IFATS07

INTERNATIONAL FEDERATION OF ADIPOSE THERAPEUTICS AND SCIENCE

ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Hyatt Regency Hotel - Indianapolis, Indiana

Fifth Annual Meeting Program

October 18-20, 2007

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Mission

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

Description

The International Federation of Adipose Therapeutics and Science (IFATS), a 501 (c) (3) non-profit organization incorporated in August of 2002. IFATS' current scientific areas of interest relate to facilitating the use of adipose tissue as a source of adult stem cells that have the potential to regenerate and repair many different tissues in the body; the generation of new fat tissue for reconstruction after cancer or birth-related defects; and the development of treatments for excess body fat. It is a leading source of information about adipose biology and related technology. IFATS' goal is to identify new technology and bring 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.

ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Welcome

From This Year's President

I look forward to welcoming you to the 5th annual meeting of the International Federation of Adipose Therapeutics and Science (IFATS)!

This is the only organization that focuses on the understanding and treatment of disease by probing into the characteristics of adipose tissue and its rich repository of stem cells.

A multidisciplinary group of investigators from around the world will come together from October 18-20, 2007, at the Hyatt Regency in downtown Indianapolis, to share knowledge, ideas, and a vision for the future of this rapidly advancing area of discovery, translation, and therapy

This meeting will be more exciting than ever before, with the field of adipose stem cells having demonstrated a truly remarkable growth in interest over the last year. This is based on a progressive recognition of the host of possibilities for addressing diseases that affect very many people, including those with heart disease, problems with circulation to the legs, stroke, neurological disorders, diabetes, obesity, hemophilia, autoimmune diseases, kidney disease, bone and joint problems, and others.

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.

Enriching this meeting from a scientific perspective about closely related work, we are looking forward to special keynote lectures from scientists who have made truly seminal contributions in the translational science of other important types of adult stem cells: Dr. Anthony Atala (leader in the field of amniotic-fluid derived stem cells), Dr. Hal Broxmeyer (discoverer of umbilical cord-blood derived stem cells), and Dr. Katarina LeBlanc (leader in the field of bone-marrow derived mesenchymal stem cells). Biosketches of each of these speakers are available on the IFATS web site.

Indianapolis is a city very familiar with the importance of teamwork! From the yearly extravaganza of the Indy 500 race to the recent winning of the Superbowl, we see that team spirit is a characteristic of the successful efforts of many grand endeavors. The worldwide assembly of researchers that make up IFATS is indeed a group that will achieve its goals most rapidly by working together closely, and accordingly the theme of the 2007 IFATS meeting is TEAM.

Team IFATS will be regaled by a special keynote address delivered by the outstanding and famous Indy 500 race car driver Sarah Fisher, who will speak on the importance of teamwork in winning a race, whether with engines or adipose stem cells.

Race to IFATS 2007! We look forward to welcoming you to Indianapolis!!!

Regards,

Keith L. March, M.D., Ph.D.
President, IFATS 2007

IFATS07

International Federation of Adipose Therapeutics and Science

October 18 - 20, 2007

Hyatt Regency Hotel - Indianapolis, Indiana

Wednesday, October 17, 2007

3:30 - 7:00 PM	Registration and Poster Set Up
5:30 - 7:30 PM	Social Gathering and Welcome Hyatt Regency (Sponsored Event: Heavy Hors D'oeuvres and Cash Bar)

Thursday, October 18, 2007

7:00 - 8:00 AM	Breakfast – Regency Ballroom Foyer
8:00 - 5:00 PM	Exhibitors on Site
8:00 - 8:15 AM	Opening Remarks from Organization Committee
8:15 - 8:30 AM	Welcome *D. Craig Brater, M.D.,* *Vice President, Indiana University* *Dean and Walter J. Daly Professor,* *Indiana University School of Medicine*
8:30 - 9:30 AM	Keynote Address I: Umbilical Cord Stem Cells *Hal Broxmeyer, Ph.D., Walther Oncology Center* *Indiana University School of Medicine*
9:30 - 11:45 AM	**Symposium I** Basic Science: ASC Biology Moderators: J. Gimble and A. Katz
9:30 - 9:45 AM	I-1 In Vitro Clonal Analysis of Murine Common Pluripotent Stem Cells Isolated From Skeletal Muscle and Adipose Stromal Cells *Srour, E, Indiana University School of Medicine, Indianapolis, IN*
9:45 - 10:00 AM	I-2 In Vitro and In Vivo Analyses of Mesenchymal Stem Cells Derived from Adipose Tissue and Bone Marrow *Bunnell, B, Tulane National Primate Research Center, Covington, LA*
10:00 - 10:15 AM	I-3 Migration of Transplanted Rat GFP$^+$ Bone Marrow Cells into Adipose Tissue *Marra, K, Univ. of Pittsburgh, Pittsburgh, PA*
10:15 - 10:30 AM	I-4 Adipose Derived Stem Cells: A Novel Source of Stem Cell for Bone Marrow Transplant *Schipper, B, Univ. of Pittsburgh, Pittsburgh, PA*
10:30 - 10:45 AM	Coffee Break
10:45 - 11:00 AM	I-5 Cultured Adipose Stromal Cells Maintain a High Level of Genomic Stability *Grimes, B, Indiana University School of Medicine, Indianapolis, IN*
11:00 - 11:15 AM	I-6 Finger Printing Cell Surface Proteins and Gene Expression Patterns in Human Adipose Stromal Cells (hASCs) *Blaesius, R, Becton-Dickinson and Company*
11:15 - 11:30 AM	I-7 Silencing Hepatocyte Growth Factor Secretion Severely Impairs the Potency of Pluripotent Adipose Stromal Cells *Cai, L, Indiana University School of Medicine, Indiana, IN*
11:30 - 11:45 AM	I-8 Adipose Tissue-derived Stem Cells as a Source of Hepatocytes: Genetic and Functional Studies **Banas, A, National Cancer Center Research Institute, Tokyo, Japan*
11:45 - 1:00 PM	Lunch – Regency Ballroom Special Address: Racing with a Team *Sarah Fisher, INDY 500 Driver*
1:00 - 2:00 PM	Poster Session I: Translational Science
2:00 - 3:00 AM	Keynote Address II: Amniotic Stem Cells *Anthony Atala, MD., Wake Forest University Baptist Medical Center, Winston-Salem, NC*
3:00 - 4:00 PM	**Symposium II** Basic Science: ASC and Adipogenesis Moderators: J. Kirkland and Y. Yoshimura
3:00 - 3:15 PM	II-1 Inflammation Causes Neo-adipogenesis - What Adipose Tissue Engineering Can Learn from the Pathogenesis of Obesity ***Hemmrich, K, University Hospital Aachen, Aachen, Germany*
3:15 - 3:30 PM	II-2 Direct Co-culture of Human Adipocytes, Resident Macrophages and Adipocyte Stem-progenitor Cells Promotes Generation of New Preadipocytes *Chazenbalk, G, Cedars Sinai Medical Center, Los Angeles, CA*
3:30 - 3:45 PM	II-3 Adipogenic Differentiation of Adipose Stromal Cells is Repressed by Endothelial Cell Contact *Clauss, M, Indiana University School of Medicine, Indianapolis, IN*
3:45 - 4:00 PM	II-4 Paracrine effects of Macrophages on the CD34$^+$/CD31$^-$ Progenitor Cells in the Human Adipose Tissue *Maumus, M, INSERM, Toulouse, France*
4:00 - 4:15 PM	Coffee Break

*Finalist for IFATS 2007 Pre-doctoral Student Award. **Finalist for IFATS 2007 Post-doctoral Fellow Award.

ADIPOSE-DERIVED STEM CELLS FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

4:15 - 5:30 PM **Symposium III**
Translating to Clinic: Funding, Regulation and Hot Topics
Moderators: J. Gimble and A. Katz

NIH Perspective
Sonia Skarlatos, PhD,
Division Director (Acting) NHLBI Gene Therapy Coordinator, Division of Cardiovascular Diseases

FDA Perspective
Mercedes Serabian, Chief, Pharmacology/ Toxicology Branch, FDA/CBER/OCTGT/DCEPT

Featured Presentations:
III-1 Clinical Experiences of Cell-assisted Lipotransfer for Soft Tissue Augmentation
Yoshimura, K, University of Tokyo, Japan

III-2 First-in-man Experience of Adipose-derived Stem Cell Transplantation in the Treatment of Patients with Acute Myocardial Infarction (APOLLO)
Duckers, HJ, Thoraxcenter, Rotterdam

III-3 Adipose-derived Stem and Regenerative Cells Improves Lameness and Pain in Dogs with Osteoarthritis: A Summary of Results from Three Clinical Trials
Harman, R, Vet-Stem Inc, Poway, CA, USA

5:30 - 6:30 PM Public Educational Forum
6:45 - 10:00 PM *Oktoberfest at the Rathskeller*

Friday, October 19, 2007

7:00 - 8:15 AM IFATS Board of Directors Meeting Breakfast

7:15 - 8:15 AM Breakfast – Regency Ballroom Foyer

8:00 - 5:00 PM Exhibitors on Site

8:30 - 8:45 AM Welcome to Indiana

8:45 - 9:45 AM Keynote Address III: Bone Marrow MSC
Katarina LeBlanc, MD,PhD.
Karolinska University Hospital, Stockholm

9:45 - 10:00 AM Coffee Break

10:00 - 11:30 AM **Symposium IV**
ASC and Skin
Moderators: R. Llull and P. Rubin

10:00 - 10:15 AM IV-1 Development of a Delayed Wound Healing Model and its use for Testing a Novel Cell-Based Therapy
Hadad, I, Indiana University School of Medicine, Indianapolis, IN

10:15 - 10:30 AM IV-2 The Effect of Stem Cells on Acute Wound Healing
Uysal, C, Nippon Medical School, Toyko, Japan

10:30 - 10:45 AM IV-3 Wound Repair by Implantation of ASCs in Full Thickness Wound of Mouse
Lew, D, Yonsei University College of Medicine, Seoul, South Korea

10:45 - 11:00 AM IV-4 Adipose Stem Cells for Wound Healing: Effect of Cell Delivery and Cell Enrichment
Katz, A, University of Virginia, Charlottesville, VA

11:00 - 11:15 AM IV-5 Treatment of Radiation Skin Damage with Coleman Fat Grafting
Chang, C, New York University School of Medicine, New York, NY

11:15 - 11:30 AM IV-6 Using Human Adipose-derived Cells for the Production of New Skin Substitutes
Fradette, J, LOEX, Laval University, Quebec, Canada

11:30 - 12:30 PM IFATS Business Meeting

12:30 - 1:30 PM Lunch – Regency Ballroom

Speaker: Social Networking and Science of the Future
Bryan Gray, MediaSauce, Carmel, IN

1:30 - 2:30 PM Poster Session 11: Basic Science

2:30 - 3:30 PM **Symposium V**
ASC and Heart
Moderators: L. Casteilla and J. Fraser

2:30 - 2:45 PM V-1 Differentation of Human Adipose-derived Stem Cells Towards Cardiomyocytes; the Role of the ECM Molecules Fibronection and Laminin
VanDijk, A, VU Medical Center, Amsterdam, the Netherlands

2:45 - 3:00 PM V-2 Adipose-derived Cardiomyogenic Cells: In Vitro Expansion and Engraftment in Mice Heart Infarct
Casteilla, L, Institut Louis Bugnard, Toulouse, France

3:00 - 3:15 PM V-3 Human Adipose Tissue-derived Stem Cells Potently Limit Tissue Damage Resulting from Myocardial Ischemia
**Cai, L, Indiana University School of Medicine, Indianapolis, IN*

3:15 - 3:30 PM V-4 Therapeutic Effect of Human ASCs on Ischemic Heart Canine Model
Jeong, J, Yeungnam University Medical Center, Daegu, South Korea

3:30 - 3:45 PM Coffee Break

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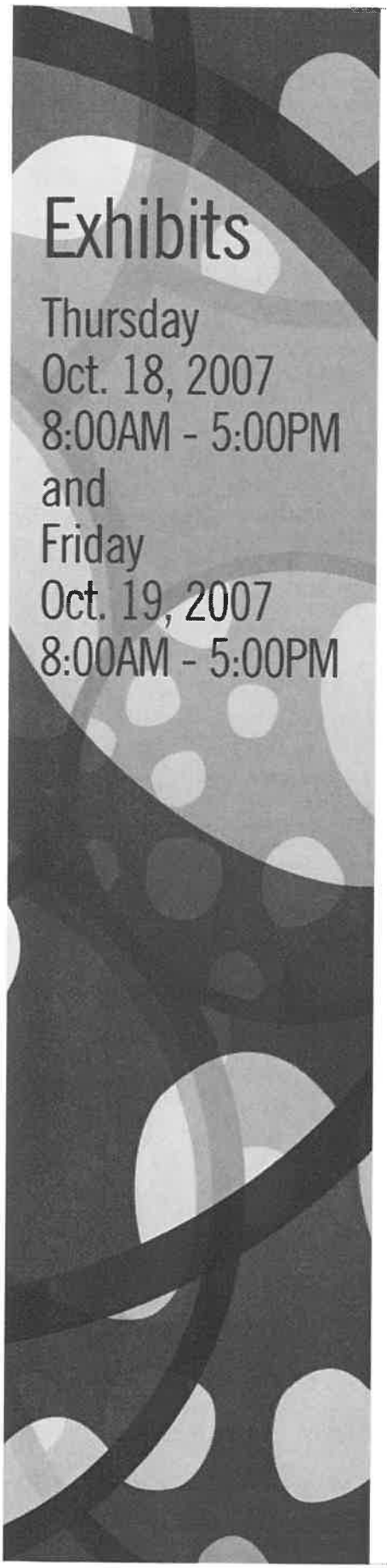
3:45 - 5:15 PM	**Symposium VI** ASC and Vasculature Moderators: J.S. Jung and A. Bouloumié
3:45 - 4:00 PM	VI-1 Adipogenic Differentiation is Accompanied by Transient Activation of a Pro-angiogenic Program of Factor Expression *Chen, J, Indiana University School of Medicine, Indianapolis, IN*
4:00 - 4:15 PM	VI-2 Functional Activation of Human Adipose-derived Stromal Cells by Hypoxia for Adhesion to Vascular Proteins *Amos, P, University of Virginia, Charlottesville, VA*
4:15 - 4:30 PM	VI-3 FGF-2 Regulates Secretion of Angiogenic Factors by Adipose-derived Stromal Cells *Suga, H, University of Tokyo, Tokyo, Japan*
4:30 - 4:45 PM	VI-4 Adipose-derived Mesenchymal Stem Cells Mobilize to Sites of Ischemia and Participate in Postnatal Neovascularization ***Thangarajah, H, Stanford University Medical School, Stanford, CA*
4:45 - 5:00 PM	VI-5 Human Adipose Tissue-derived Mesenchymal Stem Cells Improve Angiogenesis and Ischemic Limb Salvage in Mouse Model *Baek, SH, The Catholic University of Korea, South Korea*
5:00 - 5:15 PM	VI-6 Alliance of Blood Derived Endothelial Cells and Adipose Stromal Cells in Human Vasculogenesis: Timecourse and Stability ***Traktuev, D, Indiana University School of Medicine, Indianapolis, IN*
6:30 - 10:00 PM	Gala Awards Dinner Sponsored by Cytori Historic Union Station, Crowne Plaza Hotel Announcement of IFATS 2007 Pre-doctoral Student Award Announcement of IFATS 2007 Post-doctoral Fellow Award

Saturday, October 20, 2007

7:00 - 8:00 AM	Breakfast – Regency Ballroom Foyer
8:00 - 9:00 AM	Special Lecture: Aging and Adipose Stem Cells *James Kirkland, Boston University, Boston, MA*
9:00 - 9:45 AM	**Symposium VII** ASC and Cancer Moderators: R. Considine/R.C. Ogle
9:00 - 9:15 AM	VII-1 Anti-tumor Effects of Hyperthermia-treated Mesenchymal Stem Cells (MSCs) from Different Sources on SK-OV-3 Cells. *Lee, KW, Sungkyunkwan, Seoul, South Korea*
9:15 - 9:30 AM	VII-2 Cell-based Therapy of Pancreatic Cancer Using Human Adipose-derived Stromal Cells *Cousin, B, Institut Louis Bugnard, Toulouse, France*
9:30 - 9:45 AM	VII-3 Regulation of Aromatase Expression and Estrogen Biosynthesis in Adipose Stromal Cells *Ghosh, S, UT Health Science Center, San Antonio, TX*
9:45 - 10:00 AM	Coffee Break
10:00 - 11:45 AM	**Symposium VIII** ASC and Other Therapeutic Applications Moderators: K. Marra and K. McIntosh
10:00 - 10:15 AM	VIII-1 Long-term Treatments of Adipose Stromal Cells Secreted Media Protected Hypoxia-ischemia-induced Neonatal Brain Damage Du, Y, Indiana University School of Medicine, Indianapolis, IN
10:15 - 10:30 AM	VIII-2 Adipose-derived Stem Cells for Bioengineered Nerve Repair *Kingham, P, University of Manchester, Manchester, UK*
10:30 - 10:45 AM	VIII-3 Expression of Coagulation FVIII and FIX in hASC and hASC-derived Cells – Potential Autologous Cell Therapy for Hemophilia *Rosen, E, Indiana University School of Medicine, Indianapolis, IN*
10:45 - 11:00 AM	VIII- 4 Hematopoietic Colony-forming Cells Derived from the Stromal Vascular Fraction of Human Adipose Tissue *Donnenberg, A, University of Pittsburgh Cancer Institute, Pittsburgh, PA*
11:00 - 11:15 AM	VIII-5 Integrin α5/β1is a Receptor for SPARC on Adipose Stromal Cells and a Therapy Target Identified with Peptide Mimetics *Kolonin, M. University of Texas, Houston, Texas*
11:15 - 11:30 AM	VIII-6 Effect of Human Adipose Tissue-derived Stem Cells on Stress Urinary Incontinence in Rats *Lee, KW, Sungkyunkwan University, Seoul, Korea*
11:30 - 11:45 AM	VIII-7 Behavior of Adipose-derived Stem Cells in Canine Periodontal Tissue Regeneration *Tobita, M, Nippon Medical School, Toyko, Japan*
11:45 - 12:15 PM	Closing Remarks
12:15 PM	Adjournment

*Finalist for IFATS 2007 Pre-doctoral Student Award. **Finalist for IFATS 2007 Post-doctoral Fellow Award.



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Oral Abstracts

Oral Abstracts

Oral Abstracts

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-1 In vitro clonal analysis of murine common pluripotent stem cells isolated from skeletal muscle and adipose stromal cells

Edward R. Srour, Jamie Case, Tamara L. Horvath, Christopher B. Ballas, Keith L. March,
Indiana Center for Vascular Biology and Medicine, Indiana University, Indianapolis, IN

Pluripotent stem cells (PSCs) with multilineage differentiation capacity may provide useful therapeutic modalities for cellular therapy. Utility of PSCs depends on their ability to adapt to tissue-specific differentiation conditions. Previous data from our laboratory suggest that murine PSCs exhibiting an immunophenotype of CD45-Sca-1^{+}CD117^{-}CD90^{+} can be isolated from multiple tissues and may represent putative common PSCs (CoPSCs). Clonal analysis of the differentiation potential of skeletal muscle-(SM) and adipose stromal cell (ASC)-derived CoPSCs into myogenic, adipogenic and neurogenic cells was demonstrated by expanding single CoPSCs prior to specification under three separate differentiation conditions. Differentiation of SM- and ASC-derived CoPSCs into myotubes, adipocytes and neuronal-like cells was evident in clonal cultures promoting differentiation along these lineages. A total of 2.0%, 1.0% and 0.33% of SM-derived clones demonstrated unipotent, bipotent and tripotent differentiation respectively into combinations of myocytes, adipocytes and neuronal cells. As a percentage of SM-derived CoPSCs, tripotent clones comprised 0.016% of total muscle cells. Similar results were obtained in clonal analysis of ASC-derived CoPSCs, suggesting that both SM- and ASC-derived CoPSCs may be phenotypically and functionally identical. Following differentiation of single CoPSCs into three lineages, a clear and complete commitment to tissue-specific gene expression accompanied by inactivation of lineage-unrelated genes could not be demonstrated in several SM- and ASC-derived clones. These data demonstrate that phenotypically defined CoPSCs remain functionally heterogeneous at the single cell level and illustrate that morphologic lineage commitment may be independent of exclusive activation and/or deactivation of lineage specific differentiation pathways and transcription of associated genes.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-2 In vitro and in vivo analyses of mesenchymal stem cells derived from adipose tissue and bone marrow

Bruce Bunnell, Reza Izadpanah, Christopher Kriedt, Jay Hood and Cyndi Trygg
Tulane National Primate Research Center, Covington, LA

The biologic properties of mesenchymal stem cells (MSCs) isolated from two distinct tissues, bone marrow and adipose tissue were evaluated in these studies. MSCs derived from human and non-human primate (rhesus monkey) tissue sources were compared. Analyses of in vitro growth kinetics revealed shorter doubling time and significantly more population doubling for rBMSCs and hASCs. MSCs from all sources showed a marked decrease in telomerase activity over extended culture; however they maintained their mean telomere length. All of the MSCs expressed embryonic stem cell markers, Oct-4, Rex-1 and Sox-2. Early populations of both MSCs types showed similar multilineage differentiation capability, which is markedly diminished in vitro over time. The comparative analysis of the MSCs incorporated the use of Affymetrix oligonucleotide microarray data and proteomic analyses both stem cell types. The global comparison of gene expression between human and rhesus monkey mesenchymal stem cells in culture was performed by the analysis of the alterations in levels of RNA expression and protein. Analysis of the transcriptome of the MSCs from early and late passages revealed significant alterations in the patterns of gene expression (8.8% of the genes were differentially expressed in hBMSCs vs. hASCs; and 5.5% in rBMSCs vs. rASCs). Gene expression changes were much less evident within the same cell type as aging occurred (0.7% in hMSCs and 0.9% in rMSCs). Gene ontology

analysis showed that functions involved in protein catabolism and regulation of pol II transcription were over-represented in rASCs, while the regulation of IαB/NF-αB cascade were over-represented in hBMSCs. Functional analysis of genes that were differentially expressed in rASCs and hBMSCs revealed that pathways involved in cell-cycle, cell cycle checkpoints, protein-ubiquitination, and apoptosis were altered as cell age. The proteomic analysis indicates a high degree of similarity between the BMSCs and ASCs; however, the expression of distinct proteins and marked changes in the levels of some proteins were detected. Efforts are now underway to identify these novel proteins. A series of CNS transplantation experiments were performed to characterize the engraftment, migration, homing, and differentiation patterns of human MSCs. Both BMSCs and ASCs were injected into lateral ventricle of brain of NIHIII immune deficient mice by stereotaxic injection. The fate of MSCs was monitored by immunohistochemistry and PCR analyses 15, 30 and 90 days post-injection. MSCs migrated along ventricular area both rostrally and caudally from the injection site. Cells were distributed throughout the entire rostro-caudal extent of ventricular zone. Many MSCs also were detected lining the ependyma throughout the ventricle. A significant number of donor cells were distributed along the length of the spinal cord. Donor cells were dispersed into both lobes of the brain, numerous cells were detected in the cortex and different areas of cerebrum such as semilunar lobules, paraflocculus, postsuperior, fissure areas, and external and internal granular layers. Overall in vitro and in vivo characterization of MSCs from these two species and tissue sources revealed a high level of common biologic properties.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-3 Migration of transplanted rat GFP$^+$ bone marrow cells into adipose tissue

Kacey Marra, Koji Tomiyama, Noriko Murase, Donna Beer Stolz, Hideyoshi Toyokawa, Daniel R. O'Donnell, Darren M. Smith, Jason R. Dudas, J. Peter Rubin
University of Pittsburgh, Pittsburgh, PA

Following transplantation of green fluorescent protein (GFP)-labeled bone marrow (BM) into irradiated, wild-type Sprague-Dawley rats, propagated GFP$^+$ cells migrate to adipose tissue compartments. In order to determine the relationship between GFP$^+$ BM-derived cells and tissue resident GFP$^-$ cells on the stem cell population of adipose tissue, we conducted detailed immunohistochemical analysis of chimeric whole fat compartments and subsequently isolated and characterized adipose-derived stem cells (ASCs) from GFP$^+$ BM chimeras. In immunohistochemistry, a large fraction of GFP$^+$ cells in adipose tissue were strongly positive for CD45 and smooth muscle actin, and evenly scattered around the adipocytes and blood vessels, while CD45$^+$ leukocytes were found mostly in the vessels. A small fraction of GFP$^+$ cells with mesenchymal marker CD90 also existed in the perivascular area. Flow cytometric and immunocytochemical analyses showed that cultured ASCs were CD45$^-$/CD90$^+$/CD29$^+$. There was a significant difference in both the cell number and phenotype of the GFP$^+$ ASCs in two different adipose compartments, the omental (abdominal) and the inguinal fat (subcutaneous) pad; a significantly higher number of GFP$^-$/CD90$^+$ cells were isolated from the subcutaneous depot as compared to the abdominal depot. The in vitro adipogenic differentiation of the ASC was achieved; however, all cells that had differentiated were GFP$^-$. Based on phenotypical analysis, GFP$^+$ cells in adipose tissue in this rat model appear to be of both hematopoietic and mesenchymal origin; however, infrequent isolation of GFP$^+$ ASCs and their lack of adipogenic differentiation suggest that the contribution of BM to ASCs generation might be minor.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-4 Adipose derived stem cells: A novel source of stem cell for bone marrow transplant

B.M. Schipper, M.D., K. Marra, PhD, A. Donnenberg, PhD, V. Donnenberg, PhD, U. Surti PhD, A. Zeevi PhD, M. Agha M.D., J.P. Rubin, M.D
University of Pittsburgh, Pittsburgh, PA

Introduction
Many similarities between adipose derived stem cells (ASC's) and bone marrow derived stem cells have been described in the literature. Importantly, both sources can give rise to hematopoietic cells. This study aims to determine if migratory bone marrow stem cells repopulate the adipose stem cell pool, or if these stem cell pools exist in two separate compartments. If the latter is true, then autogenous ASC's might be free of the genetic mutations found in hematopoietic malignancies and possibly be used in the place of allogeneic bone marrow transplants. To answer this question we examined tissue specimens from patients with two disease states: 1) Chronic Myeloid Leukemia (CML), and 2) History of bone marrow transplant.

Methods
Adipose biopsies and peripheral blood samples were taken from bone marrow transplant recipients and short tandem repeat (STR) DNA sequences were analyzed to distinguish cells of donor and recipient origin. We also evaluated the hematopoietic cells produced from the ASC's from the bone marrow group with STR sequencing to determine their chimerism as well. Adipose biopsies were also taken from patients with CML and evaluated for the presence of the BCR/ABL mutation in ASC's via FISH and PCR. Only adherent ASC's were studied to ensure there were no "passenger" hematopoietic cells present. The adipose derived stem cells were then differentiated into hematopoietic cells using methylcellulose culture, as well as into other lineages to demonstrate multipotency. The hematopoietic cells produced by the ASC's were then checked for the presence of BCR/ABL.

Results
Bone marrow transplant patients demonstrated 100% engraftment of donor stem cells in peripheral blood samples. However, ASC's from the same patients were entirely of host origin. All hematopoietic produced from the ASC's were also of host origin. In patients with CML testing positive for the BCR/ABL mutation in blood and bone marrow, autogenous ASC's were free of this mutation by both FISH and PCR. Furthermore, these cells were successfully differentiated into monocytoid and erythroid colonies. These cells were found to be negative for BCR/ABL by PCR.

Conclusion
There appears to be no migration of bone marrow stem cells into the adipose stem cell compartment. This work suggests that the ASC population, while similar in function to bone marrow stem cells, is an independent pool of multipotent cells. This study also suggests that autogenous ASC's could be used to repopulate the hematopoietic system in the setting of blood malignancies.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-5 Cultured adipose stromal cells maintain a high level of genomic stability

Brenda R. Grimes[1], Camie M. Steiner[1], Stephanie Merfeld-Clauss[2], Keith March[2]
Indiana University School of Medicine
[1]Department of Medical and Molecular Genetics
[2]Cellular and Integrative Physiology, and Biomedical Engineering, Indiana Center for Vascular Biology and Medicine, Indianapolis, IN

Introduction
Human adipose stromal cells (ASCs) can be isolated with relative ease from fat tissue removed by liposuction. It has recently been demonstrated that ASCs can proliferate for nearly 40 population doublings ex vivo and include cells with stem-like properties. Given their potential to differentiate into cells of the adipogenic, chondrogenic, myogenic, neuronal and osteogenic lineages, they are currently being evaluated as a source of stem cells that may be suitable for genetic modification and tissue engineering. However a question that is unaddressed and is of interest for their use in functional studies and cell-based therapies, is the capacity of cultured ASCs to maintain a normal diploid chromosome number over time. In the present study, populations as well as highly proliferative clones of human ASCs were evaluated at intervals for chromosome stability, using the technique of interphase fluorescence in situ hybridization (FISH).

Methods
Five independent human ASC samples were passaged in culture to 25-30 CPD, in EGM2-MV medium. Cells were harvested and subjected to FISH analysis with probes specific to the centromeres of chromosome 17 and the X chromosome. This technique provides a measure of the frequency of diploid (2n) or tetraploid (4n) cells or aneuploid cells. In a diploid cell, 2 spots are detected with the chromosome 17 probe. In a female cell there are 2 X chromosome signals whereas males have 1 X chromosome signal. At least 200 interphase cells were scored in each sample.

Results
In initial studies, two ASC populations sampled at early and late passage were found to be mainly diploid (≥98%). Subsequently three additional ASC populations were passaged for up to 30 CPD and again most cells were diploid (98.7-99.5% of cells). Of the non-diploid cells detected, 1% (24/2555 cells) were tetraploid. The frequency of apparent aneuploidy was 0.5% (13/2555) and within the error rate of the FISH technique. Two clonal ASC lines were also examined at early and late passage using the FISH assay. Similar to the ASC populations, the two sub-clonal ASC cell lines were largely diploid at late passage (96.7 and 93.4% of cells).

Conclusions
Results of FISH analysis in five ASC populations and two ASC clonal lines indicated that cultured human ASCs maintain a remarkably high diploid content following ex-vivo expansion. These results contrast with a tetraploidy rate of up to10% reported for cultured amniotic fluid and chorion villus-derived cells, which is considered to be a cell culture artifact. The maintenance of normal chromosomal composition is a notable feature of cultured ASCs with respect to their potential for use in stem cell-based therapies.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-6 Finger printing cell surface proteins and gene expression patterns in human adipose stromal cells (hASCs)

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Flow Cytometry and microarray analysis were performed on hASCs derived from subcutaneous fat deposits from three independent donors according to standard procedures. A cell surface protein expression profile was determined by flow cytometry using a proprietary combinatorial antibody scanning panel (BD FACS™ CAP) consisting of 189 antibody reagents by flow cytometry. The BD FACS CAP™ is a screening tool that generates a surface protein expression profile through single color staining with no evaluation of surface proteins co-expression. Affymetrix HG U133 Plus 2.0 microarrays were used to determine the gene expression levels for each of these donors. We then compared the protein expression patterns to basal RNA expression levels for each donor.

Of the 189 proteins evaluated by BD FACS CAP™, 40 proteins were highly expressed (>85% of the gated population), 7 proteins were heterogeneously expressed (between 5% and 85% of the gated population) and 117 proteins were not expressed. The surface protein expression profiles were similar among the triplicate evaluation of the three donors. The remaining surface proteins had variable expression patterns which will need to be evaluated further. Microarray analysis on these same cells found ~11,000 genes expressed by at least one of the three donors, with ~9,500 (86%) common to all three donors. Using proprietary software, we identified genes encoding ~400 secreted proteins and ~600 plasma membrane proteins expressed by all three donors. Of the 47 proteins that were positive by BD FACS™ CAP, we identified probesets for 45 of the corresponding genes on the array (not all genes are represented on the microarray). Thirty one (31) of these genes had high to medium gene expression levels by array analysis (signal value 1,000 – 5,000), while the remaining 14 genes were expressed at either low levels (signal <1,000) or were not detectable in the array analysis. Of the 117 proteins not detected by BD FACS™ CAP on ASCs, we identified probe sets for 98 of the corresponding genes. Ninety one (91) of these 98 genes were either not detected or were expressed at low levels in the array analysis indicating a remarkably high degree of correlation between the RNA expression levels and the protein expression patterns. The cell surface protein expression pattern identified in this BD FACS™ CAP analysis, coupled with the microarray analysis, extends at least 9 previous reports on cell marker expression of these cells.

Author Affiliations

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SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-7 Silencing hepatocyte growth factor secretion severely impairs the potency of pluripotent adipose stromal cells

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Background
Paracrine stimulation of endogenous repair, rather than direct tissue regeneration, is increasingly accepted as the major mechanism of therapeutic stem and progenitor cell action; yet, this principle has not been fully established in vivo. Adipose-derived stem cells (ASCs) secrete many factors, including hepatocyte growth factor (HGF), and promote reperfusion and tissue repair in ischemia models. We use RNA interference to silence HGF expression, and to determine its contribution to ASC potency in vivo.

Methods and Results
A small hairpin RNA (shRNA) specific for HGF mRNA (shHGF) or a control sequence (shCtrl) were used to stably transduce ASCs (ASC-shHGF or ASC-shCtrl). ASC-shHGF secreted 5-fold less HGF, and were significantly less able to withstand the stress of serum withdrawal when incubated in EBM-2 compared to ASC-shCtrl ($p<0.05$). HGF knockdown severely impaired the ability of ASCs to promote reperfusion in a mouse hindlimb ischemia model. Perfusion of the ischemic hindlimb at 15 d in mice treated with ASC-Ctrl was 84±4%, compared to only 69±5% for ASC-shHGF ($p<0.05$). ASC-shHGF retained residual activity as indicated by greater reperfusion ($p<0.05$) than saline control (58±6%). Capillary densities in ischemic tissues from each group followed a similar rank order (ASC-Ctrl>ASC-shHGF>saline) ($p<0.05$ between each group). There was no difference in total GFP+ cells in ischemic limbs at 5 d after infusion, indicating similar homing potentials; however, by 15 d 3-fold fewer ASC-shHGF were present in ischemic tissues compared to ASC-shCtrl ($p<0.01$). The reduced persistance of ASC-shHGF was due to a higher apoptosis rate in situ as evidenced by a greater number of TUNEL-positive ASC-shHGF cells (61±0.1%) compared to ASC-Ctrl (41%±3.2%) in ischemic tissues at 5 d ($p<0.01$); suggesting that attenuated potency of ASC-shHGF was related to reduced survival in ischemic tissues.

Conclusions
These results indicate that secretion of HGF is critically important for ASC function. In addition to promoting endogenous repair, our data suggest that an important effect of HGF is autocrine promotion of ASC survival. This suggests the possibility of using other factors (either exogenous or endogenous) to promote therapeutic cell survival; thereby, increasing potency.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC BIOLOGY

I-8 Adipose tissue-derived stem cells as a source of hepatocytes: Genetic and functional studies

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We have generated in vitro hepatocyte-like cells from adipose tissue-derived mesenchymal stem cells (AT-MSCs). The newly developed cells were found to be morphologically and functionally similar to liver parenchymal cells. We used AT-MSCs from different cancer patients and found that, after incubation in the presence of specific factors (hepatocyte growth factor (HGF), fibroblast growth factor (FGF) 1 and 4, oncostatin M (OsM), and dexamethasone) the $CD105^+$ fraction of AT-MSCs exhibited a highly hepatic differentiation ability in an adherent monoculture condition. $CD105^+$ AT-MSCs-derived hepatocyte-like cells revealed several liver-specific markers such as albumin (ALB), tryptophan 2-3 dioxygenase (TDO2), transthyretin (TTR) and such functions as albumin production, low-density lipoprotein uptake, and ammonia detoxification. Microarray analysis demonstrated that AT-MSC-derived hepatocytes recapitulate the gene expression profile of adult human hepatocytes. Further analysis showed that enriched categories of genes and signaling pathways such as complement activation, blood clotting cascade, and hormone biosynthesis in the AT-MSC-derived hepatocyte-like cells were relevant to liver-specific functions. Importantly, $CD105^+$ AT-MSC-derived hepatocyte-like cells, exhibited cytochrome P-450 (CYP) activity (2C9, 1A1, 2B6, 2C19, 2D6, 3A). Interestingly, the CYP activities derived from the generated hepatocyte-like cells exhibited activities similar to the primary human hepatocytes with lower individual variations. In conclusion, AT-MSCs can be

differentiated into functional hepatocyte-like cells, which exhibit hepatocyte functions. Thus, our findings suggest a promising future potential for adipose tissue as a stem cell reservoir in liver therapy.

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SCIENTIFIC SESSION

BASIC SCIENCE: ASC AND ADIPOGENESIS

Il-1 Inflammation causes neo-adipogenesis – what adipose tissue engineering can learn from the pathogenesis of obesity

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Monocyte chemoattractant protein (MCP-1) plays a key role in the pathogenesis of obesity. It is an inflammogen that attracts monocytes to the adipose tissue and promotes their differentiation to macrophages. These tissue macrophages produce a plethora of pro-adipogenic cytokines, one of which is nitric oxide (NO). NO is produced by the inducible nictric oxide synthase (iNOS) and is suspected to be a key factor in neoadipogenesis. In this study, we mimicked the processes of fat formation in a murine adipose tissue engineering chamber model. The aim was to analyse how the pathology as found in obesity can be exploited for soft tissue reconstruction based on adipose tissue enigneering. MCP-1 and the yeast-derived inflammogen Zymosan A (Zy) were evaluated for their adipogenic potential while aminoguanidine (AG) was applied as specific iNOS inhibitor. A plastic chamber (5mm lang, 42µl) filled with Matrigel™ and fibroblast growth factor 2 was implanted around a vascular pedicle but otherwise isolated from the surrounding tissue. The following conditions were tested: 1) control (Matrigel™, bFGF only), 2) Matrigel™, bFGF, MCP-1 (0.05 ng/ml), 3) Matrigel™, bFGF, Zy (0.02µg/ml), 4) Matrigel™, bFGF, Zy, AG (40 µg/ml). Constructs were harvested after 6 weeks. Absolute volumes of fat tissue were calculated from the proportion of fat (%) and the wet volume. Matrigel-FGF-2 matrix alone was not sufficient to drive substantial adipogenesis (16.15±5.03 % and 1.55±0.63 µl adipose tissue). Zy- and MCP-1-supplemented chambers revealed dose-dependent neo-adipogenesis. Zy-treated constructs produced 44.4±5.3% fat ($p<0.005$) and volumes of 5.93±1.05 µl adipose tissue ($p<0.005$). MCP-1 induced 40.57±3.65 % ($p=0.005$) and 5.05±0.92 µl adipose tissue ($p=0.0085$). AG addition decreased percentage and volume of fat significantly (19.73±3.13% ($p<0.005$), 2.32±0.54 µl ($p<0.05$)). Histologically, all explanted MCP-1 and Zy samples showed healthy adipose tissue with infiltration of macrophages. The model presented here reveals that mimicking of the processes in obesity enables neoadipogenesis in an adipogenic matrix. Key factors in this setting are macrophages and the inflammation which they generate through their NO production. A better understanding of the processes in obesity and the Metabolic Syndrome will help to develop and test new, promising approaches in adipose tissue engineering.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC AND ADIPOGENESIS

II-2 Direct co-culture of human adipocytes, resident macrophages and adipocyte stem/progenitor cells promotes generation of new preadipocytes

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Introduction

Obesity is associated with a low-grade chronic inflammatory state that recruits macrophages to adipose tissue as the mass of tissue increases. Cross-talk between adipocytes and resident macrophages may exacerbate inflammatory changes in obese adipose tissue. Adipose tissue is composed not only of adipocytes and macrophages, but also contains adult adipocyte stem/progenitor cells (AASPCs). These cells are self-renewing and can differentiate into multiple tissue lineages, including adipocytes. Understanding adipocyte proliferation, propagation and differentiation under the influence of macrophages and AASPCs is central to unlocking the cellular and molecular biological questions posed by obesity, diabetes, wound healing, and even adipocyte stem cell plasticity.

Hypothesis

Direct co-culture between adipocytes and the macrophage/AASPC fraction promotes the generation of new preadipocytes and adipocytes.

Methods

Human adipose tissue obtained from obese patients was treated with collagenase and adipocytes (floating cells) were isolated by centrifugation. Resident macrophages and AASPCs were isolated from the stromal vascular fraction using a Ficoll gradient. Adipocytes and the macrophage/AASPC fraction were first cultured separately for 24 hours, then co-cultured for another 24 hours, and finally separated and cultured alone for an additional 48 hours.

Results

Following direct co-culture with human adipocytes, greatly enhanced proliferation of preadipocytes was observed in the resident macrophage/AASPC fraction in vitro. Actual cell-to-cell contact between the different cell types was required for this enhanced proliferation of preadipocytes to occur. This proliferation correlated with a 5- to 10-fold increase in adiponectin secretion by the resident macrophage/AASPC fraction. Immunofluorescent staining showed that the preadipocytes derived from the resident macrophage/AASPC fraction were Oil red O^+, DKL^+ and $S\text{-}100^+$. They were also $CD14^+$ and $CD68^+$ as well as $CD34^+$, $CD105^+$ and $CD146^+$. Flow cytometry studies of the macrophage /AASPC fraction revealed a population of $CD14^+$, $CD34^+$ and $S\text{-}100^-$ cells prior to co-culture, and a population of $CD14^+$, $CD34^+$ and $S\text{-}100^+$ cells after co-culture. Another important observation during our co-culture studies was the differentiation of macrophages to a preadipocyte phenotype.

Conclusion

Direct co-culture between human adipocytes and the macrophage/AASPC fraction strongly enhances generation of new preadipocytes. These findings could have far-reaching implications with adipocyte growth, weight gain and obesity, modulated at least in part by a novel cellular differentiation pathway (supported in part by NIH grants R01-DK073632 and R03-HD42077).

SCIENTIFIC SESSION

BASIC SCIENCE: ASC AND ADIPOGENESIS

II-3 Adipogenic differentiation of adipose stromal cells is repressed by endothelial cell contact

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Hypothesis and Background
Adipose stromal cells (ASC) exhibit pre-adipocytic potential while displaying functional and phenotypic overlap with the pericytic cells of microvessels in adipose tissues. We have recently shown that ASC interact strongly with endothelial cells (EC). Accordingly, we intended to test the hypothesis that physical proximity and paracrine signaling of endothelial cells (EC) to ASC regulate the adipogenic potential of ASC. We further asked whether expression levels of Wnt-signaling molecules, which are known to be key molecules in cellular differentiation, are changed under conditions of coculture, thus contributing to modulation of adipogenesis.

Procedures
We investigated the effects of EC on ASC adipogenesis in a 2D-coculture model. Adipogenic differentiation was evaluated in terms of expression of α-lipase and PPAR-γ and quantitation of lipid vesicles. To obtain insight into possible mechanisms of EC-ASC interactions, we tested gene expression of candidate genes such as Wnt-family molecules by real-time PCR comparing co-cultures versus monolayers comprised of only one cell type.

Results
We found that lipid accumulation in adipogenic differentiation media was reduced in ASC cocultured with EC, versus that seen with isolated cultured ASC. In contrast, co-culture of ASC with an equivalent number of fibroblasts rather than EC did not reduce adipogenesis. PCR revealed that canonical Wnt signaling molecules were upregulated in ASC/EC cocultures. This increase was by 2-3 fold in comparison to the summed transcript levels determined from equivalent total numbers of ASC and EC when not in co-culture. Of note, nearly all molecules known to be involved in Wnt signal transduction were found to be strongly upregulated in conditions of coculture including the Wnt receptor Fz3 but not Fz1, the intracellular signaling molecules Dv11 and TCF-7, and genes known to be expressed downstream of Wnt signaling, including Wisp1 and Sox-17.

Conclusions
These data identify the interaction between EC and ASC as a repressor of adipogenesis, and suggest the hypothesis that perivascular pericytes are protected against adipogenesis by a functional interaction with endothelial cells. Wnt signaling molecules and pathways were upregulated and activated upon contact of ASC and EC and this may play an important role in ASC fate regulation in the context of the physiologic association of ASC with EC. Finally, it is tempting to speculate that abnormal endothelial function may be associated with pathologic de-repression of adipogenesis.

SCIENTIFIC SESSION

BASIC SCIENCE: ASC AND ADIPOGENESIS

II-4 Paracrine effects of macrophages on the CD34+/CD31- progenitor cells in the human adipose tissue

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The stroma-vascular fraction (SVF) of human adipose tissue (AT) contains different cell types such as capillary endothelial cells, macrophages and CD34+/CD31- progenitor cells that are able, under appropriate culture conditions, to exhibit adipogenic and angiogenic capacities. The development of fat mass is associated with a chronic low-grade inflammation and an increased of macrophage number within AT. The aim of the present work was to evaluate whether macrophages could affect fat expansion through a paracrine action on the proliferation and/or on the adipogenic and angiogenic abilities of the CD34+/CD31- progenitor cells.

CD34+/CD31- progenitor cells and macrophages were isolated by an immunoselection/depletion approach using magnetic nanoparticules coupled to specific antibodies. Conditioned media (CM) from freshly harvested macrophages were collected. Growth responsiveness of the CD34+/CD31- cells to macrophage-CM was determined by BrdU incorporation assays on cells cultured for 48 hours and by cell counting. Furthermore the CD34+/CD31- were cultured with macrophage-CM in a "mixed medium" consisting of a combination of adipocyte and endothelial culture conditions and adipogenic and angiogenic abilities were assessed by the quantification of triglyceride (TG) content and the length of the CD31 positive network.

Macrophage-CM exerted an antiproliferative effect on the CD34+/CD31- progenitor cells since there was a decrease in BrdU incorporation and cell number of CD34+/CD31- progenitor cells treated by CM. Interestingly the antiproliferative effect mediated by AT-derived macrophages-CM was completely abolished in the presence of an antioxidant, the N-Acetyl Cystein. Moreover, in the "mixed medium", macrophage-CM modulated the adipogenic and angiogenic potentials of the CD34+/CD31- cells. Indeed macrophage-CM decreased TG storage in the CD34+/CD31- cells on one hand and on the other hand increased the CD31 length network.

The present study shows that AT-derived macrophages inhibit the proliferation of the CD34+/CD31- progenitor cells through the release of reactive oxygen species. Moreover, by the utilisation of a new culture condition which simultaneously allows to study the adipogenic and angiogenic abilities of the CD34+/CD31- cells, we show that factors secreted by the macrophages present in the microenvironment in which the progenitor cell reside might be involved in the control of adipogenesis and vascular network extension and thus in the control of fat mass development.

SCIENTIFIC SESSION

TRANSLATING TO CLINIC: FUNDING, REGULATION AND HOT TOPICS

III-1 Clinical experiences of cell-assisted lipotransfer for soft tissue augmentation

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Lipoinjection is a popular option for cosmetic and reconstructive soft tissue augmentation. However, it is difficult to predict the survival rate of transplanted fat or the risk of fibrosis and calcification. Alternatively, we tested a new

technique called cell-assisted lipotransfer (CAL), involving the concurrent transplantation of aspirated fat and adipose-derived stromal cells (ASCs). CAL is based on the observation that aspirated fat is relatively ASC-poor (56%) compared to excised fat. In CAL, aspirated fat was converted to ASC-richer fat by addition of freshly-isolated vascular stromal fraction (SVF) containing ASCs; aspirated fat acts as a living scaffold for ASCs. We expected that this technique boosts the efficacy of autologous lipoinjection; in other words, improves the survival rate and persistence of transplanted fat.

The study began in 2003 and a total of 124 patients received CAL so far: 110 patients underwent breast augmentation (including 14 breast reconstructions after mastectomy), 19 patients underwent face remodeling (including treatments for morbidities with facial lipoatrophy), 2 patients underwent hip remodeling, and 2 patients underwent hand rejuvenation. Patients were monitored for 4 to 40 months.

We experimentally found that even tissue centrifugation at 1200g alone can improve the ASC/adipocyte ratio by 14% and increases the content of adipocytes by 25% and ASCs by 43%. In clinical CAL, patients that received cosmetic breast augmentation gained 100-200 ml in breast volume at 6 months. Minimal size reduction of soft tissue volume was observed 1 to 6 months after treatment. We observed only micro-calcifications and minor cyst formation (5 to 12 mm in diameter), suggesting the safety of CAL is acceptable even with large volume augmentations. In one case in which ASCs were injected separately as cell suspensions in saline solution, fibrous breasts were observed; this suggests ASCs may differentiate into unexpected lineages under non-physiological conditions.

In patients with facial lipoatrophy, CAL clinical results suggested a positive effect of the SVF supplementation though the difference compared to non-CAL treatments was not statistically significant, probably due to the small number of patients involved (n=3 for each group).

These results suggest that postoperative atrophy of transplanted fat was prevented by the improved ASC/adipocyte ratio achieved in the CAL technique. It is known that ASCs improve the survival rate of transplanted fat by releasing angiogenic factors such as HGF and by their ability to differentiate into endothelial cells. We propose that ASCs may also preserve tissue volume by functioning as tissue specific progenitors. A quantitative volumetric evaluation of the augmentation effects of CAL, which we have been performing since 2006, and a controlled study with a larger patient cohort would provide a more thorough evaluation of the clinical efficacy of CAL in the future.

SCIENTIFIC SESSION

TRANSLATING TO CLINIC: FUNDING, REGULATION AND HOT TOPICS

III-2 First-in-man experience of adipose-derived stem cell transplantation in the treatment of patients with acute myocardial infarction (APOLLO)

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The use of adult stem cells from several sources has been shown to improve cardiac function in acute and chronic cardiac disease. A number of pilot trials using intramyocardial injection of bone marrow mononuclear cells have shown promising results in patients with chronic myocardial disease and in acute myocardial infarction. Most investigations showed a significant improvement of left ventricular ejection fraction and/or left ventricular dimensions after treatment. Many sources for adult stem cells have been discovered, including bone marrow, skeletal muscle, blood and adipose tissue.

Autologous adipose-derived regenerative cells (ADRCs) are obtained from subcutaneous adipose tissue by processing lipoaspirate. ADRCs are multipotent cells capable of differentiating into multiple lineages in vitro, such as adipocytes, osteoblasts, chondrocytes, myocytes, hepatocytes, and neurons in vitro, given the appropriate specific conditions and stimulating factors. 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 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.

The APOLLO is a first-in-man study to assess the feasibility and safety of ADRC in the treatment of patients with an acute myocardial infarction. This is a prospective, double blind, randomized, placebo-controlled, sequential dose escalation study that will enroll up to 48 patients. The study will include up to four consecutively enrolled dose cohorts of 12 patients randomized 3:1 to receive ADRC or placebo. ADRC will be delivered by intracoronary delivery within 24 hours following the onset of the myocardial infarction and primary PCI. Efficacy of the ADRC transplantation will be assessed by clinical evaluation for up to 36 months, and MRI, echocardiographic, and SPECT imaging, in addition to invasive hemodynamic analysis at 6 and 18 month follow-up.

SCIENTIFIC SESSION

TRANSLATING TO CLINIC: FUNDING, REGULATION AND HOT TOPICS

III-3 Adipose-derived stem and regenerative cells improves lameness and pain in dogs with osteoarthritis: A summary of results from three clinical trials

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Since 2003, veterinarians have treated more than 2,500 horses with adipose-derived stem and regenerative cells for orthopedic injuries. In 2006, Vet-Stem began three clinical studies to evaluate the use of this cell therapy in dogs with osteoarthritis (OA). The interim results are presented here. The first study evaluated cell therapy in OA of the canine elbow (non-blinded prospective study). A second randomized, prospective, blinded, placebo-controlled study evaluated cell therapy in OA of the canine hip. The last study, a non-blinded, prospective study, examined cell therapy in end-stage, chronic, post-surgical OA in the knee of the dog. The clinical outcome measures included lameness, pain, range of motion, and functional disability scores. All three studies demonstrated highly statistically significant improvements in dogs treated with cell therapy when compared to historical baseline values. In addition, the prospective, blinded hip study showed a statistical improvement in treated versus placebo-treated dogs. Safety, dosing, and statistical outcomes will be presented.

SCIENTIFIC SESSION

ASC AND SKIN

IV-1 Development of a delayed wound healing model and its use for testing a novel cell-based therapy

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Among the most difficult wounds to manage are those located in previously irradiated fields, in which complication rates range as high as 67%. Thus, there is a strong need for agents to facilitate wound healing in compromised regions. The aims of the present study were to create a delayed full-thickness wound healing model and examine the efficacy

of adipose stem cells (given their known pro-angiogenic characteristics), either alone or in platelet-rich fibrin gels, to improve wound healing.

Four pigs received a single 20 Gy fraction of 6 MeV electrons to the dorsal skin surface. Five weeks after radiation, dorsal hump fat was harvested and cultured to yield adipose stromal cells (ASCs). Two weeks later, 28 full-thickness, 1.5 x 1.5 cm^2 full thickness wounds were made in the irradiated skin fields. Wounds were also made in non-irradiated skin as controls. Irradiated wounds were treated with either saline, ASCs in saline, platelet-rich plasma (PRP) fibrin gel, or ASCs in PRP. On post-wounding days 4, 8, 12, 16, and 21, wound size (via open surface area) was assessed. Full-thickness biopsies were taken and histologic specimens analyzed for rate of re-epithelization and for microvessel density.

The single 20 Gy radiation dose produced a significant loss of dermal microvasculature (75% loss in vessel density, $p<0.05$), as determined by immunohistologic analysis. There was a significant difference in the rate of healing between irradiated and non-irradiated skin treated with saline ($p<0.05$), corresponding to a 10-day delay in closure. No difference in healing was seen between saline and the vehicle-control (PRP) treated wounds ($p>0.05$). The ASC in PRP treated wounds exhibited a significant improvement in wound healing, compared to saline ($p<0.05$), hastening closure by ~3 days. Interestingly, ASCs administered in saline showed no improvement in healing.

We have created a useful model that closely simulates a delayed healing environment. ASC-based therapy improves the healing rate of vascularly-depleted tissues by about 25%. The effect of ASCs appears to depend on co-administration in a gel vehicle (PRP).

SCIENTIFIC SESSION

ASC AND SKIN

IV-2 The effect of stem cells on acute wound healing

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Introduction

The mesenchymal stem cells could control the critical points in the healing processes with direct and indirect effects. The direct effect – that is the differentiation potential – of mesenchymal stem cell leads to applicability in different situations. The mesenchymal stem cells are proven to improve angiogenesis in any situation where neovascularization is necessary. We have performed an experimental study to find out the effect of mesenchymal stem cells, bone marrow derived stem cells (BSCs) and adipose derived stem cells (ASCs) on acute wound healing in rat model.

Materials and Methods

The ASCs were gathered from inguinal fat pads of wistar rats. The BSCs were gathered from the femur and tibia. After three passage in control medium (DMEM, 10% FBS), the cells were ready to be injected so that every injection included $1x10^7$ cells. The cells were labeled with Dil staining before the injection for tracing. Eight wistar rats were included in the study. Four circular skin defects with a diameter of 2cm were made on the dorsum of the rat until the muscle fascia. Injections were done depending on the group. Group 1: the control group, PBS; Group 2: ASCs; Group 3: BSCs; Group 4: full thickness skin graft. The healing was observed daily and the day of total epithelization was recorded in each group. At the day of healing, the photographs were taken to evaluate the total area of scar formation. Then the samples were examined for histology and immunohistochemistry.

Results

The healing time were recorded for each defect. The healing times were group 1: 52.50 day ±4.29 ; group 2: 47.13 day ±3.37; group 3: 47.50 day ±4.34; group 4: 44.25 day ±4.77. There was a statistical significance between the control group and the groups 2, 3 and 4 when compared one by one.($p<0.05$) The macroscopical photographs were evaluated with Adobe Photoshop and the number of pixels of the total area of scar formation was noted. The areas were group 1: 247689.88±23203.56; group 2: 311752.63±18640.06 ; group 3: 322069.13±22043.52; group 4: 392873.13±30719.40. The total area of scar was highest in group 4 and the smallest in group 1. There was a statistical significance between the

control group and the groups 2, 3 and 4 when compared one by one. ($p<0.05$) The vascular density on histological specimens among 20 fields under x20 magnification (control group 1 = 2.37±0.12; group 2 = 6.71±0.74 ; group 3= 5.94±0.78; group 4= 2.45±0.18) was statistically greater in the groups 2 and 3 when compared group 1 and 4 one by one. ($p<0.5$) Anti-VWF Ab staining revealed the differentiation of stem cells to new endothelial cells. The anti-cytokeratin Ab immunohistochemical staining indicated that some of the epithelial cells were originated from the injected stem cells.

Conclusion
The ASCs and BSCs do not only increase the healing time and help in faster primary healing but also but also could differentiate into the necessary cells and tissues in the absence of any scaffold. Despite of the fast healing, the ASCs and BSCs do not decrease scar formation in the skin. That might be due to the inhibition of myofibroblast that would lead to a better healing in quality which has been still investigated in our laboratory.

SCIENTIFIC SESSION

ASC AND SKIN

IV-3 Wound repair by implantation of adipose derived stem cell (ASCs) in full thickness wound of mouse

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Introduction
There is evidence that stem cells improve wound healing either by transforming into those cells that are native to wound, or by promoting the production of cytokines. The adipose derived stem cells (ASCs) can be easily obtained in large quantity and have high rate of replication than other adult stem cells. In order to prove that implantation of ASCs can enhance the wound healing, we established human ASCs line and were applied to the full thickness skin defect of mouse.

Methods
Adipose tissues were harvested during the abdominal liposuction, and msenchymal cells were isolated and cultured. After identifying the surface marker and several differentiation to other cells, the ASCs cell line was established and reproduced. Two dorsal full thickness wound were made on five ICR mice with 8mm punch. Each wound was covered with an occlusive dressing(Tegaderm®). $1x10^6$ ASCs suspension (0.2ml) was applied topically onto one of the two wound sites and same amount of PBS(phosphate buffer solution) applied onto the other wound as a control. Moist wound healing condition was maintained. Wound closure area was measured using the computer planimetry at post operative day 3,6,9,12 etc. Wound were harvested after 14 days for histological analysis, and numbers of vessels in wound were recorded under high powered (x400) fields. TGF-b1 and Anti human nuclei Ab(US Biological, N6925) were observed via immunohistochemistry stain.

Results
Positive surface marker on our ASCs line were CD105, CD44, CD29, CD73. The CD14, CD45, CD34, CD31 were negative. Also these cell line could differentiated into the fat cell and cartilage cell. Our data indicates that the ASCs promote the wound healing. On post operative day 3, 6,and 9, ADSCs group have reduced the wound size by 30±18, 64±18, and 78±14% compared to 12±10,42±19,and 73±19% in the control group respectively. There is a significant increase in rate of wound healing in ASCs wounds in comparison to the control ($p<0.05$). Histological examination revealed more amount of vessels in ASCs group. In addition, the TGF-b1 appeared earlier in healing process in the experimental group (ASCs) than the control group where most of TGF-b1 showed 9 days later.

Conclusion
ASCs group showed the higher wound healing rate than the control group even though the implantation was heterogenic in nature. This means its mechanism of wound healing in stem cell therapy is related to cytokines, such as TGF-β_1. Further studies are needed in investigating the exact action mechanism and impaired wound healing model to determine their efficacy as potential therapies.

SCIENTIFIC SESSION

ASC AND SKIN

IV-4 Adipose stem cells for wound healing - effect of cell delivery and cell enrichment

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Introduction

In addition to their documented developmental potential, ASCs are also known to secrete various growth factors, chemokines, and matrix-related compounds that are implicated in wound healing and tissue repair. Over the last 18 months, our multidisciplinary team has explored the ability of ASCs to enhance the healing of full thickness diabetic wounds. Diabetic wound healing is characterized by deficiency and/or dysfunction of both cells and soluble factors. In an effort to understand the impact of specific cell processing strategies and treatment parameters on ASC-mediated diabetic wound healing, we set out to:

1. explore the therapeutic impact of ASC delivery method on diabetic wound healing.
2. delineate the effect of $CD34^+$ enrichment on diabetic wound healing.

Methods

Human ASCs were isolated from adipose tissue using established methods. Depending on the specific study, ASCs were cultured in monolayer for at least 1 passage. MACS and FACS techniques were used for enrichment objectives. Three-dimensional self-organizing niche milieus ('SNiMs') were formed using a hanging drop technique and maintained in suspension culture until applied to wounds. Flow cytometry and ELISAs were used to immunophenotype cells and to assess the production of soluble factors. Diabetic (db/db) mice were monitored for the onset of diabetes via blood glucose levels, and then received dorsal 1cm full thickness excisional wounds. Non-diabetic (db/-) littermate mice were used as normal wound healing controls. Animals were randomized to receive ASC cell suspensions, ASC SNiMs or delivery vehicle only. Each wound was randomly treated on post-wounding day 1 via the topical delivery of cells (passage 3 or less). Digital images were taken of each wound every 2 or 3 days in a blinded fashion until Day 21 or until the wounds closed fully at day 12, and open wound area, expressed as a percentage of initial wound area, was quantified using Image analysis software.

Results

Our results demonstrate that ASC SNiMs statistically enhance the healing of diabetic wounds as compared to single cell suspensions. $CD34^+$ enrichment did not enhance this therapeutic effect.

Conclusion

Using a murine model of diabetic wound healing, our studies suggest that ASCs may be an effective therapy to enhance healing of abnormal wounds. Even more, our studies show that specific treatment variables – such as methods of cell preparation and cell delivery – impact in vivo biological effect. We have shown that the administration of ASCs as SNiMs accelerates the wound healing process in diabetic mice compared to those receiving no ASC treatment, or those receiving ASC cell suspensions. However, prospective ASC enrichment on the basis of CD34 expression does not enhance this therapeutic effect. The delineation of defined and reproducible treatment parameters will be critical to the successful translation of predictable and effective ASC therapies to the clinic.

SCIENTIFIC SESSION

ASC AND SKIN

IV-5 Treatment of radiation skin damage with Coleman fat grafting

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Introduction
Anecdotal clinical reports have suggested that subcutaneous fat grafting can improve fibrotic, irradiated breast tissue[1,2]. One explanation may be that adipose tissue is a source of stem cells[3]. In order to study this phenomenon, we have developed a novel mouse model of radiation-induced skin injury and used the Coleman method of fat grafting to deliver lipoaspirate beneath the irradiated skin.

Methods
The dorsal skin of 10-12 week old wild-type FVB mice was isolated with a low-pressure, non-ischemic clamp and exposed to a single dose of 45Gy XRT using a Varian 2300 Linear Accelerator. Skin changes were assessed by insonation, gross and histologic evaluation. Human lipoaspirate was harvested from healthy female donors and processed using the Coleman fat grafting technique. Refined fat was transferred to 1cc syringes for immediate transplant. Six weeks after XRT, mice with visible radiation-induced ulcers were infiltrated subdermally with 1.5mL of human fat using an 18-gauge Coleman cannula. The needle was passed subdermally in a fan-like pattern over the dorsum of the mouse and 0.033mL fat was injected per pass.

Results
All mice tolerated the radiation injury. Visible erythema, dermal thickening and skin changes were observed at 2, 4 and 6 weeks after radiation. Sirius red staining of skin biopsies demonstrated increased collagen and fibrosis of the dermal layer. Doppler imaging also showed decreased perfusion across the dorsal skin. Human fat xenograft administration caused no morbidity or mortality. Whole mount analysis showed vascular infiltration and incorporation of human tissue elements, indicating vascularity was human-derived. Histological analysis (H&E, COX-IV stains) demonstrated vascularized viable human adipose tissue with minimal peripheral fat necrosis and fibroblastic infiltration. Treatment with structural fat grafting resulted in rapid improvement in XRT skin changes compared to controls .

Discussion
We have designed a novel model for the study of irradiated skin and the effect of injected lipoaspirate grafting. We have shown persistence of fat grafting in a xenogeneic model, and integration into host tissue. In addition, we have shown presence of vascularized structures and demonstrated species-specific elements using specific immunohistochemical markers. Lastly, we have shown that subdermal fat grafting can improve overlying tissue quality and repair radiation damaged skin.

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SCIENTIFIC SESSION

ASC AND SKIN

IV-6 Using human adipose-derived cells for the production of new skin substitutes

F. Auger

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Ascorbic Acid

Stromal cells extracted from human adipose tissue offer the potential for new applications in tissue engineering, including cutaneous reconstruction. We have previously shown that adipose-derived stromal cells could be used to produce a conjunctive tissue using a self-assembly strategy. Moreover, by combining this technique with a concomitant step of adipogenic differentiation, we could recreate a functional human adipose tissue which is representative of the skin's deepest layer: the hypodermis. Our hypothesis is that adipose-derived cells could be advantageously used in skin tissue engineering to produce enhanced substitutes devoid of exogenous biomaterial. Our objective was to reconstruct both bilayered, as well as more complete trilayered skin substitutes as models to study the influence of adipocytes in cutaneous biology and wound healing. Dermis were reconstructed by the self-assembly technique using human dermal fibroblasts. Bilayered skin constructs were obtained by seeding keratinocytes directly onto either a dermis (n=3), a conjunctive tissue layer made of non-differentiated stromal cells (n=3) or an adipose tissue layer (n=3). A trilayered substitute was also produced by apposing an adipose layer (hypodermis) under a bilayered skin containing dermal fibroblasts (n=3) or stromal cells (n=3). Each reconstructed skin was cultured at the air-liquid interface for 14 days. Histological analysis of the different types of reconstructed skin was evaluated after Masson's trichrome staining. A properly regulated epidermal stratification was observed for both bilayered and trilayered reconstructed skin substitutes. Immunohistochemical labelings revealed an appropriate pattern of epithelial differentiation, with expression of K14 in the basal layer, K10 in the suprabasal layers and transglutaminase in the granular layer, similar to normal human skin. The presence of a dermo-epidermal junction (laminin 5, collagen IV), which is important for strong cohesion between the compartments, was also detected for all type of reconstructed skin. We thus suggest that easily accessible and expandable adipose-derived stromal cells could be used as an alternative cell source for dermal reconstruction. In skin featuring an adipose layer, we could see the presence of adipocytes embedded in extracellular matrix, and we are currently exploring if they could improve wound healing and reepithelialization through adipokine secretion. The influence of adipocytes on epithelial differentiation will be further investigated as function of their differentiation status. These results will determine if the new skin substitutes could represent enhanced autologous substitutes for great burn victims or patients with chronic ulcers.

SCIENTIFIC SESSION

ASC AND HEART

V-1 Differentation of human adipose-derived stem cells towards cardiomyocytes; the role of the extracellular matrix molecules fibronectin and laminin

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Myocardial infarction (MI) is a major health problem in the western world. Stem cell therapy forms a promising therapy after MI, and adipose derived stem cells (ASC) are potential candidates for stem cell therapy in MI. Although differentiation of human ASC towards cardiomyocytes has been demonstrated, its differentiation rate has been shown to be low. We hypothesized that adherence to extracellular matrix (ECM) molecules might positively affect this cardiac stem cell differentiation rate. Both laminin and fibronectin are ECM molecules present in the healthy heart and are upregulated after MI. We therefore analyzed the effect of fibronectin and laminin on the putative differentiation of human ASC towards cardiomyocytes.

ASC were cultured on fibronectin coated, laminin coated and uncoated culture plates, and stimulated with 5-aza-2-deoxycytidin for 24 hours. Cells were harvested after 1,3 and 5 weeks. Expression of cardiac markers was investigated by RT-PCR (SERCA2α, Myosin Light Chain-2α) and immunohistochemical staining of cytospin slides (desmin, an intermediate filament protein in cardiac muscle tissue and α-actinin, a subunit of microfilaments in cardiac muscle tissue.).

After 1 week, mRNA expression of Myosin Light chain, an early marker in cardiac development, was found to be increased more than 10 fold in treated cells, independent of coating, when compared to untreated cells ($p<0.05$). While mRNA expression of a late differentiation marker in cardiac development, SERCA2α, was found to be significantly increased after 5 weeks, but only in treated cells cultured on laminin.

After 3 weeks, cytospin slides showed a significantly increased number of α-actinin positive cells after treatment with 5-azacytidin, compared to the untreated cells, but only in the uncoated wells (67% versus 5%, $p<0.05$). While after 5 weeks, only in the treated cell cultures grown on laminin, the number of desmin positive cells was found to be significantly increased, when compared to the untreated cells (61% versus 3%, $p<0.05$). These results suggest that the ECM molecule laminin improves late differentiation.

Conclusion
We found that 1) a high frequency of human ASC can be differentiated towards cardiomyocytes, 2) the extracellular matrix molecule laminin, but not fibronectin, plays a role in differentiation of ASC towards cardiomyocytes, although in late differentiation only.

SCIENTIFIC SESSION

ASC AND HEART

V-2 Adipose-derived cardiomyogenic cells: in vitro expansion and engraftment in mice heart infarct

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Cells derived from stromal vascular fraction (SVF) from mouse adipose tissue can spontaneously give rise to rare functional cardiac-like cells in vitro. This study aimed to improve the production of adipose-derived cardiomyogenic cells (AD-CMG) and to assess their cardiac fate and functional outcomes after their engraftment in an acute myocardial infarction.

The culture process optimized to improve in vitro cardiac specification consisted in: (i) a primary culture of SVF cells in semi-solid methylcellulose medium, (ii) a selection of AD-CMG cell clusters, (iii) a secondary culture and expansion in BHK21 medium. AD-CMG cells were $CD29^+$,$CD31^-$,$CD34^-$,$CD44^+$,$CD45^-$,$CD81^+$,$CD90^-$,$CD117^-$ and $Flk\text{-}1^-$, expressed troponin T^+, MLC-2v, αMHC and titin. One, 2 and 4 weeks after their injection in acute myocardial infarction in mouse, strong engraftment of GFP-positive cells was identified with cardiac morphology and co-expression of MLC-2v and troponin T. Presence of these cells and the yield of chimerism were confirmed by quantitative PCR. Echocardiography showed a significant reduction of remodeling and stability of left ventricle ejection fraction in the AD-CMG cell-treated group versus controls. In conclusion, cardiomyogenic cells can be selected and expanded in large amount from mouse adipose tissue. They can survive and differentiate in an acute myocardial infarction model avoiding remodeling and impairment of cardiac function.

SCIENTIFIC SESSION
ASC AND HEART

V-3 Human adipose tissue-derived stem cells potently limit tissue damage resulting from myocardial ischemia

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Background
The use of stem cells has gained much interest as a potential therapeutic approach for repair of damage caused by a variety of cardiac insults. We and others have previously demonstrated the ability of pluripotent adipose-derived stem-derived stem cells (ASCs), to promote repair in ischemic skeletal tissues. Here we demonstrate that human ASCs also stimulate a robust functional improvement following acute myocardial infarction (MI) in athymic nude rats.

Methods and Results
ASCs were harvested from human subcutaneous adipose tissue. ASCs were characterized in vitro before in vivo testing. Growth and metabolic activity of human microvascular endothelial cells (HMVEC) cultured in growth-factor deficient minimal medium (MM) increased 1.7-fold when supplemented with a 1:1 mixture of ASC CM ($p<0.01$). Sprout formation by HMVECs and migration of endothelial progenitor cell (EPC) was enhanced by 2.1 and 2.0-fold, respectively, when ASC CM was added to MM ($p<0.01$). Following demonstration of potency in vitro, the ASCs were evaluated for the ability to protect and rescue ischemic myocardium in an athymic nude rat following permanent ligation of the proximal LAD region. Immediately after ligation 10^6 ASCs in 100 µl saline or carrier alone was injected into 2 sites of the peri-infarct region, then at 4 and 28 d heart function was evaluated echocardiography using a Visualsonics Vivo770. ASC-treated rats consistently exhibited better cardiac function at 1 month compared to the saline control group. LV ejection fraction of the ASC group was 56±7% (mean±SEM) vs 37±3% for the control ($p<0.04$). Fractional shortening was 32±5%(ASC) vs 19±2%($p<0.04$). LV volumes both at end-diastolic and end-systolic stages were lower in ASC group (311±17µl and 139±21µl, respectively) than saline group (391±30µl and 249±27µl) ($p<0.03$). Anterior wall thinning was attenuated in ASC group (1.6±0.08 mm vs 1.2±0.2, at end-diastole, $p<0.03$). Post-mortem histological analysis demonstrated that ASC treated hearts had lower fibrosis (26±6% vs 34±6%; $p<0.05$). Capillary densities in the infarcted zone were higher in ASC treated hearts (348±32/mm^2), compared to saline controls (237±41/mm^2) ($p<0.05$). Similarly, the density of small arterioles in the border infarct region was higher in ASC treated hearts (60±9/mm^2) compared to controls (27±2/mm^2) ($p<0.05$).

Conclusion
ASCs have great potential as a cell therapy to preserve heart function following ischemic insult. Given the abundant source of ASCs, therapies with these cells have a higher potential for widespread adoption compared to more rare cell types.

SCIENTIFIC SESSION
ASC AND HEART

V-4 Therapeutic effect of human ASCs on ischemic heart canine model

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As we all know, ischemic heart disease is well-known prevailing cause of death in developed countries and, for the researchers working on adipose tissue derived stem cells (ASCs), the treatment of ischemic heart disease using the human ASCs might be one of the most dramatic and challenging area among many other interesting subjects. Active clinical study using bone marrow stem cells for the heart disease is already in progression. There have been many reports showing positive effects of ASCs on ischemic heart using small animals.

In this study, we tried (1) to confirm successful cardiomyogenic differentiation of human ASCs and (2) to confirm actual therapeutic effect of human ASCs in surgically-induced acute ischemic canine model. All the adipose tissue used in this experiment was acquired from surgical specimen of human liposuction. The fat tissue specimen was rinsed with buffer solution and digested with Type I collagenase (Worthington, Chemical Co. USA) to harvest human ASCs. After culture expansion, the cells were screened for mesenchymal stem cell markers (CD13, CD29, CD44) and hematopoietic stem cell markers (CD31, CD34, CD45) using flow cytometric analysis to find out the amount and characteristics of the cells.

(1) In vitro study (cardiomyogenic differentiation): Culture expanded human ASCs were induced with 10μ mol 5-Azacytidine and β-FGF (10$\mu g/m\ell$) for 24 hours. After 4 weeks of culture expansion in DMEM with 10% FBS, immunohistochemistry was performed with anti-αactin (Sarcomeric) and RT-PCR was executed detect the expression of desmin, α-cardiac actin, β-myosin heavy chain, cardiac troponin T.

(2) In vivo study (Human ASCs therapy in canine ischemic heart model): Harvested primary ASCs, which have not been differentiated, were labeled with Feridex® and 2×10^6 cells in 0.3$m\ell$ buffered saline were injected into the ischemic myocardium of surgically-induced acute ischemic heart canine model. After 2 weeks of cell therapy, functional evaluation was done with echocardiography. Histologic study was also performed with microscopic examination and immunohistochemistry using anti-VEGF antibody, Connexin43, troponin α(H-170). Functional evaluation of ischemic heart after cell therapy using echocardiography is one of the important part of this experiment.

Evidence of Successful in vitro cardiomyogenic differentiation of human ASCs were noted in this study showing positive immunohistochemistry using anti-αactin and positive expression of desmin, α-cardiac actin, β-myosin heavy chain, cardiac troponin T in RT-PCR. Under microscopic examination, Feridex®-labeled ASCs were identified at the area of ischemic injury and the labeled cells were morphologically identical to cardiomyocytes. In vivo cardiomyogenic differentiation of Feridex®-labeled ASCs was confirmed by positive immunohistochemistry using Connexin43, troponin (H-170). The objective data of the echocardiography is going to be reported at the presentation.

SCIENTIFIC SESSION

ASC AND VASCULATURE

VI-1 Adipogenic differentiation is accompanied by transient activation of a pro-angiogenic program of factor expression

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Introduction
The temporal regulation of local vascular development occurs in parallel with the differentiation of adipose stromal cells (ASC)/preadipocytes into mature adipocytes. We hypothesize that a controlled interplay between the differentiating adipocyte and local microvasculature may be critical in defining key characteristics of adipose tissue. We employed the chick chorioallantoic membrane (CAM) assay to provide a quantitative in vivo analysis of the temporal control of vascular induction by ASCs during adipogenic differentiation.

Materials and Methods
Upon reaching confluence, human ASCs were differentiated into mature lipid-laden adipocytes over a two-week period using a standard differentiation protocol. Following Day 1, media was sequentially transitioned from Preadipocyte Media (PM: DMEM/Ham's F12 and 10% FBS); to Differentiation Media (PM, Dex ,PPARγ agonist, insulin, and IBMX); and finally to

Adipocyte Maintenance Media (PM, Dex, insulin). Conditioned media was collected upon media changes every three days during the course of differentiation. The CAM Assay, a well established in vivo angiogenic assay, was used to quantify the vascular effects of these media obtained during adipogenesis. The media samples were absorbed into 2 cm^3 gelatin sponges which were applied to CAMs on embryonic day 8. On embryonic day 12, the CAMs (N=5 per time point) were fixed and the number of vessels growing into the gelatin sponge were quantified.

Results

The temporal effect on the angiogenic response was found to correlate with the change in lipid content. Lipid accumulation began on day 3 and peaked at day 7. The angiogenic responses elicited from conditioned media at days 6 and 9 in the adipogenic process were significantly stronger than the angiogenic responses generated on days 0, 2, 12, and 15 ($p<0.05$). The angiogenic response elicited from day 6 was 1.3 fold higher then the response generated from fresh basal media ($P<0.05$); and the angiogenic response from Day 9 conditioned media was 1.2 fold higher than the response generated from the fresh basal media ($P<0.05$).

Conclusions

Activation of a proangiogenic transcriptional program and factor secretion occurs during adipogenesis in vitro. This response then wanes as the adipogenesis reaches stasis. This phenomenon may mimic the response of adipose expansion in vivo whereby a balance is maintained between expanding parenchyma and the enhanced requirement for nutrients and oxygen, which requires increasing vascularization of expanding tissue, but stable vascularization of tissue after expansion. These results may lead to a better understanding of the processes governing adipose expansion.

SCIENTIFIC SESSION

ASC AND VASCULATURE

VI-2 Functional activation of human adipose-derived stromal cells by hypoxia for adhesion to vascular proteins

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Introduction

A number of studies have shown that human adipose-derived stromal cells (hASCs) are able to affect microvascular growth and remodeling at sites of inflammation or injury when injected into the circulation in vivo, however; the mechanisms by which hASCs localize to these areas are unknown. It has also been shown that hypoxia can be used to activate hASCs in this regard. Therefore, the goal of this study is to identify possible molecular mechanisms by which hASC homing might occur in vivo and to determine whether hypoxic preconditioning is an effective means of increasing therapeutic cell delivery.

Methods

Liposuction- and panniculectomy-isolated hASCs were grown on culture plastic and introduced into a parallel-plate flow chamber at a flow rate of 1 dyn/cm^2 (P=3). 48 hours prior to use, cells were either kept in normal culture conditions or pre-treated by culture in an atmosphere of less than 2% O_2. Human proteins (type I collagen, fibronectin, VCAM-1, ICAM-1, E-selectin, P-selectin, and L-Selectin) were individually adsorbed to the bottom surface of the flow chamber, and hASCs were assayed for their ability to adhere both under laminar flow conditions and after 6 minutes of no-flow conditions. Alternately, rat heart microvessel endothelial cells (RHMVECs) were cultured on fibronectin and used as the bottom surface of the flow chamber instead of individual proteins. Endothelial cells were pretreated with either PBS or TNF-alpha prior to introduction of hASCs. Interactions were interpreted as being either positive or negative, while the frequency of adhesion events was quantified.

Results
Our results show that significantly more hASCs adhere to surfaces coated with type I collagen, VCAM-1, ICAM-1, and fibronectin under static (no-flow) conditions and to VCAM-1-coated surfaces under laminar flow conditions compared to TWEEN controls. After 48 hours of hypoxic culture, only cells isolated by liposuction showed an increase in frequency of adhesion events (increases were observed onVCAM-1 substrates in static adhesion assays and both VCAM-1 and ICAM-1 substrates in laminar adhesion assays). Preliminary adhesion testing on endothelial monolayers indicate that hypoxic preconditioning increases adhesion of hASCs to RHMVECs, and that this trend may be further increased by exposing the endothelial cells to TNF-alpha.

Conclusions
These results show that hASCs have the ability to bind several proteins present in areas of injured or inflamed endothelium and can use them to adhere under post-capillary venular flow conditions. Hypoxia has also been shown to be an effective means of increasing hASC binding to these proteins.

SCIENTIFIC SESSION

ASC AND VASCULATURE

VI-3 FGF-2 regulates secretion of angiogenic factors by adipose-derived stromal cells

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Background
It has been reported that adipose-derived stromal cells (ASCs) secrete angiogenic factors such as VEGF and HGF. However, regulatory mechanism and its relation to wound healing have not been well studied.

Methods
Effects of major angiogenic factors (VEGF, FGF-2, HGF, and PDGF), which are released in wound healing, on ASCs were evaluated. First, we examined the proliferative effect by supplementing each growth factor to control medium (DMEM, 10% FBS). Next, the interactive effects on secretion of VEGF, FGF-2, HGF, and PDGF were investigated with real-time RT-PCR and ELISA under normoxia (20%) or hypoxia (1%). Using several inhibitors, we also examined signaling pathways related to the effects. Finally, we performed in vivo studies, using an ischemia/injury model of adipose tissue.

Results
FGF-2 and PDGF promoted the proliferation of ASCs dose-dependently, though FGF-2 had a superior proliferative capacity to PDGF. Only FGF-2 greatly enhanced the expression of HGF protein and transcripts both under normoxia and hypoxia. Under a hypoxic condition, the expression of VEGF was also enhanced in the presence of FGF-2. The effects of FGF-2 on proliferation and HGF secretion were significantly inhibited by a JNK inhibitor. Upregulation of HGF mRNA and its relation to angiogenesis was shown in an ischemia/injury model of adipose tissue.

Conclusions
FGF-2 plays an important role in wound healing, regulating secretion of angiogenic factors by ASCs.

SCIENTIFIC SESSION

ASC AND VASCULATURE

VI-4 Adipose-derived mesenchymal stem cells mobilize to sites of ischemia and participate in postnatal neovascularization

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Introduction

Vascular disease afflicts greater than 71 million Americans and an astounding 403 billion dollars is spent annually treating resulting sequelae. Recent studies suggest a possible role for adipose-derived mesenchymal stem cells (MSCs) during postnatal neovascularization. In this study, we evaluated the response of adipose resident MSCs to a peripheral ischemic stimulus. The in vitro ability of MSCs to adopt an endothelial phenotype when exposed to appropriate environmental stimuli was also examined.

Methods

C57/Bl6 mice were subjected to a novel model of dorsal soft tissue ischemia. At 0, 3, 7, 14, and 21 days post-surgery, mice were euthanized and cells harvested from the inguinal fat pads, blood, and ischemic tissue. The prevalence of MSCs, defined as $CD45^-/Sca\text{-}1^+/Lin^-$ cells, in all tissue compartments was analyzed by flow cytometry. In order to evaluate the ultimate fate of these cells within ischemic tissue, adipose-derived MSCs cultured from a GFP positive transgenic mouse were injected into wildtype mice immediately following surgery. After 14 days, tissue was harvested, sectioned, and stained for the endothelial marker CD31. Fluorescence microscopy was used to evaluate for colocalization of CD31 and GFP. As both hypoxia and VEGF are known to be central to the recruitment and differentiation of vascular progenitors at sites of injury, we examined the influence of these factors on MSC differentiation in vitro. Adipose-derived MSCs were cultured at 2.5% oxygen and exposed to 50ng/mL of VEGF. After 5 days of treatment, cells were evaluated by flow cytometry for expression of the endothelial markers CD31 and Flk1. Cells were also evaluated for their ability to take up acLDL and bind fluorescently labeled lectin, characteristics typically displayed by vascular progenitors and endothelial cells. Data was analyzed with a student's t-test and one way ANOVA.

Results

At baseline, $CD45^-/Sca\text{-}1^+/Lin^-$ cells constitute approximately 0.1% of the total fat pad population. Following the onset of ischemia, this population increased within the fat reaching a peak at day 14 (3% of total cells) and subsequently trending towards baseline levels by day 21. Paralleling the increase of $CD45^-/Sca\text{-}1^+/Lin^-$ cells within the fat pad, were concurrent increases in the numbers of cells harboring the same marker profile within the circulation and ischemic skin. Peak levels of $CD45^-/Sca\text{-}1^+/Lin^-$ cells within the circulation (0.04%) and affected skin (2.4%) were observed at day 7, after which the prevalence of these cells gradually returned to baseline levels by day 21 for circulation (0.008%) and day 14 for skin (0.2%). GFP^+ adipose-derived MSCs injected into mice immediately following surgery homed to ischemic tissue and ultimately differentiated into $CD31^+$ endothelial cells. In vitro, MSCs grown under hypoxia and exposed to VEGF adopted an endothelial phenotype, with more than 3 times as many $CD31^+/Flk\text{-}1^+$ cells in the treated versus control group. Furthermore, similar to vascular progenitors and endothelial cells, many of these cells stained doubly positive for acLDL and lectin.

Conclusions

These data suggest that, in response to peripheral ischemia, adipose resident MSCs proliferate and migrate from adipose tissue to the ischemic site via the circulation, where they subsequently differentiate into endothelial cells. Adipose resident MSCs may thus represent a native/physiologic repository of vascular progenitors that normally participate in ischemic neovascularization. Therapeutic strategies aimed at increasing the prevalence of these cells within fatty tissue, or enhancing their recruitment to sites of injury, may be of significant utility in the treatment of vascular disease.

SCIENTIFIC SESSION

ASC AND VASCULATURE

VI-5 Human adipose tissue-derived mesenchymal stem cells improve angiogenesis and ischemic limb salvage in mouse model

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Introduction
Adipose tissue-derived mesenchymal stem cells (AdMSC) are capable of differentiating into endothelial cell lineages.

Hypothesis
We evaluated the effect of culture-expanded human AdMSC on in vitro angiogenesis in the presence of shear stress or growth factors and compared the effect of angiogenesis with three different cell numbers of AdMSC in ischemic hind limb mouse model.

Methods
AdMSC were isolated and cultured from human adipose tissue. Three or four passage of AdMSC were exposed to unidirectional laminar shear stress by cone-and- plate device. Hindlimb ischemia was induced by ligating the proximal femoral vessels of forty male BalbC/nude mice. With three different number of AdMSC treated group ($1.0X10^6$ cells/kg in LD group, $5.0X10^6$ cells/kg in MD group, $1.0X10^7$ cells/kg body weight in HD group) and saline-treated control group, cells were directly injected into ischemic muscle one day after ligation. Using lentivirus-eGFP transfected AdMSC, the differentiation of injected AdMSC were evaluated.

Results
Sphere formation during culture expansion of AdMSC was marked increased. In vitro, AdMSC could form tube formation in metrigel assay and expressed vW factor and KDR. Ac-LDL and UEA lection double-positive cells were considered as endothelial-like cell. Shear stress rather than growth factor accelerated the increments of the expression of Flt-1, V-catherin, KDR as endothelial cell marker by RT-PCR and immunocytochemistry. AdMSC pretreated with shear stress had resistance to apoptotic effect of TNF-alpha analyzed by Annexin V expression using FACS. The laser Doppler perfusion index was significantly improved in AdMSC-treated group than in the control group (61.0% in LD group, 79.5% in MD group, and 97.5% in HD group; $p<0.05$) on day 28. Histological examination showed that AdMSC transplantation increased capillary density, compared with the control group (64.6% in LD group, 73.8% in MD group, and 79.3% in HD group; $p<0.05$). The effect of AdMSC was correlated with the number of transplanted cells. A few GFP-positive cells could be detected in ischemic muscle, and were also stained for CD31.

Conclusion
Human AdMSC could be one of ideal adult stem cell source for clinical therapeutic angiogenesis in ischemic cardiovascular disease.

SCIENTIFIC SESSION

ASC AND VASCULATURE

VI-6 Alliance of blood derived endothelial cells and adipose stromal cells in human vasculogenesis: Timecourse and stability of neovasculature

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Introduction

Both Endothelial progenitor cells (EPC) and adipose stromal cells (ASC) are under investigation as therapies for cardiovascular diseases. Both cell types are capable of modulating vascular assembly and are, thereby, capable of directly promoting revascularization of ischemic tissues. We have shown that EPC differentiate into endothelial cells to form small vessels, whereas ASC have pericytic properties and naturally stabilize vessels. In this study we tested the possibility that ASC would interact with EPC to assemble de novo vessels in collagen in an in vivo chimeric implant.

Methods and Results

Collagen implants embedded with either umbilical cord blood EPC or adult ASC or a 4:1 mixture of both ($2x10^6$cells/ml) were implanted subcutaneously into NOD/SCID mice. After 14 d, implants were harvested and evaluated by immunohistochemistry. There was a pronounced difference among the groups in vascular network assembly. The majority of vessels formed in the EPC and ASC monocultures were small capillaries bounded by a single endothelial layer. Conversely, 100% of the plugs embedded with both cell types were highly invaded with multilayered arteriolar vessels. The density of the $CD31^+$ vessels in the EPC and co-culture plugs was 26.6±5.8 and 122.4±9.8 per mm^2, respectively. No $CD31^+$ cells of human origin were detected in the ASC monocultures, indicating that ASC, which do not express this EC-specific marker, engage murine EC or form pseudovessels in this system. The density of αSMA^+ vessels with lumens per mm^2 was 13.1±3.6 (EPC), 10.2±3.5 (ASC) and 124.7±19.7 (co-culture). By using GFP transduced ASC we demonstrated that in co-culture settings the majority of newly vessels were formed by human ASC. Moreover, the majority of these vessels were filled with erythrocytes (92.5±16.2 per mm^2), indicating inosculation with the native vasculature, which was confirmed by ultrasound with echogenic microbubbles and persisted to at least 4 months. Additional evaluation of vessel assembly dynamics demonstrated that the vessels begin to assemble by day 2 and are fully developed by day 6.

Conclusion

This study is the first to demonstrate that non-transformed human EPC and ASC cooperatively form mature and stable vasculature with subsequent functional integration into a host vasculature system.

SCIENTIFIC SESSION

ASC AND CANCER

VII-1 Anti-tumor effects of hyperthermia-treated mesenchymal stem cells (MSCs) from different sources on SK-OV-3 ovarian cancer cells

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Mesenchymal stem cells (MSCs), a subset of non-haematopoietic cells in the stroma, display a multipotency for self-renewal, proliferation and differentiation into precursors for bone, cartilage, connective and muscular tissue. Interestingly, they are also reported to play a role as tumor stroma providing a favorable environment for the growth or dissemination of tumor. Hyperthermia is a cancer therapy using heating beam. Although it is an effective cancer therapy, particularly in combination of radio- and/or immuno-therapy, it is rarely known about how hyperthermia on tumor stroma affects on cancer cells. Here, we investigated the effects of hyperthermia-treated MSCs from various sources on human ovarian cancer cell SK-OV-3. We isolated MSCs from processed lipoaspirates (PLA), liposuction aspirate fluids (LAF) and amniotic fluids (AF). After immunophenotyping by flow cytometric analysis, MSCs from PLA, LAF and AF were untreated or heat-treated and the culture supernatant of each condition was collected, followed by adding to SK-OV-3. The microscopic analysis showed the severe damage in the morphology of tumor cells by the culture supernatant of heat-treated MSCs. Cell proliferation assay demonstrated that the culture supernatant of heat-treated MSCs induced the significant inhibition in the growth of the cancer cells. The results of RT-PCR revealed that the culture supernatants of heat-treated MSCs induced the distinguished up-regulation of TNFR family (Fas and TNFR) and the prominent down-regulation of multidrug resistance proteins (MDR1 and MRP). These phenomenon were the most highly appeared by the culture supernatant of heat-treated AF-originated MSCs. Among the apoptosis-associated molecules, mRNA expression of bfl-1 was reduced by the culture supernatants of heat-treated MSCs. All of these results imply that combination therapy of hyperthermia on tumor stroma with chemotherapeutic drugs may further elevate the anti-tumor effects by overcoming the drug resistance mechanism of tumor cells. Furthermore, analysis of tumor cell cycles exhibited that the culture supernatant of heat-treated MSCs induced the cell cycle arrests of the tumor cells.

Taken all together, the culture supernatants of heat-treated MSCs exerted the suppressive effects on progressiveness and malignancy of cancer cells, suggesting that hyperthermia could enable tumor stroma to provide a sensitizing environment for tumor cells to death.

SCIENTIFIC SESSION

ASC AND CANCER

VII-2 Cell-based therapy of pancreatic cancer using human adipose-derived stromal cells

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Despite advances in surgery, radiation therapy, and chemotherapy, limited progress has been made for the treatment of patients diagnosed with pancreatic adenocarcinoma. Emerging evidences suggest that human stem cells can play an important role in the development and growth of human malignancies. Adipose tissue recently appeared being a

source of cells usable in cell therapy. Indeed, as bone marrow mesenchymal stem cells, cells derived from adipose tissue (ADSC) are able to differentiate towards numerous phenotypes, and display immunomodulatory functions. We investigate here whether human ADSC could impair tumor growth. We injected ADSC purified from fat from healthy donors into exponentially growing pancreatic tumors xenografted into atymice mice. GFP-transduced ADSC were readily detected within tumors. The intratumoral injection of ADSC significantly inhibited tumor growth and tumoral cell proliferation, and promoted cancer cell death by apoptosis. In vitro, co-culturing cancer cells with ADSC, or treating pancreatic cancer-derived cells with ADSC- conditioned culture medium inhibited cell viability and proliferation and induced cell death by apoptosis. In addition, ADSC-mediated antiproliferative effect was extended to colon, liver-derived cancer cell lines, and lymphoma. We latter identified secreted product as requested for the antiproliferative effect of ADSC. Altogether, these results described for the first time the antitumoral properties of ADSC for pancreatic adenocarcnoma, both in vitro and in vivo. Therefore, ADSC may constitute a potential cell-based therapeutic alternative for the treatment of pancreatic adenocarcinoma and its metastasis for which no efficient cure is available.

SCIENTIFIC SESSION

ASC AND CANCER

VII-3 Regulation of aromatase expression and estrogen biosynthesis in adipose stromal cells

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Recent study reveals that obesity has an immense impact on breast cancer development. Increased body mass is a strong risk factor for the development of invasive breast cancer among post-menopausal women. A number of hypotheses have been proposed to explain this association, including those that involve adipose tissue-derived estrogen, insulin and insulin-like growth factor, and several adipocytokines. However, the exact molecular basis of obesity-associated breast cancer risk remains to be elucidated.

Adipose tissue provides an important extragonadal source of estrogen. Aromatase (Cyp19), which converts androgen to estrogen, is a key enzyme in estrogen biosynthesis. In normal adipose tissue, transcription of the aromatase gene is initiated from a relatively weak adipose-specific promoter (I.4). However, in breast cancer, a switch of promoter utilization from I.4 to a strong ovary-specific promoter PII leads to increased aromatase expression and hence elevated estrogen production. Using primary adipose stromal cells (ASCs), we show that they exhibit great inducibility in aromatase expression. In particular, high cell density can trigger a dramatic increase in aromatase transcription. In contrast, fat-laden mature adipocytes have a very limited capability in aromatase expression. Furthermore, density-induced aromatase expression in ASCs is due to reactivation of the otherwise dormant cancer-associated promoter (PII) of the aromatase gene. In addition to aromatase expression, we also observed elevated transcription of a number of other breast cancer-associated factors. Consistent with the gene expression result, we also show that ASCs can stimulate migration of breast carcinoma cells.

In a separate study with ASCs, we also uncovered an intriguing relationship between the breast cancer susceptibility gene BRCA1 and aromatase expression in ASCs. Upon stimulation by phorbol ester or dexamethasone, increased aromatase expression in ASCs was accompanied by significant reduction of the BRCA1 level. In addition, adipogenesis-induced aromatase expression was also inversely correlated with BRCA1 abundance. Down-regulation of BRCA1 expression in response to various stimuli was through distinct transcription or post-transcription mechanisms. Importantly, siRNA-mediated knockdown of BRCA1 led to specific activation of the breast cancer-associated PII promoter. Therefore, in addition to its well-characterized activities in breast epithelial cells, a role of BRCA1 in modulation of estrogen biosynthesis in ASCs may also contribute to its tissue-specific tumor suppressor function. Taken together, our study sheds light on the cross-talk between mammary epithelial cells and adipose stromal cells in the tissue microenvironment.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-1 Long-term treatments of adipose stromal cells-secreted media protected hypoxia-ischemia-induced neonatal brain damage

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Recent studies suggest that adipose tissue contains pluripotent stem cells and the beneficial effects of adipose-derived stem cells (ASC) when delivered to diseased tissues appear to be related to their ability to secrete growth factors. Since almost all clinical trails failed based on the data obtained from the short term of pretreatment in animal models, in this study, we examined the long term neuroprotective effect of ASC-secreted media (ASC-CM) in hypoxic-ischemic (H-I) brains of rats. ASC isolated from rat subcutaneous adipose tissue were grown to confluence in neural basal medium and the conditioned medium was removed and concentrated. Unilateral (left) carotid artery ligation was performed on neonatal (day 7) rats, followed by exposure to hypoxia (7%) for 2 hours. The concentrated conditioned medium was injected iv at the same time of or 24 hrs after ischemia. 60 days later, the area of tissue in the hippocampus ipsilateral to the lesioned hemisphere was compared to the matching brain region in the contralateral to unlesioned hemisphere of the same rat. The percentage area loss was then determined for each animal. Additionally, a behavior test examining long term learning and cognition (Morris water maze) was performed on these rats too. Treatments of ASC-CM significantly protected against hippocampal volume loss induced by ischemic-hypoxic injury (60.5±3.4, n=4 vs. 97.7±10.0 in pretreatment, n=4; and 88.3±10.9, n=3, one-way ANOVA, $p<0.01$). Consistently, the behavior test demonstrated that ASC-CM significantly improved the learning and cognition in both treatment groups as compared to rats treated with H-I only. We conclude from these data that the delivery of a milieu of factors secreted by ASC should be a viable therapeutic option for treatment of hypoxic-ischemic injury to the brain.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-2 Adipose-derived stem cells for bioengineered nerve repair

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Tissue engineering using a combination of biomaterials and cell based therapies represents a new approach to nerve repair. Schwann cells play a pivotal role in peripheral nerve regeneration but technical issues prevent their mainstream clinical use. As an alternative cell source, adipose-derived stem cells offer tremendous potential, due to their easy accessibility and multi-lineage differentiation properties. We have investigated whether these cells can be differentiated into functional Schwann cells which could be used to enhance nerve regeneration following injury. Rat visceral fat was enzymatically digested to yield a heterogeneous population of cells expressing a range of stem cell markers including stro-1, CD29 and nestin. When treated with a mixture of glial growth factors (GGF, bFGF, PDGF and forskolin) the cells adopted a spindle-like morphology similar to Schwann cells. Immunocytochemical staining indicated that approximately 40% of these cells expressed the glial markers S100, GFAP and p75, indicative of differentiation. Protein expression was confirmed by Western blotting. The function of these cells was then assessed by co-culture with NG108-15 motor neuron-like cells. The differentiated stem cells significantly enhanced the number of NG108-15 cells expressing neurites, the number of neurites per cell and the mean length of the longest neurite extended.

Next we analyzed the biocompatibility of microporous poly-epsilon-caprolactone (PCL) films for the development of a nerve conduit to transplant the differentiated stem cells. Scaffolds were fabricated using dichloromethane and had pore sizes 3-10 microns in diameter and 1-4 microns in depth. The thickness of the films was easily adjustable by changing the concentrations of the casting solution. The differentiated stem cells readily adhered to PCL films and proliferated at a rate comparable to that on tissue culture plastic. Importantly, the stem cells retained their differentiated phenotype, expressing glial markers and potentiating NG108-15 neurite outgrowth on PCL.

These results indicate adipose tissue contains a pool of regenerative cells which can be differentiated to a functional Schwann cell phenotype. Furthermore, PCL films represent a suitable synthetic and biocompatible scaffold for the transplantation of these cells to treat nerve injuries.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-3 Expression of coagulation FVIII and FIX in hASC and hASC-derived cells: Potential autologous cell therapy for hemophilia

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Pleuripotent Adipose Stromal Cells (ASCs) have the capacity to differentiate into mesodermal lineage cells such as adipocytes, myocytes, osteocytes and chondrocytes. In addition, the ASCs can trans-differentiate in vitro into hepatogenic, neurogenic, endothelial and endocrine cells. Since hASCs are relatively easy to isolate in large quantity and have high proliferative potential, they provide a potential source of stem/progenitor cells for autologous cell therapy for the regeneration of injured tissue.

The goal of this study is to isolate and engraft ASCs to generate new tissue capable of secreting therapeutic plasma proteins. In particular, can one engraft genetically modified ASCs in a recipient that secrete coagulation factors for the treatment of hemophilia? Hemophilia A and B result from too low levels of plasma Factor VIII or FIX activity, respectively. The aim of the project is to generate hASC derived tissue capable of secreting FVIII or FIX to support hemostasis in hemophilic mice.

Unlike murine ASCs, undifferentiated human ASCs express FVIII at low but significant levels (0.03ug/ml per 10^5 cells in 48 hours; ~30% of normal human plasma FVIII level). ASCs were transfected with a plasmid expressing a modified B-domainless FVIII cDNA. Following differentiation into preadipocytes, the level of FVIII secretion increased 3-fold. In preliminary studies, expression of FIX is detectable in hASCs and the level increases following differentiation into preadipocytes. These results indicate that ASCs and preadipocytes are capable of gamma carboxylation, a post-translational modification required for FIX activity. FIX expression was further increased following infection with a lentivirus carrying a FIX expression cassette.

Since HGF has been shown to elicit a potent proangiogenic response (Cai et al., in press), hASCs predifferentated into preadipocytes were mixed in hydrogel containing HGF and injected subcutaneously into SCID mice. Oil red O staining cells were recovered in a cellular nodule at the injection site 3 weeks post-transplantation.

In summary, hASCs and hASC-derived preadipocytes cultured ex vivo express low levels of coagulation Factors VIII and IX. These levels can be increased following transfection with FVIII or FIX expression vectors. Furthermore, transplantation of hASCs cultured in adipogenic media results in the formation of preadipocyte/adipocyte containing nodules. These results suggest it may be possible to generate tissue derived from genetically modified hASC capable of secreting coagulation factors for the treatment of hemophilia.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-4 Hematopoietic colony-forming cells derived from the stromal vascular fraction of human adipose tissue

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Introduction
Adipose tissue has the ability to rapidly expand or contract in accordance with nutritional constraints. In so doing it requires rapid adjustment in its vascular supply and supporting stroma. The stromal vascular fraction of adipose tissue has been shown to contain a multipotent stem cell, which has been identified with pericytes, cells surrounding small vessels. In this report we asked whether the stromal vascular fraction of freshly isolated or culture expanded human adipose tissue could give rise to hematopoietic cells, given the proper microenvironmental cues.

Materials & Methods
The stromal/vascular fraction was isolated from whole fat or liposuction aspirate by collagenase digestion, centrifugation and treatment with ammonium chloride erythrocyte lysis buffer. Cells were cultured for 6 hours, after which nonadherent cells were discarded. The adherent fraction was expanded in culture in DMEM/F12 medium containing 10% FBS and 0.1M dexamethasone. Adherent cells were removed with trypsin EDTA and cultured in MethoCult (Stem Cell Technologies, semi solid hematopoietic culture medium containing FBS and rh Stem Cell Factor, GM-CSF, IL-3 and Epo) for 21-42 days. Cultures were harvested for 8-color Flow Cytometry (CD33, CD34, CD41, CD45, CD71, DAPI, Glycophorin A, HEA) and cytocentrifuge preparations.

Results & Discussion
Identifiable BFU-E and CFU-M were detected in MethoCult culture at low frequency. Mature erythrocytes were rare but clearly visualized on Wright Giemsa cytocentrifuge preparations. A spectrum of hematopoietic cells was detected by flow cytometry (Table). These included members of all lineages, at varying stages of differentiation. In particular both early and mature erythroid cells were observed. $CD34^+$ low side scatter cells were observed at approximately the same frequency as is present in freshly isolated bone marrow. Sorted $CD34^+$ cells gave rise to hematopoietic colonies at a frequency of 1/300 at 6 weeks in culture.

Conclusions
These data indicate that hematopoietic progenitor cells (or cells that can give rise to them in culture) are resident in adipose tissue. Control experiments (not shown) demonstrate that this was not due to contamination with peripheral blood. It is not known whether adipose derived stem cells give rise to blood cells in vivo and are in a common pool with circulating and bone marrow resident progenitors. We speculate that they are a distinct lineage with sufficient plasticity to give rise to the formed elements of blood when cultured in an artificial cytokine milieu. If correct, autologous adipose derived stem cells may be of value to reconstitute the bone marrow of patients undergoing dose intensive therapy for hematologic malignancies.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-5 Integrin α5/β1 is a receptor for SPARC on adipose stromal cells and a therapy target identified with peptide mimetics

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Adipose stromal cells (ASC), which can be obtained in high abundance through minimally invasive procedures (lipoaspiration or abdominoplasty) from white fat, present an attractive potential alternative to bone marrow-derived mesenchymal stromal (stem) cells (MSC) for autologous cell transplantation. To identify ASC markers, we initiated a screen for peptide ligands binding to cell surface receptors of patient-derived cells of the stromal-vascular fraction (SVF) of white adipose tissue (WAT). We demonstrate that human and mouse ASC express a conserved receptor targeted by peptides mimicking SPARC, a matricellular protein that is required for the maintenance of normal WAT content. A signaling receptor for SPARC has not been definitively characterized. We used SPARC-mimicking peptides CMLAGWIPC and CWLGEWLGC, isolated from binding assays on human and mouse cells, respectively, to identify an integrin α5/β1 complex as the receptor for SPARC. Our results establish that extracellular SPARC binds to integrin α5/β1 at sites of focal adhesions, a recognition that induces focal adhesion and actin network reorganization. To test integrin α5/β1 as a cell therapy target, we demonstrated that agents directed to cells with SPARC-mimicking peptides are actively internalized. Moreover, SPARC-mimicking peptides can be used to deliver pro-apoptotic compounds into cells for their targeted ablation, as well as gene therapy vectors for modulation of cell physiology. Our study identifies integrin α5/β1 as the receptor for SPARC in ASC and as a prospective therapeutic target on adult stem cells.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-6 Effect of human adipose tissue-derived stem cells on stress urinary incontinence in rats

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Stress urinary incontinence (SUI) states involuntary urinary leakage during activities such as exertion, sneezing, and coughing that increase abdominal pressure, and it is very common in women over middle age. For the treatment of urinary incontinence, many therapeutic trials such as surgical support and bulking agents have been performed. Recently, the effects of several kinds of tissue-derived stem cells for the incurable diseases have been examined and verified. In the present study, we investigated the effect of human adipose tissue-derived stem cells on SUI in rats.

Female Sprague-Dawley rats were anesthetized and their periurethral tissues were dissected (urethrolysis) to induce the rat model of urinary incontinence.

The adipose aspirates was collected from female patients undergoing conventional liposuction and the adipose aspirates were precessed to yield a pluripotent population of processed lipoaspirate (PLA) cells. After immunophenotyping by flow cytometric analysis, PLA cells were fluorescent labeled and injected on proximal urethra of the rats. The rats were divided into three groups: sham-operation group, urethrolysis group, urethrolysis & PLA cells injection group. At 8 weeks after injection of stem cells, leak point pressure was measured by using the pressure transducer after spinal cord transection at the level of T12-T13 to block the reflex of bladder contractions.

In the present results, leak point pressure was significantly decreased in the rats of urethrolysis group, while injection of human adipose tissue-derived stem cells increased the leak point pressure of urethrolysis-rats.

These results showed the possibility that human adipose tissue-derived stem cells can be used for the effective therapeutic modality to ameliorate the symptoms of urinary incontinence.

SCIENTIFIC SESSION

ASC AND OTHER THERAPEUTIC APPLICATIONS

VIII-7 Behavior of adipose-derived stem cells in canine periodontal tissue regeneration

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Objectives
Periodontal disease causes the tooth loss by the destruction of the periodontal tissue. We think that the stem cell might be necessary for periodontal tissue regeneration. We have studied rat periodontal tissue regeneration using Adipose-derived stem cells (ASCs) with platelet-rich plasma (PRP), and suggested that ASCs and PRP could contribute to periodontal tissue regeneration in immunohistochemically. In this study, we sought to determine if ASCs could be available as an alternative cell source for periodontal tissue regeneration in canine model.

Methods
ASCs were prepared from inguinal fat pads of beagle dog (six month, weight 7-10 kg), the primary culture was performed in control medium (DMEM+10%FBS). Class III furcation defects were created in the mandibular premolars with dental round bar, ASCs with PRP from autologous blood were transplanted in the experimental group (n=20). While no treatment and PRP independent were performed in the controls (n=20). After one, two and three month implantation, X-ray, histologic examination, immunostaining of Osteocalcin and type I collagen were done.

Results
After one month of implantation, in the experimental groups, alveolar bone regeneration was seen in top of class III defect, invasion of gingiva were not observed. In the control groups, alveolar bone regeneration was seen in basal part of class III defect, invasion of gingiva were observed. After two month of implantation, in the experimental groups, exasperation of radiopaque were confirmed in class III defect. Histologically, the regeneration of alveolar bone, periodontal ligament and cementum were confirmed. In control groups, exasperation of radiopaque in class III defect was present on a low level. Histologically, plenty of regeneration were not observed in periodontal tissue of class III defect.

After three month of implantation, in the class III defect of experimental groups, periodontal tissue was evidently seen in histological observations. Immunochemically, Osteocalcin positive cells were shown on surface of regenerated alveolar bone, type I collagen positive cells were seen in the alveolar bone and periodontal ligament like structure. Control groups, high density regenerated bone were not observed.

Conclusions
These findings suggest that ASCs with PRP could contribute to periodontal tissue regeneration in canine model.

Translational Posters

Definition of clinical conditions for the cryopreservation of adipose tissue

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The adipose tissue is usually used in autologous settings, in plastic and reconstructive surgery. Classically injected immediately after the harvest, repeated lipoaspiration remains necessary when the reconstruction require several interventions Therefore development of techniques for fat tissue conservation is of great interest as it would reduce surgery procedure and risks. The objective of this study was to develop a technique of cryopreservation applicable to a clinical use. We had endeavour to respect handling in closed circuit, to carry out the experiments with volumes compatible with the clinic. Methods: Adipose tissue (AT) of healthy subjects (BMI < 30) was obtained as by-products from liposuccion. The AT (8ml or 50ml) was transferred in sterile freezing bags (50ml or 750ml) using syringes and specific devices. The cryoprotectant solution was added (same volume than AT) and the bags were freeze. After a given period, the bags were rapidly thawed. Cryoprotectant was removed by 3 washes. To control the quality of the cryopreservation, we compared freshly harvested to cryopreserved AT in functional and phenotypic tests. Functional test consisted in measuring cell parameters of the stomal vascular fraction (SVF) obtained after collagenase digestion of each AT and adipose derived stromal cells (ADSC) corresponding to cultured SVF cells. The SVF and the ADSC were phenotyped by flow cytometry using directly coupled monoclonal antibodies (CD13, CD45, CD14, CD34, and CD90). Results: In a first set of experiments we used 3 concentrations of DMSO (high, medium and low) diluted in albumin (HA). The quantity of cells (expressed /g of AT) in the stromal vascular fraction (SVF) obtained after collagenase digestion was 3 times less for AT after cryopreservation as compared to fresh AT (fresh=$7,6x10^5 \pm 1,5x10^5$, highDMSO=$2,9x10^5 \pm 1,4x10^5$, mediumDMSO=$3,3x10^5 \pm 1,2x10^5$, lowDMSO=$2,9x10^5 \pm 1,1x10^5$). We observed no significant differences between the 3 cryopreservation conditions. The viability of the cells in the SVF and their phenotype were very similar for all the conditions. The cell expansion was also comparable whatever the condition tested. Thus cryopreserving AT in low DMSO solution was effective. In a second set of experiments we tried to further decrease the DMSO concentration and changed the HA to a vegetal macromolecule solution. We obtained very good results with the lowest concentration of DMSO admixed with the vegetal macromolecule solution. On a functional point of view, the expansion of ADSC was very similar with a little advantage to the highest DMSO concentrations. The cell viabilities were always excellent, and the phenotype did not vary.

Conclusion

The present work shows that adipose tissue could be cryopreserved with low concentration of DMSO in a protocol that respects clinical scale conditions in a closed circuit.

hASC are an useful tool in screening scaffolds for bone regeneration

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The emerging field of regenerative medicine will require a reliable source of stem cells in addition to scaffolds and growth factors. Adipose tissue represents an abundant and accessible source of adult stem cells which possess similar properties of mesenchymal stem cells derived from bone marrow. We isolated hASCs (human Adipose-derived Stem Cells) from the stromal–vascular fraction of subcutaneous adipose tissue from 23 adult donors undergone a liposuction procedure. hASCs highly proliferated in culture and were able to efficiently differentiate into cells of the chondrocytes, osteocytes and adipocytes lineage demonstrating their multipotency. Here we would like to present one of our studies on hASCs osteogenic differentiation improvement on several scaffolds, some prototypes and some already used in clinical application. Indeed we believe that the development of successful scaffolds for bone tissue engineering requires a concurrent engineering approach that combines different research fields. In order to limit in vivo experiments and reduce trials and error research, a scaffold screening has to be well performed in vitro. The elevated availability of adipose tissue, usually discarded, and the relative high yield of multipotent cells combined with the well-known osteogenic potential, make hASCs an excellent candidate for an accurate screening of many scaffolds for bone regeneration application, at first in vitro and then in a selected in vivo model. hASCs have been induced to differentiate into osteoblasts by culturing them in different inductive media, which allowed us to identify the best osteogenic inducer medium.

We tested interaction between hASCs with hydroxyapatite granules, titanium screws, deproteinized bovine bone, calcium alginate fibers, silicium carbide (SiC-PECVD) and bone fragment (from bone bank) and polyurethanes. hASCs loaded on scaffolds maintained their consistent proliferation rate, without the appearance of cellular toxicity. The abundant colonization by hASCs appeared evident by optical microscopy either on the surface of all tested scaffolds and, when it possible, inside the pores. A more abundant extracellular calcium deposition has been also produced by cells seeded on scaffold in comparison to the same number of cells cultured for the same time in plastic adherence condition, with increase value ranging from 60% and 500% ($p<0.01$), depending of the scaffolds. In particular bone fragments and polyurethanes seem to be the most rapid osteoinductive biomaterials, since both allow high level of calcium deposition already after two weeks instead of three as showed by cell loaded on other scaffolds or differentiated in plastic adherence condition.

SEM analysis also allows observing a good cell adherence and some secretory vesicles above cells' surface, proportionally to calcium matrix revealed. Moreover osteogenic induction of pre-differentiated and on scaffold-directly-differentiated hASC was compared: no significant difference was detected in term of adhesive properties and proliferation activity, and of osteogenic differentiation process (alkaline phosphatase activity and calcium matrix formation) between the two cell conditions.

Our results confirm the strong osteogenic potential of hASCs and show that the ensemble of inductive media and scaffolds produces a synergistic effect, whose magnitude appears to depend on the biomaterial features, like chemical properties, porosity, roughness and others. Human adipose-tissue-derived MSCs may therefore be considered an efficient, easily obtainable and renewable cellular source useful in understanding the biology of differentiation processes and, very soon, in regenerative medicine application.

Green tea extract (EGCG) – a possible tool to manage weight gain under psychopharmacotherapy?

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A common side effect of antipsychotic treatment with atypical neuroleptics is a significant weight gain. This stabilizes in the short to medium term but with the neuroleptic clozapine often continues beyond the first year leading to weight gains of up to 20-30 kg. This increase in weight is significantly correlated with clinical improvements but the causes of treatment-emergent weight gain in schizophrenic patients are currently unknown. The aim of this study was to identify the cause of this treatment-related weight gain and to investigate whether the green tea extract epigallocatechine gallate (EGCG) could be a potential remedy.

Preadipocytes were isolated from freshly excised human subcutaneous adipose tissue (n=10) and cultured in Dulbecco's modified Eagle medium (DMEM)/ Ham's F12 until confluence. Adipogenic conversion was then promoted for 14 d in DMEM/F12, supplemented with insulin, dexamethasone, IBMX, pioglitazone, triiodo-L-thyronine, and transferrin. Clozapine (5µM and 20µM), EGCG (10µM), and LiCl (2mM) were added on days 1, 3, 5 during differentiation. To evaluate preadipocyte differentiation, the activity of glycerol-3-phosphate dehydrogenase (GPDH), a molecular key marker of adipogenic conversion, was determined after 14 d. Reverse Transcription (RT) and Polymerase Chain Reaction (PCR) were carried out applying the RNeasy- and the Taq Core-Kit from Qiagen. PCR-products were subjected to electrophoresis on 1.8% agarose gels and bands visualized by ethidium bromide staining.

Preadipocytes treated with clozapine (5 µM) showed a significantly higher percentage of differentiated cells (126±12%) compared to controls (100%) ($p<0.01$). LiCl and the typical neuroleptic drug haloperidol did not increase GPDH-activity, but rather inhibited differentiation slightly. EGCG reduced differentiation by 80% ($p<0.001$) and in the presence of clozapine by 50-60%, compared to standard differentiation ($p<0.01$). The mitochondrial Manganese-Superoxide dismutase (Mn-SOD) was upregulated by LiCl and the combination of EGCG and clozapine, whereas EGCG alone had hardly any effect on Mn-SOD expression.

Our findings present a new explanation for weight gain in patients under neuroleptic treatment - weight gain due to enhanced differentiation of adipose tissue precursor cells to mature adipocytes. Also, our findings demonstrate that EGCG can effectively inhibit preadipocyte differentiation and counteract clozapine-induced adipogenesis by upregulating Mn-SOD. This mitochondrial enzyme diverts superoxide anions as produced under clozapine treatment to hydrogen peroxide, which is known to inhibit adipocyte differentiation. Our results suggest an effective way to prevent clozapine-induced weight gain. EGCG in green tea or as capsules could counteract the enhancement of adipogenesis.

Tissue harvesting site influences yield of adipose tissue derived stem cells: Implications for cell based therapies

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Background
Osteoarthritis is a serious disease affecting millions of people worldwide. Since current therapies lack regenerative capacity, stem cell therapies are being developed as a promising alternative. Adipose tissue is an attractive source of stem cells for these therapies since they can easily be isolated and provide a rich source of adipose tissue-derived mesenchymal stem cells (ASCs). The aim of this study was to compare two different adipose tissue harvesting sites, the abdomen and hip/thigh region, for yield, proliferation and differentiation capacity of adipose tissue-derived stem cells to determine which site is most promising for tissue engineering purposes, especially for a one-stage surgical procedure for the regeneration of cartilage tissue.

Methods
ASCs in the stromal vascular fraction of adipose tissue harvested from the abdomen and hip/thigh region were compared for:
1. stem cell yield using limiting dilution and CFU-F assays.
2. osteogenic differentiation using CFU-ALP assay.
3. proliferation by determining growth kinetics of cultured ASCs and surface marker expression profile by FACS characterization.
4. differentiation potential of cultured ASCs into osteogenic and chondrogenic pathways, using RT-PCR and (immuno) histochemistry.

Results
The ASC yield of adipose tissue from the abdomen was significant higher than adipose tissue harvested from the hip/thigh region (P=0.0009). The frequency of CFUs having osteogenic differentiation potential was the same for both tissues. When cultured, homogeneous cell populations were obtained with similar growth kinetics and surface marker expression. No differences were detected in differentiation capacity between ASCs from both tissue harvesting sites.

Conclusion
We conclude that the yield of ASCs, but not the total amount of nucleated cells per volume and ASC proliferation and differentiation capacities, are dependent on the tissue-harvesting site. Regarding this, the abdomen seems to be preferable to the hip/thigh region for harvesting adipose tissue, in particular when considering SVF cells for stem cell based therapies in one-step surgical procedures for skeletal tissue engineering.

Effect of harvesting technique in autologous fat grafting evaluated through vital staining: A pilot study

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Autogenous fat transfer is a relatively common procedure performed by plastic and reconstructive surgeons1. The goal of fat grafting is to provide the patient with a predictable, long lasting autogenous soft tissue augmentation. This is achieved by maximizing the transfer of viable adipocytes. This process can be divided in three phases: harvest, preparation and application2. The harvest of fat cell is the critical portion of the procedure. At the completion of the harvest portion, the maximal number of viable adipocytes is present. In an attempt to maximize the percentage of viable cells, centrifugation has become, for many surgeons, part of the preparation of the harvested fat. Unfortunately most of the evidence supporting centrifugation of the harvested fat is anecdotal or based on clinical observation. Autogenous fat transfer has been used extensively as an adjunct to facial rejuvenation. As well it has been applied to body contouring and augmentation of the hips, trochanteric areas, thighs and buttocks, back, torso and breast 3,4,5. Fat grafting used for body contouring represents a unique challenge in that larger volumes need to be transferred. In the clinical setting, this means a longer extracorpeal time of the graft and longer anesthesia time. Liposuction aspirate is an attractive alternative to the time consuming syringe assisted fat harvest in cases where a larger volume is planned to be transferred. The goal of this study is to evaluate the effect of different aspiration technique and centrifugation on the viability of adipocytes through the analysis of their structural integrity.

Material and Method

Harvest of Fat A total of five patients were recruited for this study. All were healthy and consulted our senior author (KK) for cosmetic abdominal suction lipectomy. Written consent was obtained for the procedure and verbal consent was obtained for the study. Following induction of general anesthesia, the abdomen was prepped with betadine in the usual fashion. Tumescent solution (lidocaine 0.1%, epinephrine 1:1000000) was then infiltrated in the abdominal area. A total of 20 minutes were allowed to maximize the vasoconstrictive effects of epinephrine. Aspiration was then performed using a Coleman aspirator (3mm) attached to a 60, 30 and 10 syringe. Then, using a 5mm canula, conventional liposuction was performed at low (-10mmHg), medium (-15mmHg) and high (-20mmHg). All specimens were transferred to a 10cc syringe and allowed to separate through sedimentation. Lastly, 2 specimens harvested at medium constant negative pressure were submitted to short (10 sec) and long (3 minutes) centrifugation at 3000rpm. The fluid layer composed of cellular debris, red blood cell and tumescent solution was then evacuated and the specimens were processed for vital staining as described below.

Processing of Specimen To evaluate for structural integrity of the cells Hoechst 33342 and propidium iodide vital stains were used. Hoechst 33342 is a lipophilic dye which can easily cross intact cell membranes and bind the chromatin of normal and apoptotic cells. When bound to DNA the dye will fluoresce at a wavelength of 461nm. Propidium iodide, a hydrophilic molecule, will only penetrate compromised cell membranes to bind chromatin. When bound to DNA, propidium iodide will fluoresce at a wavelength of 617nm. Therefore, by examining the fluorescence under a microscope, it is possible to distinguish viable and dead cell populations. Five hundred microliter of aspirate was obtained from the syringe specimen. This was gently mix with chilled (4oC) 1 mL PBS, 1µL working Hoechst solution, and 1µL working Propidium iodide solution. Incubation was allowed for 20 to 30 minutes. Image fluorescence using Chroma filter sets: exciter HQ560/55x, dichroic Q595LP, emitter HQ645-/75m for propidium iodide and exciter 350/60x, dichroic 400DCLP, emitter E420LPv2 for Hoechst; three images were captured by sample at 100x magnification. Using SlidebookTM deconvolution software (Intelligent Imaging Innovations) PI:Hoechst cell ratio was calculated. A mask based on Hoechst staining was created to count the total number of cells in each field. A second mask was created based on PI staining to count cells with compromised cell membranes. The number of cells staining for Hoechst but not propidium iodide will determine percent of structurally intact cells.

Statistical Analysis Analysis of variance was performed to determine whether a harvest technique yielded an increase percentage of viable cells compared to another technique. Paired t-tests were used to evaluate the effect of centrifugation on the percentage of viable cells. Statistical significance is defined as $p<0.05$.

Rapid adherence of non-cultured adipose stem cells to a bioresorbable scaffold: possible implications for a one-step operational procedure

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Introduction
Bone marrow mesenchymal stem cells are often purified and expanded using lengthy and costly in vitro culturing procedures prior to clinical application[1]. Since the stromal vascular fraction (SVF) of adipose tissue provides an abundant source for adipose tissue derived stem cells (ASCs), culturing for expansion is not needed[1]. However, the SVF is a heterogeneous cell population and culturing for purification is required. Rapid and possibly selective adherence of ASC-like cells to a scaffold would circumvent these procedures and allow a one-step surgical procedure in which the SVF is obtained via liposuction, seeded onto a scaffold and then directly returned into the patient[2].

Methods
SVF cells were allowed to attach (5' up to 8 hrs) to a 70:30 Poly(D,L-lactide-co-caprolactone) scaffold. Attached cells were allowed to proliferate and differentiate. Confocal microscopy was used to characterize the phenotype of the attached cells and differentiation rate after 14 days. Kinetics of SVF cell adherence and the proliferation capacity was assessed using DNA CyQUANT®.

Results
After 1 hour, 77.8% of the attached cells had an ASC-like phenotype ($CD34^+$, $CD31^-$, $CD45^-$, $CD146^-$). The number of attached cells to the scaffold was significantly higher for all time-points compared to polystyrene culture wells. After attachment, the cells were able to proliferate with a population-doubling time of 2,4 days. Osteogenic differentiation was confirmed with the expression of bone sialoprotein and osteonectin.

Conclusion
Predominantly ASC-like cells of the SVF rapidly adhere (~10 minutes) to the scaffold and this subpopulation is capable of proliferation and differentiation towards the osteogenic lineage.

[1]Zuk et al. Tissue Eng. 2001
[2]Helder et al. Tissue Eng. 2007

Collagen II synergistically induces chondrogenesis in adipose stem cells with nucleus pulposus cells by shaping cells

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Background
Degenerated disc disease (DDD) is one of the most prevalent causes for chronic low back pain. One strategy might be to inject adipose tissue-derived mesenchymal stem cells (ASCs) into the nucleus pulposus (NP) of the DDD intervertebral disc, in order to regenerate the tissue and restore disc function.

Aim
To investigate whether and how the main components within the NP microenvironment, collagen type II and NP cells, affect chondrogenic differentiation of ASCs in a 3D environment.

Materials and Methods
Human ASCs were cultured with monolayer as control. Firstly, we compared the effects of collagen type II and collagen type I 3D environment on chondrogenic differentiation of ASCs. Secondly, we co-cultured ASCs and NP cells in 0.4 µm-transwell systems, and compared the effects of collagen type II and collagen type I 3D environment on chondrogenic differentiation of ASCs in the presence of NP cells. After the cells were cultured for 4, 14 or 21 days, they were harvested and chondrogenesis-related gene expression and alcian blue staining were analyzed. Moreover, cell shape characterized by actin cytoskeleton visualization was compared between ASCs in collagen type I and collagen type II gels.

Results
In the absence of NP cells, the collagen type II and I gels had similar effects on the ASCs, showing only up-regulation of collagen type X (day 4), but not of collagen type I, IIA (marker for prechondrocytes), IIB (marker for mature chondrocytes) and aggrecan gene expression in ASCs. In the presence of NP cells, collagen type X gene expression of ASCs was shown to be further up-regulated in both collagen type I and II gels, whereas collagen type I gels only induced collagen type IIA gene expression in ASCs at day 4. Strikingly, collagen type II gels could act synergistically with NP cells, inducing gene expression of both collagen type IIA and IIB at day 4 and/or day 14. The strongest alcian blue staining was also observed in ASCs embedded in collagen type II gels when co-cultured with NP cells for 21 days. At day 4, only part of ASCs in collagen type I gels displayed round cell shapes with relaxed actin fibres while the others had stressed fibres crossing the cells; however, most of ASCs in collagen type II gel displayed round cell shapes characterized by less stressed actin fibres, and most of which distributed underneath the cellular membrane.

Conclusion
In this study, we demonstrated that collagen type II can act synergistically with NP cells on chondrogenic differentiation of ASCs, and round cell shape remodelling by collagen type II might be involved in this process. This finding enables us to proceed to in vivo experiments in which ASCs could be used for the injection treatment for mildly degenerated intervertebral discs.

Tissue engineering of pliable vocal folds with adipose derived stem cells on 3D hydrogels

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Introduction
Regeneration of pliable vocal folds using engineered autologous stem cells is an attractive strategy for treating scarred vocal folds. Injection of bone marrow derived stem cells in scarred vocal folds has shown some augmentation. Adipose derived stem cells (ADSCs) are an alternative cell source for rebuilding pliable vocal fold tissue. Our goal was to investigate differentiation of ADSCs in various soft hydrogel matrices that might eventually be used for vocal fold implantation.

Method
Human ADSCs were isolated from abdominal adipose tissue using collagen digestion and series of filtration procedures. The ADSCs were characterized by FACS, real time PCR, and immunohistochemistry. The multipotential ability of ADCSs was tested by cell culture in lineage specific media (osteogenic, adipogenic). The ADSCs were seeded in 3D gel scaffold materials (HA, collagen, fibrin) and cultured for up to 10 days. Cells were imaged and gene expression was analyzed by real time PCR.

Result
According to our FACS analysis, over 90% of isolated ADSCs expressed adult stem cell markers. Cells differentiated into osteoblasts and adipocytes according to the differentiation media environment, indicating their multipotentiality. Higher expression of stem cell genes (CD105, CD44) was found in cells grown in HA gels than in cells grown in collagen gel, while collagen gel induced higher expression of the decorin gene. Cells grown on fibrin and collagen gels showed elongated morphology and stained positively for CD31.

Conclusion
In this study we report that tissue engineering of fibrin-HA-collagen 3D gel scaffolds with ADSCs enhances expression of stem cell and fibroblast genes. Our data suggest that tissue engineering of gel scaffolds with ADSCs may provide insights for understanding of differentiation of ADSCs to vocal fold fibroblasts

Human adipose tissue xenografts in nude mice: a pilot study examining the effect of stromal vascular fraction cells

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Surgeons have employed adipose tissue autografts for over a century; however, the technique remains challenging due to issues of reproducibility. Often, tissue implants display evidence of fibrosis and necrosis due to inadequate angiogenesis. To address these concerns, recent studies by Yoshimura and others have combined stromal vascular fraction cells with lipoaspirate adipose tissue fragments to improve engraftment (Moseley et al. Plast Reconstr Surg 118(3 Suppl):121S, 2006). We have performed a pilot study examining lipoaspirates implants prepared from a 58 year old, female African-American subject with a BMI of 28.2. Lipoaspirated tissue fragments were washed with PBS and either left intact or digested with collagenase (0.1%) for 1 hr to isolate stromal vascular fraction (SVF) cells. The SVF cells isolated from a unit volume of tissue were combined with intact tissue fragments at the following ratios: 1:10, 1:3.3, 1:1, and 3.3:1. Duplicate immunodeficient outbred NU/NU mice were injected with a 0.25 ml volume containing the SVF cell:lipoaspirate tissue combination on their left dorsal flank. An injection of 0.25 ml volume of intact tissue alone on the right dorsal flank of each animal served as a control. After 41 days, animals were euthanized and the implants harvested for histological evaluation. The intact tissue alone controls maintained their morphology as a discrete adipose depot upon gross morphology and only 1/8 showed

evidence of cyst formation. While the SVF cell: tissue implants were well vascularized, 4/8 contained cysts filled with clear or yellow fluid. Additional histological evaluations of the tissue explants are underway and future studies will examine the SVF cell dose dependence of adipose tissue xenograft formation.

The frequency of proliferative stromal cells in adipose tissue varies between inbred mouse strains

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Stromal cells derived from adipose tissue (ASCs) can proliferate as undifferentiated cells with a fibroblast-like morphology in cell culture or can be induced to differentiate into a variety of cell types including, adipipogenic, myogenic, neurogenic, osteogenic, chondrogenic and hepatic cells. There is increasing interest to understand the factors controlling the proliferation of ASCs since these cells might provide a readily available source of autologous stem/progenitor cells for cell therapy applications. To explore potential genetic factors that modify the properties of ASCs, we tried to identify relevant properties of ASCs that differ between inbred mouse strains. Plating cells in a modified colony forming assay indicated that the percentage of high proliferative cells among ASCs differs more than 2-fold between 129x1/svj and C57Bl/6J mice. The identification of genetic factors affecting the proliferative capacity of stem cell populations could improve the efficacy of cell therapy.

Numerical measurement of viable and non-viable adipocytes and other cellular components in aspirated fat tissue

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Background
A reliable method to assay viability and number of adipocytes and other cellular components in adipose tissue remains to be established.

Methods
We assessed cell viability and number obtained from 1g suctioned adipose tissue and respective layers (the top, middle, and bottom layers) before and after digestion and centrifugation, using cell staining with Hoechst 33342 and propidium iodide (PI) and the XTT and glycerol-3-phosphate dehydrogenase (GPDH) assays (n=10). The correlation between the number of prepared cells (adipocytes, adipose stromal cells [ASCs], and WBCs) and the resulting values from the XTT and GPDH assays was also examined (n=5). The cell composition of the stromal vascular fraction isolated from the same adipose tissue was determined by multicolor flow cytometry (n=5).

Results
Hoechst 33342 and PI staining allowed distinguishing of viable adipocytes from lipid droplets, dead adipocytes, and cells other than adipocytes. We obtained 6.9×10^5 non-ruptured adipocytes from 1g suctioned adipose tissue; 30% of the original adipocytes appeared to have been ruptured. Both the XTT and GPDH assays provided good correlations between the number of viable adipocytes and resulting values, but only the GPDH assay was strictly specific for adipocytes. The ratio of ASCs to adipocytes was found to be much larger than previously desribed.

Conclusions
Single use or a combination of the viability assays used in this study can appropriately determine the number of adipocytes and other cells, although it remains difficult to assess original cells directly without tissue dissociation.

A murine model to study diffusely injected human fat

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Introduction
The study of human autologous fat grafting has been primarily anecdotal. Few studies have scientifically analyzed the fate of grafted fat or its effect on the tissues into which it is placed. We developed a murine model to study fat harvested from a human donor, then refined and infiltrated into a mouse using the Coleman method of fat grafting.

Methods
Fat Harvesting Our method of fat harvesting and refinement utilizing the Coleman method has been outlined in detail previously[1]. Fat was harvested from healthy donors at various sites using a two-hole Coleman harvesting cannula with a blunt tip. The syringes were then placed in a centrifuged at 3000 rpm for 3 minutes. The isolated fibro-fatty layer was then transferred to 1 ml syringes for injection.

Lipoaspirate Placement 8-week old, Foxn1^{nu}/Foxn1^{nu} nude male mice, weighing approximately 20-25 grams were utilized in this study. The dorsum of the mouse was utilized as the recipient bed for the processed lipoaspirate. The lipoaspirate was infiltrated using a blunt-tip cannula through a 2mm caudal midline incision. The infiltration cannula was advanced and withdrawn in a fan-like pattern across the dorsal surface of the mouse. Fatty tissue was only injected as the cannula was withdrawn, in small volumes of approximately 1/30th ml per withdrawal. A total of 2 ml of fatty tissue was infiltrated per mouse.

Tissue Collection and Analysis
The dorsal skin and adherent fat was excised at 2, 4, 6 and 8-week timepoints. It was excised en bloc, and fixed immediately in 10% formalin. Standard processing and sectioning techniques were utilized. H and E and Cox-IV staining was performed and the sections were analyzed. At the 8-week timepoint, fat was dissected from the dorsal skin and volume analysis was performed using volume displacement.

Results
All mice tolerated the procedure well and were viable at the 8-week timepoint. The injected lipoaspirate was visible and persistent in the injected areas. Volumetric analysis of the injected fat at the 8-week timepoint showed strong persistence of infiltrated fat, with 80-85% persistence of the original volume. Gross analysis of the fat showed it to be healthy, non-fibrotic and vascularized. H and E analysis of the injected lipoaspirate revealed there to be minimal inflammatory or capsular reaction with viable adipocytes that were clearly distinct from the native mouse adipocytes. Fat grafted areas were vascularized with multiple blood vessels. Cox-IV human specific stain revealed these vessels to be of human origin.

Discussion
In this study we have translated the Coleman method of fat grafting into a nude mouse model. We have demonstrated a high degree of persistence to the 8-week timepoint. There is minimal inflammatory reaction and visible and documented incorporation of the lipoaspirate into the mouse dorsal bed. The fat is viable and vascularized, with human-derived blood vessels in a mouse model. This model may provide a platform from which we can not only study the fate of injected fat, but also investigate the components of fat (pre-adipocytes, stem cells, cytokines, hormones et cetera) and their effects on the surrounding tissues.

[1]Coleman S. Facial Augmentation with Structural Fat Grafting. Clinics in Plastic Surgery. 2006 October 2006 33(4)

Effect of adipose-derived stem cells on rat periodontal tissue regeneration - Immunohistochemical study

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Objectives
Several therapeutic approaches for periodontitis such as guided tissue regeneration (GTR) method or enamel matrix derivative (EMD) have been clinically available, although there are still some limitations. We think that the stem cell is necessary for extensive periodontal tissue regeneration. We have studied periodontal tissue regeneration using Adipose- derived stem cells (ASCs) with platelet-rich plasma (PRP) in rat model. In this study, the regenerated periodontal tissue was reviewed immunochemically, the origin of regenerated tissue was investigated in rat model.

Methods
ASCs were prepared from inguinal fat pads of GFP transgenic rat Crj:Wistar-Tgn(CAG/GFP), the primary culture was performed in control medium (DMEM+10%FBS). The Periodontal tissue defect was made for the upper jaw palate side molar of cognate rat with dental round bar, ASCs with PRP from autologous blood were transplanted in the experimental group (n=8). While no treatment and PRP independent were performed in the controls. After eight weeks of implantation, histologic examination, immunostaining of Osteocalcin, Osteopontin, type I collagen and GFP were done.

Results
After eight weeks of transplantation, in H.E. staining of the experimental groups, the alveolar bone and periodontal ligament like structure were regenerated, and alveolar crista was seen on periodontal tissue defect. In immunostaining, Osteocalcin positive cells were shown on surface of regenerated alveolar bone, Osteopontin positive cells were seen in bone cavity of regenerated alveolar bone, and type I collagen positive cells were seen in the alveolar bone and periodontal ligament like structure. GFP positive cells were evident in surface of alveolar bone, intrabone and periodontal ligament like structure. Especially double positive cells which were stained anti GFP antibody and anti osteocalcin antibody were encountered. In PRP independent group, although regenerated alveolar bone was seen, alveolar crista was not evident clearly, periodontal ligament was not seen. In no treatment group, no regenerated alveolar bone around dental root was observed; granulation tissue was invaded between dental root and alveolar bone.

Conclusions
These findings suggest that ASCs and PRP could contribute to periodontal tissue regeneration in immunohistochemically.

Adipose tissue-derived stem cells (ASCs) improve liver function in mouse with CCl_4 induced liver damage.

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Background
Regenerative medicine using adult stem cells is an attractive and promising therapy for liver disease. We have reported that ASCs can differentiate into hepatocytes and would be source of hepatocytes for cell therapy. We investigate the capability of undifferentiated ASCs for liver disease.

Methods
ASCs were isolated from inguinal adipose tissue of female green fluorescent protein (GFP) transgenic mice. Six-weeks olds male C57BL/6 mice were treated with 1 ml/kg carbon tetrachloride (CCl4) dissolved in olive oil. Twenty-four hours after injection of CCl4, $1x10^5$ GFP positive ASCs or same volume of Hanks' balanced salt solutions (HBSS) as a control were injected into the tail vein. Then the mice were anesthetized and serum and liver tissue were harvested twenty-four hours after cell transplantation. Serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were measured to assess the extent of liver damage.

Results
The mice which were received ASCs implantation tended to show the lower level of serum AST, ALT and LDH than the control group. Although the difference was not apparently significant at present but no side effect related to cell transplantation was seen. The present results indicate that ASCs transplantation probably have protective effect to the liver ability and improve liver function in damaged liver.

The effect of adipose derived stem cells on flap viability after ischemia reperfusion injury

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Introduction:
The stem cells are known to have angiogenesis potential in any situation where ischemia is evident. The BSCs were presented to improve viability of random pattern flaps. We have performed an experimental study to find out the effect of adipose derived stem cells (ASCs) on flap viability after ischemia reperfusion injury.

Materials and Methods
The ASCs were gathered from inguinal fat pads of 6 weeks old ICR mouse. After three passage in control medium (DMEM, 10% FBS), the cells were ready to be injected so that every injection included $1x10^7$ cells. The cells were labeled with DiI staining before the injection for tracing. Symmetrical cranial based double flaps were elevated with a dimension of 1cm to 5cm in mice. Each flap was injected either ASCs in 1 cc phosphate buffered saline (PBS) or 1cc PBS only. Then the flaps were subjected to 6 hours of ischemia and subsequently reperfusion. On the postoperative 7^{th} day, the flap survival area was measured depending on the length of the flaps. (n=12).

Results
The viable flap length in the control group was 15,2±3,4 mm whereas the flap length was 24,4± ,9 mm. There was a statistically significant difference between the groups. ($p<0.05$) Histological examination revealed that the number of capillaries per 20 fields under microscope in the control group and the experimental group were 4.50±0.80 and 7.50±0.75 respectively and the average number vascular density has significantly increased in the ASCs group($p<0.05$).This finding was confirmed with anti Von Willebrand Factor (factor VIII related antigen) antibody immunohistochemical staining.

Discussion and Conclusion
The ASCs have a direct angiogenesis effect around 30% that is differentiation to the endothelial cells and indirect effect of 70% that is mainly by differentiation to other specialized cells, important in healing cascades and respectively the secretion of growth hormones and cytokines. The ASCs not only improve the survival of the ischemia reperfusion mediated damaged tissue, but also could differentiate into the necessary cells and tissues in the situations despite of the absence of any scaffold.

The hair growth promoting effect of adiponectin

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Introduction
Hair has growth cycle, which is characterized by distinct stages of active growth (anagen), apoptosis-driven regression (catagen) and relative quiescence (telogen). Many factors have been implicated in modulating these processes in hair follicles. Adipose tissues are observed to be located around Hair follicles especially during agagen (active growth period) stage. However, except the spatial relationship between hair and adipose tissue, any functional role of adipose tissue on hair growth was not investigated yet. We evaluated the hair growth promoting effect of adiponectin, one of the well known adipokines, and its molecular mechanism.

Methods
After treatment with adiponectin, proliferation of the outer root sheath (ORS) cells and dermal papilla (DP) cells was evaluated by MTT assays. Hair organ culture with the treatment of adiponectin was performed. RT-PCR analysis was performed to search for growth factors from dermal papilla cells that were affected by adiponectin. We measured the expression levels of IGF-1, KGF, HGF, VEGF (stimulating mediator on hair growth), and TGFβ1 (inhibitory mediator on hair growth).

Results
We found increased proliferation of ORS cells and DP cells. Hair growth in organ culture elongated with adiponectin. There were no significant changes in the expression of growth factors from ORS cells. However, the expression of VEGF, HGF, IGF-1 from DP cells increased significantly with the exposure of adiponectin.

Conclusion
Adiponectin, one of the adipokines may stimulate the hair growth by increasing the secretion of IGF-1, HGF, VEGF in dermal papilla cells. Adipose tissue may play a role in hair growth partially via the secretion of adiponetin.

Evaluation of Medpor® as a scaffold for tissue engineering of chondrocytes cultured from rabbit ADSCs

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Objective
Many biocompatible scaffolds have previously been investigated for tissue-engineering and reconstructive surgery. Medpor® has been widely used by surgeons in a variety of forms and shapes as surgical implants, indicating its capability of case-specific scaffold structure construction with the absence of systemic or cytotoxic effects. This study was conducted to examine the possibility of utilizing Medpor® (Porex, Newnan GA) as a 2-D scaffold for the implantation of chondrocytes cultured from adipose-derived stem cells (ADSCs).

Methods
ADSCs were obtained from scapular fat pads from New Zealand white rabbits (2-4 kg) and were cultured for 2 passages before chondrogenesis was performed. Pictures of the control (Medpor® alone) as well as Medpor® seeded with chondrocytes were taken with phase contrast and scanning electron microscopy (SEM). Alcian blue staining was performed to confirm the chondrogenesis of the cultured cells. Four scaffolds seeded with autologous chondrocytes were implanted subdermally at the flank of the rabbit. At eight weeks, two of the scaffolds were taken out and Alcian blue staining was performed.

Results
Positive results were obtained from all staining procedures indicating successful chondrogenic and osteogenic differentiation of ADSCs into chondrocytes and osteocytes. Both phase contrast and SEM pictures showed positive cell attachment and growth into the pores of the scaffold. The implanted scaffolds that were removed exhibited cartilage tissue with micromass-like structure and a height of 3-4 mm.

Conclusions
These findings demonstrate the plausible usage of Medpor® as a scaffold for tissue engineering in reconstructive surgery. Implications for future research include optimization of cell seeding and implantation efficacy.

Isolating adipose stem cells for aesthetic plastic surgery applications

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Background Information
Stem cells are distinguished from other type of cells because they are unspecialized and therefore can renew themselves for long periods through division. They can also be coaxed to become cells with special functions such as forming heart muscle, repairing cartilage or regenerating functional tissue. In aesthetic plastic surgery applications benefits from this new technology can probably include augmenting tissue after mastectomy or filling soft tissue defects, using the patients own adipose stem cells obtained from liposuctioned fat aspirate.

Objective
To gain knowledge and experience on this important field, a tissue acquisition protocol was designed to understand the handling of adipose tissue and isolation of the progenitor cell fraction.

Materials & Methods
A tissue acquisition protocol was established with IRB approval wherein adipose tissue was obtained from fresh abdominoplasty flap specimens and liposuctioned fat aspirate during aesthetic surgical procedures performed in a fully accredited outpatient surgical facility. All patients (N=20) involved to the present time agreed and signed an informed consent to study factors that affect the yield and viability of progenitor cell fraction. Up to this date, time between harvest and processing, tissue dissociation methods, and removal of large debris and adipocytes have been investigated.

Results
Results obtained until now under present optimal conditions, indicate a yield of 125,000 +/- 55,000 plastic adherent cells per g fresh of lipoaspirate. Cells within this fraction expressed the surface markers CD44, CD105, and NG2 associated with progenitor cells. Conditions for enzymatic digestion of the fresh tissue were found to be an important determinant of viable cell yield. Studies are on-going and findings on additional variables such as patient demographics, microscopic analysis and viability after cryopreservation of tissue , will be presented..

Conclusion:
We are very optimistic with these initial results that will serve to extrapolate laboratory findings with future clinical applications.

ASC media protect against 6-OHDA-induced neuronal death

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Recent studies suggest that the delivery of adipose stromal cells (ASC) to damaged tissue is emerging as a novel therapeutic option for patients with several diseases such as stroke or cardiovascular disease. However, the role of ASC in neurodegenerative disorders remains unknown. In this study, we investigated whether or not ASC could protect neurons in a Parkinson's disease model, the in vitro 6-hydroxydopamine (6-OHDA) lesion neuronal model. Rat ASC were cultured in EGM2MV media to confluence and then switched into Basal Medium Eagle (BME) for additional 24 hours. The ASC Conditioned Medium (ASC-CM) was collected and subsequently added to the cultured rat cerebellar granule neurons (CGN). Neuronal viability was quantified by either counting fluorescein (green) positive neurons or staining living neurons with MTT. Since neurotoxicity induced by 6-OHDA was believed to be due, at least in part, to the production of reactive oxygen species (ROS), we also investigated the levels of free radical generation in our model by using dihydroethidium (DHE) and dihydrorhodamine 123 (DHR). We have found that exposure of CGN to 50mm 6-OHDA resulted in significant increases in free radical production and CGN neuronal death. Pretreatment with ASC (30% replacement of the culture medium) for 2 h significantly prevented both 6-OHDA-induced neuronal toxicity (Neuronal viability: 17.6±1.1 in 6-OHDA only vs. 51.3±0.3 in 6-OHDA+ASC, $p<0.01$) and free radical generations (DHE fluorescent intensity (% of Control): 156±4.5 in 6-OHDA only vs. 120±9.2 in 6-OHDA+ASC, $p<0.01$; DHR fluorescent intensity (% of Control): 135±3.6 in 6-OHDA only vs. 120±4.6 in 6-OHDA+ASC, $p<0.05$). Furthermore, ASC also attenuated H_2O_2-induced neurotoxicity (Neuronal viability (% of Control): 49.8±3.3 in H_2O_2 only vs. Vs +BME 70.6±3.9 in +ASC, $p<0.01$). Our results strongly suggest that ASC-CM blocks 6-OHDA-induced neuronal death and 6-OHDA-induced free radical generation. Additionally, ASC-CM also directly blocked free radical-induced neurotoxicity. Both antioxidative and neuroprotective effects of ASC-CM may be beneficial in the therapy for Parkinson's disease and other neurodegenerative diseases.

Basic Science Posters

Adipocyte differentiation of adipose tissue-derived stem cells in vitro: The effect of extracellular matrix substrata

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Preliminary Observations
Recently, fat tissue-derived stem cells have been cultured and differentiated to various cell lineages, and they represent a promising option for novel cell-based tissue engineering applications. To be able to set up an effective way to utilize autologous adipose-derived stem cells for this new technology, it will be necessary to obtain a good proliferative and differentiating capacity on cell culture. The objective of this study was to characterize the adipocyte differentiation of human and rat adipose tissue-derived stem cells in vitro and to study the effect of commercial extracellular matrix (ECM) substrata on cell proliferation and differentiation.

Both human and rat stem cells exhibited adipogenic potential as demonstrated by the intracellular staining of lipid vacuoles. The differentiation was also characterized by quantitative real time RT-PCR analysis of the expression of the adipocyte differentiation marker genes aP2 and ACBP during the adipose conversion. The relative mRNA expression of the adipocyte fatty acid-binding protein aP2 was markedly enhanced throughout the culture period of three weeks in the adipogenic rat cell cultures and slightly enhanced in the adipogenic human cell cultures when compared to the control. The expression of the acyl-CoA binding protein (ACBP) was slightly increased in the adipogenic rat cell cultures when compared to the control, whereas it showed no difference between the adipogenic and control human cell cultures. A basement membrane extract (Matrigel) and a soluble ECM extract of human placenta (Human ECM) were used as a thin coating on culture surfaces or applied in the adipocyte medium. The for Human ECM-treated human cell cultures when compared to adipocyte conversion without any ECM treatment. The PCR analysis did not show a notable effect of the ECM treatments on adipocyte differentiation process at the mRNA expression level on human and rat cell cultures. Additional experiments are necessary to diminish the variation between the results of different experiments. The application of ECM substrata for cell culture and differentiation makes it possible to mimic more closely the actual in vivo conditions and could have therapeutic potential and utility for soft tissue engineering strategies.

Patterns of gene expression related to the stemness and senescence in long-termed adipose-derived stem cells

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The age-related symptoms of stem cells including adipose-derived stem cells (ADSCs) have given rise to a result which the regenerative or recovery ability in defected tissue is on the decrease. To obtain an enormous number of stem cell for effective therapies, the majority of researchers were expanded cells in vitro. Though the ADSCs could abundantly obtain by liposuction compared with bone marrow mesenchymal stem cells, the expansion process is accomplished to secure a number for success therapy. Therefore, the present study examined differentiation ability of ADSCs (from

several sexagenarian volunteers) into adipocytes and characterization by reverse transcriptase-polymerase chain reaction and immunocytochemistry at the regular passage of each cell-line. To understand the relation between stemness and ageing, we investigated the mRNA expression of senescence related genes such as Bmi1 (a member of polycomb group), p16INK4A, p19ARF4d (inhibitor of cell cycles), Oct-4, SCF (stem cell related genes), p53 and HLA class 1 & 2 (immune-response related genes). The expression of surface markers by immunocytochemistry was not changed between the 4th and 10th passages. However, the result of mRNA analysis showed internal turns of gene expression about the cell fate in long-term cultured ADSCs. These results suggest the information of cell expansion in vitro and the ageing process of ADSCs and they will be useful in developing process of cell therapy.

Equine adipose tissue–derived mesenchymal stem cells: Cell growth characteristics and differentiation potential

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Objectives
To characterize equine adipose tissue-derived mesenchymal stem cells (ATSCs) and to evaluate their adipogenic and osteogenic differentiation potential.

Introduction
Mesenchymal stem cells (MSCs) have the ability to differentiate into different mesodermal tissues like cartilage, bone, muscle, tendon and ligament. This ability makes them an attractive cellular source to treat different injuries. These cells are usually obtained from bone marrow. However, the adipose tissue represents a new interesting source of MSCs because of its abundance and easy isolation. Therefore stem cells obtained from this new source are termed adipose tissue-derived mesenchymal stem cells (ATSCs).

Materials and Methods
The sample was obtained from subcutaneous adipose tissue from different regions of 16 horses of different breed, age and sex. Between 10 and 20 grams of subcutaneous adipose tissue were extracted. The sample was put in a sterile tube with transport medium to be carried to the laboratory. At the laboratory, the samples were processed to obtain ATSCs through enzymatic digestion and plastic adherence. Cell viability was evaluated with fluorescent DNA binding dyes ethidium bromide and acridine orange. Sample extraction and processing quality was determined through microbiologic analysis of transport and wash media. Adipogenic and osteogenic differentiation was made with different lineage-specific inductive agents and growth factors. After 20 days of differentiation, the cells were fixed with 4% paraformaldehyde to stain them with Oil Red O and Alizarin Red to detect lipid vacuoles and calcium deposits.

Results
Equine ATSCs presented a good dish adherence and a viability of 81.2 ± 4.8 %. The cell morphology is spindle-shaped and it was possible to culture the cells until 10 or 12 passages. In all samples, microbiologic analysis demonstrated absence of aerobic and anaerobic microorganisms. Morphologic analysis of the cells was made during 20 days of differentiation. Significant changes characteristic of adipose and bone tissue were found, like the presence of lipid vacuoles during adipogenic differentiation and nodule formation with some mineralization during osteogenic differentiation. On the one hand, with Oil Red O it was seen that 31.4 ± 7.5 % of equine ATSCs managed to differentiate into adipogenic lineage. On the other hand, with Alizarin Red stain it was detected that 60.3 ± 4.8 % of equine ATSCs differentiated into osteogenic lineage.

Conclusions
Equine adipose tissue is an abundant source of MSCs, which have the ability to adhere and proliferate in monolayer cultures. Equine ATSCs have a morphology and a differentiation potential similar to those documented in other mammalian species.

Adipocyte but not ASC interaction with EC promotes angiogenesis

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Hypothesis and Background
It has been recently shown that differentiation of pre-adipocytes to adipocytes can influence blood vessel growth (angiogenesis) and vice versa that angiogenesis can effect adipocyte differentiation. Further studies have demonstrated that fat tissue mass can be reduced with applying inhibitors of angiogenesis suggesting that adipose tissue is dependent on an angiogenic and immature vasculature. Based on the functional and phenotypic overlap of adipose stroma cells (ASC) with the pericytic cell of microvessels in adipose tissues, we tested the effects of adipogenic differentiation on angiogenesis when ASC and endothelial cells (EC) are in close physical proximity. Procedures. To assess the influence of ASC differentiation to (pre)adipocytes on angiogenesis, we used a spheroid coculture model with human microvascular endothelial cells and ASC. In this model ASC cells build the inside and the endothelial cells cover the outside to form an inverse vascular lumen. Sprouting angiogenesis can be monitored by quantification of sprouts formed from these spheroids with and without addition of VEGF. Results. ASC cells in the undifferentiated (pericyte like) form prevent the endothelial cells from sprouting out of the spheroid structure and into the surrounding 3-dimensional collagen gel, even in the presence of VEGF. In contrast, exposure of this spheroid coculture model to adipogenic differentiation medium increased sprout formation in response to VEGF significantly. Furthermore, when ASC cells were exchanged with differentiated adipocytes also increased angiogenesis was observed. Conclusions. These data identify the close proximity between EC and ASC as a negative regulator of angiogenesis. When ASC differentiate into adipocytes this negative regulation is lost and angiogenesis with and without VEGF is increased indicating that the perturbation of ASC-EC communication contributes to increased angiogenesis observed during fat tissue growth.

The characterization of islets formed during adipocyte differentiation of human adipose tissue-derived stem cells

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As the possibility for treatment of incurable disease is increasing, diversified attempt using human adipose tissue-derived stem cells (hADSCs) has been accomplished. The hADSCs have the advantage of multipotency, high proliferation rate, and harvest of plenty. On induction to adipocyte differentiation, we observed the self-assembled islets in hADSCs from different donors. Length of the maximal axis of the islets which is visible to the naked eye was 0.173~0.834 mm (n=11). The islets had grown until they reached to irregularly specific size and maintained the size and morphology up to the date artificially ending (about 8 months). Interestingly, the new islet formation was started when formerly established islet was removed in the same chamber. For that reason, we had hypothesized that the islet would secrete some factors that regulate adipocytes population such the rate of differentiation of hADSCs. To investigate the factors that can inhibit of differentiation of hADSCs or accumulation of mature adipocytes, the islet-derived conditioned medium (AI-CM) and adipocytes islet were analyzed. Another hADSCs was differentiated into adipocytes with differentiation induction medium and exposed to AI-CM at every 3 days. After four weeks, we assessed a differentiation degree by oil red O staining and RT-PCR against the mature adipocyte-specific markers. Isolated islets were observed a histological appearance and analyzed mRNA expression of adipokines such as interleukin-6, adiponectin, leptin, and tumor necrosis factor alpha. In conclusion, the islet was an aggregation of mature adipocytes and its cytokines has regulatory effects on hADSCs differentiation. These results might be offered a clue for maintenance of hADSCs in vitro and obesity researches which related to size regulation of adipocyte population.

Adipogenic differentiation of human adipose-derived adult stem cells isolated from cryopreserved adipose tissue

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Background
Although frozen adipose tissue is often used for soft tissue augmentation, its availability is still controversial. Previously, we have shown that adipose-derived adult stem cells (ADAS cells) can be a promising source for adipose tissue engineering. Optimal preservation of adipose tissue is an important issue for future use of ADAS cells as well as adipocytes.

Methods
To assess if long-term preservation of adipose tissue requires cryopreservation techniques, adipose tissue was frozen directly or with cryoprotectant at -20°C or -80°C. For viability of adipocytes, acridine orange/ethidium bromide staining was performed. Functional differentiation of ADAS cells isolated from frozen-thawed adipose tissue was assessed by using oil red O staining and RT-PCR of adipogenic related genes.

Results
Viability of adipocytes directly frozen at -20°C was decreased steeply during 2 months storage and addition of cryoprotectant hardly affected cell viability. Mature adipocytes were almost viable at -80°C regardless of addition of cryoprotectant during short-term storage of 7 days, however, viability of adipocytes frozen without cryoprotectant was gradually decrease with storage time, and lower than 50% in 2 months. There was no significant decrease of cell viability in adipocytes frozen with cryoprotectant at -80°C during 2 months storage. Moreover, ADAS cells were successfully isolated from adipose tissue stored with cryoprotectant at -80°C and differentiated into adipocytes containing lipid-rich vacuole, however, ADAS cells were not found in adipose tissue frozen directly or stored at -20°C.

Conclusions
These results indicate that adipose tissue cryopreserved with cryoprotectant and stored at optimal temperature could be a reliable source of human ADAS cells as well as mature adipocytes.

Analysis of VEGF expression in different populations of adipose derived stem cells

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Adipose derived stem cells (ASC's) have been shown to secrete various growth factors with different stimulating forces. VEGF is one of the factors to have high levels of secretion under hypoxic conditions, and ASC's are able to improve limb survival in an ischemic rodent model. Since adipose stem cells are a heterogenous population we sought to determine which sub-populations of cells are responsible of VEGF expression. In this study, ASCs were harvested and sorted by flow cytometry according to different cell surface markers. Four populations were analyzed: $CD31^-CD34^+$, $CD31^+CD34^-$, $CD31^-CD34^-CD146^+$, $CD31^+CD34^+$, as well as a control group with unsorted cells. Different groups of cells were grown in either normoxic conditions with 21% oxygen or hypoxic conditions with 0% oxygen for 72 hours in an incubator. Media was collected from each group and ELISA performed. VEGF expression under hypoxic conditions in a mixed (unsorted) low passage population is 5 times higher than the same cells grown under normoxic conditions. In sorted cells, the $CD31^+CD34^-$ cell population showed the highest expression of VEGF under normoxic conditions compared to other groups. Although all the populations had higher expression of VEGF under hypoxia condition, $CD31^+CD34^-$ and $CD34^+CD31^-$ population showed a greater increase of VEGF expression compared with other sorted groups. The $CD31^+CD34^-$ population showed the highest amount of VEGF expression under hypoxic conditions. The $CD31^+CD34^-$ cell population of ASCs could be a better choice to promote new vessel growth in tissue repair and reconstruction, or in the treatment of tissue or organ ischemia.

Osteogenic potential of human adipose-derived stem cells treated with rat infant dural conditioned medium

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The objective of this project was to examine the osteogenic potential of human adipose-derived stem cells (ASCs) when treated with conditioned medium from rat infant dural cells. Consistent with the infant dura's osteoinductive potential noted in clinical practice, it has been shown that co-culture of preosteoblasts with infant dura leads to enhanced osteogenic differentiation of the preosteoblasts as compared to preosteoblasts cultured in control conditions. Therefore, we hypothesized that treatment with dural cell culture conditioned medium would induce osteogenic differentiation of mesenchymal stem cells derived from adipose tissue. To test this hypothesis, we treated passage 2-4 human ASCs from three patients with medium from rat infant dural cell cultures in the following concentrations: 0% dural cell medium: 100% plating medium; 20% dural cell medium: 80% plating medium; 50% dural cell medium: 50% plating medium; 80% dural cell medium: 20% plating medium. An additional control group was treated with osteogenic medium containing dexamethasone, ascorbic acid and beta-glycerophosphate. After 3 weeks in culture, we stained the ASCs for Alizarin Red, Alkaline Phosphatase, and Oil Red O. We also examined osteocalcin expression via ELISA. Our results indicate that all media formulations containing dural cell conditioned medium did induce osteogenesis, comparable to control osteogenic media. This study indicates a potential role for both ASCs and the dura mater in treating calvarial defects. Further studies to identify the specific molecules in the dural cell conditioned medim that promote osteogenesis are currently underway.

Signaling pathways in ASC differentiation

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Hypothesis and Background
Recently, cells derived from the stromal fraction of adipose tissue have been shown to possess pluripotent differentiation potential in vitro. These adipose stromal cells (ASC) have been differentiated in a number of laboratories including ours to myogenic and adipogenic cell phenotypes. Here we aimed to identify signaling pathways which inhibit differentiation to adipocytes and promotes differentiation to skeletal myocytes.

Procedures
We used ASC clones, demonstrated to differentiate into adipocytes or skeletal muscle cells, respectively. Relevant clones were subjected to adipogenic or myogenic differentiation medium in the absence or presence of signal transduction pathway inhibitors or agonists. Adipogenic and myogenic differentiation was assessed by real time PCR of differential marker gene expression or by phenotypical analysis (Oil Red O staining for increased accumulation of lipids). Results. We identified canonical Wnt and PKC signaling pathways leading to diverse effects in adipogenic versus myogenic differentiation. A chemical canonical Wnt signaling agonist (a GSK-3 inhibitor) and soluble Wnt-3a agonist both decreased adipogenic but increased myogenic differentiation. Conversely, a broad spectrum antagonist of protein kinase C (PKC) activity inhibited myogenesis but enhanced adipogenesis.

Conclusions
These results demonstrate the ability of canonical Wnt and PKC signaling to suppress adipogenic and to increas myogenic differentiation of ASC. Employing these pathways may be helpful to manipulate the distribution of myogenic versus adipogenic cells in disease and therapeutic cell therapy.

Stem cell antigen-1 is necessary for adipogenic differentiation in EMSC (ear mesenchymal stem cells)

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Adipocytes arise from multipotent stem cells of mesodermal origin. Our laboratory has shown that the outer ears of mice contain a population of mesenchymal stem cells (ear mesenchymal stem cells – EMSC) that posses the ability to differentiate into adipocytes, osteoblasts, chondrocytes and spontaneously contracting myocytes. Our analyses have demonstrated that EMSC abundantly expressed stem cell antigen-1 (Sca-1). In our previous studies we found that Sca-1 positive but not Sca-1 negative EMSC showed a robust accumulation of lipid droplets after 9 days of adipogenic differentiation. In the present study, we have analyzed the expression of adipogenic transcription factors and adipocyte expressed genes in Sca-1 enriched and Sca-1 depleted EMSC fractions. In addition, we have determined the concentration of leptin in the culture media.

Materials and Methods
EMSC were isolated from external ears of 3-week-old C57BL/6J mice. Cells were cultured in DMEM/F12 media containing 15% FBS. Subconfluent cells were detached followed by magnetic cell sorting. The Sca-1 MicroBead Kit (Miltenyi Biotec, Auburn, CA) was used to select Sca-1 positive and Sca-1 depleted fractions of cells. The sorted cells were plated at 10^5/well in 12-well plate and cultured until confluency. From this point cells were stimulated with adipogenic differentiation media (insulin, dexamethason and IBMX) for 2 days and than replaced by maintaining media (insulin, TZD) for next 7 days. On days 0, 3, 6 and 9 cells and medium were collected for RNA purification and leptin concentration assay, respectively. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA) and column-purified with RNeasy and RNase-Free DNase kits (Qiagen, Valencia, CA). cDNA synthesis was performed with 500 ng of total RNA using the high capacity cDNA archive kit (Applied Biosystems, Foster City, CA). Endogenous mRNA levels for Pref-1, Wnt-10b, C/EBPβ/δ, C/EBPα, C/EBPζ, PPARγ2, aP2, LPL were measured with Taqman probes using the ABI Prism 7700 Sequence Detection System (Perkin Elmer, Boston, MA). Leptin concentration in culture medium was determined using DuoSet ELISA Development kit (R&D Systems Inc., Minneapolis, MN).

Results
The mRNA levels of Pref-1 and Wnt-10b (inhibitors of adipocyte differentiation) were high during the undifferentiated stage of cells (Day 0) and abruptly downregulated during adipogenic stimulation (Day 3, 6 and 9). The expression of transcription factors C/EBPβ, C/EBPδ and C/EBPζ showed similar patterns, with highest expression at Day 0 which gradually decreased during the course of the differentiation process (Days 3-9). In contrast, C/EBPα and PPARγ2 expressions were almost undetectable on Day 0, however they were substantially upregulated during adipogenic stimulation. Late markers of adipogenic diffemedia was detected only in Sca-1 enriched population of EMSC but not in Sca-1 depleted.

Conclusion
The presented data suggest that EMSC expressing stem cell antigen-1 are necessary for adipogenic differentiation.

Hepatocyte growth factor regulates adipocyte function: Effects on proliferation and survival

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Hepatocyte Growth Factor (HGF) is a mitogenic and angiogenic factor that regulates target cell function via activation of the tyrosine kinase receptor c-Met. HGF is produced in adipose tissue; therefore we tested if HGF could regulate adipose tissue function. In human subcutaneous adipocytes c-Met mRNA expression is inversely correlated with BMI ($r=-0.612$; $P=0.0007$). There was no relationship between c-Met expression and BMI in human omental adipocytes. Expression of c-Met is greater in omental adipocytes than subcutaneous adipocytes isolated from the same obese subjects (BMI range 37.8-51.1 kg/m2). c-Met is also present on 3T3-F442A preadipocytes and fully differentiated adipocytes. HGF (10 ng/ml; 48 h) stimulates proliferation of 3T3-F442A preadipocytes 145.3±13.8% of control ($p<0.05$). In 3T3-F442A preadipocytes, TNFα stimulates caspase 3 cleavage in a dose- and time-dependent fashion. HGF (100 ng/ml) significantly inhibits TNFα (10 ng/ml)-induced caspase 3 cleavage 53.0±8.8% with 6 h of treatment. HGF also inhibits caspase 3 cleavage induced by 1 ng/ml TNFα by 52.3±3.9% with 3 h of treatment. These findings demonstrate that adipocytes express the HGF receptor c-Met and that HGF acts in a paracrine and/or autocrine manner in adipose tissue to regulate preadipocyte function. The elevated levels of HGF characteristic of the obese state may contribute to expansion of adipose tissue by promoting the proliferation and survival of preadipocytes within the tissue.

Controlled growth factor delivery system to induce adipogenesis of adipose-derived stem cells

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Introduction
The current options for soft tissue reconstruction include autologous tissue flaps and prosthetic implants. Both have disadvantages such as foreign body reaction, donor site morbidity, and implant migration. An injectable system utilizing autologous stem cells offers a promising alternative to the previous therapies. Our objective was to maintain a controlled delivery of adipogenic factors to induce differentiation of adipose derived stem cells (ASCs) into adipocytes. We encapsulated dexamethasone and insulin in poly (lactic-co-glycolic acid) (PLGA) microspheres. The in vitro release kinetics were assessed. The ability of the release factors to induce ASC adipogenic differentiation was assessed.

Materials and Methods
Dexamethasone was encapsulated in PLGA utilizing a single emulsion solvent extraction technique. Insulin was encapsulated in PLGA utilizing a double emulsion technique. The microspheres were characterized by scanning electron microscopy. The in vitro release kinetics of dexamethasone and insulin was assessed spectrophotometrically at 242nm and utilizing a commercially available ELISA kit for dexamethasone and insulin, respectively. Adipose derived stem cells (ASCs) were isolated from extracted adipose tissue. Human ASCs were seeded and treated with one of the treatment groups and their respective controls for 2 weeks. After 2 weeks, the cells were fixed and stained with Oil Red O.

Results
Dexamethasone was encapsulated in PLGA, and the average diameter of the microspheres was 9.85 m. Release was maintained in vitro over 52 days. Dexamethasone in media was successfully replaced with dexamethasone microspheres. There was a significant increase in the amount of cells with lipid inclusions with the addition of dexamethasone microspheres compared to cells treated without dexamethasone. Insulin was encapsulated in PLGA, and the average diameter of the microspheres was 272.4 μm. Release was maintained over a 14 day period of incubation with the human ASCs. Insulin microspheres successfully replaced insulin in media with no significant differences in cell differentiation between the two groups. There was a significant increase in cell differentiation in the group treated with the insulin microspheres compared to the cells treated without insulin. The combination of dexamethasone and insulin microspheres alone can induce differentiation of the ASCs. The differentiation was significantly higher compared to cells treated with no dexamethasone or insulin. No difference in cell differentiation was seen when treated with empty microspheres.

Conclusions
Dexamethasone and insulin were successfully encapsulated. The microspheres were characterized. Dexamethasone microspheres can replace dexamethasone in media; insulin microspheres successfully replace insulin in media. The addition of dexamethasone and insulin microspheres can induce ASC differentiation. Future murine studies will determine the efficacy of this system in vivo.

The effects of EGF and bFGF on cell proliferation and adipogenic differentiation potential of human adipose-derived stem cells

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Studies by Hauner (Eur J Clin Invest. 25:90, 1995), Zaragosi (Stem Cells 24:2412, 2006), and Quarto (Tissue Eng 12:1405, 2006) have demonstrated that EGF and bFGF maintain the stem cell properties of proliferating human adipose-derived stem cells (hASCs) in vitro. While tissue engineering applications of ASCs will require both the expansion and cryogenic preservation of isolated cells, these manipulations can significantly weaken both their proliferative and adipogenic differentiation function. This study examined cryogenically preserved hASCs (n = 4 donors), with respect to these functions, after culture with basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) at varying concentrations (0 to 10 ng/ml). Relative to the control, cells supplemented with EGF and bFGF significantly increased cell proliferation rates by up to 3-fold with substantial improvement in oil red O staining. This was accompanied by significantly increased levels of several adipogenesis-related mRNAs: adiponectin, aP2, C/EBPδ, lipoprotein lipase, PPARγ, and PPARγ Co-activator-1 (PGC1). In contrast, the absolute activity of the adipogenic enzyme marker, glycerol phosphate dehydrogenase, was not induced. These findings indicate that bFGF and EGF can be used as culture supplements to optimize the adipogenic differentiation potential and proliferative capacity of cryopreserved human ASCs. These agents merit consideration for inclusion in cGMP medium for ASC expansion in clinical protocols.

PPARγ2 regulates a molecular signature of marrow mesenchymal stem cells

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Bone formation and hematopoiesis are anatomically juxtaposed and share common regulatory mechanisms. Bone marrow mesenchymal stromal/stem cells (MSC) contains a compartment that provides progeny with bone forming osteoblasts and fat laden adipocytes, as well as fibroblasts, chondrocytes, and muscle cells. In addition, marrow MSC provide an environment for support of hematopoiesis, including the development of bone resorbing osteoclasts. The PPARγ2 nuclear receptor is an adipocyte-specific transcription factor that controls marrow MSC lineage allocation toward adipocytes and osteoblasts. Increased expression of PPARγ2 with aging correlates with changes in the MSC status in respect to both their intrinsic differentiation potential and production of signaling molecules that contribute to the formation of a specific marrow micro-environment. Here, we investigated the effect of PPARγ2 on MSC molecular signature in respect to the expression of gene markers associated exclusively with stem cell phenotype, as well as genes involved in a formation of stem cell supporting marrow environment. We found that PPARγ2 is a powerful modulator of stem cell-related gene expression. In general, PPARγ2 affects the expression of genes specific for the maintenance of stem cell phenotype, including LIF, LIF receptor, Kit ligand, SDF-1, Rex-1/Zfp42, and Oct-4. Moreover, antidiabetic PPARγ2 agonist, TZD rosiglitazone, specifically affects the expression of "stemness" genes, among them ABCG2, Egfr, and CD44. Our data indicate that aging and anti-diabetic TZD therapy may affect mesenchymal stem cell phenotype through modulation of PPARγ2 activity. These observations may have important therapeutic consequences and indicate a need for more detailed studies of PPARγ2 role in stem cell biology.

Osteogenesis vs. chondrogenesis of adipose-derived stem cells using BMP-2

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The role of the culture environment can be significant when differentiating adult stem cells into mesenchymal lineages. In this study, we examined a 2D culture of human adipose-derived stem cells (ASCs) and compared to a 3D pellet culture of ASCs. By delivering different dosages of bone morphogenetic protein-2 (BMP-2), we were able to control differentiation of human ASCs into osteoblasts vs. chondrocytes. Osteogenesis was identified by staining with Alizarin Red and Alkaline Phosphatase while chondrogenesis was identified by staining with Alcian Blue and Safranin O. Additionally, Oil Red O staining was utilized to examine possible adipogenic differentiation under the culture conditions. Our results demonstrate that the environment (2D vs. 3D) can be a major factor in the mesenchymal differentiation of adult adipose-derived stem cells and that BMP-2 is a potent stimulator of both osteogenesis and chondrogenesis of ASCs.

Adipogenic differentiation of ASCs is associated with markedly increased glucose consumption and release of the vasculotropic factors VEGF and MCP-1

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Maintanence of a balance between fat mass and its supporting vasculature is hypothesized to be important in modulating the systemic effects of adipose tissue, in part via factors which it secretes. We have been studying this regulation at a cellular level in the context of adipogenic differentiation of ASCs using a model characterized by a 100-fold increase in lipid accumulation/cell and an increase from 0.1 ng/ml to 25 ng/ml leptin in conditioned media, over a 14 day period. Correlating with this differentiation was a remarkable increase in glucose consumption from ~ 0.1 pmol/cell/hour in an undifferentiated state up to a maximum of ~ 1.4 pmol/cell/hour with differentiation. Interestingly, analysis of conditioned media obtained throughout the timecourse of differentiation demonstrated a molar ratio of lactic acid produced to glucose consumed of 2:1. Analysis of proteins secreted into the medium during differentiation demonstrated an increased accumulation of angiogenic factors VEGF and MCP-1 as the ASCs mature into adipocytes (5 and 10 fold increase respectively). The relation of vascular remodeling mediated by angiogenic factors secreted during adipogenesis, to the increased energy demands of expanding fat mass may play a critical role in modulating both local and systemic effects of adipose tissue.

Accumulation of fibronectin in the heart after myocardial infarction: a putative stimulator of adhesion and proliferation of adipose derived stem cells

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Myocardial infarction (MI) is a major health problem in the western world. Stem cell therapy forms a promising therapy after MI, and adipose tissue derived stem cells (ASC) are a potential candidates for stem cell therapy. A major problem in stem cell therapy, however, is that only a small proportion of stem cells applied to the heart, survive and differentiate into cardiomyocytes. We hypothesized that the presence of fibronectin in the heart after MI may positively affect stem cell adhesion and proliferation at the site of injury. Therefore, we investigated the kinetics of attachment and proliferation of ASC on fibronectin, and analyzed the time frame and localization of fibronectin accumulation in the human heart after MI. ASCs were seeded onto fibronectin-coated and uncoated culture wells. Numbers of ASC adhered were quantified after different incubation periods (5 min to 1hr), using DAPI-staining. Proliferation of ASC was quantified after culturing ASC for different periods (0-9 days), using DNA assays. 4 Fibronectin accumulation after MI was quantified by immunohistochemical staining of heart sections from 35 patients, with different infarction periods (0-14 days old). We found that ASC attachment and proliferation on fibronectin-coated culture wells was significantly increased compared to uncoated wells (attachment: $p=0.025$, proliferation: $p=0.009$, repeated measures, $n=3$). Fibronectin deposition was found to be significantly increased from 12hrs-14days post infarction, both in the infarction area and the borderzone, compared to the uninfarcted heart. Our results suggest that a positive effect of fibronectin on the retrieval of stem cells in the heart, can only be achieved when stem cell therapy is applied at least twelve hours after MI, when accumulation of fibronectin in the infarcted heart occurs.

Dexamethasone activation of circadian transcriptional mechanisms in human and murine mesenchymal stem cells

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While circadian transcriptional mechanisms have been associated historically with the suprachiasmatic nucleus in the brain, their role in peripheral tissues has now been appreciated. We have previously established human adipose-derived stem cells (ASCs) as an in vitro circadian model. In this study, we demonstrate that both human and murine bone marrow derived mesenchymal stem cells (MSCs) can serve in a comparable capacity. A two hour exposure to dexamethasone induces the temporal oscillatory expression of the *bmal1*, *Per3*, and *Rev-erbα* mRNAs in huMSCs and muMSCs. The presence of the glycogen synthase kinase 3β inhibitor, lithium chloride, lengthens the oscillatory period, as previously demonstrated in huASCs. These findings are consistent with microarray studies demonstrating the oscillatory expression of >20% of genes in murine calvarial bone, including those encoding the circadian transcription factors. Thus, primary cell cultures can be used to define circadian regulatory mechanisms in adipose, bone, and related metabolic tissues.

Chitosan microspheres for stem cell culture and tissue engineering applications

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In the present study we have optimized in vitro adipocyte mesenchymal stem cells (MSC) cell culture methods and developed a strategy to capture and release the pluripotent stem cells from chitosan microspheres in situ. The isolated adipose stem cells were cultured with chitosan microspheres and allowed to attach and proliferate in vitro. To measure cell viability, an MTT assay was performed using 2, 4, 6 and 8 mg of microspheres and showed that the chitosan microspheres were able to support cell growth and that the cells not only attached, but were inside the microspheres. The cells inside the microspheres were metabolically active and viable cells could be retrieved from the spheres. The present investigation provides a model to capture pluripotent stem cells in vitro, expand their cell number in the biomaterial microspheres during culture and possibly allow infiltration into damaged tissue upon transplantation.

Adipose tissue stromal cells as in vitro angiogenesis inductors

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The delivery of autologous progenitor cells to improve angiogenesis is emerging as a treatment measure for patients with cardiovascular diseases, but may be limited by the accessibility of sufficient cell numbers. The effects of delivered cells appear to be related to their multipotency and ability to secrete angiogenic growth factors[1]. We examined adipose stromal cells (ASCs) from rat subcutaneous fat tissue for their angiogenic potential in coculture model with postnatal rat cardiomyocyte mixed fraction (CMF). ASCs were co-cultured with CMF from postnatal rat hearts in concentration 1:4, respectively. ASCs and CMF were labeled with lipophilic dyes PKH26/DiI and PKH2, correspondently. In 5-10 days of co-culture there appeared CD31 positive vessel-like structures of mixed origin. CD 31 positive vessel-like structures were also formed by CMF fraction alone, but these structures were less branched and disappeared in 5-6 days. ASCs alone didn't form any CD31 positive vessel-like structures in our culture conditions. In co-culture ASCs seem to stabilize and support vessel-like structures and their branching up to 21 days. Consequent experiments with supernatant media taken from ASCs culture suggest that angiogenic growth factors in the media provide only half of the vessel-supporting effect observed in co-culture of ASCs and CMF. We assume that ASCs support and stabilize the CD31-positive structures formed by postnatal cardiac cell fraction. The important feature of this interaction besides ASCs secreted factors is the cell-to-cell contact between ASCs and CD31-positive cells. We suppose that ASCs can be a source of pericytes involved in vessel stabilization during neo-angiogenesis.

[1]Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation (2004)16;109(10):1292-8

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