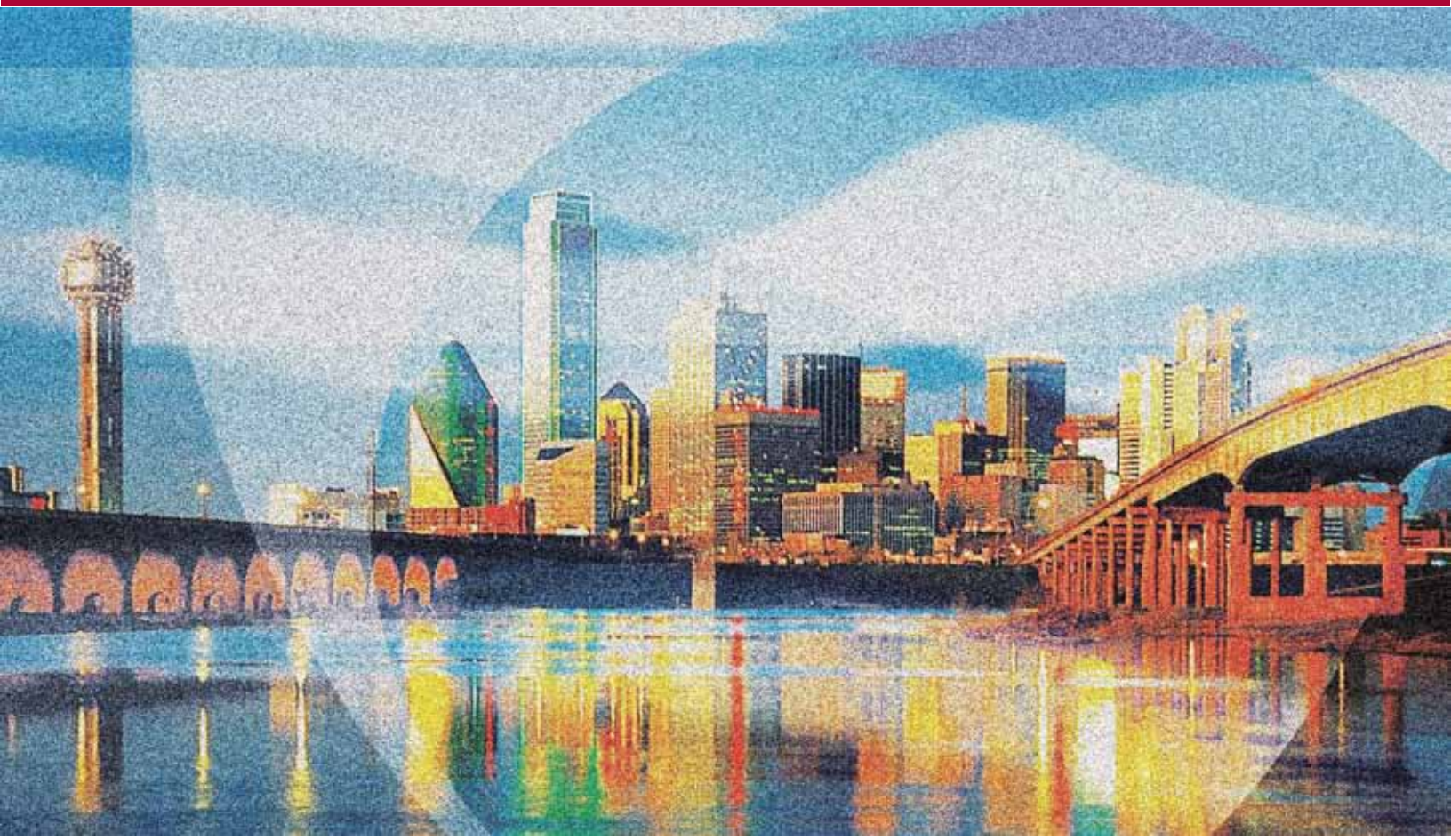
Dallas IFATS 2010 Scientific Program



October 22–24, 2010
Westin Galleria • Dallas, Texas

IFATS
The First Global Fat Applied Research Association

Save a Limb. Save a Life.

by Chronic Disease Specialists (CDS)

Every thirty seconds, a foot amputation occurs somewhere in the world; 100,000 amputations are performed in the U.S. each year. Chronic Disease Specialists (CDS) through its innovative program, "**Save a Limb. Save a Life**." is dedicated to reducing the epidemic of diabetic foot amputations worldwide.

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IFATS

The First Global Fat Applied Research Association

We are pleased to host the International Federation of Adipose Therapeutics & Science (IFATS) Conference on October 22-24, 2010 at the Westin Galleria in Dallas, Texas.

This program has developed around the concept of presenting new information from leading scientists in the field of adult adipose stem cells research and learn about the latest scientific, medical, and technological advances in the clinical arena. As the majority of the program is determined by the abstracts submitted, the abstract review committee had a difficult task as over 120 abstracts were submitted from all over the world. The program format has retained the linear structure without breakout sessions in order to have all speakers available to all attendees.

As the information and clinical applications have increased, so has the IFATS program. This year's program provides new data on adipocytes, tissue engineering, and preclinical and clinical treatments. Keynote speakers from industry in the US and foreign countries, US Armed Forces as well as the FDA will provide overviews and the discussion panels will serve to answer questions.

The speakers will meeting the program goal of providing new knowledge, clinicians using adipose and adipose stem cells, and technology developers who are creating new and cost-effective devices, procedures and biological scaffolds to move the application of these cells as a regenerative tool.

On behalf of the IFATS Board of Directors, we look forward to a successful meeting and give you a Texas-style welcome to Dallas.

With best wishes,

Spencer A. Brown, PhD
President Elect

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Professor and Chairman
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University of Texas Southwestern Medical Center

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University of Texas Southwestern Medical Center

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University of Texas Southwestern Medical Center at Dallas

Kotaro Yoshimura, MD
University of Tokyo

PROGRAM SCHEDULE

Friday, October 22, 2010

7:00 - 7:30 am	Breakfast
7:00 - 4:00 pm	Registration
7:30 - 7:40 am	**Welcome and Introductions** Spencer Brown, PhD, J. Peter Rubin, MD, Michel Saint-Cyr, MD
7:40 - 7:50 am	**IFATS: History - Past & Future** Ramon Llull, MD *Founder of IFATS*
7:50 - 8:05 am	**Adipose & Stem Cells: Overview** Spencer Brown, PhD & J. Peter Rubin, MD

SESSION I: BASIC SCIENCE

Moderators: Spencer Brown, PhD & Paul DiMuzio, MD

8:05 - 8:10 am	Opening Remarks: Overarching Theme
8:10 - 8:25 am	**SeXXy Fat** Deborah Clegg, PhD, RD *University of Texas - Southwestern Medical Center at Dallas*
8:25 - 8:40 am	**Facial Fat Pads and Aging – What is the Potential Role of ASC's** Joel E. Pessa, MD University of Texas Southwestern Medical Center at Dallas
8:40 - 8:55 am	**Adipogenic and Proliferative Effects of Neuropeptide Y on Primary Cultured Human Adipose-Derived Stem Cells** Brian Philips, PhD *University of Pittsburgh*
8:55 - 9:10 am	**Angiogenetic Growth Factor Production in Adipose Tissue-Derived Mesenchymal Cells** Timm P. Wolter *University Hospital of the RWTH Aachen*
9:10 - 9:25 am	**Differential Gene Expression in Human Adipose Stem Cells Cultured in Allogeneic Human Serum Versus Fetal Bovine Serum** Bettina Lindroos, PhD *University of Tampere*
9:25 - 9:40 am	**Dedifferentiated Fat (DFAT) Cells Convert into Cardiomyocytes Phenotype and Repair Infarcted Cardiac Tissue in Rats with Myocardial Infarction** Tomohiko Kazama, PhD *Nihon University School of Medicine*

9:40 - 9:55 am	**Flow Conditioning and Enos Transfection Improve Function of a Tissue-Engineered Vascular Graft (TEVG) Created with Adipose-Derived Stem Cells (ASC)** Paul DiMuzio, MD, FACS *Thomas Jefferson University Hospital*
9:55 - 10:10 am	**Isolated and Cultured Human Expression Profiles** Spencer A. Brown, PhD *UT Southwestern Medical Center*
10:10 - 10:25 am	Panel Discussion
10:25 - 10:40 am	Coffee Break

SESSION II: DELIVERY SYSTEMS (SCAFFOLDING)

Moderators: Adam Katz, MD & Jae-Ho Jeong, MD, PhD

10:40 - 10:45 am	Opening Remarks: Overarching Theme (Cell Biology)
10:45 - 11:00 am	**Evaluation of a Defined Medium for the Culture of Adipose-Derived Stromal Cells (Asc)** Philippe Bourin, MD, PhD *Etablissement Français du Sang Pyrénées-Méditerranée*
11:00 - 11:15 am	**Clinical Use of ASCs in Cranio-Maxillofacial Bone Reconstruction** Susanna Miettinen, MD *Institute for Regenerative Medicine*
11:15 - 11:30 am	**Soft Tissue Engineering Using Modular, Serum-Free Methods** Hulan Shang, MS *University of Virginia*
11:30 - 11:45 am	**Hyaluronic Acid-based Scaffold for Growing Human Adipose-Derived Stem Cells in Immunodeficient Mice** Sin-Daw Lin, MD *Kaosiung Medical University Hospital*
11:45 am - 12:00 pm	**Tissue-Engineered Human Tissues as In Vitro Models for Prevascularized Substitutes: Impact of Adipocytes on the Formation of Capillary Networks** Maryse Proulx, MSc *Université Laval*
12:00 - 12:15 pm	**Improving the Viability of Diced Cartilage Grafts: Effects of Adipose Derived Stem Cells and Slow-Release bFGF** Hakan Orbay, MD *Nippon Medical School*
12:15 - 12:30 pm	**Soft Tissue Engineering with Decellularized Adipose Tissue** Lauren E. Flynn *Queen's University*

12:30 - 12:45 pm	**Adipogenesis of Human Adipose-Derived Stem Cells within 3D Hollow Fiber-Based Bioreactors** Yen-Chih Lin, PhD *University of Pittsburgh*
12:45 - 1:00 pm	Panel Discussion
1:00 - 3:00 pm	Lunch - Electronic Papers

SESSION III: CLINICAL TRANSLATION

Moderators: Keith March, MD, PhD & Ali Mojallal, MD

3:00 - 3:10 pm	Opening Remarks: Overarching Theme (Cell Biology)
3:10 - 3:25 pm	**Augmentation of Fat Graft Survival with Progenitor Cell Mobilization in a Murine Model** Parag Butala, MD *New York University Langone Medical Center*
3:25 - 3:40 pm	**Local Delivery of Adipose Derived Mesenchymal Stem Cells for Cutaneous Wound Healing in a Porcine Model** Summer E. Hanson, MD *University of Wisconsin School of Medicine and Public Health*
3:40 - 3:55 pm	**Intravenous Xenogeneic Transplantation of Human Adipose-Derived Stem Cells Improved Myocardial Function in Swine Acute Myocardial Infarction Model** Soon Jun Hong, MD, PhD *Indiana University School of Medicine*
3:55 - 4:10 pm	**ASC Survival Within a Novel, Biodegradable Polysaccharide Hydrogel for Adipose Tissue Engineering** Kacey Marra, PhD *University of Pittsburgh*
4:10 - 4:25 pm	**In Vivo Study of Adipose Dermal Equivalent Seeded by Porcine BrdU Labelled Adipose derived Stromal Cells** Charlotte Lequeux *University of Texas Southwestern Medical Center*
4:25 - 4:40 pm	**Pronounced Anti-Inflammatory Effects of Adipose Stem Cells in the Central Nervous System** Bruce A. Bunnell, PhD *Tulane University School of Medicine*
4:40 - 4:55 pm	Panel Discussion

SESSION IV: ASC – CLINICAL APPLICATIONS

Moderators: Michel Saint-Cyr, MD & Stuart Williams, PhD

4:55 - 5:10 pm	Opening Remarks: Overarching Theme (Cell Biology)
5:10 - 5:25 pm	**Genetically Engineered Human Mesenchymal Stem Cells as Development Candidates for Tissue Repair and Disease Correction** Jan Nolta, PhD *UC Davis School of Medicine*
5:25 - 5:40 pm	**Biochemical Analysis of Crude Collagenase Products Used in Adipose Derived Stromal Cell (ADSC) Isolation Procedures and Development of a Purified Tissue Dissociation Enzyme (TDE) Mixture** Robert C. McCarthy, PhD *VitaCyte LLC*
5:40 - 5:55 pm	**Stem Cell Based Strategies to Enhance Pancreatic Islet Survival in Type 1 Diabetes Mellitus** Tatsuyoshi Kono, PhD *Indiana University School of Medicine*
5:55 - 6:10 pm	**Cross-talk Between Subcutaneous Adipose Tissue and Skin & Utilization of Ischemic Adipocyte in the Healing of Burns** Marco Aurelio Pellon, MD *Clínica São Vicente*
6:10 - 6:25 pm	**Human ADSC Cell-Based Therapy For Skin Burn Wound Healing: Efficiency and Mechanisms** Valérie Planat-Benard, MCF *UMR5241 UPS/CNRS*
6:25 - 6:35 pm	Panel Discussion
6:45 - 8:00 pm	Reception - Pool Terrace (Fourth Floor)

Saturday, October 23, 2010

SESSION V: Clinical Fat Grafting: Innovation and Science Behind Fat Grafting Supplemented with ASCs

Moderators: Rod Rohrich, MD, FACS & J. Peter Rubin, MD

7:00 - 7:30 am	Breakfast
7:00 - 4:00 pm	Registration
7:30 - 7:40 am	Opening Remarks
7:40 - 7:55 am	**Density Fractions of Lipoaspirate : Implication in Fat Graft Survival** Sydney Coleman, MD *Langone Medical Center New York University*

7:55 - 8:10 am	**Comparison Of The Fat Graft Survival Following Centrifugation And Telfa®-Rolling** Orlando Canizares, MD *Langone Medical Center New York University*
8:10 - 8:25 am	**Standardization of Analytical Methods for Autologous Human Fat Graft: Learning to Speak the Same Language when Evaluating Acquisition and Preparation Techniques** Kevin C. Hicok, MS *Cytori Therapeutics Inc.*
8:25 - 8:40 am	**Stromal Vascular Fraction Isolation with an Automated System; Analysis of Cells and Clinical Use in Cell-Assisted Lipotransfer** Kentaro Doi, MD *University of Tokyo*
8:40 - 8:55 am	**Mechanical Factors Influencing Fat Cell Transplants Quality** Djaffar Ould-Ali, MD *La Conception Hospital*
8:55 - 9:10 am	**Processing and Passaging of Human Adipose-Derived Stromal/Stem Cells: Use of Animal Free Products and Extended Storage at Room Temperature** Pedro Carvalho, DVM *Pennington Biomedical Research Center*
9:105 - 9:25 am	**Bioengineered Breasts: Combining Implants, Scaffolds, and Cells** G. Patrick Maxwell, MD *Maxwell Aesthetics*
9:25 - 9:40 am	Panel Discussion
9:40 - 9:55 am	Coffee Break

SESSION VI: Fat Grafting: Innovative Technology and Role of Stem Cell Supplementation
Moderators: Rod Rohrich, MD, FACS & Val Lampros, MD, FACS

9:55 - 10:10 am	Opening Remarks
10:10 - 10:25 am	**Reconstructive and Regenerative Potential of Fat Grafting** Sydney R. Coleman, MD *Tribeca Plastic Surgery*
10:25 - 10:40 am	**Soft Tissue Volume Augmentation with ASCs and Scaffolds** Hy Lee, MD *Medi-Khan, Inc.*
10:40 - 10:55 am	**Fat Grafting with Adipose Stem Cells; Stem Cell Grafting with Dying Tissue (Signals)** Kotaro Yoshimura, MD *University of Tokyo*

10:55 - 11:10 am	**Optimizing "Workflow" - Mega-Volume Fat Grafting Using the "Large Syringe" Technique** Daniel Del Vecchio, MD *North Shore Medical Center, Back Bay Plastic Surgery*
11:10 - 11:25 am	**Fat Grafting Experience in Breast Reconstruction** Michel Saint-Cyr, MD *University of Texas - Southwestern Medical Center at Dallas*
11:25 - 11:40 am	**Mammographic Changes after Stem Cell Supplemented Fat Transfer Compared with Changes after Breast Reduction** J. Peter Rubin, MD *University of Pittsburgh*
11:40 - 11:55 am	**Fat Grafting for Implant Failure Patients** Kamran Khoobehi, MD *Advanced Plastic Surgery*
11:55 am - 12:20 pm	Panel Discussion
12:20 - 2:30 pm	Lunch - Exhibits - Electronic Papers

Session VII: Commercialization of ASC: Worldwide Perspective
Moderators: Spencer Brown, PhD & Jan Nolta, PhD

2:40 - 2:45 pm	Open Remarks
2:45 - 3:00 pm	Neil Riordan, PhD *Medistem*
3:00 - 3:20 pm	Bill Paspaliaris, PhD *Adistem*
3:20 - 3:40 pm	William Cimino, PhD *GID Stem Cell Center*
3:40 - 4:00 pm	**FDA Regulation of HCT/P (Human Cells, Tissues and Cellular or Tissue-Based Products)** Yong Fan, MD *FDA/CBER*
4:00 - 4:25 pm	Panel Discussion

SESSION VIII: Military Applications of ASC: Present and Future Needs
Moderators: J. Peter Rubin, MD & William Futrell, MD

4:30 - 4:50 pm	**Craniomaxillofacial Battle Injuries, Current Treatment Challenges** Col. Robert Hale, DDS *US Army - Institute of Surgical Research*

4:55 - 5:05 pm	**Autologous Fat Transfer for Scar Prevention And Remodeling (Aft-Spar): Design of a Randomized, Blinded, Prospective Clinical Trial** Adam Katz, MD *University of Virginia*
5:05 - 5:15 pm	**Adipose Based Therapies for Military Trauma** J. Peter Rubin, MD *University of Pittsburgh*
5:15 - 5:25 pm	Panel Discussion

SESSION IX: Device & BioPharmaceutical
Moderators: Spencer Brown, PhD & Rod Rohrich, MD, FACS

5:30 - 5:35 pm	Opening Remarks
5:35 - 6:00 pm	**J&J Global Commercial Strategies for ASC** Joseph F. Amaral, MD *Surgical Technology*
6:00 - 6:15 pm	Panel Discussion
6:30 - 10:00 pm	IFATS Dinner - Three Forks Restaurant *Buses will depart from the Westin Hotel at 6:30 pm and will return by 10:00 pm*

Sunday, October 24, 2010

SESSION X: Wound Healing
Moderators: Adam Katz, MD & Philippe Bourin, MD, PhD

7:00 - 7:30 am	Breakfast
7:00 - 4:00 pm	Registration
7:30 - 7:40 am	Opening Remarks
7:40 - 7:50 am	**Improvement in Radiation Injury Following Fat Graft** Sydney R. Coleman, MD *Tribeca Plastic Surgery*
7:50 - 8:05 am	**Effects of Fat Grafts and ASC in Radiation Rodent Model** Lucas Rifkin, MD *University of Michigan*
8:05 - 8:20 am	**Adipose-Derived Stromal Vascular Fraction Patch Improves Coronary Blood Flow after Myocardial Infarct** Stuart Williams, PhD *University of Louisville*

8:20 - 8:35 am	**Adipose Stem and Regenerative Cell Therapy of Canine Immune-mediated Polyarthritis** Robert Harman *Vet-Stem, Inc.*
8:35 - 8:50 am	**Automated Point of Care Isolation of Adipose Derived Regenerative Cells for Tissue Engineered Vascular Grafts** Stuart Williams, PhD *Cardiovascular Innovation Institute*
8:50 - 9:15 am	Panel Discussion

SESSION XI: Stem Cells, Cancer and Late Breaking Presentations
Moderators: Ali Mojallal, MD & Michel Saint-Cyr, MD

9:20 - 9:25 am	Opening Remarks
9:25 - 9:40 am	**Adipose Tissue Increases Dramatically the Tumour Growth when Co-Injected with Breast Cancer Cell Lines: A Prudence Recommendation for Autologous Fat Transfer in Breast Parenchyma** Ali Mojallal, MD *University of Lyon*
9:40 - 9:55 am	**Gene-Modified Adipose Derived Progenitors as Anti-Cancer Therapy** Massimo Dominici *University-Hospital of Modena and Reggio Emilia*
9:55 - 10:05 am	**Optimized Autologous Fat Grafting with Adipose Derived Stem Cells within the Operating Room** Hebert Lamblet, MD *Vikaara Klinik*
10:05 - 10:20 am	**Analyses of Hematopoietic Cells Resident in Human Subcutaneous Adipose Tissue** Hitomi Eto *University of Tokyo*
10:20 - 10:35 am	**Adipogenic Factor-Loaded Microspheres to Augment Lipoaspirate for Soft Tissue Repair** Arta Kelmendi-Doko, MSc *University of Pittsburgh*
10:35 - 10:55 am	**Adipose Remodeling After Non-Vascularized Grafting and Therapeutic Potential of Systemic Hyperoxygenation** Harunosuke Kato *University of Tokyo*

10:55 - 11:15 am	**Enhancement of Myogenic Differentiation of VEGF-Treated Adipose Stem Cell Graft by Using Thermo Sensitive Hydrogel** Min Hwan Kim *Asan Institute for Life Sciences*
11:15 - 11:30 am	**Therapeutic Potential of Adipose Derived Stem Cells on Alzheimer's Disease** Yoo-Hun Suh *The Nat'l Creative Research Initiative Center for Alzheimer's Dementia*
11:30 - 11:45 am	Mike Hutchinson, MD *Medivet America LLC*
11:45 am - 12:00 pm	Panel Discussion
12:00 - 12:15 pm	**Metabolic Profiling of Stem Cells** Hesham Sadek, MD, PhD *University of Texas Southwestern Medical Center at Dallas*
12:15 - 12:30 pm	Meeting Summary/IFATS Business Meeting/Future Sites

SESSION I
BASIC SCIENCE

ADIPOGENIC AND PROLIFERATIVE EFFECTS OF NEUROPEPTIDE Y ON PRIMARY CULTURED HUMAN ADIPOSE-DERIVED STEM CELLS

Presenter: Brian Philips, PhD
Authors: Philips BJ[1], Bhaumik M[1], McAtee J[1], Marra KG[1], Fernstrom JD[2], Rubin JP[1]
[1]*Department of Surgery, Division of Plastic Surgery, University of Pittsburgh;* [2]*Department of Psychiatry, University of Pittsburgh*

Introduction: A current unmet need for rendering soft tissue reconstructive therapies more effective is the identification of pharmacologically-active agents that can be used in vivo. Such compounds might help to promote mature adipose-derived stem cell (ASCs) differentiation, as well as adequate revascularization within the transplanted fat. To date, very few agents are known that promote de novo adipogenesis, angiogenesis or proliferation for adipose tissue regeneration.

Purpose: Neuropeptide Y (NPY) has shown promising results in established cell lines for soft tissue survival regarding adipogenesis and proliferation, though to our knowledge, no conclusive data has been reported utilizing human ASCs. The aim of this study was to ascertain the potential effects of Neuropeptide Y on cell differentiation and proliferation of primary cultured human ASCs.

Materials & Methods: ASCs were isolated from abdominal fat obtained from human, non-diabetic female patients (Age: 39-55, BMI: 23-30). ASCs were treated with 10^{-14}M-10^{-6}M NPY and assessed for differentiation (14d) via lipid accumulation using the AdipoRed Assay Reagent, and for proliferation (72h) with the CyQUANT Cell Proliferation Assay Kit.

Results: Preliminary results from 2 female ASC samples indicated NPY modestly simulated adipogenesis and strongly inhibited ASC proliferation in a dose-dependent manner.

Discussion: Because of their capacity to differentiate into multiple cell lineages and to secrete various growth factors, ASCs offer remarkable potential for clinical application. Our studies indicate that NPY can variably stimulate adipogenesis, and not proliferation, of human ASCs. For increased sample size/statistical power, NPY dose-response assays are currently being conducted on additional female ASC samples.

ANGIOGENETIC GROWTH FACTOR PRODUCTION IN ADIPOSE TISSUE-DERIVED MESENCHYMAL CELLS

Presenter: Timm P. Wolter, MD
Authors: Wolter TP, Pallua N
Department of Plastic Surgery, Hand and Burn Surgery, University Hospital of the RWTH Aachen

Introduction: Optimal vascularization of the fat graft after transplantation is important. Adipose tissue, specifically the stromal vascular fraction (SVF), is known to regulate its own vascular network. To analyze angiogenetic growth factor production we have investigated the content of insulin growth factor (IGF), vascular endothelian growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), leptin and metallothioneine-9 (MMP-9) in the SVF after incubation in differentiation or proliferation media.

Methods: The SVF was isolated from human adipose tissue. Cells were either cultured in DMEM/F12 supplemented with 10% FCS and 10 ng/mL bFGF (proliferation medium, PM) or in DMEM/F12 (1:1) without serum addition, supplemented with 66 nM insulin, 100 nM dexamethasone, 0.5 mM IBMX, 0.1 mg/mL pioglitazone, 1 nM triiodo-l-thyronine and 10 mg/mL human transferrin (differentiation medium, DM). After three and six days the samples were analyzed for the respective factors by ELISA.

Results: The GF levels showed a distinctive characteristic depending on the culture medium and over time. IGF, PDGF and MMP-9 levels decrease with PM and increase with DM. VEGF levels were increased in both media. Leptin showed a decrease in both media. Except for PDGF and Leptin using differentiation medium there was a high interindividual distribution in growth factor production.

Conclusion: Incubation with DM produces a more reliable increase of selected growth factors than incubation with PM. Enrichment of fat grafts with growth factor-activated SVF samples could ensure a better ingrowth of the transplanted tissue. Scattering of the results could explain the interindividual differences regarding the outcome.

DIFFERENTIAL GENE EXPRESSION IN HUMAN ADIPOSE STEM CELLS CULTURED IN ALLOGENEIC HUMAN SERUM VERSUS FETAL BOVINE SERUM

Presenter: Bettina Lindroos, PhD
Authors: Lindroos B[a], Aho KL[b], Suuronen R[a], Miettinen S[a,]
[a] *REGEA, Institute for Regenerative Medicine, University of Tampere and Tampere University Hospital, Biokatu 12, 33520 Tampere, Finland;* [b] *Institute of Signal Processing, Tampere University of Technology, Finland*

Human adipose stem cells ASCs are an attractive and abundant source of multipotent progenitor cells residing in the adipose tissue. ASCs have been shown to have therapeutic relevancy in diverse clinical applications, but a standard expansion method remains yet to be established. Current expansion protocols include medium containing fetal bovine serum (FBS) which is an unsuitable option due to the risk of infection and severe immune reactions in the recipient. By expanding the ASCs in medium containing human serum (HS) the problem can be reduced.

As FBS is the most commonly used serum for stem cell expansion, we compared the cell proliferation frequency, multilineage differentiation capacity, gene expression and surface marker expression profiles of ASCs cultured in FBS vs. allogeneic HS.

Our results show, that the choice of serum affects ASCs in several ways. Based on genome-wide microarray, several transcripts involved in cell cycle and differentiation were differentially regulated between ASCs in FBS and HS. However, no major differences were seen in the proliferation rate and surface marker profile.

Several reports have been published on characteristics of BM-MSC cultured in FBS vs. HS; however, to our knowledge no equally extensive studies have been reported performed on ASCs. Here we report comparative genome-wide microarray analysis and extensive flow cytometric analysis performed on ASC in FBS vs. HS that has not been reported previously. Thorough characterisation is momentous for utilisation of adipose stem cells in cell therapy applications. The characterisation and differentiation data presented here is an important step towards this goal.

DEDIFFERENTIATED FAT (DFAT) CELLS CONVERT INTO CARDIOMYOCYTES PHENOTYPE AND REPAIR INFARCTED CARDIAC TISSUE IN RATS WITH MYOCARDIAL INFARCTION

Presenter: Tomohiko Kazama, PhD
Authors: Matsumoto T[1], Jumabay M[1], Kazama T[1], Kano K[2], Fukuda N[3], Mugishima H[4]
[1]*Department of Medical Science, Division of Cell Regeneration and Transplantation, Nihon University School of Medicine, Tokyo, Japan;* [2]*Department of Animal Sciences, College of Bioresource Sciences, Nihon University, Fujisawa, Japan;* [3]*Advanced Research Institute of Science and Humanities, Nihon University, Tokyo, Japan;* [4]*Department of Pediatrics, Nihon University School of Medicine, Tokyo, Japan.*

Introduction: We have established dedifferentiated fat (DFAT) cells from mature adipocytes that are capable of differentiating along multiple lineages. In the present study, we investigated whether the DFAT cells differentiate to cardiomyocytes in vitro and contribute to myocardial regeneration in vivo.

Methods: DFAT cells derived from GFP-transgenic rats are directly or indirectly cocultured with neonatal SD rat cardiomyocytes or cultured in semisolid medium MethoCult GF M3534. Differentiating ability of DFAT cells into cardiomyocytes was evaluated by detection of the cardiac phenotype markers in immunocytochemical and RT-PCR analysis. In vivo, GFP-labeled DFAT cells were injected intramuscularly of ischemic heart area at 3 hours after left coronary artery ligation in rats. Cardiac function, scar size, and vascular density in infarcted area were measured and compared to saline-injected control rats. Differentiation of transplanted cells into myocardium was evaluated immunohistochemically.

Results: DFAT cells expressed several cardiac phenotype markers when cocultured with cardiomyocytes and also when grown in MethoCult medium in the absence of cardiomyocytes. In a rat acute myocardial infarction model, DFAT cell transplantation significantly improved cardiac function, reduced scar size, increased vascular density in the infarcted area. Transplanted DFAT cells were efficiently accumulated in infarcted myocardium and expressed cardiac sarcomeric actin at 8 weeks after the cell transplantation.

Conclusions: DFAT cells have ability to differentiate to cardiomyocyte-like cells in vitro and in vivo. Transplantation of DFAT cells led to neovascularization and improved cardiac function. We propose that DFAT cells represent a promising candidate cell source for cardiomyocytes regeneration in severe ischemic heart disease.

FLOW CONDITIONING AND ENOS TRANSFECTION IMPROVE FUNCTION OF A TISSUE-ENGINEERED VASCULAR GRAFT (TEVG) CREATED WITH ADIPOSE-DERIVED STEM CELLS (ASC)

Presenter: Paul DiMuzio, MD
Authors: DiMuzio P, McIlhenny S, Bagameri G, Fernandez S, Comeau J, Shapiro I, Tulenko T
Department of Surgery, Thomas Jefferson University

Introduction: We recently reported intraluminal thrombosis within TEVG composed of a vascular tissue scaffold seeded with autologous ASC differentiated into endothelial cells (EC). Herein, we hypothesize that flow conditioning prior to graft implantation, along with transfection of ASC with eNOS, improves graft function.

Methods: TEVG were created by seeding autologous ASC cultured in EC Growth Supplement (to induce EC differentiation) and transfected with eNOS (adenovirus) onto decellularized vein. After seeding, the grafts were placed within a bioreactor where intraluminal flow was increased linearly (9dynes over 5d). The grafts were implanted into rabbit aorta and harvested at 2wk/2mo. Graft seeding was evaluated by confocal microscopy and ELISA (integrin expression); eNOS transfection was evaluated by PCR, immunoblot, chemiluminescence, and relaxation of aortic rings.

Results: Linear flow conditioning significantly improved ASC retention compared with no or step-wise conditioning. Shear force up-regulated $\alpha_v\beta_1$ integrin expression and selective blockade eliminated cell retention. After EC induction of ASC, no eNOS was detected; following adenoviral transfection, ASC expressed eNOS up to 3wk, and produced nitric oxide after stimulation (Ca ionophore or bradykinin). Conditioned media from transfected ASC produced vasorelaxation of aortic rings. All TEVG remained patent at 2wk (n=4) and 2mo (n=4). Compared with unseeded controls, we observed reduced mural thrombus and intimal hyperplasia within the TEVG.

Conclusions: Linear flow conditioning improves stem cell retention via up-regulation of the $\alpha_v\beta_1$ integrin. ASC transfected with eNOS produce functional NO. Both improvements in TEVG creation result in a durable small-diameter bypass graft with anti-thrombogenic properties and reduced intimal hyperplasia.

SESSION II
DELIVERY SYSTEMS (SCAFFOLDING)

EVALUATION OF A DEFINED MEDIUM FOR THE CULTURE OF ADIPOSE-DERIVED STROMAL CELLS (ASC)

Presenter: Philippe Bourin, MD, PhD
Authors: Duluc C*, Peyrafitte J*, Airola E*, Caillot L§, Bourin P*
**Laboratoire d'Ingénierie Cellulaire, Etablissement Français du Sang Pyrénées-Méditerranée, §Abcell Bio, Paris, France*

Introduction: Human or animal serum additives present batch-to-batch variability and may be a source of pathogen contamination and immunoreactivity. Thus using a defined culture medium would be an advantage to produce ASC for clinical applications. We have tested the defined medium SPE that is devoid of xenogenic proteins.

Methods: Stromal vascular fraction (SVF) was obtained from adipose tissue according to conventional enzymatic method. SVF cells (4000/cm²) were cultured 8 days in either our medium containing a human substitute (RM) or in SPE (primary culture = P0). Then ASC were subcultured during periods of 6 days in the same media (passage 1, 2, 3, 4 = P1, 2, 3, 4). At the end of each passage, cell expansion, phenotype and CFU-F content were evaluated. Osteoblastic, adipocytic and chondrocytic differentiation were assayed at the end of P1.

Results: At the end of every passages, expansion was 2.9 to 6.5 fold higher with SPE as compared to RM. This difference was statistically significant at P0, P2, P3 and P4 ($p<0.05$). Progenitor content was always greater in the ASC population grown in SPE ($p<0.05$). Indeed, CFU-F frequency varied from 15.4±2.7% versus 9.6±2.8% at P0 to 12.1±3% versus 6.6±1.2% at P4 for SPE and RM respectively. The differentiation potential of ASC toward the 3 lineages was less pronounced when cultured in SPE. Except for mesenchymal markers, we observed significant variation in the cell surface phenotype, especially for adhesion molecules (i.e. CD49c, CD146).

Conclusion: SPE allows a strong proliferation of ASC and seem to better maintain the progenitor population.

SOFT TISSUE ENGINEERING USING MODULAR, SERUM-FREE METHODS

Presenter: Hulan Shang, MS
Authors: Shang, H, Wang, X, Yun S, Yang N, Katz A
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Introduction: ASCs have a clear capacity for adipogenic differentiation, and therefore have potential to provide insights and solutions to both soft tissue reconstruction and obesity-related challenges. Adipogenesis occurs in vivo within a 3-dimensional (3-D) context. We therefore hypothesized that ASCs formulated as self-assembling spheroids in suspension would demonstrate more robust adipogenic differentiation than cells cultured as monolayers. We further hypothesized that modular spheroids would support defined, serum free culture and differentiation better than monolayer conditions. Finally, we postulated that cells formulated as spheroids would demonstrate more localized and efficient engraftment and differentiation in vivo than cells delivered as monolayer-derived suspensions.

Methods: Human ASCs were isolated using established techniques and culture expanded. On day 0, 3-D (MAs) were fabricated and placed into suspension culture and parallel groups of cells were plated into adherent monolayer (ML) culture in either serum-free medium (SFLM) or DMEM/F12+10%FBS culture medium (D10). Adipogenic differentiation was initiated by adding adipogenic supplements (Dexamethasone, Insulin, IBMX and Indomethacin) to each of the base medias (SFLM, D10). Differentiation was evaluated by Oil Red-O staining for intracellular lipid and by quantitative RT-PCR for lineage-related genes (LPL, PPARr2, and FABP; N=5). Collagen production was evaluated by Sirius Red F3B dye binding assay and picro-sirius red staining of cryosections. In vivo studies evaluated cell survival using PCR techniques, histology using H&E and Oil Red O, and cell tracking using BrdU labeling.

Results: After adipogenic induction, ASCs in 3-D culture demonstrate significantly higher adipogenic gene expression levels than parallel cells in 2-D (monolayer) culture. Varying levels of self-generated collagen was detected in the extracellular matrix, depending on specific culture conditions. Implantation studies demonstrate robust engraftment of ASC MAs by fluorescent and light micrsoscopy. PCR quantification studies suggest more localized survival of ASCs delivered as modular aggregates, and histology demonstrates the clear development of ASC-derived 'fat pads'.

Conclusion: ASCs cultured as 3-D MAs display more robust adipogenic differentiation than ASCs in monolayer culture, both in serum-containing and serum-free conditions. Initial pilot findings support the notion that 3-D formulation and delivery provides a favorable environment for efficient and localized cell engraftment and adipo-differentiation in vivo. These findings have implications for the mechanistic study of adipogenic differentiation, as well as for translational therapeutic objectives.

HYALURONIC ACID-BASED SCAFFOLD FOR GROWING HUMAN ADIPOSE-DERIVED STEM CELLS IN IMMUNODEFICIENT MICE

Presenter: Sin-Daw Lin
Authors: Lin SD, Huang SH, Lee SS, Lin TM, Chang HW, Lai CS, Chai CY, Lin YN

Background: Engineered tissue substitutes are used to facilitate the regeneration of subcutaneous adipose tissue after reconstructive plastic surgery. Commercially available injectable fillers, however, have demonstrated limited durability. This report proposes the in vitro culture of human adipose-derived stem cells (hASCs) on RESTYLANE® for in vivo growth of de novo adipose tissue . The proposed soft tissue filler is sufficiently long-lasting to enable the gradual displacement of the RESTYLANE¨ by new adipose tissue.

Materials and Methods: For in vitro studies, hASCs were isolated from human adipose tissue and cultured on RESTYLANE®, a commercially available hyaluronic acid. The effectiveness of cell attachment and proliferation on RESTYLANE® was surveyed by light microscopy. For in vivo studies, suspensions of RESTYLANE¨ containing hASCs were subcutaneously injected into nude mice. At eight weeks post-injection, the implants were harvested for histological examination by hematoxylin and eosin (H&E) staining and by Oil Red O staining. The human-specific Alu gene was also examined by RT-PCR.

Results: Adipose-derived stem cells (hASCs) were well established on the RESTYLANE®. In vivo grafts showed well-organized new adipose tissue constructs with no signs of tissue necrosis, cystic spaces, or fibrosis. The experimental groups exhibited growth of human-specific Alu gene-expressed neo-adipose tissues.

Conclusion: Commercial hyaluronic acid provides an effective injectable scaffold for adipose tissue engineering, and hASCs grown on a hyaluronic acid scaffold provide a long-lasting soft tissue filler.

TISSUE-ENGINEERED HUMAN TISSUES AS IN VITRO MODELS FOR PREVASCULARIZED SUBSTITUTES: IMPACT OF ADIPOCYTES ON THE FORMATION OF CAPILLARY NETWORKS

Presenter: Maryse Proulx, MSc
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Centre LOEX de l'Université Laval, Génie tissulaire et régénération : LOEX - Centre de recherche FRSQ du Centre hospitalier affilié universitaire de Québec, and Département de Chirurgie, Faculté de Médecine, Université Laval, Québec

Tissue-engineered substitutes designed to stimulate rapid revascularization after implantation represent good candidates to enhance long-term volume retention of grafted adipose substitutes. We report the in vitro production of human "endothelialized" reconstructed tissues devoid of exogenous matrix components. Human stromal cells were used to produce connective and adipose tissues using the self-assembly approach of tissue engineering.

This method stimulates cells to organize their own extracellular matrix environment resulting in manipulatable connective sheets. By including an adipogenic differentiation step, adipose sheets can be produced. Thicker tissues are obtained by layering multiple cell sheets. Addition of human microvascular endothelial cells (HMVECs) prior to sheet superposition resulted in endothelialized connective and adipose constructs. Immunolabelings and confocal analyses revealed a network of PECAM-expressing capillaries only within tissues enriched with HMEVCs.

In comparison to connective tissues, adipocytes impacted on the percentage of PECAM+ structures featuring a well-defined lumen on tissue sections (2-fold increase) and a tendency towards 20% greater lumen diameter. This increase in capillary-like structures was correlated with an increased secretion of many pro-angiogenic molecules from adipose constructs, namely a 30-fold increase in leptin level, 3.8 fold for angiopoietin-1, 1.8 fold for HGF and 1.7 fold for VEGF as assessed by ELISA assays.

Finally, a short-term pilot study indicated that endothelialized adipose constructs implanted onto nude mice may promote revascularization within the first 7 days after grafting. The use of autologous cells combined to endogenous human matrix elements make these tissue-engineered adipose tissues unique and natural substitutes for future clinical applications.
Supported by CIHR.

IMPROVING THE VIABILITY OF DICED CARTILAGE GRAFTS: EFFECTS OF ADIPOSE DERIVED STEM CELLS AND SLOW-RELEASE BFGF

Presenters: Hakan Orbay, MD
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Introduction: This study aims to examine the effects of ASCs & slow-release bFGF on the viability of diced cartilage grafts wrapped both with fascia or Surgicel® and hopefully to improve clinical results.

Materials and Methods: 30 Fischer rats were divided into 6 groups: diced cartilage grafts wrapped with fascia (group I), Surgicel® (group II), fascia + slow-release bFGF hydrogel (10 µgr/graft) (group III), Surgicel®+ slow-release bFGF hydrogel (group IV), fascia + ASCs ($1x10^6$cells/graft) (group V), Surgicel®+ ASCs (group VI). ASCs were harvested from the inguinal fat pads of rats, fascia from the abdominal region and cartilage from ears of the rats. Grafts were placed to dorsal pockets, weights recorded. Histological examination was done at 8 weeks with H&E, picrosirius red staining, safranin O, Van Gieson, and GFAP immunohistochemical stainings.

Results: Lowest weight resorption ($p<0.05$) and highest vessel count ($p>0.05$) were in group V. Number of nucleated lacunae per unit area was significantly higher in group V then in other groups ($p<0.05$). Inflammation score was highest in Surgicel® groups and lowest in group V ($p<0.05$). Proteoglycan content was highest in group V ($p<0.05$). Elastic fibers around regenerating cartilage islands were noted in Van Gieson staining in fascia groups but not in group II. GFAP positive chondrocytes were commonest in group V ($p<0.05$).

Conclusion: ASCs significantly increased the viability of the grafts while slow-release bFGF hydrogel also provided an improvement and fascial tissue seems to be a better option to wrap the diced cartilage grafts.

SOFT TISSUE ENGINEERING WITH DECELLULARIZED ADIPOSE TISSUE

Presenter: Lauren E. Flynn
Author: Flynn LE
Department of Chemical Engineering, Queen's University, Kingston; Human Mobility Research Centre, Kingston General Hospital, Kingston; Department of Anatomy and Cell Biology, Queen's University, Kingston

Introduction: Towards engineering an optimized microenvironment for adipogenesis, this study focused on developing a decellularization strategy for human fat, to produce clinically relevant, 3-D scaffolds for soft tissue regeneration.

Hypothesis: Adipose tissue is a rich source of matrix components that can be isolated with minimal compositional and architectural changes, to produce non-antigenic scaffolds that promote adipogenesis in human adipose-derived stem cells (ASC).

Materials and Methods: A decellularization protocol was developed, involving mechanical disruption, enzymatic digestion, and polar solvent extraction.1 The decellularized adipose tissue (DAT) was characterized by electron microscopy, histology, and immunohistochemistry. DAT scaffolds (200 mg) were seeded with 1 x 106 human ASC (P2), and cultured under proliferation or adipogenic differentiation conditions. Tissue culture and cellular aggregate cultures were included as controls. The expression of key adipogenic markers and ECM components was assessed by RT-PCR. GPDH enzyme activity was quantified at 72 h and 7 days. (n=3, N=3).

Results and Discussion: The 5-day, detergent-free extraction method yielded an intact matrix with significant integrity. The characterization confirmed that no cells or macroscopic cellular debris were present, and demonstrated the conservation of matrix organization. The basement membrane was preserved, including intact vascular structures. In the in vitro studies, the DAT provided an inductive microenvironment for adipogenesis, where the master regulators, PPARγ and CEBPα, were expressed without exogenous differentiation factors. In addition, the highest levels of GPDH activity were observed in the DAT samples, supporting that the DAT provided a uniquely permissive environment for fat formation in the current studies.

References: [1] Flynn LE. Biomaterials 2010;31(17):4715-4724.

ADIPOGENESIS OF HUMAN ADIPOSE-DERIVED STEM CELLS WITHIN 3D HOLLOW FIBER-BASED BIOREACTORS

Presenter: Yen-Chih Lin, PhD
Authors: Lin YC, Brayfield CA, Minteer D, Li H, Gerlach JC, Rubin JP, Marra KG
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Introduction: Traditional culture and adipogenic differentiation of adipose-derived stem cells (ASCs) in 2D flasks result in lipid inclusions within cells resembling adipocytes. However, long-term 2D culture of adipocytes is not plausible. In order to further differentiate ASCs into mature adipocytes and create 3D adipose tissue in vitro, we have used 3D, hollow fiber-based bioreactor systems.

Hypothesis: We hypothesize that a 3D hollow fiber bioreactor will result in the long term (e.g., 3 month culture) of human adipocytes in vitro, providing metabolically active tissue that serves as an experimental model for screening drugs to treat diabetes

Methods: ASCs were isolated from discarded human abdominal subcutaneous adipose tissue, then inoculated into hollow fiber-based bioreactors to differentiate. Insulin stimulated glucose uptake from the medium was assessed with and without TNF-alphs.

Results: A 3D adipose tissue was generated in the bioreactors. AdipoRed immunofluorescence staining showed 3D-bioreactors treatment displayed multiple mature adipocyte markers with more unilocular morphologies compared to 2D-cultures. The results of real-time PCR showed 3D-bioreactor treatment had a more efficient differentiation in FABP4 and PPAR gamma expression. Repeated insulin stimulation resulted in increased glucose uptake, with a return to baseline between tests. Importantly, TNF-alpha blunted the glucose uptake, rendering the bioreactor tissue "diabetic."

Conclusions: Hollow fiber-based bioreactors provide allow more mature adipocyte differentiation of ASCs, generating adipose tissues in vitro for up to 3 months. Reproducible Metabolic activity of the adipose tissue in the bioreactor was demonstrated, making this model potentially useful for drug discovery.

NOTES

SESSION III
CLINICAL TRANSLATION

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