and immunohistochemical analyses. Quantitative measurements of angiogenesis and osteogenesis were evaluated.

### Results:

Angiogenesis was compared within each group. There was statistical significance between the prefabrication groups and the non-prefabrication groups. Osteogenesis was also compared within each groups. There was no statistical significance within groups Ax,Ay and Bx,By.

### Conclusions:

Our observation indicates that angiogenesis could be successfully introduced into the engineered bone tissue. Osteogenesis could also be maintained after flap prefabrication.

## Characterization of aspirated adipose tissue: differences in structure and cellular components from intact adipose tissue

Hitomi Eto; Hirotaka Suga; Kotaro Yoshimura

### Introduction:

For adipose tissue harvesting, two clinical procedures are available: excision and aspiration. Lipo-aspiration, which removes cosmetically-unwanted adipose without leaving visible scars, is actually the only practical method for harvesting adipose tissue in large volume, though it is hard to characterize aspirated adipose tissue using animals. To determine the differences between aspirated and intact adipose tissue, we have harvested and characterized both human tissues.

### Methods:

Freshly aspirated or intact human subcutaneous adipose tissue samples were evaluated as follows; 1) Whole-mount staining: three-dimensional visualization of living adipose tissue with a confocal microscopy that enables observation of cellular and structural events in adipose tissue, 2) glycerol-3-phosphate dehydrogenase (GPDH) assay, 3) multicolor flow cytometry: CD34, CD31 and CD45, and 4) cell yield of stromal vascular fraction (SVF) cells including adipose-derived stromal cells (ASCs).

### Results:

Whole-mount staining revealed presence of capillaries running beside adipocytes and non-adipocyte cells such as endothelial cells and ASCs comprising over half of total cells in both intact and aspirated adipose tissue. Although there were vascular structures including vessels with a diameter of over 50 $\mu$ m in intact adipose tissue, aspirated adipose tissue had only small and short blood vessels and small lipid droplets presumably as a result of a mechanical damage. Dead cells, which were distinguished from viable cells by cell staining with propidium iodide, were 45-70% of total cells in aspirated adipose tissue, while 20-50% in excised adipose tissue. Aspirated adipose tissue showed a lower value of total GPDH activity, which correlated with the total number of adipocytes and a higher value of extracellular GPDH released from ruptured adipocytes than intact adipose tissue. Although the total SVF cell yield isolated from aspirated adipose tissue was higher than excised adipose tissue, flow cytometric analysis revealed that aspirated adipose tissue contained a much higher percentage of blood-derived cells (CD45+) and a lower number of ASCs (CD45-CD31-CD34+) than intact adipose tissue. Nevertheless, a sufficient number of adherent ASCs were obtained by 1 week culture.

### Conclusions:

We confirmed that both aspirated and excised adipose tissues were vasculature-rich tissues, but in aspirated adipose tissue, a large number of cells were dead, and vessels were generally disrupted and large vessels were lost, suggesting a substantial mechanical damage.

## Threatened Facial Hardware Salvage Using Fat Grafting

William Joseph Casey III, Daniel Krochmal; Alanna Rebecca; Kristen Kalkbrenner

### **Introduction:**

Bony hardware placed in areas of minimal soft tissue coverage are at risk for extrusion, exposure, surface irregularity and patient dissatisfaction. These complications traditionally necessitate removal of hardware, local flap coverage or free tissue transfer. The use of fat grafting may obviate the need for more invasive procedures in some cases.

### **Methods:**

A sixty seven year old female sustained profound facial injuries during a plane crash twenty five years prior to presentation. Multiple facial reconstructive surgeries resulted in iatrogenic globe injury with enucleation and extensive plating. The patient lost the majority of subcutaneous tissue around the orbit due to scarring and repeated procedures. At presentation the contours of plates could be easily visualized through the skin. No exposure was noted. Fat grafting was attempted to provide improved hardware coverage and to prevent extrusion.

### **Results:**

55 ml of harvested abdominal fat was prepared in standard fashion and placed into the subcutaneous plane around the orbital rim. Three months post operatively, a significant amount of grafted adipocytes remained viable. The periorbital plates remain covered and no longer appear at risk for exposure. The patient is pleased with the functional and aesthetic result.

### **Conclusions:**

In carefully chosen cases of threatened hardware exposure, free fat grafting may present a viable alternative to traditional flap procedures for adequate coverage.

## Clinical Review of Autologous fat injection

Choi young woong

### Introduction:

Fat graft was first reported by Dr. Neuber in 1893. Last two decade, many materials-liquid silicone, polyurethane, collagen, hyaluronic acid and others-was used for filling of soft tissue defect and contour deformity of face and trunk. Autologous fat graft is simple and safe method which has less complication. So it became one of most popular method for correction of contour deformity, and we reviewed our experiedces.

### Methods:

We performed autologous fat injection 24 patients. The most frequently injected lesions were nasolabial fold, periorbital area, glabella and forehead. The donor sites were lower abdomen, buttock. The remaining fat after injection were kept under -20°C for 6 months and were used for the repeated injection. Patients were follow up first, 5th day, 2 month and 6 month after injection.

### Results:

Most patient were satisfied with the outcome. 1 patient was given reinjection because of under correction on 20th day after injection. Complication was trivial - skin erythema(3cases), firm nodule(1 case)- and they were treated conservative treatment.

### Conclusions:

Autologous fat injection has many advantages. it is simple and effective for correction of facial contour deformity. Precise anatomic position and place and surgeon's knowhow from repeated injection can improve the result.

## Autologous fat implantation for tissue regeneration

Adrien Aiache

### Introduction:

The harvesting of autologous fat is designed for tissue increase in different body areas

### Methods:

Harvesting the fat is performed with non traumatic techniques,with minimal manipulation and exposure

### Results:

Scar elevation and softening, increase in volume of different areas and tissues is obtained with sub cutaneous, intramuscular, and subperiosteal injections. Excess aspirated fat is frozen for successive implantation

### Conclusions:

Autologous fat contains stem cells able to regenerate some chosen tissues

## Optimization of Cartilaginous Tissue Formation Using Human Adipose Tissue-derived Stem Cells and PLCL/Hydrogel Scaffolds

Sang-Hee Kim; Youngmee Jung; Sang-Heon Kim; Jong-Won Rhie; Young Ha Kim; Chul Geun Kim; Soo Hyun Kim

### Introduction:

In our previous studies, we have developed a porous poly (L-lactide-co- $\epsilon$ -caprolactone) (PLCL) scaffold that has mechanical properties similar to natural cartilage. We have investigated the cartilaginous tissue formation of hASC-seeded PLCL scaffold prepared by a combined seeding of hASCs and fibrin hydrogel which was used to make 3-dimensional environment in the interior of PLCL scaffold. The purpose of this study is to find optimal conditions of hASC/fibrin-incorporated PLCL scaffold for in vivo cartilage formation with respect to the induction period of in vitro chondrogenic differentiation of hASCs and the concentration of fibrin used for hydrogel formation.

### Methods:

PLCL scaffolds with 85% porosity and 300-500  $\mu$ m pore size were fabricated by a gel-pressing method. For examining cartilaginous tissue formation in vivo, hASCs were mixed with 1% or 5% fibrinogen solution having thrombin, seeded in the scaffold, and subcutaneously implanted on the dorsa of nude mice after being cultured for 14 and 21 days in chondrogenic media. Specimens were harvested at 4, 8 weeks after implantation and analyzed by histological and biochemical methods, SEM images, and RT-PCR. For histological analysis, specimens were fixed for 3 days in 10% (v/v) buffered formalin and stained with Haematoxylin and Eosin (H&E), Masson's trichrome (M-T), alcian blue, and Safranin O.

### Results:

To investigate the effect of the gel concentration and in vitro induction time for chondrogenic differentiation on the formation of in vivo cartilage of hASC/fibrin-incorporated PLCL scaffold, four different groups were tested as follows: induction for 14 days in 1% fibrin gel (14d/1%), induction for 14 days in 5% fibrin gel (14d/5%), induction for 21 days in 1% fibrin gel (21d/1%), induction for 21 days in 5% fibrin gel (21d/5%). Immunofluorescence analysis for collagen type II revealed that cartilaginous phenotypes were much more expressed in the groups of 14d/1% and 21d/5%. These tendencies were also confirmed from the quantitative analysis of sulfated glycoaminoglycans (s-GAGs) using 1, 9-dimethylmethylene blue assay. Figure 1 shows the content of s-GAGs for the four groups. It is showed that GAG contents increased with an increase in the implantation time and the groups of 14d/1% and 21d/5% have greater GAGs contents at 8 weeks as compared with other groups.

### Conclusions:

In conclusion, we demonstrated that the highest cartilage formation were observed in hASC/fibrin-incorporated PLCL scaffolds prepared with the following condition; induction for 14 days in 1% fibrin gel (14d/1%) and induction for 21 days in 5% fibrin gel (21d/5%). Finally, a hASC/fibrin-incorporated PLCL scaffold might be a meaningful system for 3-dimensional tissue formation for cartilage tissue engineering.





















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