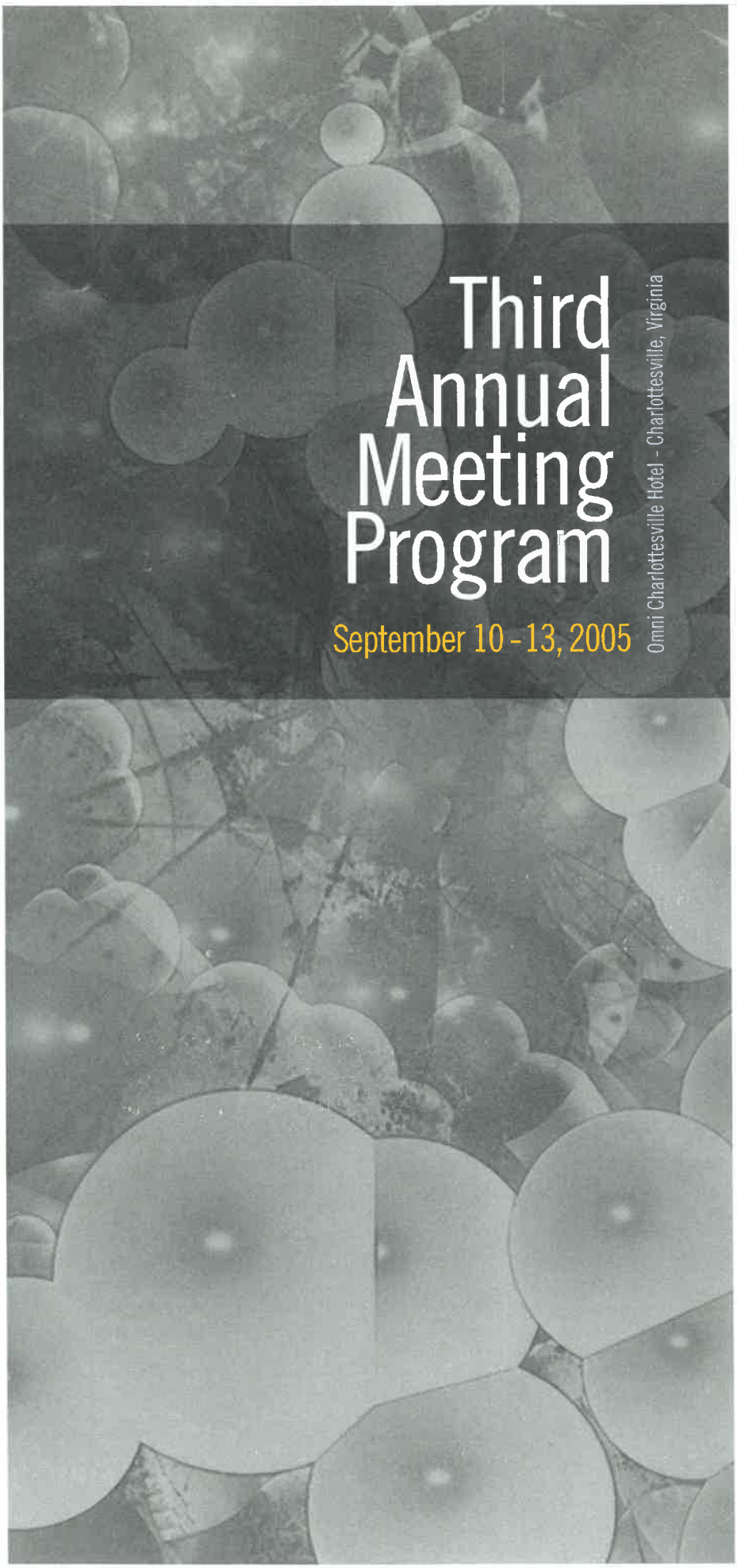

Third Annual Meeting Program

September 10 - 13, 2005

Omni Charlottesville Hotel - Charlottesville, Virginia

IFATS 2005

International Fat Applied Technology Society

THE ROLE OF ADIPOSE TISSUE IN REGENERATIVE MEDICINE: Opportunities for Clinical Therapy

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Mission

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

Description

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

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Welcome

From This Year's President

It is my great pleasure to announce the 3rd annual IFATS meeting, to be held at the Omni Charlottesville Hotel, in Charlottesville, Virginia, September 10-13, 2005. IFATS is a unique multidisciplinary adipose research society with an emphasis on cutting edge translational research. Based on the overwhelming interest in, and success with last year's meeting, the scientific program this year will again focus on adipose stem cells (ASC's) for tissue engineering and regenerative medicine. Leading experts from around the world - representing varied backgrounds and disciplines - will gather to present their work and learn from one another. We anticipate participants from academia, private industry, and government to provide a broad perspective and encourage new collaborations. This year's scientific program will consist of peer-reviewed abstracts/papers juried by a well-rounded, international scientific committee, with the addition of a select few keynote speakers to compliment and emphasize major themes and to highlight opportunities with exceptional translational potential. Finally, the program will include a plenary session on adipose and inflammation, and an FDA-guided panel on regulatory hurdles facing this burgeoning field.

This year's meeting will take place in beautiful Charlottesville, Virginia. Charlottesville is located in central Virginia at the base of the Blue Ridge Mountains, 90 miles southwest of Washington D.C., and home to the historical and nationally recognized University of Virginia. The meeting hotel is conveniently located in historic downtown on the pedestrian mall, in easy walking distance to many fine restaurants, shops, theaters, an indoor ice skating rink, and an outdoor amphitheater.

On behalf of this year's sponsors, the scientific committee and the IFATS board of directors, I invite you to join us and be a part of this exciting event that represents the best of this burgeoning scientific and commercial field. We look forward to seeing you in C'ville!

Sincerely,
Adam J. Katz, M.D.
President, IFATS 2005

IFATS 2005

International Fat Applied Technology Society

September 10 - 13, 2005

Omni Charlottesville Hotel - Charlottesville, Virginia

Saturday, September 10, 2005

2-6 PM	Registration
2-5 PM	Set Up Posters
5-7:30 PM	Meet and Greet Wine and Cheese Reception Poster Viewing
7:30 PM	Adjourn Attendees Encouraged to Explore Charlottesville

Sunday, September 11, 2005

7-8:30 AM	Buffet Breakfast
8:30-11:45 AM	**Symposium 1** Adipose Tissue and Systemic Inflammation: Current Perspective. Moderator: Dr. Barry Sears
8:30-8:45 AM	Moderator Overview and Comments
8:45-9:15 AM	Dr. Barry Sears: "When Good Fats Turn Bad."
9:15-9:45 AM	Dr. David Ludwig: "The Low Glycemic Index: A Perfect Compromise Between Low Fat & Low Carb"
9:45-10:15 AM	Dr. Armour Forse: "The Role of Surgery In Treating Diabetes and Metabolic Syndrome."
10:15-10:30 AM	Open Discussion
10:30-10:45 AM	Coffee Break
10:45-11:45 AM	Keynote Address: "The Courage To Create" Chic Thompson
11:45 AM	Adjourn
11:45 AM-1:15 PM	Lunch. Atendees on Their Own
1:30-4:30 PM	**Symposium 2** Clinical/Commercial Applications of ASC's Moderators: Yasuo Kitagawa, Nagoya, Japan, & Ramon Llull, Madrid, Spain.
1:30-1:45 PM	Moderator Overview and Comments
1:45-2:15 PM	Commercial Orthopedic Use of Fat-Derived Stem Cells In The Horse. *Bob Harman, Vetstem*
2:15-2:30 PM	Adipocyte-Specific Laminin Á4 G Domain Fragments Suppress De Novo Adipogenesis at the Site of Injection of Matrigel and Fibroblast Growth Factor (FGF)-2. *Toriyama K, Goto C, Yamashita H, Tajima R, Narita R, Torii S, Kitagawa Y.*
2:30-2:45 PM	A Clinical Trial of Soft Tissue Augmentation by Lipoinjection with Adipose-Derived Stromal Cells (ASC's). *Yoshimura, K; Matsumoto, D; Gonda, K.*
2:45-3:00 PM	FGF-2 Delivery Enhances Adipose-Derived Stem Cell Survival and Improves Vascularization in Soft Tissue Formation. *Defail, AJ; Clavijo-Alvarez, JA; Marra, KG; Taieb, A; Badylak, SF; Bennett, Jm; Rubin, J.*
3:00-3:15 PM	Coffee Break
3:15-3:30 PM	Achieving Optimal Long-Term Preservation of Human Adipose Tissues. *Pu, LLQ.*
3:30-3:45 PM	Short-Term & Long Term Effects of Transplanted Fat On Aging and Scarred Skin. *Coleman, SR.*
3:45-4:00 PM	Histologic and Stem Cell Evaluation of Fat Overgrowth After Structural Fat Grafting to the Face. *Coleman, SR; Fraser, JK.*
4:00-4:15 PM	Therapeutic Angiogenesis for Ischemic Tissue by Autologous Transplantation of Adipose-Derived Adult Stem Cells. *Rigotti, G; Marchi, A; Galliè, M; Baroni, G; Benati, D; Sbarbati, A.*
4:15-4:30 PM	In Vivo Differentiation of Human Adipose Derived Stem Cells is Independent of Cell Fusion. *Zhang, R; Jack,GS; Rao,N; Zuk,PA; Rodriguez,LV.*
4:30 PM	Concluding Remarks & Adjournment
4:30-5:30 PM	Poster Viewing Session
6:30 PM	Group Dinner, Details TBA

Monday, September 12, 2005

7-8:00 AM	Buffet Breakfast and Business Meeting for IFATS Members *Adam Katz, President;* *Peter Rubin, Past President*
8:15-10:00 AM	**Symposium 3** Culture Conditions and Yield of ASC's. Moderators: Jin Jung, Pusan, Korea, & James Kirkland, Boston, MA.
8:15-8:30 AM	Moderator Overview and Comments

THE ROLE OF ADIPOSE TISSUE IN REGENERATIVE MEDICINE:

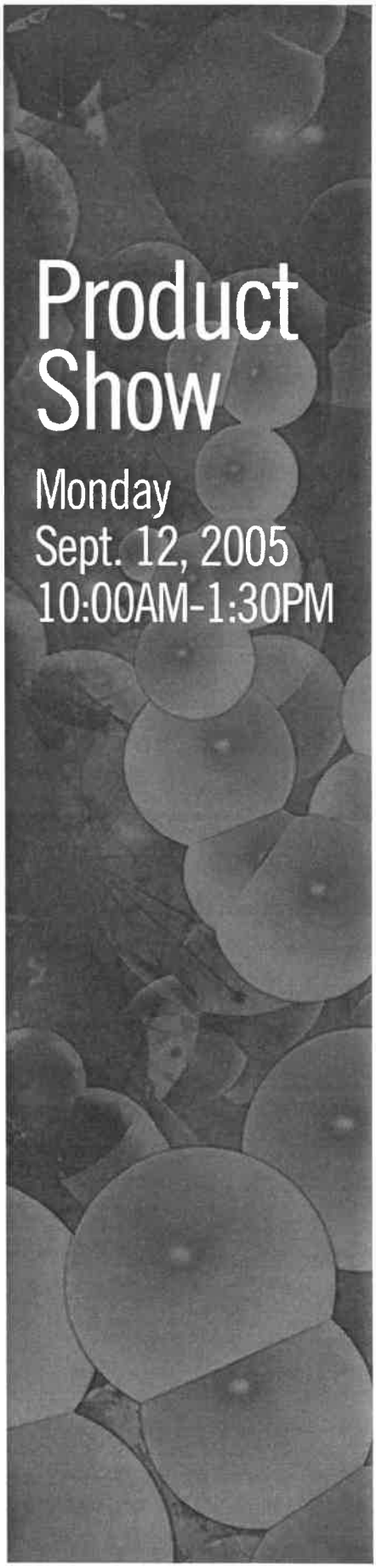
Opportunities for Clinical Therapy

8:30-8:45 AM	Macrophage-Like Behaviour of Adipose-Derived Stem Cells (ASC's) in Co-Culture with Lipid Droplets Prepared by Collagenase-Digestion of Adipose Tissues. *Miyazaki, T, Toriyama K, Torii, S, Kitagawa, Y.*
8:45-9:00 AM	Selective Expansion of Mesenchymal Stem Cells from Adult Adiposederived Stromal Vascular Fraction Cells by Low-Serum Culture and Humanization of Culture System of Autogenous Human Stem Cells. *Kobori, M, Omae, K, Toriyama, K, Torii, S, Kitagawa, Y.*
9:00-9:15 AM	Adipose Tissue-Derived Mesenchymal Stem Cell Yield and Growth Characteristics are Affected by the Tissue Harvesting Procedure. *Oedayrajsingh-Varma, MJ; Helder, MN; Klein-Nulend, J; Van Ham SM; Ritt, MJPF; Van Milligen FJ.*
9:15-9:30 AM	Low Serum and Serum Free Culture of Multipotential Human Adipose Stem Cells. *Parker, AM; Shang, H; Khurgel, M; Katz, AJ.*
9:30-9:45 AM	Cell Cycle Characterization Under Defined Media Conditions. *Sefcik, LS; Parker, AM; Shang, H; Katz, AJ; Botchwey, EA.*
9:45-10 AM	Effects of Uniaxial Cyclic Strain on Adipose-Derived Stem Cell (ASC) Morphology, Proliferation, and Differentiation. *Lee, WC; Rubin, JP; Maul, TM; Vorp, DA; Marra KG*
10:00-10:15 AM	Developing Isolation and Characterization Techniques for Porcine Adipose Stem Cells. *Blanton, MW; Johnstone, BH; Merfeld-Clauss, S; Li, J; Patel, NS; Rogers, PI, Sturek, M; March KI.*
10:15-10:30 AM	Coffee Break
10:30-Noon	**Symposium 4** Immunophenotypic Features of ASC's. Moderators: Anne Bouloumie, Frankfurt,Germany, & Louis Casteilla, Toulouse, France.
10:30-10:45 AM	Moderator Overview and Comments
10:45-11:00 AM	The Immunophenotype of Human Adipose Derived Cells: Temporal Changes in Stromal- and Stem Cell-Associated Markers. *Mitchell, JB; Mcintosh, M; Zvonic, S; Garrett, S; Floyd, ZE; Kloster, A; Halvorsen, YD; Storms, RW; Gimble JM.*
11:00-11:15 AM	The Immunogenicity of Human Adipose Derived Cells: Temporal Changes in Vitro. *Mcintosh, K; Zvonic, S; Garrett, S; Mitchell, JB; Floyd, ZE; Hammill, L; Halvorsen, Y; Ting, J; Storms, R; Gimble, JM.*
11:15-11:30 AM	Adipose Tissue-Derived Mesenchymal Stem Cells Do Express the Stem Cell Markers Cd34 and Cd117, but Expression is Lost Upon in Vitro Propagation. *Van Milligen, FJ; Oedayrajsingh Varma, MJ Helder, MN; Klein-Nulend, J; Van Ham, SM; Meijer CJLM.*
11:30-11:45 AM	Immuno-Characterization, Clonal Analysis and Differentiation of Prospectively Sorted Human Adipose-Derived Stem Cells. *Shang, H; Khurgel, M; Parker, AM; Katz, AJ.*
11:45-12:00 PM	Identification and FACS Purification of an Ubiquitous Population of Myogenic Stem Cells in Human Tissues, Including White Adipose Tissue. *Yap, S; Crisan, M; Zheng, B; Sun, B; Logar, A; Rubin, JP; Huard, J; Giacobino, JP; Peault, B.*
12:00-1:30 PM	Lunch
1:30-3:00 PM	**Symposium 5** Differentiation Potential of ASC's. Moderators: Patricia Zuk, Los Angeles, CA, & Jeff Gimble, Baton Rouge, LA.
1:30-1:45 PM	Moderator Overview and Comments
1:45-2:00 PM	Neural Differentiation of Adipose-Derived Stem Cells Harvested From GFP Transgenic Mice and Rats. *Fujimura, J; Ogawa, R; Mizuno, H; Fukunaga, Y; Suzuki,H.*
2:00-2:15 PM	Neural Differentiative Response in ASC's. *Tholpady, SS; Mcglynn, KA; Spano, A; Ogle, RA; Frankfurter, A; Katz, AJ, and Ogle, RC*
2:15-2:30 PM	Adipose Tissue Derived Mesenchymal Stem Cell Enhances Motor Function in Rats with Cerebral Infarction. *Jeong, JH; Kim, YW; Kim, YJ; Jang, HS; Kim, HS; Chang,Y.*
2:30-2:45 PM	Hascs in a Rat Mesenteric Model of Angiogenesis: Positional, Histomorphic, and Functional Evidence of a Perivascular Phenotype. *Peirce, SM; Bailey, AM; Shang, H; Katz, AJ.*

2:45-3:00 PM	A Population of Cd34-Positive Adipose Stromal Cells Share Hematopoietic, Mesenchymal, and Pericyte Surface Markers and Reside in a Perivascular Niche. *Traktuev, D; Li, J; Feng, D; Merfeld-Clauss, S; Johnstone, BH; March, KL.*
3:00-3:15 PM	Adipose Tissue-Derived Stem Cells Enhance Cardiac Function Following Surgically-Induced Myocardial Infarction. *Strem, BM; Jordan, MC; Daniels, EJ; Roos, EP; Schreiber, RE; Hedrick, MH; Fraser, JK; Maclellan, RM.*
3:15-3:30 PM	Preservation of Heart Function Following MI Using an Abundant Source of Autologous Stem Cells Derived from Adipose. *Johnstone, BH; Tsokolaeva, Z; Youssef, EA; Cai, L; Traktuev, D; Cook, Tg; Bolli R; March, KL.*
3:30-3:45 PM	Coffee Break
3:45-5:45 PM	**Symposium 6** Regulatory Affairs & FDA Perspective Moderators: John Fraser, San Diego, CA, & Keith March, Indianapolis, IN.
3:45-4:30 PM	"Regulatory Issues Surrounding the Clinical Use of ASC's." *FDA Representatives, Steven Bauer, Chief, Laboratory of Stem Cell Biology, FDA, Center for Biologics Evaluation and Research; and Bruce F. Schneider, Medical Officer, FDA, Center for Biologics Evaluation and Research*
4:30-4:50 PM	Industry Perspective "Translation of Potential Cell-Based Therapies To Commercial Products" *Alan Smith, Cognate Therapeutics*
4:50-5:10 PM	Industry Perspective *Mark Hedrick, Cytori Therapeutics*
5:10-5:45 PM	Discussion
5:45 PM	Adjourn. Dinner, Attendees on Their Own.

Tuesday, September 13, 2005

7-8:00 AM	Buffet Breakfast
8:15-10:15 AM	**Symposium 7** Bone and Cartilage Moderators: Roy Ogle, Charlottesville,VA, & Kacey Marra, Pittsburgh, PA.
8:15-8:30 AM	Moderator Overview and Comments
8:30-9:00 AM	Adipose-Derived Adult Stem Cells for Cartilage Tissue Engineering. *Farshid Guilak, Duke University*
9:00-9:15 AM	Impact of Gender and Harvest Depot on the Induction of Oseogenic Differentiation of Human Adipose-Derived Stem Cells. *Aksu, AE; Rubin, JP; Dudas, JR; Marra, KG.*
9:15-9:30 AM	Mesenchymal Stem Cells Derived From Bone Marrow and Adipose Tissue Possess Distinct Biologic Properties. *Bunnell, BA; Izadpanah, Trygg, C; Kriedt, C; Gimble JM.*
9:30-9:45 AM	Microdistraction of Osteoblast Precursors and Adipose-Derived Stem Cells Affects Their Osteogenic Differentiation Kinetics. *Gabbay, J; Mitchell, S; Zuk, PA; Bradley, JP.*
9:45-10:00 AM	Differentiation of ASC's to Osteoclasts in Vivo and in Vitro. *Tholpady, A; Tholpady, SS; Katz, AJ; Ogle, RC.*
10:00-10:15 AM	Co-Cultures of Adipose Stem Cells (ASC's) and Nucleus Pulposus Cells Under Chondrogenic Conditions Can Induce The Nucleus Phenotype in ASC's. *Helder, MN; Lu, ZF; Zandieh Doulabi, B; V. Milligen, FJ; Hoogendoorn, RJW; Bank, RA; Wuisman, PIJM.*
10:15-10:30 AM	Coffee Break
10:30-Noon	**Symposium 8** ASC's and ESC's: Memory and Synergy. Moderators: Adam Katz, Charlottesville, VA, and Peter Rubin, Pittsburgh, PA.
10:30-10:45 AM	Moderator Overview and Comments
10:45-11:00 AM	Adipose-Derived Stem Cells (ASC's) Inherit Adipose Tissues Under Strong Influence of Mature Adipocytes. *Kitagawa, Y; Miyazaki, T; Kobori, M; Omae, K; Takamatsu, S.*
11:00-11:15 AM	Adipose-Derived Stem Cells Express Multiple Genes in Common with Embryonic Stem Cells: Are Adult Stem Cells More Plastic Than We Think? *Zuk, PA, Hedrick, MH, and Benhaim, P.*
11:15-11:30 AM	Adipose Stem Cells as Autologous Feeder Cells for Human Embryonic Stem Cells. *Kokai, LE; Sammak, PJ; Rubin, JP; Jane, E; Marra, KG.*
11:30-11:45 AM	A Hierarchy of Proliferative Cells is Observed in Populations of Non-Hematopoietic, Cd34+ Pluripotent Cells Isolated from Adipose Tissues. *Merfeld-Clauss, S; Johnstone, BH; Ingram, DA; March, KL.*
11:45 AM	Closing Remarks and Adjourn



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Oral Abstracts

Oral Abstracts

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

Adipocyte-specific laminin α4 G domain fragments suppress *de novo* adipogenesis at the site of injection of Matrigel and fibroblast growth factor (FGF)-2.

Kazuhiro Toriyama, Chie Goto, Hironobu Yamashita, Rie Tajima, Ryo Narita, Shuhei Torii and Yasuo Kitagawa. Nagoya University, Japan

Appreciation of ubiquity and plasticity of adult stem cells has changed dramatically and adipose tissues are found to contain abundant progenitor cells (adipose-derived stem cells; ASCs) with the potential of angioblasts and mesenchymal stem cells. Thus, the mechanism suppressing ASCs to be quiescent in adipose tissues need to be elucidated. Gene expression of laminin α4 _chain increases depending on the adipose conversion of 3T3-L1 fibroblasts. Laminin G-like domain (LG) 4 module of α4 chain has strong affinity for syndecans. Finding that α4 LG3-5 is cleaved from laminin-8 (α4β1γ1) *in vivo*, we explored its potential function in suppressing ASCs using our test system of *de novo* adipogenesis at the site of injection of Matrigel and fibroblast growth factor (FGF)-2.

We have previously shown that subcutaneous injection into mice of Matrigel in combination with FGF-2 causes migration of endogenous stem cells into Matrigel space and their proliferation and differentiation result in *de novo* formation of adipose tissues. In this process, invading endothelial cells first form microcapillary network within three days and preadipocytes migrated to the vicinity of microcapillary then accumulate intracellular lipid droplets within a week. These processes are strictly dependent on the addition of FGF-2 to Matrigel. In this *de novo* adipogenesis, addition of α4 LG4-5 at 20 nM suppressed the adipogenesis completely. Activity of LG4-5 was cryptic within the whole structure of G domain since α4 LG1-5 showed only weak effect. Suppressive effect of α4 LG4 was lost in its mutants with reduced heparin-binding activity. The suppression was observed also by antithrombin III and counteracted by heparin. Antibodies against extracellular (but not cytoplasmic) domain of syndecan-2 and 4 suppressed the adipogenesis.

Many lines of evidence obtained in our research group suggested that ASCs inherit adipose tissues under strong influence of mature adipocytes, and this may explain the reason why ASCs deposit specifically in adipose tissues. We here suggest that laminin α4 LG modules may be the element of suppressive influence on ASCs as a component of the basement membrane coating every mature adipocyte.

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

A clinical trial of soft tissue augmentation by lipoinjection with adipose-derived stromal cells (ASCs).

Kotaro Yoshimura, M.D., Daisuke Matsumoto, M.D., and Koichi Gonda, M.D. Department of Plastic Surgery, University of Tokyo, Japan

Cosmetic soft tissue augmentation is one of big demands in the field of cosmetic surgery, but lipoinjection, a most common option, has yet several issues to be resolved such as a low survival rate of transplanted fat and a risk of fibrosis or calcification.

We found that a significant amount of ASCs can be isolated not only from the fatty portion of liposuction aspirates but also the fluid portion of it, and that suctioned fat is relatively stem-cell-deficient compared to the whole fat. Freshly-isolated ASCs were found as a CD34+CD31-CD45- fraction in SVF. ASCs could be cultured at least up to 20

weeks and about 20% of the ASCs maintained CD34 expression. Animal experiments showed that ASCs can boost the effectiveness of lipoinjection when transplanted with suctioned fat and the transplanted ASCs can survive and locate in the interstitial space of fat or differentiate into vascular endothelial cells.

Under permission of our IRB, we performed soft tissue augmentation with lipoinjection in combination with transplantation of freshly isolated ASCs (SVF) in 23 cases (22 females and 1 male) so far. SVF, which contained CD34+ ASCs (30-50%), CD31+CD45- endothelial cells (2-5%), and CD45+ white blood cells (40-60%) other than red blood cells, were injected with suctioned fat into the face or breasts for cosmetic augmentation. Patients have been followed up for 4 to 26 months, and satisfactory clinical results were obtained in all cases. Gradual reduction of the augmented volume usually seen after lipoinjection was minimal. Except for one case which showed fibrosis at the central area of the chest, no complications were found.

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

FGF-2 Delivery Enhances Adipose-derived Stem Cell Survival and Improves Vascularization in Soft Tissue Formation

DeFail, AJ; Clavijo-Alvarez, JA; Marra, KG; Taieb, A; Badylak, SF; Bennett, JM; Rubin, JP, University of Pittsburgh Division of Plastic and Reconstructive Surgery

Introduction: We have been assessing the controlled release of FGF-2 from biodegradable microspheres to determine the effect of FGF-2 on the enhancement of cell survival *in vivo*. Previously we examined the injection of small intestinal submucosa (SIS) particles seeded with adult adipose derived stem cells (ASCs) to form adipose tissue *in vivo*, utilizing a mouse subcutaneous model. The injection resulted in very few cells surviving *in vivo* after 90 days. H&E staining demonstrated the absence of adipocytes at the site of implantation in our previous experiment. The local release of FGF-2 has been shown to enhance stromal cell proliferation1 and promote adipogenesis2. Controlled-release and local delivery of FGF-2 is advantageous due to the short half-life of FGF-2 *in vivo*.

We hypothesize that the local delivery of FGF-2 from poly(lactic-co-glycolic acid), (PLGA), microspheres will increase cell survival *in vivo*. The *in vitro* release of FGF-2 from the microspheres at 37°C over two weeks was examined. We have combined FGF-2-loaded microspheres with SIS particles in an attempt to enhance cell survival in the mouse model and thus, increase soft tissue formation.

Materials and Methods: FGF-2 Encapsulation. FGF-2 was encapsulated in PLGA (75:25) using a double emulsion/solvent evaporation technique. Briefly, PLGA was dissolved in CH_2Cl_2 and mixed with FGF-2 and PVA solution to form an emulsion. The first emulsion was added to a stirring PVA solution followed by the addition of isopropyl alcohol. The microspheres were collected by centrifugation and lyophilized. Encapsulated microspheres were characterized in terms of size, morphology, encapsulation efficiency, and release profile. The *in vitro* release of FGF-2 from the microspheres in PBS was examined over 14 days and was quantified by ELISA.

in vitro studies. ASCs were seeded onto 24-well tissue cultured plates and incubated for 24 hours. The cells were subjected to one of the following four treatments for 72 hours: 1. 10% FBS media, 2. 10% FBS media + 1 ng/mL FGF-2, 3. 10% FBS media +FGF-2 microspheres, 4. 10% FBS media + empty microspheres. Cell viability was determined using CyQuant(TM) cell proliferation assay.

in vivo studies. Athymic nude mice were used. Fluorescently-labeled ASCs were seeded onto SIS particles in a spinner flask and incubated at 37°C and 5% CO2 for 6 days. The SIS particles were diluted in 200 µL of DMEM and injected with 1 cc syringes and 22G needles. The treatment groups were: 1. SIS particles + ASCs, 2. SIS particles + ASCs + FGF-2 microspheres, and 3. SIS particles + 1ng FGF-2. The animals were euthanized 14 days after implantation. Samples were collected by isolating the full thickness of the dermis and sectioning each sample for analysis. One half of each sample was used for H&E staining and the second half of the sample was prepared for fluorescent analysis to identify implanted cells.

Results and Discussion: The FGF-2 loaded PLGA microspheres exhibited a smooth surface morphology and were an average diameter of 9 μm. A burst release was observed in the first 24 hours of FGF-2 release *in vitro*. After 3 days, the release had reached steady-state, and the remaining FGF-2 had been released by day 14. *in vitro*, the addition of 1 ng/mL FGF-2 or FGF-2 loaded PLGA microspheres resulted in a significant increase in viable cell number. With the addition of FGF-2 microspheres, the injected SIS and ASCs resulted in greater quantities of labeled cells present and increased vascularization of the surrounding tissue.

Conclusions: We have encapsulated FGF-2 in PLGA microspheres and maintained release *in vitro* for 14 days. We have injected FGF-2 microspheres with ASC/SIS constructs into the mouse model. Injected labeled cells were identified within the site of implantation, and vascularization around the constructs was enhanced. The microsphere/ASC/SIS constructs represent an ideal modality for injectable therapies for soft tissue repair.

References :

[1] Yuksel, E. W., et al., Plastic and Reconstructive Surgery (2000) **105**; 1712-1720.
[2] Tabata, Y, et al., Tissue Engineering (2000) **6**; 279-289.

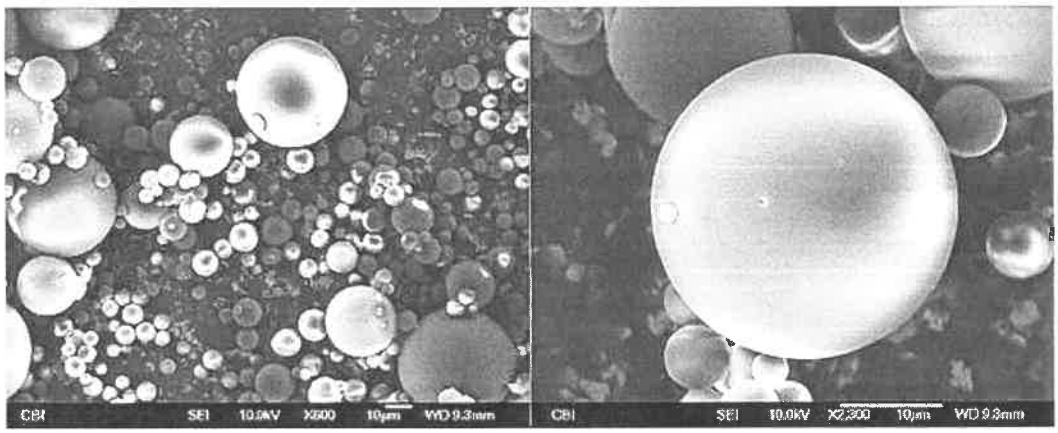

Figure 1. a) Scanning electron microscope image of FGF-2 encapsulated PLGA microspheres. b) SEM image higher magnification.

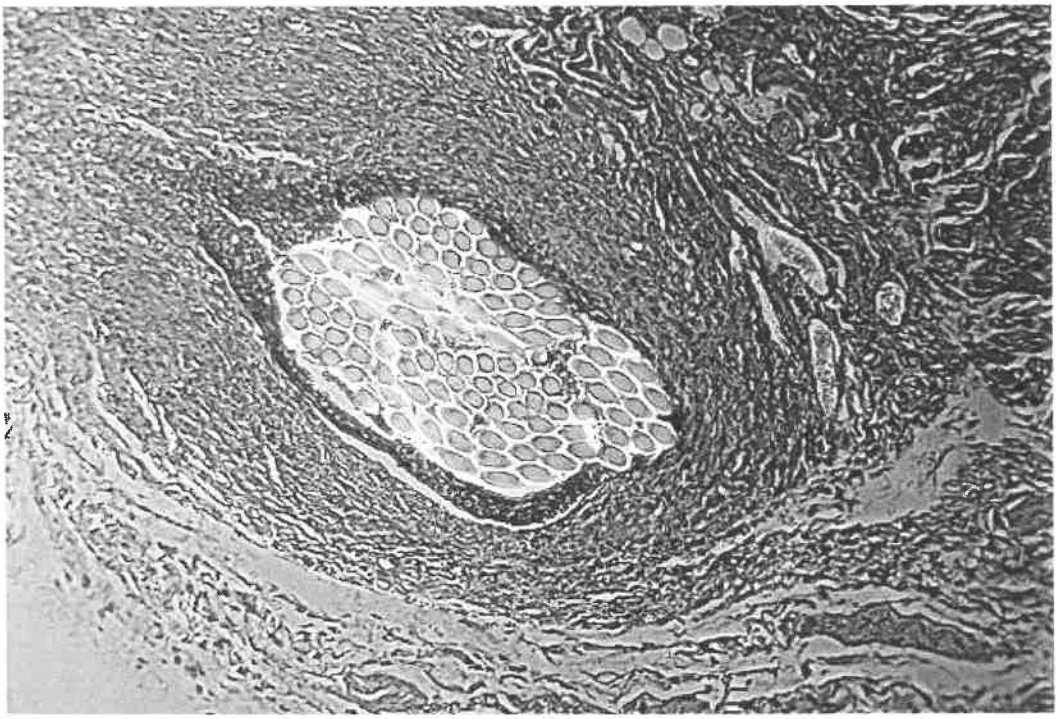

Figure 2. H&E staining of SIS + ASCs + FGF-2 loaded PLGA microspheres (100x).

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

Achieving Optimal Long-term Preservation of Human Adipose Tissues

Lee L.Q. Pu, MD, PhD, FACS, Division of Plastic Surgery, University of Kentucky, Lexington, KY, USA

Liposuction is one of the most common cosmetic surgical procedures performed in the United States and its popularity is increasing. However, adipose aspirates can only be used for immediate autologous fat transplantation during the same setting of the liposuction and adipose aspirates obtained from the procedure are usually discarded because there is no successful method available at present time. It has been a strong desire of both plastic surgeons and patients to be able to preserve the adipose aspirates after liposuction for possible future applications. Adipose aspirates from conventional liposuction can also be an excellent source of human stem cells confirmed by several recent studies. The modern cryopreservation technique has recently made the long-term storage of living cells or tissues possible for future clinical applications. In the past two years, a successful method (the combination of DMSO and trehalose as CPAs and the slow freezing and fast thawing protocol) for optimal long-term preservation of adipose tissues was developed in our laboratory. Further, the previously cryopreserved adipose aspirates were also shown by us to be a potential source of human stem cells because these adipose aspirates could still be processed later for processed lipoaspirate cells after optimal long-term preservation. Our novel approach appears to provide reliable long-term preservation of human adipose tissues and may indeed have a potential to develop a new era in cosmetic and reconstructive plastic surgery.

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

Short-term & long term effects of transplanted fat on aging and scarred skin

Sydney R. Coleman, MD, New York University Department of Surgery

The author has been grafting fat for restoration or creation of fullness in the face and body since 1987. He presents his observations of the changes which occur in skin overlying the grafted sites over time (for instance, immediately after implantation, in the first months, at one year, three years, five years, eight years et cetera). The volume of the fat appears to stabilize four months after the procedure, but the texture of sun damaged or scarred skin continues to improve dramatically in the ensuing months. The improvement usually continues up to five years and in many cases for almost a decade.

Our recent increased understanding of stem cell activity in normal adipose tissue leads the author to believe that such dramatic aesthetic improvements of skin overlying transplanted fat may be an effect of underlying stem cell activity. Coordinating these clinical observations with relevant research in adipose derived stem cells may promote our treatment of aging and scarred skin.

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

Histologic and Stem Cell Evaluation of Fat Overgrowth after Structural Fat Grafting to the Face

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This study looks at a case of remarkable fat overgrowth after structural fat grafting in a healthy, athletic male patient who did not undergo weight gain after the procedure. The overgrown fat and a control area of facial fat were extracted and evaluated histologically and for non-buoyant cell number, cell frequency, and yield of stem cells and progenitor cells with adipocytic or osteocytic potential.

The tissue extracted from the area of grafted fat overgrowth contained a three-fold higher number of non-buoyant (stromal) cells per unit volume of tissue. Within this expanded population there was a further expansion in progenitor and stem cells evidenced by a three-fold higher frequency of adipo-progenitor cells and a 50% increased frequency of stem cells in the absence of a simultaneous selective increase in osteo-progenitor cell frequency.

The data described in the present study do not support the hypothesis that injection site overgrowth was due to cell hypertrophy (increased volume of individual adipocytes) or to the accumulation of free lipid cysts, inflammatory infiltrate, or connective tissue. Rather, the data are consistent with the hypothesis that overgrowth observed at the site of injection is associated with increased number of adipocytes and an associated expansion of adipocyte precursor and progenitor cells.

This suggests an alternative explanation in which proliferation of stem and adipo-progenitors within the donor tissue, or post-transfer recruitment of cells, was activated by the process of transfer either through local release of paracrine growth factors or by the loss of enervation as a consequence of transfer. That is, the process of transfer may have changed the responsiveness of cells to local growth control cues or initiated recruitment of additional stem and progenitor cells.

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

Therapeutic angiogenesis for ischemic tissue by autologous transplantation of adipose-derived adult stem cells.

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Purpose: There are evidences that stem cells contribute to restore tissue vascularization and organ function. Significant clinical results were obtained with bone marrow stem cells after ischaemic events in limbs and myocardium. Human adipose tissue was proved to represent a rich source of mesenchymal stem cells (AMSCs) exhibiting multilineage potential and featuring secretion of angiogenic and antiapoptotic factors in animals.

Methods: Twenty consenting patients exhibiting a previously never described scleroderma-like chronic microangiopathy after radiotherapy were treated with transplants of lipoaspirate containing AMSCs directly injected into the radio-damaged areas. A computer-based procedure was used to plan the optimal patterns of lipoaspirate injection. Ultrastructural studies were performed on the lipoaspirate and on tissue samples from the radio-damaged areas, before transplantation and after 1, 2, 4-6 and 12 months.

The technique for AMSCs purification and injection within their natural scaffold is based on centrifugation. Cells culture is not necessary. Clinical results were evaluated with LENT-SOMA scale.

Results: At cytofluorimetric-based examination, human lipoaspirates resulted to contain at least $1.8x10^3$ AMSCs/cc. Ultrastructural analysis demonstrated that radiation-induced alterations of microcirculation were solved by a newly formed microcirculation after AMSCs transplant, leading to the restitutio ad integrum of the treated tissues.

Significant clinical improvements were constantly observed. In the group of 11 patients with LENT-SOMA scale grade 4 damage, 4 progressed to grade 0; 5 to grade 1 and 2 to grade 2. In the group of 9 patients with grade 3 damage, 5 progressed to grade 0 and 4 to grade 1.

Conclusions: This work is focused on human adipose mesenchimal stem cells, which have been recently found to secrete angiogenic factors in mices but have never been previously used for ischemic tissue neovascularization in humans. The study demonstrates the therapeutic efficacy of AMSCs, for the treatment of radiation-induced damages through a process of replacement of damaged tissue with reconstructed normal tissue. AMSCs procurement is easy and safe. The technique for AMSCs purification and injection within their natural scaffold is simple and low-cost and exhibits the lowest risk of micro-organism contamination.

SCIENTIFIC SESSION

CLINICAL/COMMERCIAL APPLICATIONS OF ASC's

In vivo differentiation of human adipose derived stem cells is independent of cell fusion

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Human adipose derived stem cells have been isolated and shown to have multileneage differentiation capacity. Although both plasticity and cell fusion have been suggested as mechanisms for cell differentiation *in vivo*, few have studied the effect of the local *in vivo* environment on the differentiation of adipose derived stem cells. We previously reported the *in vitro* capacity of smooth muscle differentiation of these cells. In the current study we evaluated the effect of an *in vivo* smooth muscle environment in the differentiation of these cells. We studied this by two experimental designs: 1) *in vivo* evaluation of smooth muscle differentiation of hASCs injected into a smooth muscle environment, 2) *in vitro* evaluation of smooth muscle differentiation capacity of hASCs exposed to bladder smooth muscle cells. Our results indicate a time dependent differentiation of hASCs into mature smooth muscle cells when these cells are injected into the smooth musculature of the rat urinary bladder. Similar findings were seen when the cells were co-cultured *in vitro* with primary bladder smooth muscle cells. Chromosomal analysis demonstrated that microenvironment cues rather than fusion are responsible for this differentiation. We conclude that the stem cell plasticity is present in hASC's and differentiation of hASCs to other cell types is *in vivo* and *in vitro* is accomplished in the absence of cell fusion.

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SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Macrophage-like behavior of adipose-derived stem cells (ASCs) in co-culture with lipid droplets prepared by collagenase-digestion of adipose tissues

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When unilocular mature adipocytes prepared by collagenase-digestion of adipose tissue and differential centrifugation of the dispersed cells were maintained with attachment to the ceiling surface of culture flasks filled with medium (ceiling culture), two fibroblastic cell populations appeared at the ceiling and the bottom surface. Both populations exhibited potential of unlimited proliferation, and differentiated along mesenchymal linage to produce adipocytes, osteoblasts and chondrocytes. These observations showed that the cells attached to unilocular adipocytes could generate stem cells with mesenchymal potential. We have recorded this process by a microscope focused to the ceiling surface and equipped with a time-lapsed video recorder.

After starting ceiling culture, a limited population of intact unilocular adipocytes slowly attached to the ceiling surface and showed weakly spread and extended morphology. Such extended unilocular adipocytes remained at the ceiling surface without further change. When we prepared the same adipocytes from mice expressing green fluorescent protein (GFP) in cytoplasm of all body, however, most adipocytes were found to be deprived of their plasma membranes and could not retain GFP in the cytoplasm. Such peeled adipocytes released its large lipid droplet, which was tentatively broken into smaller droplets. Fibroblastic cells contained in the floating layer of differential centrifugation together with adipocytes showed strong affinity to the peeled adipocytes and the released lipid droplets, and actively migrated at the surface of droplets. Such fibroblasts often gathered scattered smaller lipid droplets, which fused to each other to form larger droplets. The fibroblastic cells did not proliferate when attached to lipid droplets but proliferated actively when freed from lipid droplets. Similar behavior of fibroblastic cells was also observed in a ceiling culture system prepared by combining fibroblastic cells with lipid droplets generated by passing mature adipocytes through filter having pores of 0.45 μm. Thus, we found that adipose-derived stem cells (ASCs) exhibit macrophage-like behavior having affinity to lipid droplets.

In many previous reports on ceiling culture of mature adipocytes, unilocular adipocyte was suggested to partition their large lipid droplets to multiple and to lose its droplets gradually. It has been believed that unilocular adipocyte ultimately changes into fibroblastic cells and recovers the ability of mitotic proliferation. Although this morphology change of mature adipocytes has been understood with a keyword of "de-differentiation", we suggest that the macrophage-like behavior of ASCs has been overlooked in the previous reports and mature adipocyte was misunderstood to "de-differentiate".

SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Selective expansion of mesenchymal stem cells from adult adipose-derived stromal vascular fraction cells by low-serum culture and humanization of culture system of autogenous human stem cells

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Adipose tissue is a deposition site of adult stem cells (adipose-derived stem cells; ASCs) having high potential of differentiation. For the isolation of such stem cells, stromal-vascular fraction (SVF) cells, which are precipitated cells after collagenase-digestion of adipose tissue, have been cultured at low density for single colony isolation of stem cells that exhibit the potential of mesenchymal differentiation. We have previously established another method referred to as "ceiling culture". In this method, unilocular adipocytes in the floating fraction of the cells dispersed by collagenase-digestion of adipose tissues are maintained with attachment to the ceiling surface of culture flasks filled with medium. After a week of culture, two fibroblastic cell populations appear at the ceiling, and the bottom surface and both exhibited potential of unlimited proliferation and differentiated along mesenchymal linage. In these isolation methods, the media contain 10-20 % fetal bovine serum (FBS). Considering the unlimited self-renewing potential generally retained in stem cells, however, addition of high concentration of serum can be toxic since contained growth factors may induce uncontrolled differentiation of the stem cells. We report here that reduction of serum concentration to 2% is favorable for selective expansion of stem cells from SVF cells. This finding allowed us to humanize the isolation system of autogenous stem cells for direct clinical applications.

We cultured SVF cells in a high-serum (20 % of FBS) medium or a low-serum (2 % of FBS) medium containing hFGF-2, and compared growth and differentiation potential of the proliferated cells. Low-serum cultured cells exhibited more rapid growth rate than high-serum cultured cells, and they could be sub-cultured over 15th passages, which corresponded to the 40th population doubling level. SVF cells cultured in the low-serum medium showed high frequency of differentiation into adipocytes, osteoblasts and chondrocytes than the high-serum cultured SVF cells. We thus suggest that the stem cells in adipose tissue maintain their self-renewing cycle in the low-serum medium retaining the high potential of multiple differentiation. Low-serum cultured cells, as well as high-serum cultured cells, are positive for CD13, CD90 and CD105, and are negative for CD34, CD45 and CD117, indicating typical characteristics of mesenchymal stem cells.

The SVF cells from patients with ages ranging from 1 to 82 years old grew in the high-serum medium but the potential of osteogenic differentiation dropped markedly depending on the age of patients above 40 years old. When expanded in the low-serum medium on the other hand, the stem cells with osteogenic potential could be obtained even from a patient at 82 years old. This suggested that the low-serum medium is preferable for selective expansion of mesenchymal stem cells, of which population markedly dropped depending on the age.

We confirmed that 2 % human adult serum can substitute for 2 % FBS to give even better proliferation of the cells and to maintain the mesenchymal potential. Thus, we could completely humanize our culture system for direct clinical applications of autogenous human cells.

SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Adipose Tissue-Derived Mesenchymal Stem Cell Yield and Growth Characteristics are Affected by the Tissue Harvesting Procedure

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Adipose tissue contains a stromal vascular fraction which can be easily isolated and provides a rich source of adipose tissue-derived mesenchymal stem cells (ASCs). These ASCs are a potential source of cells for tissue engineering. We studied whether the yield and characteristics of ASCs were affected by the type of surgical procedure used for adipose tissue harvesting, i.e. resection, tumescent liposuction, and ultrasound-assisted liposuction. Frequencies of ASCs in the stromal vascular fraction were assessed in limiting dilution assays. The phenotypical marker profile of

ASCs was determined, using flow cytometry, and growth kinetics were investigated in culture. The number of viable cells in the stromal vascular fraction was not affected by the type of surgical procedure. After all three surgical procedures, similar subpopulations of stromal vascular cells did express a $CD34^{high}/CD31^{-}/CD105^{+}/CD45^{-}$ ASC phenotype. However, ultrasound-assisted liposuction resulted in a lower frequency of proliferating ASCs in culture (figure 1). In the stromal vascular fraction of resected adipose tissue 6.3 ± 1.7% (mean ± SEM, n=4) of the cells did adhere and showed proliferative capacity. In the stromal vascular fraction obtained by tumescent liposuction 2.9 ± 1.9% (mean ± SEM, n=4) of the cells did adhere and proliferate, and by ultrasound-assisted liposuction 0.4 ± 0.2% (mean ± SEM, n=5). Also the the population doubling time of ASCs obtained by ultrasound-assisted liposuction, 26.1 ± 4.6 days (mean ± SEM, n=4), was significantly longer when compared to the population doubling time of cells obtained by resection (2.4 ± 0.34 days (mean ± SEM, n=4) or tumescent liposuction (2.8 ± 0.64 days (mean ± SEM, n=4) (figure 2A, B, C and D). We conclude that yield and growth characteristics of ASCs are affected by the type of surgical procedure used for adipose tissue harvesting. Resection and tumescent liposuction seem to be preferable above ultrasound-assisted liposuction for tissue engineering purposes.

SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Low serum and Serum Free Culture of Multipotential Human Adipose Stem Cells

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Adipose tissue provides an easily accessible and abundant source of putative stem cells, ASCs, for translational clinical research. Currently prevalent methods of culturing these cells include the use of fetal bovine serum, a highly variable and undefined component, which brings with it the potential for adverse patient reactions to foreign proteins. In an effort to eliminate the use of animal products in human ASC cultures, we have developed two new culture methods, a very low human serum expansion media and a completely serum free media. The first method requires ½ human serum, an amount readily obtainable from a small blood draw. It yields population-doubling times averaging 1.41 days in early passage. The second, a completely serum free method, is a much slower culture system, with doubling times averaging 6.12 days. However, this method allows for the study of cells without the interference of undefined components that are present in all mammalian serum.

With the exception of a lower expression of CD34, averaging 12.4% in early passage, ASCs cultured in low serum or serum-free conditions express levels of ALDH, HLA, CD133, CD184, and CD31 that are comparable to those cells cultured in 10% Fetal Bovine Serum. The ASCs in these new culture conditions also maintain their ability to differentiate into adipo-, chondro-, and osteogenic lineages *in vitro*. These newly developed culture conditions provide a unique environment to study ASCs without the interference of animal serum and allow for the rapid expansion of autologous ASCs in culture in an animal product free environment for use in human clinical trials.

SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Cell Cycle Characterization under Defined Media Conditions.

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One of the main obstacles in stem cell research is the heterogeneity of the cells grown in culture. Recent work in the hematopoetic stem cells field suggests that "stemness" is a transitory state, which can fade and subsequently return in part as a function of the cell cycle. We have developed new low serum and serum free culture systems, which drastically affect the proliferation rates and, consequently, cell cycle characteristics of cultured ASCs.

In an effort to better understand these behavioral changes, we have examined the cell cycle of ASCs in three different culture medium conditions: DMEM with 10% FBS, serum free culture medium, and 0.5% human serum media. Data accumulated over three different patient donors all showed the same trends. Cells cultured in DMEM 10% FBS were found to be mostly in the G1 phase (75.68%), the cell cycle stage some have proposed to be optimal for inducing differentiation. Both serum supplemented and serum free culture conditions resulted in greater cell percentages in the G2+S phases (as great as 45%), indicating greater DNA replication and thereby suggesting more robust cell proliferation. Assuming that all cells entering the S-phase go on to divide, as many as half of the cells in 0% serum are actively dividing, whereas the number of dividing cells in DMEM 10% is less than 20% (Figure 1). These results suggest that our new defined culture medium conditions have the potential to significantly improve the *ex vivo* expansion potential of ASCs in low serum and serum free culture environments.

We aim now to relate the cell cycle distribution of a given population of multipotent ASCs with our defined media conditions to identify and optimize potentially fluctuating "stemness." Specifically, we will determine the appropriate media conditions needed to enhance the optimal cycle phase for differentiation along different phenotypic pathways for use in tissue engineering applications.

SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Effects of Uniaxial Cyclic Strain on Adipose-derived Stem Cell (ASC) Morphology, Proliferation, and Differentiation

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Introduction: There is a need for cellular therapies in vascular tissue engineering. An attractive source of autologous cells is adipose tissue. Adipose tissue is an abundant, accessible, and replenishable source of multipotent adult stem cells that can differentiate into a variety of cell types, including vascular smooth muscle cells (SMCs). Vascular SMCs populate the media of blood vessels and play important roles in the control of vasoactivity and the remodeling of the vessel wall. It is well documented that the phenotype and functions of vascular SMCs are regulated not only by chemical factors such as TGF-β1 but also by mechanical factors such as uniaxial strain. The purpose of this work was to explore the effects of TGF-β1 alone and in combination with cyclic strain on adipose-derived stem cell (ASC) morphology, proliferation, and differentiation.

Methods: Adipose-derived stem cells were isolated from human adipose tissue obtained from patients undergoing abdominoplasty via enzymatic digestion. Low passage ASCs were cultured on collagen-coated silicon membranes and were subjected to 10% uniaxial cyclic strain at 1Hz for 7 days, using a FX-4000T strain unit (Flexcell Corp., McKeesport, PA). Controls consisted of unstrained ASCs prepared in an identical manner. In order to study the combined effect of chemical and mechanical stimulation on ASCs, controls and strained ASCs were also cultured in media supplemented with 1 ng/mL TGF-β1 (R&D Systems, Minneapolis, MN) for the duration of 7 days, with medium changes every other day. Cell morphology and proliferation were examined, and the expression of SMC-specific markers was assessed via immunofluorescence staining and Western blot analysis.

Results: Our initial results indicated that cyclic strain significantly inhibited cell growth, and resulted in alignment of the cells perpendicular to the direction of strain. Immunofluorescence staining and Western blot analysis detected protein expression of early smooth muscle cell markers such as β-SMA and h1-calponin in both strained cells and controls. Uniaxial cyclic strain alone reduced the expression of both β-SMA and h1-calponin. Cells cultured under static conditions with TGF-β1 demonstrated a significant increase in the expression of β-SMA and h1-calponin, marked by the presence of pronounced actin stress fibers. ASCs cultured under strained conditions with TGF-β1 expressed β-SMA and h1-calponin in levels nearly comparable to that of the static controls treated with TGF-β1. Currently, we are reproducing these experiments to examine the expression of mature SMC-specific markers such as h-caldesmon, smoothelin and smooth muscle myosin heavy chain.

Conclusion: While the response of SMCs and other progenitor cells such as bone marrow stromal cells (BMSCs) to mechanical forces has been well-documented, the roles of these forces on ASCs remain unexplored. This work advanced our understanding of the mechanical regulation of ASCs and demonstrates the potential of combined chemical and mechanical stimulation in the differentiation of ASCs to SMCs. Future studies include examination of mechanically stimulated ASCs in murine cardiac infarct models.

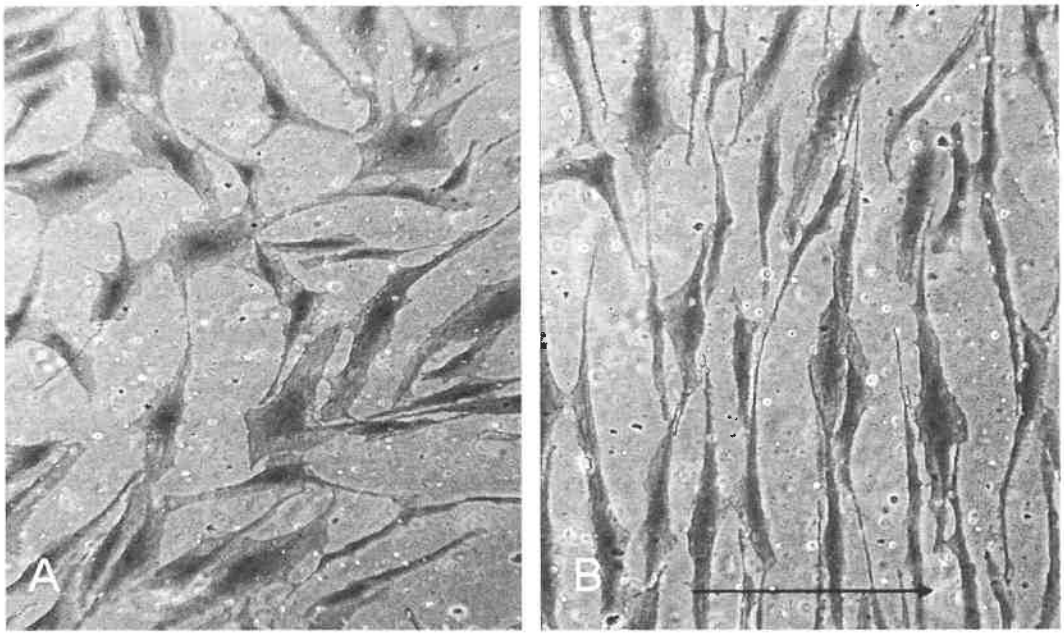

Figure 1: Coomassie Blue stained samples of ASCs cultured for 7 days under A) static conditions, and B) dynamic conditions

SCIENTIFIC SESSION

CULTURE CONDITIONS AND YIELD OF ASC's

Developing isolation and characterization techniques for porcine adipose stem cells.

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Introduction: Standardized isolation methods have been described to recover ASCs from subcutaneous adipose tissues of human, mouse and rat origin; however, the texture of subcutaneous swine fat is significantly different, primarily due to high fibrous tissue content, which prompted us to evaluate several ASC isolation methods as well as to compare the composition and processing requirements of a range of various porcine fat depots. In addition, we explored alternative tissue depots to determine if a source of fat with similar consistency as human existed in pig. Finally, we performed a limited characterization of the ASCs to determine the population make-up and to compare similarity to ASCs from other species. .

Methods: We obtained fat from domestic Yorkshire-cross (45-90kg) fed normal chow as well as Ossabaw swine that had been fed normal (65-75kg) or high fat (80-90kg) diets for approximately 2 years. Adipose tissue was collected post-mortem from the abdominal, inguinal, dorsal hump, omental, leaf, and jowl anatomical regions. Each fat sample

was weighed and placed in a 50ml conical tube, enzymatically digested with 2mg/ml collagenase + 10% FBS using a 1:1 (w/v) ratio at 37°C in a water bath for 3 hours with agitatation every 30 minutes. The samples were centrifuged once, the pellet was resuspended and filtered through a 100µm nylon cell strainer (BD Biosciences, Bedford, MA) before centrifuging again. The supernatant was discarded and the cell pellets were resuspended in EGM-2MV medium (Clonetics). Cells were counted and viability was determined using trypan blue staining. Each sample was plated in culture medium to determine appropriate ASC attachment, morphology, and cell growth. Flow cytometry was performed to CD90 and CD45 to determine the percentage of mesenchymal and leukocytic cells.

Results and Discussion: We screened many depots of fat obtained from Yorkshire-cross swine for ease of acquisition and cell yields. There was no significant differences in ASCs obtained from any of the regions tested; although, the dorsal hump region trended higher ($4.24x10^5 \pm 3.17x10^5$ cells/g; N=13) and was the easiest to access, with predicted lowest morbidity, compared to subcutaneous fat from the inguinal ($2.80x10^5 \pm 3.53x10^5$ cells/g; N=12) and the abdominal subcutaneous ($2.65x10^5 \pm 1.20x10^5$ cells/g; N=13) regions. A similar screen was performed on Ossabaw swine fat, with the inclusion of high fat and normal diets ($1.40x10^5 \pm 1.76x10^5$ cells/g; N=23 vs. $1.55x10^5 \pm 1.87x10^5$ cells/g; N=20 respectively). The yield from different anatomical locations was $1.65x10^5 \pm 2.71x10^5$ cells/g; N=6 (omentum), $1.45x10^5 \pm 1.84x10^5$ cells/g; N=3 (jowl), $1.39x10^5 \pm 1.67x10^5$ cells/g; N=9 (leaf), $1.00x10^5 \pm 1.74x10^5$ cells/g; N=7 (inguinal), $8.95x10^4 \pm 9.46x10^4$ cells/g; N=10 (abdomen), and $8.11x10^4 \pm 1.45x10^5$ cells/g; N=5 (dorsal hump).

The morphology of the ASCs was similar to that seen from other species, and the cells exhibited robust growth in culture and expanded by over 1013 cells after 3 months in culture. The majority of ASCs were positive for CD90 (63.92 ± 7.60%); while 16.11 ± 11.21% of initially-isolated cells expressed the leukocytic marker CD45. This data suggests that the porcine ASCs are similar to those obtained from other species.

The effect of adiposity on the yield of ASC was determined by inducing obesity through high fat diet in the pre-diabetic Ossabaw swine. Diet did not significantly influence the yield of ASCs obtained from any region, even though it was shown that the high fat-diet induced a substantial increase in overall adiposity.

Conclusions: In this study ASC yields from different depots or anatomical locations were examined in domestic swine as well as Ossabaw swine predisposed to the metabolic syndrome. Based on cell morphology, growth *in vitro* and initial flow cytometry, porcine ASCs appear similar to those isolated from human, mouse and rat. These data support the study of autologous porcine ASCs in wound healing and tissue injury models.

SCIENTIFIC SESSION

IMMUNOPHENOTYPIC FEATURES OF ASC's

The immunophenotype of human adipose derived cells: Temporal changes in stromal- and stem cell-associated markers

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Adipose tissue represents an abundant and accessible source of multipotent adult stem cells and is used by many investigators for tissue engineering applications; however, not all laboratories use cells at equivalent stages of isolation and passage. We have compared the immunophenotype of freshly isolated human adipose tissue-derived Stromal Vascular Fraction cells (SVFs) relative to serial passaged Adipose-derived Stem Cells (ASCs). The initial SVFs contained Colony Forming Unit-Fibroblasts (CFU-F) at a frequency of 1:30. Colony Forming Unit-Adipocytes (CFU-Ad) and –Osteoblasts (CFU-Ob) were present in the SVF at comparable frequencies (1:40 and 1:12, respectively). With progressive passage, all CFU frequencies increased. The immunophenotype of the adipose derived cells based on flow cytometry changed progressively with adherence and passage. Stromal cell associated markers (CD13, CD29, CD44, CD63, CD73, CD90, CD166) were initially low on SVFs and increased significantly with successive passages. The stem cell associated marker CD34 was at peak levels in the SVFs and/or early passage ASCs and remained present, although at reduced levels, throughout the culture period. Aldehyde dehydrogenase (ALDH) and the multidrug resistance transport protein (ABCG2), both of which have been used to identify and characterize

hematopoietic stem cell, are expressed by SVFs and ASCs at detectable levels. Endothelial cell associated markers (CD31, CD144 or VE-cadherin, VEGF receptor 2, von Willebrand factor) were expressed on SVFs and did not change significantly with serial passage. Thus, the adherence to plastic and subsequent expansion of human adipose-derived cells in fetal bovine serum supplemented medium selects for a relatively homogeneous cell population, enriching for cells expressing a "stromal" immunophenotype, as compared to the heterogeneity of the crude stromal vascular fraction.

SCIENTIFIC SESSION

IMMUNOPHENOTYPIC FEATURES OF ASC's

The immunogenicity of human adipose derived cells: Temporal changes *in vitro*

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Regenerative medical techniques will require an abundant source of human adult stem cells that can be readily available at the point of care. The ability to use unmatched allogeneic stem cells will help achieve this goal. Since adipose tissue represents an untapped reservoir of human cells, we have compared the immunogenic properties of freshly isolated human adipose tissue-derived Stromal Vascular Fraction cells (SVFs) relative to passaged Adipose-derived Stem Cells (ASCs). The expression of hematopoietic associated markers (CD11a, CD14, CD45, CD86, HLA-DR) on adipose-derived cells decreased with passage. In mixed lymphocyte reactions (MLRs), SVFs and early passage ASCs stimulated proliferation by allogeneic responder T cells. In contrast, the ASCs beyond passage P1 failed to elicit a response from T cells. Indeed, late passage ASCs actually suppressed the MLR response. Thus, the adherence to plastic and subsequent expansion of human adipose-derived cells selects for a relatively homogeneous cell population based on immunophenotype and immunogenicity. These results support the feasibility of allogeneic human ASC transplantation, although confirmatory *in vivo* animal studies will be required.

SCIENTIFIC SESSION

IMMUNOPHENOTYPIC FEATURES OF ASC's

Adipose tissue-derived mesenchymal stem cells do express the stem cell markers CD34 and CD117, but expression is lost upon *in vitro* propagation.

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Adipose tissue contains a stromal vascular fraction that can easily be isolated. This stromal vascular fraction is a rich source of adipose tissue stem cells (ASCs), that have the capacity to differentiate into several cell types of the mesodermal lineage. We are interested in using these freshly isolated, non-cultured ASCs for tissue engineering purposes. Therefore we characterized the phenotype of ASCs directly after isolation and upon culture, using flow cytometry. Of the stromal vascular fraction cells 78% ± 2.3% (mean ± SD) stained positive for CD34. Based on the level of CD34 expression, and the expression of endothelial marker CD31, cell populations were isolated by FACS®, and culture expanded. The $CD34^{bright}CD31^{-}$ cell fraction represented 34.6% ± 17.8% (mean ± SD, n=5) of the total amount of cells and contained cells exhibiting growth characteristics of ASCs. These cells demonstrated a homogeneous expression of CD105, HLA-ABC, HLA-DR, CD90, CD117 and CD54. Expression of CD166 was very low,

and cells were negative for CD31, CD146 and CD106. When SVF cells were expanded *in vitro*, the ASCs did typically grow as adherent cells, and exhibited a fibroblast-like morphology. The mean population doubling time of the cells in passage 3 was 2.4 ± 0.34 days (mean ± SEM, n=4). Upon culture of ASCs, expression of HLA-DR, CD34 and CD117 decreased, whereas expression of CD105 and CD166 increased. Our results demonstrate that freshly isolated ASCs do express the stem cell markers CD34 and CD117, which are lost upon culturing of the cells. The high CD34 expression can be used as a tool for the development of an ASC isolation procedure.

SCIENTIFIC SESSION

IMMUNOPHENOTYPIC FEATURES OF ASC's

Immuno-characterization, clonal analysis and differentiation of prospectively sorted human adipose-derived stem cells

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Clonal analysis of putative stem cells is widely considered a critical test of "stemness". To better characterize populations of promising adult adipose stem cells, we employed initially flow cytometry to identify specific cell surface markers, and subsequently FACS sorted for the putative stem cell markers CD34 and ALDH. Sorted cells were used in single cell assays to assess the clonal and differentiation potential of their respective populations.

Using flow cytometry, we found hASC populations to contain cells positive for CD34 (mean of initial plating =94.1% and SD=2.97, n=5) and ALDH (mean=11.86% and SD=2.92; n=7). The level of CD34 staining was highly dependent on the antibody used, and the time in culture. In addition, early passage (P≤2) hASCs included a small percentage (1.0-2.4%) of cells expressing lineage markers (such as CD31, CD144, CD62P, CD106 or CD133), consistent with an endothelial phenotype. The low-level detection of those markers decreased further with successive culture expansion. A majority (97-98%) of hASCs were positive for CD29, CD49e, CD51, CDw90, and CD105. The presence of those latter markers remained at similar levels in late passages.

Using the putative stem cell markers CD34, and ALDH, four target populations ($CD34^+ALDH^+$, $CD34^-ALDH^+$, $CD34^-ALDH^+$, and $CD34^-ALDH^-$) were subsequently single sorted into 96 well plates to measure clonal and differentiation potential. Each population demonstrated similar (14-17%) colony-formation efficiency. When the resulting colonies were expanded and subsequently grown in adipogenic or osteogenic medium, all clonal populations demonstrated a similar differentiation potential. Therefore, our results suggest that hASCs contain a subset of cells that stain for the stem cell markers CD34 and/or ALDH. However, prospective enrichment of subpopulations based on these markers failed to demonstrate any significant differences in colony formation or directed differentiation.

SCIENTIFIC SESSION

IMMUNOPHENOTYPIC FEATURES OF ASC's

Identification and FACS Purification of an Ubiquitous Population of Myogenic Stem Cells in Human Tissues, including White Adipose Tissue

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The presence of multipotent stem cells in diverse tissues of the adult has been indirectly documented. Notably, the stromal vascular compartment within human white adipose tissue (hWAT) contains elusive multi-lineage "adipose stem cells" (ASC). The origin, identity and anatomic distribution of ASC within hWAT, as well as those of other multipotent progenitors such as MAPC, MSC or MDSC in other tissues, remain unknown. We have recently postulated that cells within the walls of adult blood vessels can be at the origin of these primitive progenitors. To test this hypothesis, we have first tested the myogenic capacity of endothelial cells and endothelium-related cells such as pericytes, which are closely adherent to the non-luminal aspect of endothelial cells, in a number of adult human tissues. In the case of hWAT, tissues were obtained from abdominal subcutaneous fat in the course of cosmetic surgery. After thoroughly digesting the tissue, stromal vascular cells (SVC) were separated from mature adipocytes by centrifugation. On average, we obtained about 3.5 x 10^5 SVC per gram of hWAT. We then performed the FACS analysis of total SVC, and separated pericytes and endothelial cells from the other cell populations within the SVC by cell sorting using anti-CD34, anti-CD144, anti-CD45 and anti-CD146 antibodies. Endothelial cells were selected on the basis of CD34 and CD144 expression and absence of CD45, and comprised up to 70% of the total SVC. Pericytes were selected on CD146 expression and lack of CD34 and CD45. They comprised approximately 15% of the total SVC. Endothelial cells sorted from hWAT did not exhibit any myogenic potential *in vivo*. Conversely, pericytes (CD146+ CD45- CD34-) sorted from hWAT differentiated into spectrin-positive myofibers when transplanted into the muscles of NOD-SCID mice prealably conditioned by injection of cardiotoxin. Quantitatively, the myogenic potential of fat-derived pericytes outclassed that of myogenic progenitors sorted from human skeletal muscle. Most importantly, none of the other cell subsets constituting the stromal fraction of hWAT exhibited myogenic potential. We suggest that the progenitor cell potential previously evidenced within hWAT is associated with a subset of peri-vascular cells. The fact that we have obtained the same results with pericytes sorted from other human tissues such as pancreas, muscle and bone marrow further indicate that these peri-vascular cells represent an ubiquitous reserve of primitive progenitors.

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

Neural Differentiation of Adipose-Derived Stem Cells Harvested from GFP Transgenic Mice and Rats

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Background: In previous studies about neural differentiation using adipose-derived stem cells (ASCs) or bone marrow-derived stem cells, there are a few negative opinions that the morphological changes resulted from rapid disruption of the cytoskeleton due to the cytotoxic medium. About neural differentiation using mesenchymal stem cells, there are many opinions. Thus, using not only immunocytochemial stainings but also electron micrographs, we assessed the ASCs after neural induction. Moreover, we tried to establish the experimental system using GFP transgenic animals for the further studies *in vivo*.

Methods: ASCs were isolated from inguinal fat pads of GFP transgenic mice and rats. The cells were cultured in control medium (DMEM and 10% FBS) for several passages, after that the medium was changed into neural induction medium (DMEM, insulin, indomethacin and isobutylmethylxanthine). The cells were assessed using electron micrographs and immunocytochemical stainings.

Results: After neural induction, the ASCs developed characteristic round cell bodies with several branching extensions and also expressed GFP fluorescence. Electron micrographs revealed that most of the neurally induced cells had many microtubules in their soma and processes. Immunocytochemical studies showed that the neurally induced cells expressed positive for neuronal and glial markers.

Conclusions: Using the electron microscopic images, we proved that neurally induced cells possessed microtubules in their soma and processes, which provides strong evidence that the neurally induced cells in the present study had neuronal structures. Furthermore, neural differentiated cells harvested from GFP transgenic animals were detected clearly under fluorescent microscope. Therefore, it was considered that this cell population was useful for the further experiments *in vivo*. We are now trying to make the animal models which have neurological damages and to make use of the ASCs derived from GFP transgenic animals for therapy.

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

Neural Differentiative Response in ASCs

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Adipose stem cells (ASCs) can be induced to differentiate *in vitro* into cells that express a dendritic morphology and gene expression profile characteristic of neuronal cells by treatment with a variety of antioxidants, agents that elevate cAMP or serum withdrawal. Human subcutaneous ASCs and rat visceral fat ASCs were treated by the beta-mercaptoethanol induction procedure (Woodbury et al) and analyzed for neural morphology (number and length of neurites) and expression of neuronal markers including beta 3 tubulin, neural specific enolase, intermediate filament M, microtubule associated protein 2, and microtubule associated protein tau (Tau). The cellular morphology of differentiated cells was highly dependent on the type of extracellular matrix (laminin, collagen, vitronectin or fibronectin) and fibroblast growth factor (FGF-2 or FGF-10) employed. Unlike other types of neuroblastic cells, the ASCs show no preference for laminin substrates in promotion of neurite formation. Analysis of mRNA expression for neural-specific genes by Affymetrix arrays and RT/PCR showed up regulation of transcripts in the differentiation-treated cells. Post-translational modifications and correlation of morphological changes and expression of markers were followed by immunofluorescence microscopy and Western analysis employing monoclonal antibodies. Beta 3 tubulin, intermediate filament M and neural specific enolase could be detected in undifferentiated ASCs and were clearly upregulated in cells treated to differentiate prior to formation of neurites. This observation may reflect the requirement to synthesize a pool of cytoskeletal structural proteins prior to their assembly into microtubules and intermediate filaments. These results also suggest that ASCs could serve as a valuable cell culture model for neural differentiation and studies of the cytoskeleton of neuronal cells.

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

Adipose Tissue Derived Mesenchymal Stem Cell Enhances Motor Function in Rats with Cerebral Infarction

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Objective: To investigate whether the adipose tissue derived stem cells (ADSC) enhance the functional recovery of the cerebral infarction and to know the fate of the treated stem cell, we have evaluated the recovery of motor function and also tracked the cells after intravenous or perilesional administration of ADSC in rats with cerebral infarction.

Method: The cerebral infarction was induced by the MCA occlusion in 30 rats. The cerebral infarcted rats were randomly divided into three groups; vehicle treated control group, intravenous ADSC treated experimental group 1, perilesional ADSC treated experimental group 2. The ADSC were obtained from inguinal fat pad of each rat and culture expanded in DMEM with 10% FBS for two weeks. At 2 weeks of cerebral infarction, approximately 1x106 cells were injected intravenously or directly into the perilesional area of each rat. The rotor rod test for motor function evaluation was done at -2, 0, 2, 4, 6, and 8 weeks of the ADSC treatment. For tracking of the injected ADSC *in vivo*, cultured ADSC were tagged by magnetic labeling technique before injection and serial MR image was taken, which visualize the location of the Feridex transfected ADSC.

Results: The motor function assessment by rotor rod test at 0 day of the cell therapy were nearly zero second among the groups. However, the motor function was recovered in experimental group 1 (intravenous treated) at 2 to 3 weeks of ADSC treatment, while there was no recovery in experimental group 2 as well as in control group at that time. The motor function in experimental group 2 (perilesional treated) was recovered to pre-infraction level at 4 weeks of ADSC treatment. The degree of recovery of motor function in control group was less than half of the pre-infraction level until 8 weeks of the experiment. Serial MR imaging after Feridex transfection in experimental group 1 (intravenous treated) showed progressive accumulation and migration of ADSC from contralateral normal cerebral hemisphere to infarcted area across the corpus callosum. In experimental group 2, also revealed migration of ADSC from superficial perilesional treated area to the deeper part of infarction.

Conclusion: ADSC therapy improved motor function recovery in the rats with cerebral infarction. In serial MR imaging with Feridex transfection technique showed migration of the treated ADSC into the damaged area.

Key Words: Adipose tissue derived mesenchymal stem cell, Cerebral infarction, *in vivo* tracking of stem cell

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

hASCs in a Rat Mesenteric Model of Angiogenesis: Positional, Histomorphic, and Functional Evidence of a Perivascular Phenotype

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Introduction: Human adipose-derived stromal cells (hASCs) have been shown to differentiate into endothelial cells, form vascular-like sprouts in matrigel, enhance neovascularization in an ischemic hindlimb model, and secrete angiogenic and anti-apoptotic growth factors, suggesting a potential for this cell population in therapeutic vascularization. In a rat model of angiogenesis, we investigate, at the single cell level, the potential of hASCs to contribute to microvascular remodeling when injected *in vivo*, and we have found that injected hASCs migrate to the abluminal surface of microvessels, alter their cell morphology to conform to the curvature of the microvessel in a manner that is consistent with perivascular cell behavior, augment vascular density, and express perivascular cell markers.

Methods: Human ASCs and lung fibroblasts were isolated, expanded in culture, labeled with the fluorescent marker DiI, and injected into adult nude rat mesenteries stimulated to undergo microvascular remodeling via Compound 48-80, a mast cell degranulating agent that induces inflammation. 10, 30, and 60 days after cell injection, mesenteric tissues were harvested and processed with immunohistochemical techniques to determine hASC quantity, positional fate in relation to microvessels, expression of endothelial and perivascular cell markers, as well as vascular density.

Results: After 60 days, 29% of total hASCs exhibited perivascular morphologies compared to 11% of injected human lung fibroblasts (hLFs). Total vascular density was increased 46% in tissues treated with hASCs over age-matched controls that did not receive cell injections. Some hASCs exhibiting perivascular morphologies also expressed markers characteristic of vascular pericytes: smooth muscle a-actin (10%) and NG2 (8%).

Conclusions: This study demonstrates that hASCs can exhibit perivascular morphology when injected *in vivo*, express pericyte lineage markers, and contribute to increases in microvascular density during angiogenesis associated with inflammation.

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

A Population of CD34-positive Adipose Stromal Cells Share Hematopoietic, Mesenchymal, and Pericyte Surface Markers and Reside in a Perivascular Niche.

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Introduction: It has been shown by our group and others that short pre-plating (1-3 days) of freshly-isolated adipose stromal cells (ASC) on plastic results in an enrichment of the $CD34^+$ population (>98%), while further cell culturing results in decrease of the number of $CD34^+$ cells. While data has also demonstrated a high level of ASC plasticity, the secretion of pro-angiogenic factors, protective effects on ischemic tissue, relatively little is understood about the normal functions and responses of these cells, and their physiological roles in adipose tissue. Accordingly, we are evaluating co-expression of endothelial, mesenchymal, and pericyte/smooth muscle cells markers by flow cytometry and western blot in a $CD34^+$ cell population, evaluated effects of single growth factors on their proliferation, and determined their location in tissues in situ by immunohistology.

Methods: Enzymatically-isolated ASC plated for 1-3 days in EGM-2MV medium were evaluated by flow cytometry, with multiple staining of the cells for CD34 along with key mesenchymal (CD10, CD13, CD90, CD105), endothelial (CD31, CD144), and pericytic (CD140b) markers. We also employed western blot for analysis of expression by $CD34^+$ cells antigens of additional pericytic (NG2) and SMC (calponin, caldesmon) proteins. Further experiments were designed to test the proliferative responses of ASC to individual growth factors: VEGF, HGF, bFGF, EGF, IGF and PDGF-BB. Freshly isolated and passage 1 cells were cultured for 6 days, followed by cell counts.

Results: After 3 days in culture, 98% of ASC express CD34 and are negative for CD45. Dual staining of $CD34^+$ cells revealed that more than 95% of the cells were positive for CD10, CD13, CD90, markers of mesenchymal stromal / stem cells, as well as for $CD140b^+$ which is the marker of pericytes, while typically less than 10% of the cells express markers of endothelial cells - CD31 and CD144. After day 3, cells enter into a proliferative stage with a period of doubling of 18-24 hours. Subsequently, the intensity of CD34 expression is progressively attenuated, while the other markers (CD10, CD13, CD90, CD140b) are conserved. Western blot analysis shows that the $CD34^+$ cells also express caldesmon, calponin, and NG2 - markers of pericyte and SMC. Both fresh and passaged cells respond with mitogenic effect to PDGF-BB (280%), bFGF (200%) and EGF (200%), in comparison with control medium (EBM-2/5%FBS), while VEGF and HGF were not able to stimulate their proliferation.

Conclusion: Based on the results of our experiment, we conclude that: 1) the majority of freshly isolated preplated cells share the CD34 marker with markers of mesenchymal stromal cells as well as pericytes/SMC; 2) entrance of $CD34^+$ cells into the logarithmic stage of proliferation is associated with a dramatic decrease in cell surface CD34, which disappears entirely at late passage; 3) PDGF-BB and bFGF and EGF have significant influence on ASC proliferation, supporting that the cognate surface receptors for these factors are functional on ASC. Histologic evidence links this defined population with a perivascular niche in situ.

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

Adipose Tissue-Derived Stem Cells Enhance Cardiac Function Following Surgically-Induced Myocardial Infarction

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Background: Adipose tissue has recently been identified as a source for adult stem cells. The aim of this study was to determine whether adult adipose tissue-derived stem cells (ADCs) can improve left ventricular (LV) function after an acute myocardial infarction (AMI).

Methods: An AMI was surgically-induced by occluding the left coronary artery for 60 minutes in female Lewis rats (225-275 g, n=18). Following 15 minutes of reperfusion, 5x10^6 freshly isolated, syngeneic ADCs (n=10) or saline (n=8) were injected into the LV chamber, an approximation of intracoronary delivery. Two-dimensional echocardiography (echo) was used to evaluate LV function prior to AMI and 12 weeks post AMI. In addition, left ventricular pressures (LVP) were obtained via Millar catheterization at the time of sacrifice. Statistical analysis was performed using the student's t-test and reported as mean ± SEM.

Results: Echo at 12 weeks showed a significant improvement in ejection fraction in ADC-treated animals (76.0 ± 0.9 % vs. 68.3 ± 1.9 %, p=0.008) and a reduction in ventricular septal thickness (1.22 ± 0.03 mm vs. 1.46 ± 0.12 mm, p=0.027). LVP measurements showed improved contractility and relaxation in the cell treated group (+dP/dT: 5494.46 ± 550.76 mmHg/s vs. 2837.61 ± 301.19 mmHg/s, p=0.015; -dP/dT: -6323.28 ± 544.61 mmHg/s vs. -2716.49 ± 331.83 mmHg/s, p=0.003). Conclusion: Cellular cardiomyoplasty using adipose tissue-derived stem cells significantly improves ejection fraction and contractility parameters following an acute myocardial infarction. In addition, cell treated animals demonstrated a reduction in LV remodeling and the compensatory hypertrophy post AMI, evidenced by a reduction in septal thickness. These data provide the basis for continued functional and mechanistic investigation of ADCs for the treatment of ischemic heart disease.

SCIENTIFIC SESSION

DIFFERENTIATION POTENTIAL OF ASC's

Preservation of Heart Function Following MI Using an Abundant Source of Autologous Stem Cells Derived from Adipose

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Background: Stem cell-based therapies hold tremendous promise for treating many cardiac syndromes. Adipose tissue stroma is a source of abundant pluripotent, CD34+/CD45- cells (adipose stem cells; ASCs). Here we report on the ability of ASCs to improve cardiac function following myocardial infarction in rats.

Methods and Results: Male Lewis rats underwent 30 min LAD occlusion and reperfusion, followed by randomization to 4 groups (n = 5-6). At 5 min post-reperfusion, saline or 10^6 cells was injected into the border zone. The cells were freshly isolated ASCs (fASCs), cultured ASCs (cASCs) or lethally irradiated ASCs (LI-ASCs). GFP-labeled fASC and cASC were detected in the myocardium at 3 days, suggesting that both cell types were competent for early tissue incorporation. At 1 month, only cASC-treated rats exhibited significant improvement in heart function over controls (serial echocardiography). The LV ejection fraction of rats given cASCs was 46.4%+7.2 (mean + SEM) vs. 36.9%+3.0 for saline and 30.9%+4.6 for LI-ASCs ($P<0.01$ for each). Fractional shortening, cardiac output and stroke volume were also significantly improved with cASC treatment ($P<0.02$ for all). Interestingly, by 2 months these effects diminished, whereas hearts treated with fASCs were significantly better than saline control ($P<0.04$ for EF and FS). Anterior wall thinning was attenuated in the fASC group (2.84+0.12 mm vs. control 2.44 + 0.11 mm, $P<0.03$). Systolic pressures and +dP/dT were both greater in the fASC group at 2 months ($P<0.04$). These results may be explained by the fact that fASCs exhibit a lag before proliferating *in vitro* and express very low levels of growth factors such as VEGF during this quiescent period, implying support of ischemic tissues or promotion of myocardial regeneration may be delayed.

Conclusions: These data demonstrate the feasibility of preserving cardiac function following MI by treating with ASCs, an easily accessible and abundant source of autologous stem cells that may possess broad utility for treating cardiac syndromes. The observed influence of cell processing on effect may have broad implications for the translation of ASC therapy, and cell therapies in general. These data provide a rationale for investigating the usefulness of this approach in patients with ischemic heart disease.

SCIENTIFIC SESSION

BONE AND CARTILAGE

Impact of Gender and Harvest Depot on the Induction of Osteogenic Differentiation of Human Adipose-Derived Stem Cells

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Introduction: Adipose tissue represents an abundant, practical, and appealing source of donor tissue for autologous cell replacement. It has been demonstrated that stem cells within the subcutaneous adipose tissue display multilineage developmental plasticity, and under appropriate conditions, can mineralize their extracellular matrix, and thus, can undergo osteogenesis. The aim of this study is to examine *in vitro* osteogenic differentiation properties of adipose-derived stem cells (ASCs), to assess the role of gender and fat depot as variables linked to osteogenic differentiation, and to demonstrate the optimal time for differentiation. Drawing conclusions from the *in vitro* experiments, then, provides us with the understanding of the best combination of variables for differentiation. This data will be preparatory for the application of these combinations to differentiation modalities on scaffolds, particularly for an *in vivo* athymic mouse calvarial defect osteogenesis model.

Methods: Human ASCs, isolated from superficial and deep adipose layers of the abdominoplasty specimens of different individuals, were grown under appropriate conditions. Early P1 passages were cultured in osteogenic media (OM). After 1, 2 and 4 weeks of differentiation, ASC cultures were assessed for markers of osteogenesis. Alkaline phosphatase (AP) staining for osteoblastic transformation, Alizarin Red (AR) staining for matrix mineralization, Masson's Trichrome staining for collagen production, ELISA for the detection of Gla-Osteocalcin and Western Blot Analysis for Osteonectin protein expression were performed.

Results: Osteogenic differentiation began as early as 1 week post-treatment with OM. Cell behavior and morphology, tissue morphology, and matrix components changed with differentiation. Cells exhibited a vertical growth pattern; lacunar spaces formed in the cultures; matrix volume increased and mineralization was observed. Differences in AP staining was most evident during the 1st week. However, AR activity progressively increased over four weeks, particularly after the 2nd week. Collagen was detected in the extracellular matrix (ECM) secreted by the differentiated ASCs, but not in the ECM of control ASCs. There was no signifcant difference in differentiation between the ASCs of superficial and deep depots in the female. In the male, ASCs from the superficial depot differentiated faster and more efficiently than those of the deep depot. Male ASCs from both depots differentiated at a faster rate and more efficiently than female ASCs from both depots.

Conclusion: We have studied the effect of both gender and harvest depot on the osteogenic differentiation properties of human ASCs. Those derived from male superficial abdominal adipose tissue were observed to be more efficient than male deep abdominal adipose tissue, and both female depots in achieving osteogenesis. In achieving osseous healing by using autologous stem cells, the importance of the gender and depot differences will be a guide in clinical settings. Our next study is to examine the healing potential of both male and female ASCs in a murine calvarial defect model.

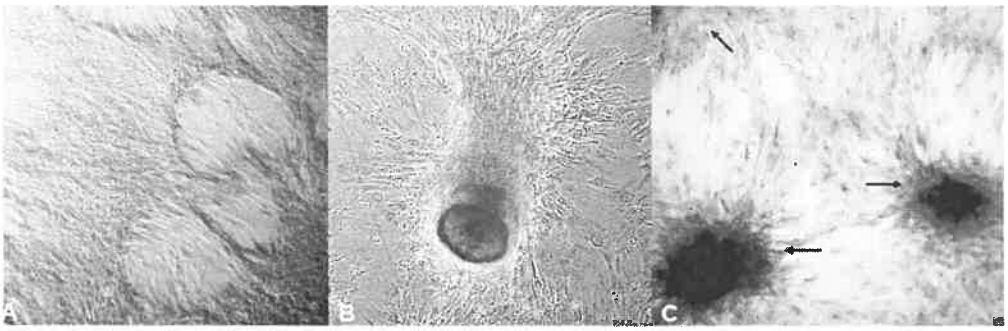

Figure 1. Osteogenic differentiation of MALE superficial abdominal depot adipose-derived stem cells at 4 weeks. **A.** All culture is strongly stained with Alkaline Phosphatase. Note the lacunae formation (5X). **B.** Alizarin Red (AR) staining. Note the vertically grown culture and the osteoid-like structure positively stained with AR (10X). **C.** Masson's Trichrome staining. Arrows indicate the depositions of collagen, especially prominent on the regions where cells are more clustered (10X).

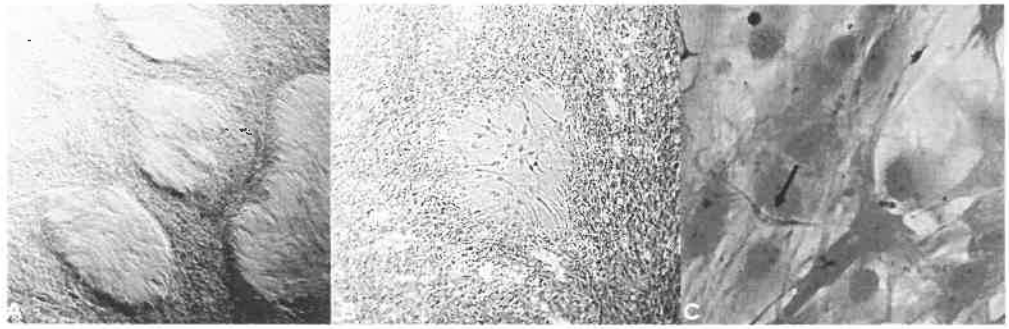

Figure 2. Osteogenic differentiation of FEMALE superficial abdominal depot adipose-derived stem cells at 4 weeks. **A.** Culture is faintly stained with Alkaline Phosphatase stain. **B.** Matrix mineralization demonstrated with Alizarin Red staining. No osteoid-like structures appeared and the mineralization level is lower than that is seen with male cells. **C.** Masson's Trichrome staining. Only a few number of fibrillar collagen encountered (arrow).

SCIENTIFIC SESSION

BONE AND CARTILAGE

Mesenchymal Stem Cells Derived From Bone Marrow and Adipose Tissue Possess Distinct Biologic Properties

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Adult mesenchymal stem cells (MSCs) are believed to be multipotent which can develop into other cell lineages such as bone, fat, cartilage, and neuron. MSCs have become the center of attention as an ideal stem cell source, since it would be expected to contribute to practical use for regenerative medicine. In our research, the biologic properties adult MSCs isolated from both adipose tissue and bone marrow of humans have been investigated both *in vitro* and *in vivo*.

The data demonstrate that MSCs derived from adipose tissue (AMSCs) have a more rapid doubling time when compared to MSCs derived from bone marrow (BMSCs). Analysis of the growth kinetics revealed that the AMSCs underwent significantly more population doublings than BMSCs, and retain multilineage differentiation potential markedly longer. RT-PCR analysis demonstrated the expression of the MSC related genes such as Oct-4, Rex-1 and Sox-2 in both MSC populations in both cell populations. Alterations in Oct-4 and Sox-2 protein expression as passage number increases have been confirmed using immunocytochemistry and Western blot analyses. Flow-cytometric analysis of the cell surface antigen profiles showed marked commonalities between these two types of MSCs, such high levels of expression of CD90 and CD133 markers and no expression of hematopoietic lineage markers (CD3, CD4, CD8 and CD34). Our data indicate that both MSC lineages persist more than 200 days in culture without detectable levels of telomere shortening, suggesting that they do not senesce.

Early populations of AMSCs and BMSCs showed relatively similar differentiation capability and efficiency. However, AMSCs showed higher differentiation efficiency in higher passages (over passage 30) while BMSCs fail to differentiate after 20 passages. Overall *in vitro* characterization of MSCs from these two tissue sources demonstrated that AMSCs retain their stem cell properties longer than BMSCs *in vitro*.

We are presently comparing the homing, engraftment and differentiation ability of both young and older passages of AMSCs and BMSCs in the central nervous system. Early data indicate that AMSCs efficiently engraft and demonstrate neural differentiation potential as indicated by the expression of both neural and glial antigens on the transplanted cells. In addition, our data indicate that a significant number of AMSCs engraft and differentiate when transplanted into the brain after cerebral ischemia damage.

SCIENTIFIC SESSION

BONE AND CARTILAGE

Microdistraction of osteoblast precursors and adipose-derived stem cells affects their osteogenic differentiation kinetics

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Distraction osteogenesis has been used to correct hypoplastic and asymmetric bony deformities in the growing patient and has been used extensively to stimulate bone growth after bony trauma. However, the underlying cellular mechanisms of distraction osteogenesis are poorly understood. In order to determine these mechanisms, an *in vitro*

microdistractor model was employed using an osteoblast precursor cell line. In addition, the possible use of this system in stimulating osteogenesis by stem cells was assessed. Both MC3T3 and ASCs (Adipose-derived Stem Cells) were polymerized in a three-dimensional collagen gel (Vitrogen) and placed in the microdistraction device under osteogenic stimulation. Two distraction conditions were set up: 1) Active Distraction: 0.35mm distraction every 24 hours using the microdistractor and 2) Static Distraction: maintenance of gel dimension (i.e. prevention of gel shrinkage) through the use of a static distraction device that imparts constant, low level tension (n=30). The effect of active and static distraction on proliferation was determined by cell counting, real-time PCR for the cell cycle genes *PCNA, cyclin D1* and *histone H4* and by using a spectrophotometric MTT proliferation assay. Apoptotic levels under these two conditions were also determined using a colormetric caspase assay. The effect of distraction on cellular homeostasis was assessed through real-time quantitation of multiple housekeeping genes, including *GAPDH, 18S rRNA* and *b-actin.* To assess the effect of distraction on osteogenic differentiation, alkaline phosphatase assays and real-time PCR for alkaline *phosphatase, CBFA-1, osteopontin, osteocalcin, osteonectin* were performed. Finally, quantitative Westerns for the cytoskeletal proteins *rho* and *rac* were performed in order to quantify the relative stress levels active and static distraction impart upon pre-osteoblasts and stem cells. Preliminary results suggest that pre-osteoblasts subjected to distraction forces upregulate their expression of osteogenic genes. Similar results were seen in ASC samples, however, the differentiation kinetics in this cell population appeared to be distinct from that of the osteoblast precursors. Interestingly, the static distraction set-up resulted in significant changes in osteogenic gene expression by ASCs, suggesting that a low-level constant tension is sufficient to influence osteogenesis in stem cells. Taken together, the results suggest that an *in vitro* microdistraction system can be used to influence the osteogenic differentiation program of both osteoblast precursors and multipotent stem cells.

SCIENTIFIC SESSION

BONE AND CARTILAGE

Differentiation of ASCs to Osteoclasts *in vivo* and *in vitro*

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Osteoclasts, the resorptive cells of bone, are known to be derived from monocyte/macrophage precursors of blood and bone marrow, a lineage thought to be distinct from that giving rise to mesenchymal stem cells. FACS analysis of human adipose stem cells (ASCs) from passages 3-7 determined between 1-2% expressed CD11a or CD11b, markers for the monocyte-macrophage lineage, suggesting possible progenitors of osteoclasts are included among the ASC population. Other markers of osteoclast precursors such as CD51 and CD61 were expressed by higher percentage of the ASCs. Cells were implanted in 8mm critical sized defects in the parietal bones of NIH-nude rats, either with or without 10 day pretreatment with osteoblastic differentiation stimuli (dexamethasone, beta-glycerol phosphate and ascorbate). Analysis by 3D reconstruction of CT showed an irregular pattern of bone regeneration in animals with undifferentiated ASC--some showed areas of significant bone resorption extending beyond the margin of the original defect. Histological studies with human-specific antibodies showed that the regenerating bone in both groups contained labeled osteoblasts, periosteal and endosteal cells, which were of human origin. The group receiving undifferentiated cells also displayed numerous resorption lacunae containing labeled osteoclasts, particularly in the regions of the defects showing high resorptive activity. The osteoclasts expressed the characteristic multicellular morphology and specific markers of the osteoclast phenotype including calcitonin receptor and tartaric acid resistant acid phosphatase (TRAP). These results suggest caution and further study of local application of undifferentiated ASCs *in vivo*. To evaluate the potential of ASCs to differentiate into osteoclasts *in vitro*, cells were plated at 50,000 cells/ cm^2 and treated with dexamethasone, RANKL and macrophage colony stimulating factor over a 30-day period. Within 14 days cells in treated cultures began to express TRAP. Within 21 days cells began to fuse to form multinuclear giant cells, which were positive for TRAP, calcitonin receptor and prominent actin rings characteristic of osteoclasts *in vitro*. It is currently unclear whether the fraction of the adherent ASCs giving rise to osteoclasts is restricted to the CD11a+/11b+ sub-population. In summary, this study indicates that the adherent cells, presumably ASCs, prepared from human adipose tissue can differentiate into osteoclasts *in vivo* and *in vitro*. Thus adipose tissues could be a valuable resource for osteoclasts for tissue engineering and cell replacement therapies for diseases like osteopetrosis. The abundance of ASCs and ease of producing these cells could facilitate testing studies of osteoclast biology, osteoporosis and other *in vitro* applications.

S C I E N T I F I C S E S S I O N

BONE AND CARTILAGE

Co-cultures of Adipose stem cells (ASCs) and nucleus pulposus cells under chondrogenic conditions can induce the nucleus phenotype in ASCs

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Introduction: Treatment of degenerative disc disease (DDD) with multipotent adult cells has the potential for regeneration of the nucleus pulposus (NP). Human adipose contains a population of stem cell-like cells (adipose stem cells-ASCs), however, it is not known if ASCs can differentiate towards the NP cell phenotype. We hypothesize that co-culture of NP cells with ASCs will generate a microenvironment in which the NP differentiation capacity of ASCs will be exhibited. This hypothesis was tested in a transwell setting using co-cultures of ASCs and NP cells in different modalities (micromass vs. monolayer).

Materials and methods: Human NP- and ASC cells were obtained from the same patient. The NP cells were isolated using the procedure of Gan et al. (1), the ASCs were isolated according to Zuk et al (2). Both cell types were cultured in DMEM/F12 (1:1) supplemented with 10% fetal calf serum (FCS) and antibiotics (Streptomycin, Penicillin, and Amphotericin). Passage 2 or 3 cells were used for all experiments.For groups 1 and 2 (see below) NP cells w ere precultured for 1 week in a micromass configuration; the other NP cells and all ASCs were freshly started from monolayer cultures. Co-cultures were performed in 8 groups in transwells: (1) NP micromass (precultured), ASC monolayer; (2) NP micromass (precultured), ASC micromass; (3) NP ("fresh") micromass, ASC monolayer; (4) NP ("fresh") micromass, ASC micromass; (5) NP and ASC monolayers; (6) NP monolayer, ASC micromass; (7) ASC monolayer (ASC control); and (8) NP monolayer (NP control). After 4 and 14 days of culture, total RNA was isolated, and cDNA synthesis and real-time PCR were performed to quantify the relative expression levels of the early (common chondrogenic/osteogenic) markers cbfa1/runx-2, aggrecan (late chondrogenic marker) and collagen type I (late osteogenic marker). Expression of each gene was normalized to that of the 18S housekeeping gene.

Results: *cbfa1*: It was found that in all experimental groups(gr. 1-6), up to two-fold upregulations of cbfa1 mRNA were observed in the ASC cultures, at both time points. No clear differences were observed between the micromass and monolayer cultures, with one exception; at the 14 day time point, culturing of ASCs as a micromass showed 4-fold higher cbfa1 induction when compared to monolayer.

collagen type I: No induction of collagen type I mRNA was observed in all groups, however, some groups showed even downregulations (two-fold in groups 2, 4 at 4 days, as in groups groups 2,4, 5 and 6 at day 14; in group 1 of day 14, virtually no collagen type I message could be detected).

aggrecan: At the 4-day time point, some upregulation of aggrecan mRNA was observed in ASCs when cultured in combination with NP micromasses, with the highest upregulation in group 4 (5.3 fold). Strikingly, a consistent upregulation was found in all ASC-NP micromass combinations (groups 1-4), with highest inductions in the ASC micromass configuration. Only control levels were observed in the other groups.

Discussion/Conclusion: Our data indicate that NP cell monolayers induce only early chondrogenic differentiation in ASCs, characterized by cbfa1/runx-2 induction and control (low) levels of aggrecan mRNA. By contrast, when NP cells are cultured under micromass conditions, induction progresses to later chondrogenic differentiation phases, characterized by upregulated expression of aggrecan mRNA, and a continued low and often downregulated expression of the "bone" collagen marker collagen type I. The data suggest that culturing ASCs under "chondrogenic" conditions (mimicked by micromass cultures) may enhance the responsiveness of the ASCs

In conclusion: these data suggest that in an appropriate microenvironment, such as present in the intervertebral disc nucleus, ASC are capable of differentiating towards a NP-like phenotype and hence may enhance regeneration of DDD-affected intervertebral discs.

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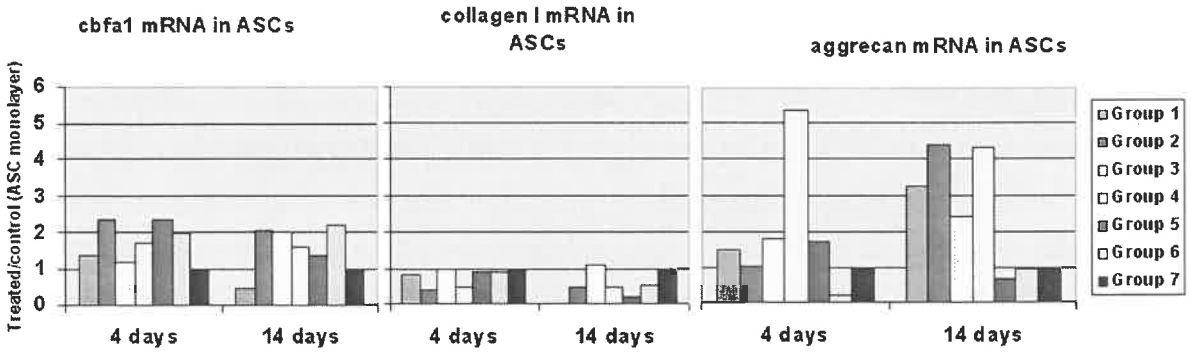

SCIENTIFIC SESSION

ASC's AND ESC's: MEMORY AND SYNERGY

Adipose-derived stem cells (ASCs) inherit adipose tissues under strong influence of mature adipocytes.

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Recent focus on adipose-derived stem cells (ASCs) is their large content in adipose tissues. Many groups have claimed their content to be more than 30% of the cells in stromal vascular fraction (SVF), which is sediment from the cell populations dispersed from adipose tissues by collagenase-digested. Considering that bone marrow-derived mesenchymal stem cells (MSCs) account for only 0.002% of total cells in bone marrow fluid, important questions to be addressed to ASCs are; 1) why ASCs deposit specifically in adipose tissues with such a high content, 2) how ASCs remain quiescent in adipose tissues, and 3) how could we culture ASCs *ex vivo* maintaining their high potential of differentiation along with not only mesenchymal linage but also endothelial and hemopoietic lineages. Based on the following lines of interpretation, we suggest that ASCs may inherit adipose tissue at immature and quiescent stage under a strong influence of adipocytes.

In contrast to averaged human body tissues composed of about 10^9 cells per gram, adipose tissues are exceptional in that most of the space is occupied by small number of adipocytes having extremely large size. Our repeated dispersion of the cells from human adipose tissues by collagenase-digestion gave round numbers of the component cells per gram to be one million each for floating adipocytes (intact or peeled), de-nucleated blood cells, nucleated blood cells non-attachable to culture dishes and attachable cells containing endothelial cells, pericytes and fibroblast-like ASCs. Such cell counting gives us a 3D image of adipose tissue in which most space is occupied by large adipocytes delivered with space micro-vascular system, and ASCs are hiding among adipocytes with cell ratio less than 1:1. Thus, ASCs seem to inhabit adipose tissues under overwhelming influence from adipocytes.

When the floating layer of cell suspension dispersed from adipose tissues by gentle collagenase-digestion was transferred to culture flasks filled with medium (ceiling culture) and observed by a microscope focused to the ceiling surface, ASCs migrate actively at the surface of apparently intact unilocular adipocytes and lipid droplets released from adipocytes. ASCs did not proliferate when attached to lipid droplets but proliferated actively once they were freed from lipid droplets after migration to the ceiling surface of flasks. Since we found that the unilocular adipocytes to which ASCs attached had missed intact plasma membranes, we cannot be sure whether similar suppressive influence from adipose tissues is functioning in adipose tissues. However, this observation is competent with a model that mature adipocytes are nursing ASCs at immature stages.

We have previously shown that a stem cell population with multiple potential of mesenchymal differentiation selectively proliferates from the cells in SVF in a medium containing 2% serum and FGF-2 as far as cultured on dishes coated with fibronectin. When we tested the same culture on monolayer of adipocytes differentiated from above stem cells, the cells in SVF remained unproliferated.

Thus, three line of interpretation suggested that ASCs inherit adipose tissues under suppressive influence of mature adipocytes and this may explain the reason why ASCs deposit specifically in adipose tissues but remain quiescent despite their high content in adipose tissues.

SCIENTIFIC SESSION

ASC's AND ESC's: MEMORY AND SYNERGY

Adipose-derived Stem Cells Express Multiple Genes in Common with Embryonic Stem Cells: Are Adult Stem Cells More Plastic Than We Think?

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Recent analysis of embryonic and adult stem cells using microarray technology has identified multiple genes in common, leading researchers to propose that all stem cells may possess a common stem cell "signature" or profile. These "stemness" genes may imply that adult stem cells are more plastic than previously thought and may possess differentiation potentials similar to ES cells. To determine if Adipose-derived Stem Cells/ASCs express elements of this stem cell signature, ASCs were analyzed by microarray technology. In order to circumvent the problem of heterogeneity with the ASC population, microarray analysis was also performed on single ASC-derived clone possessing adipogenic, osteogenic and chondrogenic potentials (AOC clone). ASCs and the AOC clones were analyzed using the Clontech Human Atlas Gene Array, an array of 1186 genes comprising multiple categories, including signal transduction, apoptosis, cell cycle and matrix adhesion/remodeling. The resulting data was corrected and analysed using ScanAnalyze, according to the manufacturer. Multiple arrays for each cell population were performed and the data normalized and averaged. A gene profile was generated for both ASCs and the AOC clones and the genes classified according to function. Both ASCs and AOC clones expressed genes from several categories thought to be important to the stem cell phenotype, including: 1) resistance to stress, 2) a sensitive mechanism controlling apoptosis, 3) a well-regulated cell cycle mechanism, 4) expression of multiple adhesion and matrix proteins and 5) expression of signal transduction pathways known to be involved in stem cell fate decisions. The microarray data was verified at the gene and protein level using RT-PCR, indirect immunofluorescence and Western analysis. Comparison of the AOC gene profile with that previously published for ES cells identified many genes in common, suggesting that the ASC population may share commonalities with totipotent stem cells. Supporting this, RT-PCR analysis confirmed expression of multiple "ES-specific" genes, including *TDGF-1*, *TERF-1* and

OCT4. IF analysis also confirmed expression of the ES markers SSEA-1, SSEA-4 and embryonic alkaline phosphatase, in addition to the multi-drug resistance gene ABCG2 and OCT4. In addition, putative totipotency in ASCs was also assessed by analyzing these stem cells for expression of genes and proteins characteristic of the endoderm and ectoderm lineages. RT-PCR, IF and Western analysis confirmed expression of multiple endoderm markers, including *alpha feto-protein*, *liver aldolase*, *prealbumin/transthyretin* and *cytokeratin 18*. Similarly, expression of the ectoderm lineage genes, *NeuroD*, *NeuN*, *nestin* was also detected together with dopamine and serotonin transporters. Taken together, the data indicates that ASCs and their AOC clonal derivatives share many commonalities with other adult stem cells and totipotent ES cells, suggesting that these stem cells could be used for the repair of tissues other than the mesodermal lineage.

SCIENTIFIC SESSION

ASC's AND ESC's: MEMORY AND SYNERGY

Adipose Stem Cells as Autologous Feeder Cells for Human Embryonic Stem Cells

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Background: Human embryonic stem cells (hESCs) are one of a few cell types that can be utilized in cell transplantation therapy for human neural diseases. However, for successful treatment of diseased tissue, hESCs must be capable of surviving, integrating and functioning in the recipient tissue while avoiding an immunological rejection by the host [1]. A current barrier to the use of hESCs is their unique requirement of animal cells and products for maintenance in an undifferentiated state or differentiation to defined fates and therefore their potential exposure to nonhuman pathogens [2-4]. In absence of a feeder layer, ESCs die or unpredictably differentiate to somatic cells rendering them useless for future applications [5]. While hESC can be maintained on human fibroblasts, current protocols for differentiation to neuronal lineages typically use animal derived stromal cells. We evaluated the use of human adipose-derived stem cells (hASCs) as feeder cells for hESC. hASC are easily obtained from patients, have a known immunosuppressive ability [6] along with a strong similarity to currently employed mouse preadipocytic stromal feeder cells [7]. The purpose of this study was to use hASCs in lieu of animal cells, thus providing a xeno-free co-culture system with the potential adaptation to a patient-specific culture environment. We evaluate patient-specific adult stem cell- embryonic stem cell co-culture for the ability to provide a niche favorable for the generation of neural stem cells and neurons from hESCs.

Methods: hASCs were isolated by collagenase digestion of adipose tissue collected from patients undergoing elective surgical procedures. Each derived cell line was characterized using fluorescence activated cell sorter. hESCs, line HSF-6 from UCSF and H7 from Wisconsin were maintained on mouse derived feeders in DMEM high glucose with 20% Knock-Out Serum replacer as recommended by the provider and then transferred to different density feeder layers composed of either mitomycin-C treated hASC feeder cells or treated human fibroblasts. The cells were cultured together for a period of 19 days or 2 months. No additional growth factors were added to the normal hESC culture media. The differentiation of hESCs was analyzed with immunocytochemistry.

Results: hASCs support the development of mature neurons and neural networks from hESCs after two months in coculture. Immunostaining with β-III tubulin, a neuron-specific tubulin isoform, shows development of long neuronal processes and interdigitated networks and bundles after two months demonstrating promotion of complex neural networks (Figure 1a, 1b).

For further detail we evaluated neural development at 19 days. There was no detectable expression of Oct4 from hESCs cultured with hASCs, therefore all hESCs cultured with hASCs differentiated. However, hESCs cultured with human fibroblasts as a control retained some undifferentiated cell populations as determined by the presence of Oct4. Some hESCs retained early neural stem cell qualities (Pax6 expression) when cultured with hASCs. The detection of Pax6 increased with ASC feeder layer density. Additionally, hESCs differentiated to oligodendrocytes, glia and neurons as determined by A2B5, GFAP, nestin, and beta-III tubulin expression, when culture with hASCs (Figure 1c, 1d, 1e). In addition, the level of hESCs differentiation to neurons increased with statistical significance with an increased feeder layer density (Figure 2). hESCs grown on the control fibroblast feeder layer also sporadically expressed mature neural markers indicating spontaneous differentiation occurred.

Conclusion: Human fibroblast derived feeder layers maintained an undifferentiated hESC stem cell population while hASC derived feeder layers encouraged neuronal differentiation of hESCs. Furthermore, the extent of neural differentiation was controlled by the variable hASC feeder layer density. Because hASCs directed all hESCs to differentiate past potential teratoma forming cells and promoted neurite outgrowth and network formation, these findings support the potential use of patient specific feeder layers for culture and differentiation of hESCs to neural subpopulations for use in therapy for degenerative diseases.

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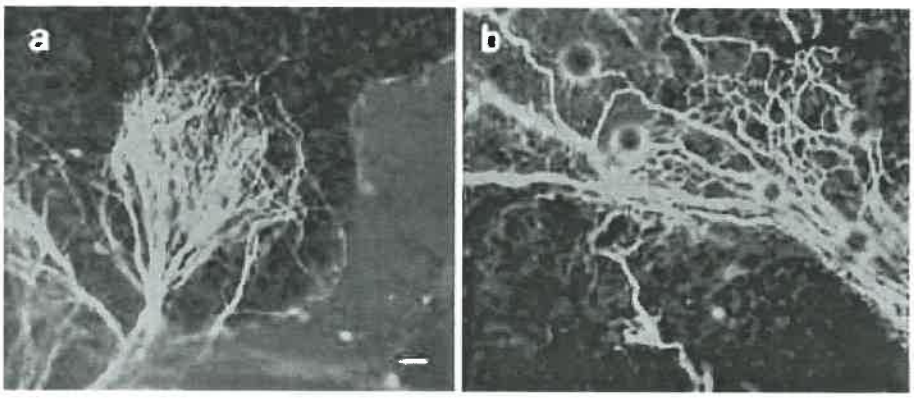

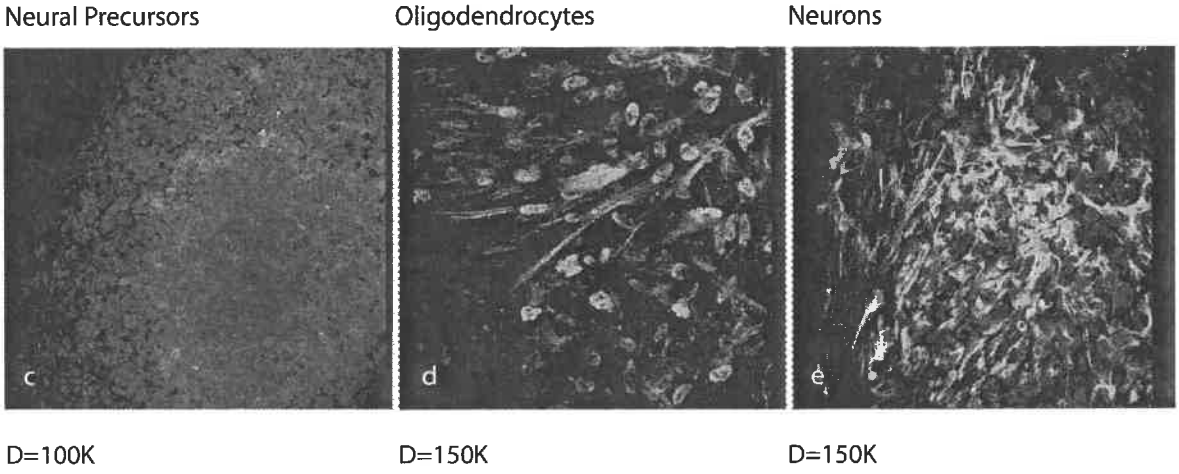

Figure 1. hASCs promoted neural outgrowth after two months coculture with hESCs (a and b). After 19 days, hESC differentiation into neural progenitors, oligodendrocytes and neurons (c,d and e). Mitomycin treated hASCs from a 38 yr old patient were plated at 50K, 100K and 150K cells per well in a 6-well plate. After 24 hours, hESCs were passaged at 1:10 and plated in colonies of 50-100 cells. All hESCs differentiated without added growth factors. At high feeder layer density, (c), there was Pax6 expression for neural precursor cells. hESCs also differentiated to oligodendrocytes when cultured in monolayers (d) with A2B5 expression strongest in highest density feeder layer conditions and neurons when cultured in colonies (e) with nestin expression highest density feeder layers.

SCIENTIFIC SESSION

ASC's AND ESC's: MEMORY AND SYNERGY

A Hierarchy of Proliferative Cells is Observed in Populations of Non-Hematopoietic, CD34+ Pluripotent Cells Isolated from Adipose Tissues

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Background: The recent description of a rare population of highly proliferative and clonogenic $CD34^+/CD45^-$ endothelial progenitor cells in adult blood led us to search for tissue depots where similar cells may reside in greater abundance. Adipose tissue contains a large number of $CD34^+/CD45^-$ pluripotent progenitor / stem cells that can be induced to adopt aspects of an endothelial phenotype. This study describes the identification and characterization of cells with high proliferative potential and a high frequency of clonogenicity resident within adipose tissues.

Methods and Results: Fresh lipoaspirates were obtained from 3 human patients undergoing elective surgery. After filtering through a 20 micron mesh, the solution was digested with collagenase for 2 hr and then centrifuged to obtain the stromal cell pellet. For clonogenic assays, cells were diluted to 0.5 cells / well of a 96 well plate containing EGM-2MV (Clonetics). Every second day ½ of the media was exchanged, after which cells in each well were counted. Nearly half (49 + 10%; mean + sd) of the cells underwent cell divisions. Furthermore, the population was highly

clonogenic, with 53 + 4 % of the proliferating single cells forming colonies of >10,000 cells after 3 weeks of culturing. Some clones were exceptionally proliferative, expanding to1012 from a single cell in 44 days. Two of the most highly proliferative clones and 2 low proliferative clones-- at an early stage of passage (20 - 22 population doublings)-- were replated again as single cells. As with the proliferative EPCs from the blood, most of the highly proliferative clones retained a robust growth potential; almost 80% of the singly plated cells formed secondary colonies of >10,000 by 17 days. Conversely, the low proliferative clones were only capable of modest regrowth and none formed colonies of >10,000.

Conclusions: We have identified a population of highly proliferative and clonogenic cells resident in the stromal fraction of adipose tissues. Clonogenicity and self renewal are characteristic of primordial cells, supporting the concept that the adipose stromal cells are comprised of a hierarchy of stem and progenitor cells. Furthermore, the ability to obtain large populations of clones opens up additional options for therapeutic or tissue engineering applications with these cells.

Poster Abstracts

Osteogenic differentiation of human adipose-derived stem cells within Poly (D,L-lactic-co-glycolic acid) PLGA scaffold in the nude mouse

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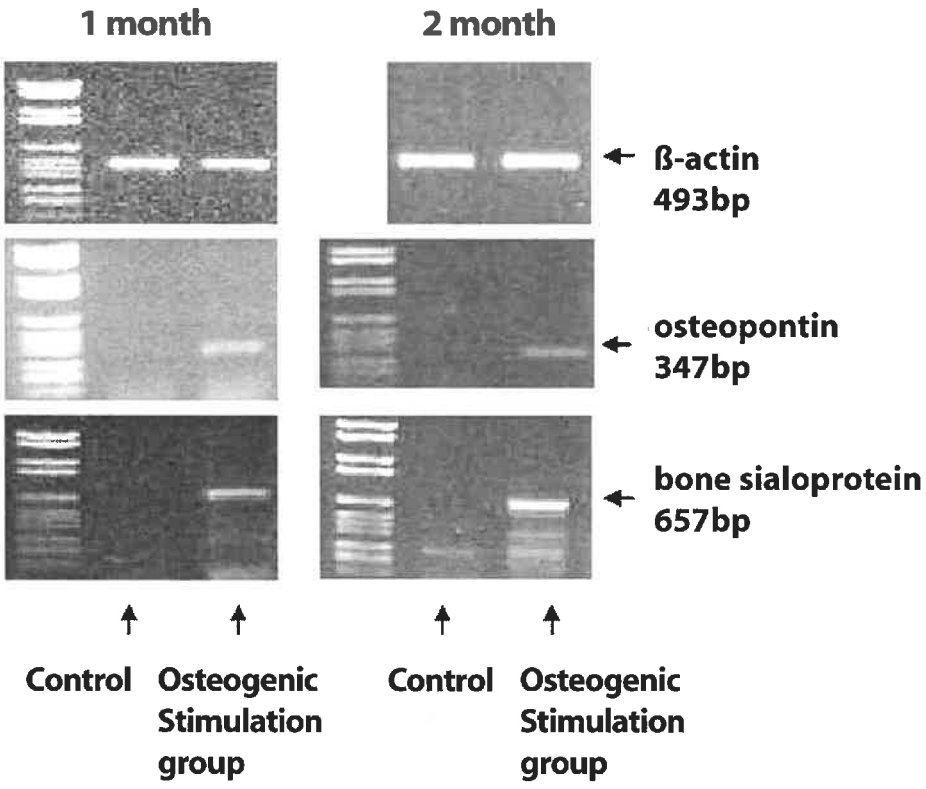
Many studies have recently performed for tissue-engineered bone formation using adipose-derived stem cells within scaffold, but not enough yet. To make *in vivo* tissue-engineered bone, the author implants the poly (D,L-lactic-co-glycolic acid) (PLGA) scaffold with adipose- derived stem cells in subcutaneous layer of nude mouse after osteogenic stimulation *in vitro* for 1 month. Osteogenesis was assessed by RT-PCR for mRNA of osteopontin and bone sialoprotein (BSP), and immunohistochemistry for osteocalcin, and von Kossa staining for calcification of extracellular matrix at 1 and 2 months.

The results were as follows :

1. Implanted PLGA scaffold with adipose-derived stem cells were well vascularized, and PLGA scaffolds degraded and were substituted by host tissues.
2. mRNA of osteopontin and BSP was detected by RT-PCR in both osteogenic stimulation group and also osteocalcin was detected by immunohistochemistry at osteogenic stimulation 1 and 2 months, but no calcified extracellular deposit in von Kossa stain was found in all groups.

In conclusion, it could also maintain *in vivo* the charateristics of osteogenic differentiation that adipose-derived stem cells within PLGA scaffold after stimulation of osteogenic differentiation *in vitro*, but did not develop calcification in extracellular matrix.



In vivo periodontal tissue regeneration by adipose-derived stem cells

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Introduction: Periodontal diseases with/without alveolar bone absorption represent a clinically significant problem in the field of oral and maxillofacial surgery. Several therapeutic approaches such as guided tissue regeneration (GTR) method or enamel matrix derivative (EMD) have been applied for it. Recently, however, bone marrow derived stem cells have been introduced for their regenerative capability of periodontal tissue including alveolar bone and periodontal ligament. In this study, we sought to determine if adipose-derived stem cells (ASCs) could be available as an alternative cell source for periodontal tissue regeneration.

Materials and Methods: ASCs were isolated from inguinal fat pads of Wister rat according to the previous publication. After incubation into 2 passage in control medium (DMEM, 10% FBS), cells were trypsinized and collected for implantation. 1 x 10^7 ASCs were subsequently mixed with platelet rich plasma (PRP), obtained from rats of the identical race by double spin method, followed by injected into the critical size periodontal tissue defect created using surgical round bar. After 2, 4 and 8 weeks of implantation, tissue specimen was harvested for histological analyses. Periodontal tissue defect without any treatment was served as a control group.

Results: After 2 and 4 week of injection, alveolar osteogenesis was found histologically and surrounded by many cells, which were considered as ASCs. Moreover, periodontal ligament-like structure was regenerated between regenerated alveolar bone and dental root after 8 weeks of injection. In contrast, above noted findings were not recognized at any time point in the control group.

Conclusions: These findings suggest that ASCs could contribute to periodontal tissue regeneration *in vivo*. Because of the availability of human liposuctioned fat in large quantity and in the low morbidity, ASCs may be a useful tool in future clinical cell-based therapy for periodontal diseases.

Bone Marrow Regeneration Using Adipose-Derived Stem Cells: The 2nd Report

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Backgrounds: We have reported bone marrow regeneration using adipose-derived stem cells (ASCs) since 2003. In our study using mice, we showed that not only bone matrix but also bone marrow could be regenerated subcutaneously *in vivo*. Transplanted ASCs proliferated and differentiated into osteoblasts in micropores on the surface of scaffolds made of hydroxyapatites (HA). We have not yet obtained incontrovertible proof that the ASCs differentiated into hematopoietic cells. However, we could regenerate a subcutaneous microenvironment in which hematopoietic cells survived, which may lead to new treatments for fibrotic bone marrow diseases, such as idiopathic myelofibrosis and osteopetrosis.

Purposes: In this report, we examined the optimal shapes of scaffolds for bone and bone marrow regeneration using ASCs.

Methods: Taking advantage of homogeneously marked cells from green fluorescent protein (GFP) transgenic mice, ASCs were isolated from the inguinal fat pads of GFP transgenic mice. ASCs were cultured *in vitro* and after three passages were seeded into the small pores of scaffolds of various shapes made of HA. The ASC-containing scaffolds were then subsequently implanted into immunocompetent mice subcutaneously. Two months later, the scaffolds were extirpated for histological, immunohistochemical and flow cytometry analyses.

Results: Histological examination showed that the pores were filled with bone matrix and that the bone marrow was composed of adipocytes, hematopoietic cells, and vasculatures. Bone marrow regeneration on small, thin scaffolds of high porosity was better than on other scaffolds. Immunohistochemical analysis confirmed that the GFP+ ASCs that had differentiated into osteoblasts were composed of bone matrix. Flow cytometry analyses showed that the bone marrow was composed of blood cells, including populations of monocytes, lymphocytes and granulocytes.

Conclusions: Bone marrow engineering has great potential for hematopoietic disease therapy. The regeneration of the hematopoietic system using ASCs might be useful in the future for treating hematopoietic stem cell diseases such as leukemia as well as fibrotic bone marrow diseases. We plan to continue looking for suitable scaffolds and methods for bone and bone marrow regeneration using ASCs.

Comparison of Stem Cells Harvested from Adipose Tissue and Bone Marrow

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Backgrounds: We have studied tissue engineering using both adipose-derived stem cells (ASCs) and bone marrow-derived stem cells (BSCs), and previously demonstrated that these stem cells differentiate into a variety of cell lineages both *in vitro* and *in vivo*.

Purposes: In this study, we investigated the biological differences between ASCs and BSCs harvested from mice. We studied the differences from the viewpoint of 1. morphology, 2. cell surface antigens, 3. proliferation potency, 4. pluripotency, and 5. gene transfer efficiency.

Methods: ASCs and BSCs were isolated from 5-week-old C57BL/6 mice. After subculture in a control medium, the cell surface markers expressed on the ASCs and BSCs were studied by flow cytometry. The cells were also incubated in osteogenic, chondrogenic and adipogenic media and differentiation was assessed by electron microscopy, special staining and RT-PCR. Gene transfer efficiency was studied using adenoviral, lentiviral, retroviral, adeno-associated viral (AAV) type 1, type 2, and type 5 vectors.

Results: BSCs were generally much more anaplastic than ASCs. However, ASCs and BSCs had generally similar properties as targets of viral vector mediated gene transfer, except that ASCs were particularly sensitive to lentiviral and oncoretroviral mediated gene transfer. The proliferation potency of ASCs was better than that of BSCs, but pluripotency and cell surface antigens were similar. Conclusions: ASCs can be easily harvested and proliferated; ASCs may be better than BSCs for further experiments on stem cell biology and tissue.

Osteogenic Potential of Adipose-Derived Stem Cells for Repair of the Pediatric Cranial Vault

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Introduction: Limited autologous bone for craniofacial reconstruction necessitates the development of engineered hard tissue. This is especially true for children aged two to seven, who have lost the dural osteogenic potential of infancy, but who have not yet developed a diploic space. Adipose-derived stem cells (ASCs) possess osteogenic potential, and promote bone formation *in vivo*. This study sought to establish a rabbit model of ASC osteogenesis for calvarial defect repair

Methods: ASCs were isolated from female New Zealand White rabbits, and cultured in osteogenic media (for up to 4 weeks), then assessed for markers of osteoblastic differentiation: alkaline phosphatase activity, matrix mineralization, and expression of osteogenic transcripts/proteins. ASCs were seeded on gelatin foam, and attachment and proliferation were quantified with Cell Titer Blue. For implant construction, $1x10^5$ male ASCs were seeded onto gelatin foam, induced in osteogenic media for seven days, and placed in female rabbit calvarial defects (8 mm). Thirty defects were randomly treated with cellular or acellular implants, autograft, or left empty. Calvaria were harvested at six weeks, and assessed radiographically and histologically.

Results: ASCs exhibited *in vitro* osteoblastic differentiation, as evidenced by increases in alkaline phosphatase expression and extracellular matrix mineralization. Addition of BMP-2 to the osteogenic media enhanced differentiation. The optimal seeding density of ASCs per 8-mm gelatin disk was 1x105 cells, which saturated the scaffold within 36 hours (steady-state = 2.4×10^5 cells). Radiographs of defects treated with ASCs indicated mineralized bone tissue in the defect sites, and histology confirmed the presence of osteoid. Fluorescence *in situ* hybridization studies on the sex mis-matched tissues to determine donor and recipient cell contribution to healing, as well as statistical analyses of healing, are currently underway and will be presented.

Conclusion: We have demonstrated the *in vitro* osteoblastic differentiation of rabbit ASCs, and we are the first to show their potential for calvarial defect repair in rabbits. Our preliminary data suggest that this may be an efficacious and clinically applicable cellular therapy for promoting post-operative osteogenesis. Furthermore, this study is the first to consider whether pre-implantation osteoinduction of ASCs confers additional osteogenic capacity *in vivo*.

Figure: Radiographic and histological analyses of calvarial defect healing by osteoinduced ASCs on a gelatin foam scaffold. Radiograph (left) shows mineralized tissue in the defect site, both peripherally and as bony islands. H&E staining shows bony infiltration into the defect site, as well as residual scaffold material.

Effect of Wnt3a and histone deacetylase activities on proliferation and differentiation of human adipose-derived stromal cells

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Multipotential adult mesenchymal stem cells (MSC) are able to differentiate along several known lineages, and lineage commitment is tightly regulated through specific cellular mediators and interactions. Human adipose tissues contain cell populations that have similar characteristics with bone marrow stromal cells. Wnt proteins and the modulation of histone deacetylase (HDAC) activities have been reported to be involved in proliferation and differentiation of stem cells. In this study, the effects of HDAC inhibitors and Wnt3a on differentiation and proliferation of human adipose tissue-derived stromal cells (hADSC) were determined.

To analyze the role of β-catenin signaling in human adipose stromal cells (hADSC), we examined the effects of β-catenin short hairpin RNAs (shRNA) expression and Wnt3a conditioned media in hADSC. The decrease of β-catenin expression in hADSC by transduction of β-catenin RNAi lentivirus was demonstrated by Western blot and immunohistochemistry. β-catenin RNAi lentivirus-transduced cells showed lower CFU numbers and lower cell numbers per colony than control lentivirus-infected cells, but did not show any changes in attachment of hADSC. The downregulation of β-catenin levels by shRNA expression induced an increase in osteogenic differentiation. The treatment of Wnt3a conditioned media increased cellular β-catenin levels and proliferation, but inhibited osteogenic differentiation. Transduction of β-catenin RNAi lentivirus blocked the Wnt3a effect on proliferation of hADSC.

Valproic acid (VPA), an inhibitor of HDAC, increased osteogenic differentiation of hADSC in a dose-dependent manner. The pretreatment of VPA before induction of differentiation also showed stimulatory effects on osteogenic differentiation of hADSC. Trichostatin A (TSA), another HDAC inhibitor, also increased osteogenic differentiation, whereas valpromide (VPM), a structural analog of VPA which does not possess HDAC inhibitory effects, did not show any effect on osteogenic differentiation on hADSC. RT-PCR and Real time PCR analysis revealed that VPA treatment increased osterix, osteopontin, BMP-2 and Runx2 expression. The addition of noggin inhibited VPA-induced potentiation of osteogenic differentiation. VPA inhibited proliferation of hADSC in a dose-dependent manner.

Taken together, these findings indicate that endogenous Wnt3a and HDAC activities play important roles on the regulation of proliferation and osteogenic differentiation of hADSC.

Chondrogenic differentiation of human adipose-derived stem cells in alginate sponge scaffolds

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The differentiation and growth of stem cells within engineered tissue constructs are hypothesized to be influenced by cell-biomaterial interactions. Recently, it has been proven that population of stem cells obtained from stromal vascular fraction of fat tissue have the multilineage potential which is a capability of differentiation into bone, cartilage, fat, and muscle. In addition, a surgeon can easily harvest human adipose tissue using by minimal invasive technique such as a liposuction.

In this article, I hypothesized that human adipose tissue contains the stem cells and has an capability of chondrogenic differentiation in alginate sponge scaffolds. Alginate sponge scaffolds were made by lyophilizing of alginate throughout alginate gel state and human adipose tissue was obtained from a liposuction. In experimental group, human adipose-derived stem cells in alginate sponge scaffolds, were differentiated with chondrogenic medium consisted of transforming growth factor-β1 and dexamethasone. Control group was defined as a culture with complete medium. After differentiation at 1, 2, and 3 weeks, gene expression was examined by reverse transcriptase polymerase chain reaction(RT-PCR) for collagen type II and link. In addition, content of glycosaminoglycan and Alcian blue stain were carried out for confirming of chondrogenesis.

RT-PCR results showed gene encodings of collagen type II and link in experimental group. The content of glycosaminoglycan in experimental group was greater than that in control group. In Alcian blue stain, sulfated proteoglycan was stained with blue color in experimental group.

Consequently, it was proven that adipose-derived stem cells have a capability of chondrogenic differentiation in alginate sponge scaffolds. In a view point of cell therapy, tissue engineering of adipose-derived stem cells is able to apply for reconstruction of tissue defects, congenital, and esthetics.

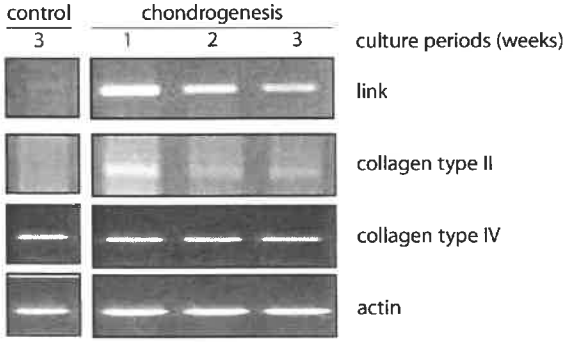

Fig. 1.

RT-PCR, confirming the expression of collagen type II and link. Human adipose-derived stem cells cultured for 1, 2, 3 weeks in chondrogenic medium or control medium were analyzed, with RT-PCR, for the expression of collagen type II and link in micromass culture samples.

Osteogenic Differentiation of Human Adipose-derived Stem Cells in the Three Dimensional PLGA Scaffolds

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Recent studies have shown that human liposuction aspirates contain pluripotent adipose-derived stem cells (ASCs) that have previously been shown to differentiate into various mesodermal cell types, including osteoblasts, myoblast, chondroblast and preadipocyte. To develop an autologous research model of bone tissue engineering, the author isolated ASCs form human liposuction aspirates and induced osteogenic differentiation in three dimensional PLGA scaffold.

Human liposuction aspirates underwent proteolytic digenstion and centrifugation to obtain ASCs. After primary culture in a control medium and expansion to three passages, the cells were either incubated in a two dimensional osteogenic medium or seeded onto a three dimensional PLGA scaffold with pore size of 200-300_m and thickness of 2mm and incubated in osteogenic medium for 4 weeks.

In two dimensional culture, osteogenesis was assessed by RT-PCR, alkaline phosphatase (ALP) staining and von Kossa staining. In three dimensional culture, osteogenesis was assessed by von Kossa and alizarine red S staining.

ASCs incubated in two dimensional osteogenic medium were stained positively for von Kossa and alkaline phaphatase staining. Expression of osteogenic specific bone sialoprotein gene was detected by RT-PCR. PLGA scaffold seeded with ASCs shows calcified extracellular matrix nodule for von Kossa and alizarine red S staining after two weeks of incubation with osteogenic medium

In conclusion, the author identified the osteogenic potential of ASCs and demonstrated bone formation in PLGA scaffold seeded with ASCs. After more investigation of the applicability of these results to the clinical setting, the clinical implication of this article may translate cell-based therapies for conditions of bone defect that would result in minimal donor site morbidity, easier procurement, minimized risk of contamination during *ex vivo* expansion.

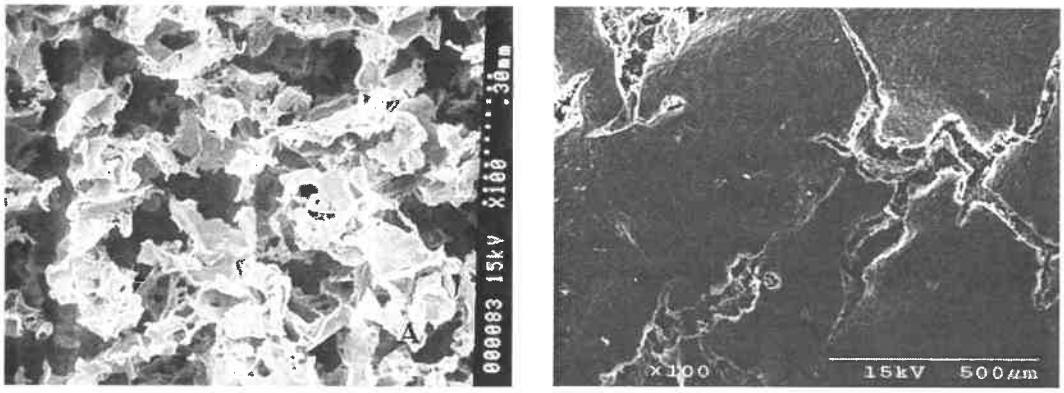

Fig.1. Scanning electron microscopic (SEM) findings of three dimensional PLGA scaffold prior to cell seeding (left) and 4 weeks after osteogenic differentiation (right). (original magnification,100x)

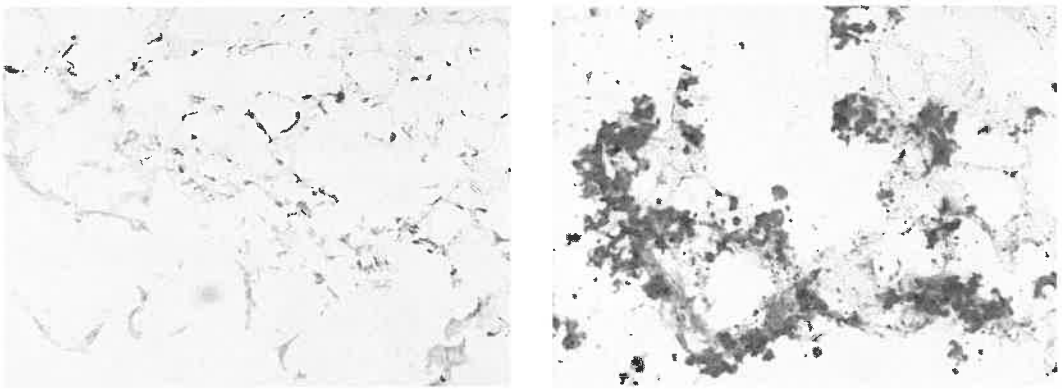

Fig.2 PLGA scaffold seeded with ASCs was stained using alizarine red S staining method. ASCs cultured in control media (left) for 2 weeks demonstrate the absence of calcified nodules. ASCs cultured in osteogenic media (right) for 2 weeks demonstrate numerous calcim nodules.

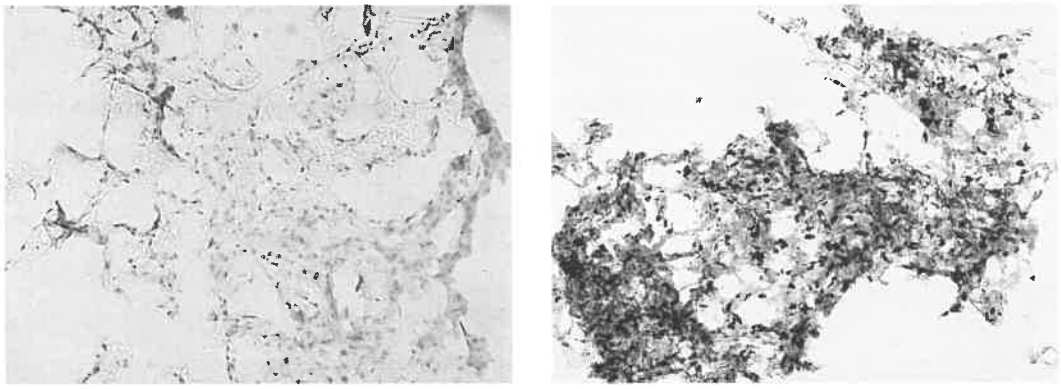

Fig.3. PLGA scaffold seeded with ASCs was stained using von Kossa staining method. ASCs cultured in control media (left) for 4 weeks lack formation of calcified nodule, whereas ASCs cultured in osteogenic media (right) for 4 weeks demonstrate numerous calcified nodules.

Fat tissue differentiated from stromal cell in chitosan bead

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Successful transplantation of cells depends on the isolation of individual cells and seeding in a suitable matrix and long term cell viability. The purpose of this study was to investigate chitosan bead as an injectable scaffold containing stromal cells derived from isolated fat tissue for subsequent fat cell transplantation. A 600 μm sized chitosan bead with multiple pores(30-80 micron of pore size) pass through 18 gauge needle with ease. The culture of fat stromal cell on chitosan beads *in vitro* showed that the beads were permissive for stromal cell adhesion and migration into the bead. Human fat –stromal cell were mixed with chitosan beads and injected immediately into athymic mouse subcutaneous sites. Fat-stromal cell transplantation without chitosan beads and chitosan beads implantation without fat-stromal cells served as controls.

Fat-stromal cells cultured in chitosan beads were differentiated into mature adipocytes after 2 weeks. Adipocyte differentiation factor, PPAR-gamma and LPL genetic factors were expressed by RT-PCR. Adipocytes were found in chitosan beads under confocal microscopic examination. Four weeks after implantation of beads in athymic mouse, fat-stromal cell implanted with beads transformed to fat tissue with matured adipocytes stained with Oil-red-O. Under confocal microscope, chitosan scaffold contained mature adipocytes.

Fat-stromal cells in injectable chitosan bead were differentiated into mature adipocyte in medium supplement, and *in vivo* implantation.

In conclusion, these results suggest that porous chitosan bead could serve as an injectable scaffold for fat-stromal cell conversion to mature adipocytes and future fat cell transplantation. This may have significant clinical implications for soft tissue filler in aesthetic and reconstructive surgery.

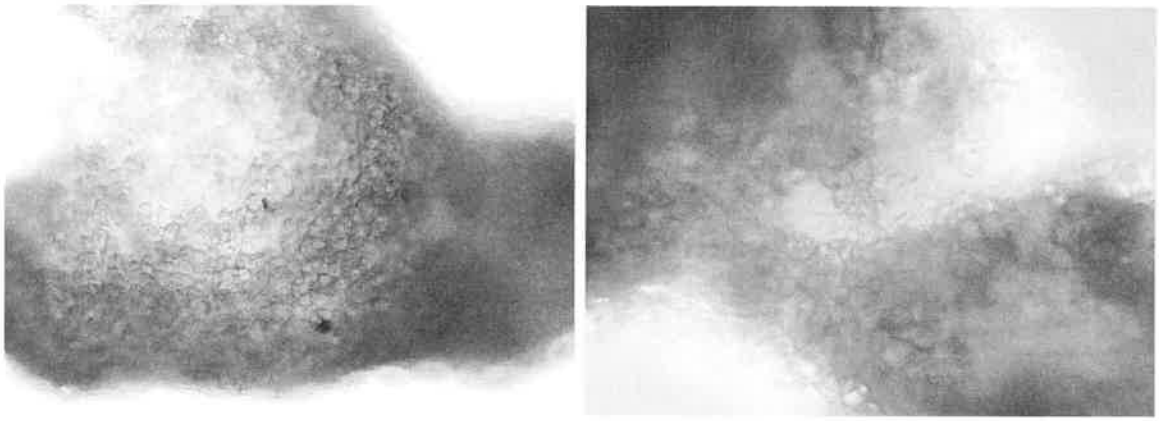

Fig. 1. Differentiation of human fat derived stromal cell in chitosan beads. Three weeks after seeded in chitosan beads, the stromal cells were differentiated into matured adipocytes, showing lipid droplets in the light micrograph (magnification A; X 100, B; X 200)

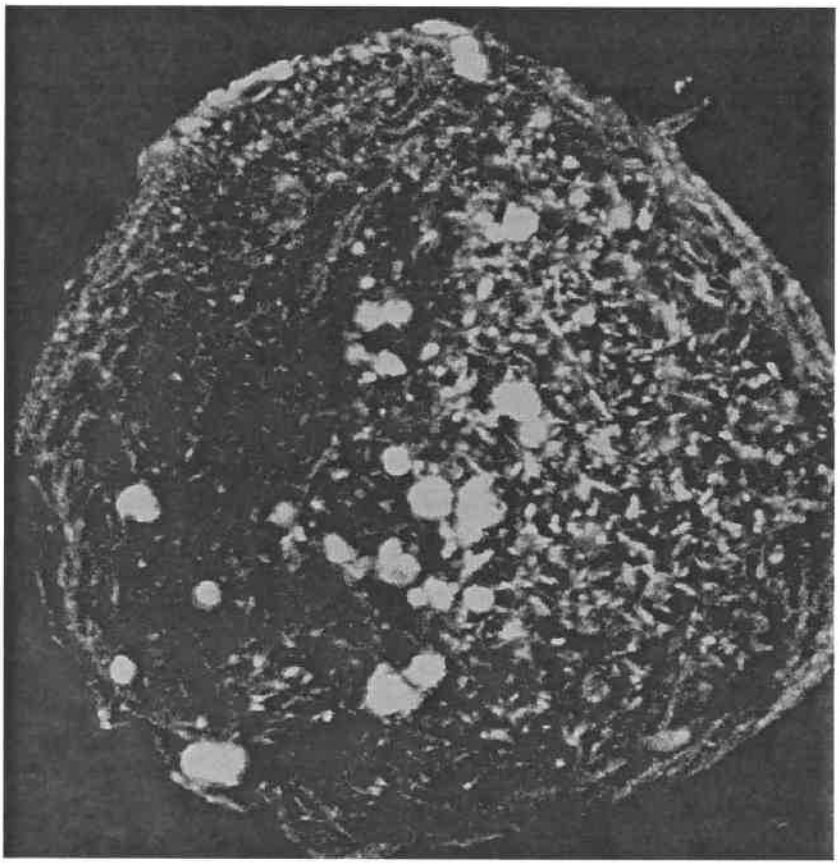

Fig. 2. Confocal microscopic observation of the surface of chitosan beads showing the distribution of fat-stromal cells after staining with fluorescence - labeled PKH67. (magnification X 200).

NG2 Expression of HUVEC and hASC in Coculture

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Introduction: It has been shown that hASCs have the ability to home to sites of injury and participate in vascular growth and remodeling through secretion of pro-angiogenic growth factors and differentiation into endothelial cells. These cells, when injected *in vivo*, also have the ability to exhibit a perivascular phenotype where they assume close contact with microvessels on the ablumenal surface. In a Transwell coculture model, it is possible to identify any association of hASCs and other cell types such as HUVECs or hSMCs mediated by contact or soluble factors. It is, therefore, the thrust of this study to determine whether physical contact or soluble factors influence hASCs into assuming a pericyte phenotype.

Methods: DiI-labeled hASCs and HUVECs were cocultured on either side of a Transwell membrane system in cell-cell contact arrangement using early passage cells (P3 and P2, respectively) seeded at 10k cells/cm^2 and 70k cells/cm^2, respectively. Cells were cultured in DMEM/F12 with 10% FBS for 24 hours before being fixed with 0.4% paraformaldehyde and stained for PECAM and NG2. Images were then analyzed with a confocal microscope and quantified by counting cells in apparent contact, and cells showing positive NG2 immunofluorescence.

Results: Approximately the same percentages of HUVECs and hASCs exhibited NG2 positive staining (45.5% and 53.5%, respectively), when cocultured on either side of the membrane (N=2). 11.1% of all hASCs observed were in direct morphological contact with HUVECs, and 66.7% of these cells expressed NG2 positive staining.

Conclusions: The observed percentages of NG2 expression for HUVECs and hASCs, especially in cases where cells were in apparent contact, suggest that there is a correlation between hASC/HUVEC contact coculture and pericyte recruitment, thus implicating hASCs as an active participant in blood vessel function and stability.

Characterization of hASC Adhesion Under Static and Flow Conditions

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Introduction: Although previous studies have shown that i.v.-injected human adipose-derived stromal cells (hASCs) have the ability to travel through the circulation and home to remote sites of tissue injury, there has been no previous work addressing their functional adherence to endothelial ligands and extracellular matrix proteins. Furthermore, cultured hASCs are responsive to hypoxia and, when injected *in vivo*, partially recover ischemic hindlimbs in animal models. Here, we characterize the differential adhesion characteristics of hypoxic versus normoxic cells. This will lead to a better understanding of the mechanisms of mobilization and extravasation of native hASCs, present at a site of injury in fat depots, to nearby vasculature, and may offer a mode through which to increase the efficiency of targeted therapeutic cell delivery.

Methods: Early passage hASCs were isolated, and expanded in culture under normoxic or hypoxic (0% oxygen) conditions for 48 hours before performing adhesion assays in a parallel plate flow chamber at a flow rate of 1 dyne/cm^2 (cells assayed at P=2). Either Type I Collagen, fibronectin, fibrinogen, V-CAM1, I-CAM1, E-selectin, P-selectin, or L-selectin was absorbed to the bottom plate of the chamber, and the cells were tested for their ability to initially capture to the surface under flow conditions and for their ability to firmly adhere under static conditions. Interactions were interpreted as being either positive or negative, while the frequency of capture events was quantified.

Results: The control TWEEN, fibrinogen, E and L-selectins failed to adhere either cell population under static or laminar flow conditions. Type I collagen and fibronectin positively adhered both cell populations under laminar flow and static conditions (100% of cells adhered), although only weak evidence for initial capture and adherence under laminar conditions was seen for either cell population's interaction with fibronectin. Interestingly, pre-conditioning hASCs in hypoxia increased the frequency of capture events and firm adhesion on P-selectin by 23% and on I-CAM1 by 83%. In contrast, on V-CAM1 substrates, the frequency of capture events was unchanged while the incidence of firmly adhered cells increased significantly with hypoxic pre-conditioning (77% increase in cell adherence).

Conclusions: These results suggest that hASCs can functionally bind to substrates presented by the endothelium in the presence of a physiologically relevant flow field. Furthermore, hASCs appear to alter their functional surface expression of adhesion molecules in response to hypoxia.

Oxygen Consumption and Proliferation of ADAS Cells in Hypoxia

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Massive bone defects due to trauma or tumor resection are conventionally repaired using vascularized bone grafting techniques. These methods are complicated and are limited by donor site availability and complications. An alternative method for the repair of such defects is the use of autologous adipose-derived adult stem (ADAS) cells in an appropriate scaffold material. During the initial implantation period, these constructs are initially devoid of a vascular supply, and thus the implanted cells are exposed to a potentially hypoxic environment which may adversely affect cell function and viability.

In order to characterize the effects of hypoxia on ADAS cell proliferation, primary rabbit ADAS cells were cultured in 20%, 5%, and 0.1% oxygen while differentiating in control, osteogenic, or adipogenic media. Under these conditions cellular proliferation was determined using a CyQuant cell proliferation kit (Molecular Probes, Eugene, OR). The oxygen consumption rates of ADAS cells suspended in fibrin glue (Tisseel, Baxter Biosurgery) was measured using the BD-oxygen biosensor system.

ADAS cells survived 0.1% hypoxia for at least 20 days under control, adipogenic or osteogenic conditions. ADAS cells in control media proliferated slower at 20%, 5%, and 0.1% oxygen relative to cells cultured in osteogenic or control media. The oxygen consumption rate of the control cells was 37 ± 0.5 fmol / min after 8 days of culture. ADAS cells in osteogenic media had higher oxygen consumption rates (76.9 ± 1.0 fmol / min) than cells cultured in control and adipogenic media and proliferated slower than control media cultured cells. ADAS cells in adipogenic media had very low oxygen consumption rates (18.0 ± 0.2 fmol/min), and proliferated most slowly and nearly stopped proliferating under 0.1% oxygen conditions.

These data show that hypoxia can impair ADAS cell proliferation under some differentiation conditions and that the oxygen consumption rate of these cells is also affected by these conditions.

Adipose-Derived Stem Cells in Experimental Animals: Relevance to Clinical Studies

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Adipose tissue (AT) contains a specific population of undifferentiated cells that has the capacity to give rise to several mesenchymal-related cell lineages. Because AT can be harvested in relatively large amounts with minimal morbidity it has been the subject of intense research in the field of cellular therapy using adult stem cells. Although the majority of research in the stem cell field has been done in humans and rodents, large animal models are especially useful as research progresses towards the clinic.

We examined the pelleted, cellular fraction of digested subcutaneous adipose-tissue from pigs and dogs and compared it with that obtained from human adipose. The percentual range or average found for CD45+, CD31+, CD34+, CD184+ and ABCG2+ cells in human, dogs and pigs are summarized in the table below:

Marker \ Specie	Human	Dog	Pig
CD45+	13-56%	15%	10-22%
CD31+	6-32%	NA	3-10%
CD34+	17-72%	50-60%	NA
CD184+	3-16%	19-29%	8-15%
ABCG2+	1-7%	7%	9%

The ranges of all markers examined, except CD45 and CD31, were similar between species. The range of CD45 expressing cells from human tissue was greater than that in the animal tissue, likely due to the relatively high amount of blood, which varied in quantity between lipoaspirate samples. The CD45+ and CD31+ fraction in the adipose-derived cells from dogs and pigs is lower because the tissue, collected by lipectomy, is relatively blood-free. Thus, we found that major cell populations within the adipose tissue of dogs and pigs is comparable to that of human tissue. Therefore, investigations of the therapeutic potential of adipose-derived cells in pig and dog experimental models should be useful in predicting potential utility of these cells in the clinical setting.

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