Adipose Stem Cell Literatures

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on adipose stem cell.
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Introduction

Stem cell is the origin of an orgnism's life. Stem cells have the potential to develop into many different types of cells in life bodies, that are exciting to scientists because of their potential to develop into many different cells, tissues and organs. Stem cells can be used in the clinical medicine to treat patients with a variety of diseases (Daar, 2003). Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situuition or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a tipical and important topic of life science.

The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell" (Stedman's Medical Dictionary, 2002).

Stem cell is totipotent, that means it holds all the genetic information of the living body and it can develop into a mature cell. Stem cell is a single cell that can give rise to progeny that differentiate into any of the specialized cells of embryonic or adult tissue. The ultimate stem cells (fertilized egg) divide to branches of cells that form various differentiated tissues or organs. During these early decisions, each progeny cell retains totipotency. Through divisions and differentiations the embryonic stem cells lose totipotency and gain differentiated function. During normal tissue renewal in adult organs, tissue stem cells give rise to progeny that differentiate into mature functioning cells of that tissue. Stem cells losing totipotentiality are progenitor cells. Except for germinal cells, which retain totipotency, most stem cells in adult tissues have reduced potential to produce different cells.

Aristotle (384-322 BC) deduced that the embryo was derived from mother's menstrual blood, which was based on the concept that living animals arose from slime or decaying matter. This concept was accepted in western world for over 2000 years, and it controlled western philosophy for over 2000 years either. In 1855, Virchow supposed that all cells in an organism are derived from preexisting cells. Now we know that all the human cells arise from a preexisting stem cell – the fertilized egg, that come from the mating of a man and a woman naturally but now can be produced in the laboratory tube. The counter hypothesis of spontaneous generation was accepted until 1864, when the French scientist Louis Pasteur demonstrated that there would be no microorganisms' growing after sterilizing and sealing.

The animal body has an unlimited source of stem cells, almost. However, the problem is not in locating these stem cells, but in isolating them from their tissue source.

Five key stem cells have been isolated from human: (1) Blastocysts; (2) Early embryos; (3) Fetal tissue; (4) Mature tissue; (5) Mature cells that can be grown into stem cells.

Up to today, only stem cells taken from adults or children (known generically as "adult stem cells") have been used extensively and effectively in the treatment of degenerative diseases.

Embryonic stem cells hold great promise for treating degenerative diseases, including diabetes, Parkinson's, Alzheimer's, neural degeneration, and cardiomyopathies (Bavister, 2005). Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Embryonic stem cells can replicate indefinitely. This makes it feasible to culture the cells

on a large scaled for cell transplantation therapy in clinical application. Embryonic stem cells are pluripotent and have the potential to differentiate into all three germ layers of the mammalian body including the germ cells.

Normally to say that somatic stem cells differentiate only into specific tissue cells wherein they reside. However, somatic stem cells can differentiate into cells other than those of their tissue of origin. Adult bone marrow, fat, liver, skin, brain, skeletal muscle, pancreas, lung, heart and peripheral blood possess stem or progenitor cells with the capacity to transdifferentiate. Due to this developmental plasticity, somatic stem cells may have potential in autologous regenerative medicine, circumventing problems like rejection and the ethically challenged use of embryocyte stem cells.

As the example, the following is describing the isolation and characterization of the putative prostatic stem cell, which was done by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in 2003. The detail methods have been described by Bhatt, Brown, Hart, Gilmore, Ramani, George, and Clarke in the article "Novel method for the isolation and characterisation of the putative prostatic stem cell" in the journal Cytometry A in 2003 (Bhatt, 2003).

This material gives some literatures on adipose stem cell.

Literatures

Arnalich-Montiel, F., S. Pastor, et al. (2008). "Adipose-derived stem cells are a source for cell therapy of the corneal stroma." Stem Cells **26**(2): 570-9.

In 2008, Arnalich-Montiel et al describes that most corneal diseases affect corneal stroma and include immune or infectious diseases, ecstatic disorders, traumatic scars, and corneal dystrophies. Cell-based therapy is a therapeutic method candidate cure the disadvantages of corneal transplantation. Arnalich-Montiel et al searched a cell source to repopulate and regenerate corneal stroma. They investigated the ability of human processed lipoaspirate derived (PLA) cells to regenerate corneal stroma in experimental animals. In the first set of experiments, they tested the biosafety and immunogenicity of human PLA stem cells transplanted into the corneal stroma of rabbits. No immune response was elicited even though they used immune-competent animals. PLA cells survived up to 10 weeks post-transplant, maintained their shape, and remained intermingled in the stroma without disrupting its histological pattern. Transparency was preserved even 10 weeks after the transplant, when PLA cells formed a discontinuous layer in the stroma. In the second set of experiments, regeneration of the corneal stroma by PLA cells was assessed, creating a niche by partial ablation of the stroma. After 12 weeks, human cells were disposed following a multilayered pattern and differentiated into functional keratocytes. The results showed that adipose-derived adult stem cells could be a cell source for stromal regeneration and repopulation in diseased corneas.

Astori, G., F. Vignati, et al. (2007). ""In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells." J Transl Med **5**: 55.

In 2007, Astori et al described that the stromal vascular fraction (SVF) is a heterogeneous cell population derived from the adipose tissue. This group investigated SVF by FACS analysis, cytological and "in vitro" assays. They studied CD34+ population by combining FACS with human colony-forming-cell haematopoietic assay (CFC). The endothelial fraction was investigated by quantifying the co-expression of specific markers (CD146, CD105, CD31 and UEA-1). Mesenchymal potential was assessed by CFU-F assay and cultured AT-MSC were characterized by a 5-color FACS analysis. The multipotent differentiation potential (osteogenic, adipogenic and chondrogenic) was investigated both at cellular and molecular level. Their results showed an identified fact in the SVF two CD34+ populations with a marked difference in the intensity of antigen expression, the majority of the cells expressing CD34 at low intensity.

Baglioni, S., M. Francalanci, et al. (2009). "Characterization of human adult stem-cell populations isolated from visceral and subcutaneous adipose tissue." Faseb J **23**(10): 3494-505.

As Baglioni et al described in 2009, adipose tissue is a dynamic endocrine organ with a central role in metabolism regulation. Functional differences in adipose tissue seem associated with the regional distribution of fat depots, in particular in subcutaneous and visceral omental pads. In this paper, they report for the first time the isolation of human adipose-derived adult stem cells from visceral omental and subcutaneous fat (V-ASCs and S-ASCs, respectively) from the same subject. Immunophenotyping shows that plastic culturing selects homogeneous cell populations of V-ASCs and S-ASCs from the corresponding stromal vascular fractions (SVFs), sharing typical markers of mesenchymal stem cells. Electron microscopy and electrophysiological and real-time RT-PCR analyses confirm the mesenchymal stem nature of both V-ASCs and S-ASCs, while no significant differences in a limited pattern of

cytokine/chemokine expression can be detected. Similar to S-ASCs, V-ASCs can differentiate in vitro toward adipogenic, osteogenic, chondrogenic, muscular, and neuronal lineages, as demonstrated by histochemical, immunofluorescence, real-time RT-PCR, and electrophysiological analyses, suggesting the multipotency of such adult stem cells. Our data demonstrate that both visceral and subcutaneous adipose tissues are a source of pluripotent stem cells with multigermline potential. However, the visceral rather than the subcutaneous ASC could represent a more appropriate in vitro cell model for investigating the molecular mechanisms implicated in the pathophysiology of metabolic disorders such as obesity.

Baptista, L. S., K. R. da Silva, et al. (2009). "Adipose tissue of control and ex-obese patients exhibit differences in blood vessel content and resident mesenchymal stem cell population." Obes Surg **19**(9): 1304-12.

BACKGROUND: The normal function of white adipose tissue is disturbed in obesity. After weight loss that follows bariatric surgery, ex-obese patients undergo plastic surgery to remove residual tissues and it is not known whether their adipose tissue returns to its original state. The aim of this study was to compare the white adipose tissue composition of ex-obese with control patients with regard to blood vessels and resident mesenchymal stem cells (MSC). METHODS: Quantification of blood vessels was performed on histological sections of adipose tissue stained with hematoxylin and eosin and for von Willebrand antigen. MSC were induced to the adipogenic and osteogenic lineages by specific inductive culture media. Expression of PPARgamma2 was analyzed by reverse transcription polymerase chain reaction. RESULTS: Ex-obese adipose tissue showed a higher number ($p = 0.0286$) of small (107.3 +/- 22.0) and large (22.5 +/- 6.4) blood vessels, when compared to control patients (42.0 +/- 24.4 and 7.2 +/- 2.2, respectively) and they also occupied a larger area (control versus ex-obese, $p = 0.0286$). Adipose tissue MSC from both groups of patients expressed PPARgamma2 and were equally able to differentiate to the osteogenic lineage, but ex-obese MSC showed a higher adipogenic potential when induced in vitro ($p < 0.05$). CONCLUSIONS: The higher number of adipose tissue blood vessels in ex-obese patients explains the excessive bleeding observed during their plastic surgery. The presence of more committed cells to the adipogenic lineage may favor the easy weight regain that occurs in ex-obese patients. These results show that, after extensive weight loss, adipose tissue cell composition was not totally restored.

Blande, I. S., V. Bassaneze, et al. (2009). "Adipose tissue mesenchymal stem cell expansion in animal serum-free medium supplemented with autologous human platelet lysate." Transfusion **49**(12): 2680-5.

BACKGROUND: Mesenchymal stem cells (MSCs) have been considered for human regenerative therapy applications, and safe culture and expansion protocols are needed especially in the context of interspecies contamination. Human platelet lysate (PL) has been proposed as animal serum substitute during in vitro MSC expansion. In this work, a simplified and efficient method to obtain autologous PL to replace animal serum in cell culture applications is described. STUDY DESIGN AND METHODS: PL obtained by freezing and centrifugation procedures was tested as medium supplement for human adipose mesenchymal stem cell (hASC) culture. Differential proliferation, immunophenotypic changes, and differentiation under PL or fetal bovine serum (FBS) were assessed. RESULTS: In contrast to 10% FBS supplementation, cell population doubling time was significantly lower when hASCs were cultured with the same concentration of PL (PL 22.9 +/- 1.5 hr vs. FBS 106.7 +/- 6.5 hr, t test, $p < 0.05$). Furthermore, hASCs maintained with 2.5% PL supplementation also showed satisfactory results. Immunophenotypic analysis revealed no differences between hASCs cultivated with PL or FBS supplementation and both cultures retained the potential to differentiate into adipose cells. These results demonstrate that autologous PL obtained from the same donor can be used as animal serum substitute in hASC culture. CONCLUSIONS: Taken together, evidence is provided that platelets provided by a single donor are sufficient to obtain PL for hASC propagation for clinical-scale applications mitigating the potential untoward side effects associated with the use of animal-derived reagents.

Bochev, I., G. Elmadjian, et al. (2008). "Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro." Cell Biol Int **32**(4): 384-93.

Mesenchymal stem cells (MSC) have been characterized as multipotent cells which are able to differentiate into several mesodermal and nonmesodermal lineage cells and this feature along with their extensive growth and comprehensive immunomodulatory properties establish them as a promising tool for therapeutic applications, including cell-based tissue engineering and treatment of immune-mediated disorders. Although bone marrow (BM) is the most common MSC source, cells with similar characteristics have been shown to be present in several other adult tissues. Adipose tissue (AT),

large quantities of which can be easily obtained, represents an attractive alternative to BM in isolating adipose tissue-derived MSC (AT-MSC). BM-MSCs and AT-MSCs share some immunomodulatory properties as they are both not inherently immunogenic and suppress the proliferation of alloantigen- or mitogen-stimulated T-cells. Our purpose was to comparatively examine under appropriate in vitro conditions, phenotypes, morphology and some functional properties of BM-MSCs and AT-MSCs, such as differentiation potential and especially the ability to suppress the immunoglobulin production by mitogen-stimulated B-cells. While the morphological, immunophenotypical, colony-forming and adipogenic characteristics of both types of cells were almost identical, AT-MSCs showed less potential for osteogenic differentiation than BM-MSCs. We found that AT-MSCs not only inhibited the Ig-production but also suppressed this B-cell function to a much greater extent compared to BM-MSC. This finding supports the potential role of AT-MSCs as an alternative to BM-MSCs for clinical purposes.

Boquest, A. C., A. Noer, et al. (2007). "CpG methylation profiles of endothelial cell-specific gene promoter regions in adipose tissue stem cells suggest limited differentiation potential toward the endothelial cell lineage." Stem Cells **25**(4): 852-61.

In vivo endothelial commitment of adipose stem cells (ASCs) has scarcely been reported, and controversy remains on the contribution of ASCs to vascularization. We address the epigenetic commitment of ASCs to the endothelial lineage. We report a bisulfite sequencing analysis of CpG methylation in the promoters of two endothelial-cell-specific genes, CD31 and CD144, in freshly isolated and in cultures of ASCs before and after induction of endothelial differentiation. In contrast to adipose tissue-derived endothelial (CD31(+)) cells, freshly isolated ASCs display a heavily methylated CD31 promoter and a mosaically methylated CD144 promoter despite basal transcription of both genes. Methylation state of both promoters remains globally stable upon culture. Endothelial stimulation of ASCs in methylcellulose elicits phenotypic changes, marginal upregulation of CD31, and CD144 expression and restrictive induction of a CD31(+)CD144(+) immunophenotype. These events are accompanied by discrete changes in CpG methylation in CD31 and CD144 promoters; however, no global demethylation that marks CD31(+) cells and human umbilical vein endothelial cells occurs. Immunoselection of CD31(+) cells after endothelial stimulation reveals consistent demethylation of one CpG immediately 3' of the transcription start site of the CD31 promoter. Adipogenic or osteogenic differentiation maintains CD31 and CD144 methylation patterns of undifferentiated cells. Methylation profiles of CD31 and CD144 promoters suggest a limited commitment of ASCs to the endothelial lineage. This contrasts with the reported hypomethylation of adipogenic promoters, which reflects a propensity of ASCs toward adipogenic differentiation. Analysis of CpG methylation at lineage-specific promoters provides a robust assessment of epigenetic commitment of stem cells to a specific lineage.

Chandra, V., S. G, et al. (2009). "Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells." Stem Cells **27**(8): 1941-53.

The success of cell replacement therapy for diabetes depends on the availability and generation of an adequate number of islets, preferably from an autologous origin. Stem cells are now being probed for the generation of physiologically competent, insulin-producing cells. In this investigation, we explored the potential of adipose tissue-derived stem cells (ASCs) to differentiate into pancreatic hormone-expressing islet-like cell aggregates (ICAs). We initiated ASC culture from epididymal fat pads of Swiss albino mice to obtain mesenchymal cells, murine epididymal (mE)-ASCs. Subsequent single-cell cloning resulted in a homogeneous cell population with a CD29(+)CD44(+)Sca-1(+) surface antigen expression profile. We formulated a 10-day differentiation protocol to generate insulin-expressing ICAs from mE-ASCs by progressively changing the differentiation cocktail on day 1, day 3, and day 5. Our stage-specific approach successfully differentiated mesodermic mE-ASCs into definitive endoderm (cells expressing Sox17, Foxa2, GATA-4, and cytokeratin [CK]-19), then into pancreatic endoderm (cells expressing pancreatic and duodenal homeobox [PDX]-1, Ngn3, NeuroD, Pax4, and glucose transporter 2), and finally into cells expressing pancreatic hormones (insulin, glucagon, somatostatin). Fluorescence-activated cell sorting analysis showed that day 5 ICAs contained 64.84% +/- 7.03% PDX-1(+) cells, and in day 10 mature ICAs, 48.17% +/- 3% of cells expressed C-peptide. Day 10 ICAs released C-peptide in a glucose-dependent manner, exhibiting in vitro functionality. Electron microscopy of day 10 ICAs revealed the presence of numerous secretory granules within the cell cytoplasm. Calcium alginate-encapsulated day 10 ICAs (1,000-1,200), when transplanted i.p. into streptozotocin-induced diabetic mice, restored normoglycemia within 2 weeks. The data presented here demonstrate the feasibility of using ASCs as a

source of autologous stem cells to differentiate into the pancreatic lineage.

Chaubey, A., K. J. Ross, et al. (2008). "Surface patterning: tool to modulate stem cell differentiation in an adipose system." J Biomed Mater Res B Appl Biomater **84**(1): 70-8.

There are several issues that need to be better understood before breast tissue-engineering becomes viable clinically. One of the key issues is the interaction between cells and the microtopography of the implant surface. The aim of this study was to evaluate the efficacy of D1 cells, multipotent mouse bone marrow stromal precursors, in differentiating to fat and to characterize their metabolic activity (lactic acid released and glucose consumed) and lipid production when cultured on patterned poly-L-lactide (PLLA) films. It was determined that, with appropriate stimulation, the D1 cells displayed morphological characteristics of adipocytes and produced lipid. The results show that the patterned surfaces did affect the rate of lipid production. Polynomial models were proposed to predict the metabolic activity of the cells over a period of time.

De Girolamo, L., M. F. Sartori, et al. (2008). "Human adipose-derived stem cells as future tools in tissue regeneration: osteogenic differentiation and cell-scaffold interaction." Int J Artif Organs **31**(6): 467-79.

Tissue engineering is now contributing to new developments in several clinical fields, and mesenchymal stem cells derived from adipose tissue (hASCs) may provide a novel opportunity to replace, repair and promote the regeneration of diseased or damaged musculoskeletal tissue. Our interest was to characterize and differentiate hASCs isolated from twenty-three donors. Proliferation, CFU-F, cytofluorimetric and histochemistry analyses were performed. HASCs differentiate into osteogenic, chondrogenic, and adipogenic lineages, as assessed by tissue-specific markers such as alkaline phosphatase, osteopontin expression and deposition of calcium matrix, lipid-vacuoles formation and Glycosaminoglycans production. We also compared osteo-differentiated hASCs cultured on monolayer and loaded on biomaterials routinely used in the clinic, such as hydroxyapatite, cancellous human bone fragments, deproteinized bovine bone granules, and titanium. Scaffolds loaded with pre-differentiated hASCs do not affect cell proliferation and no cellular toxicity was observed. HASCs tightly adhere to scaffolds and differentiated-hASCs on human bone fragments and bovine bone granules produced, respectively, 3.4- and 2.1-fold more calcified matrix than osteo-differentiated hASCs on monolayer. Moreover, both human and deproteinized bovine bone is able to induce osteogenic differentiation of CTRL-hASCs. Although our in vitro results need to be confirmed in in vivo bone regeneration models, our data suggest that hASCs may be considered suitable biological tools for the screening of innovative scaffolds that would be useful in tissue engineering.

De Rosa, A., F. De Francesco, et al. (2009). "A new method for cryopreserving adipose-derived stem cells: an attractive and suitable large-scale and long-term cell banking technology." Tissue Eng Part C Methods **15**(4): 659-67.

Recent studies have shown potential ways for improving stem cell cryopreservation. The major need for autologous stem cell use is a long-term storage: this arises from the humans' hope of future use of their own cells. Therefore, it is important to evaluate the cell potential of vitality and differentiation before and after cryopreservation. Although several studies have shown a long-term preservation of adipose tissue, a few of them focused their attention to stem cells. The aim of this study was to evaluate the fate of cryopreserved stem cells collected from adipose tissue and stored at low a temperature in liquid nitrogen through an optimal cryopreservation solution (using slowly cooling in 6% threalose, 4% dimethyl sulfoxide, and 10% fetal bovine serum) and to develop a novel approach to efficiently preserve adipose-derived stem cells (ASCs) for future clinical applications. Results showed that stem cells, after being thawed, are still capable of differentiation and express all surface antigens detected before storage, confirming the integrity of their biology. In particular, ASCs differentiated into adipocytes, showed diffuse positivity for PPARgamma and adiponectin, and were also able to differentiate into endothelial cells without addition of angiogenic factors. Therefore, ASCs can be long-term cryopreserved, and this, due to their great numbers, is an attractive tool for clinical applications as well as of impact for the derived market.

De Ugarte, D. A., Z. Alfonso, et al. (2003). "Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow." Immunol Lett **89**(2-3): 267-70.

Our laboratory has characterized a population of stromal cells obtained from adipose tissue termed processed lipoaspirate cells (PLAs). PLAs, like bone-marrow derived mesenchymal stem cells (BM-MSCs), have the capacity to differentiate along the adipogenic, osteogenic, chondrogenic, and myogenic lineages, In order to better characterize these two multi-lineage populations, we examined the surface phenotype of both bone marrow and adipose tissue-

derived cells from five patients undergoing surgery. PLA and BM-MSC cells were isolated, subcultivated, and evaluated for cell surface marker expression using flow cytometry. PLA and BM-MSC cells both expressed CD13, CD29, CD44, CD90, CD105, SH-3, and STRO-1. Differences in expression were noted for cell adhesion molecules CD49d (Integrin alpha4), CD54 (ICAM-1), CD34, and CD106 (VCAM-1). While markedly similar, the surface phenotypes of PLA and BM-MSC cells are distinct for several cell adhesion molecules implicated in hematopoietic stem cell homing, mobilization, and proliferation.

Estes, B. T., B. O. Diekman, et al. (2008). "Monolayer cell expansion conditions affect the chondrogenic potential of adipose-derived stem cells." Biotechnol Bioeng **99**(4): 986-95.

Adipose-derived stem cells (ASCs) are an abundant, readily available population of multipotent progenitor cells that reside in adipose tissue. Isolated ASCs are typically expanded in monolayer on standard tissue culture plastic with a basal medium containing 10% fetal bovine serum. However, recent data suggest that altering the monolayer expansion conditions by using suspension culture plastic, adding growth factors to the medium, or adjusting the seeding density may affect the self-renewal rate, multipotency, and lineage-specific differentiation potential of the ASCs. We hypothesized that variation in any of these expansion conditions would influence the chondrogenic potential of ASCs. ASCs were isolated from human liposuction waste tissue and expanded through two passages with different tissue culture plastic, feed medium, and cell seeding densities. Once expanded, the cells were cast in an agarose gel and subjected to identical chondrogenic culture conditions for 7 days, at which point cell viability, radiolabel incorporation, and gene expression were measured. High rates of matrix synthesis upon chondrogenic induction were mostly associated with smaller cells, as indicated by cell width and area on tissue culture plastic, and it appears that expansion in a growth factor supplemented medium is important in maintaining this morphology. All end-point measures were highly dependent on the specific monolayer culture conditions. These results support the hypothesis that monolayer culture conditions may "prime" the cells or predispose them towards a specific phenotype and thus underscore the importance of early culture conditions in determining the growth and differentiation potential of ASCs.

Fischer, L. J., S. McIlhenny, et al. (2009). "Endothelial differentiation of adipose-derived stem cells: effects of endothelial cell growth supplement and shear force." J Surg Res **152**(1): 157-66.

BACKGROUND: Adipose tissue is a readily available source of multipotent adult stem cells for use in tissue engineering/regenerative medicine. Various growth factors have been used to stimulate acquisition of endothelial characteristics by adipose-derived stem cells (ASC). Herein we study the effects of endothelial cell growth supplement (ECGS) and physiological shear force on the differentiation of ASC into endothelial cells. MATERIALS AND METHODS: Human ASC (CD13(+)29(+)90(+)31(-)45(-)) were isolated from periumbilical fat, cultured in ECGS media (for up to 3 wk), and exposed to physiological shear force (12 dynes for up to 8 d) in vitro. Endothelial phenotype was defined by cord formation on Matrigel, acetylated-low density lipoprotein (acLDL) uptake, and expression of nitric oxide synthase (eNOS), von Willebrand factor (vWF), and CD31 (platelet endothelial cell adhesion molecule, PECAM). Additionally, cell thrombogenicity was evaluated by seeding canine autologous ASC onto vascular grafts implanted within the canine arterial circulation for 2 wk. RESULTS: We found that undifferentiated ASC did not display any of the noted endothelial characteristics. After culture in ECGS, ASC formed cords in Matrigel but failed to take up acLDL or express the molecular markers. Subsequent exposure to shear resulted in stem cell realignment, acLDL uptake, and expression of CD31; eNOS and vWF expression was still not observed. Grafts seeded with cells grown in ECGS (+/- shear) remained patent (six of seven) at 2 wk but had a thin coat of fibrin along the luminal surfaces. CONCLUSIONS: This study suggests that (1) ECGS and shear promote the expression of several endothelial characteristics in human adipose-derived stem cells, but not eNOS or vWF; (2) their combined effects appear synergistic; and (3) stem cells differentiated in ECGS appear mildly thrombogenic in vitro, possibly related, in part, to insufficient eNOS expression. Thus, while the acquisition of several endothelial characteristics by adult stem cells derived from adipose tissue suggests these cells are a viable source of autologous cells for cardiovascular regeneration, further stimulation/modifications are necessary prior to using them as a true endothelial cell replacement.

Follmar, K. E., H. L. Prichard, et al. (2007). "Combined bone allograft and adipose-derived stem cell autograft in a rabbit model." Ann Plast Surg **58**(5): 561-5.

Currently available options for the repair of bony defects have substantial limitations. Much work has looked to the possibility of engineering bone using stem cells. These tissue-engineering efforts have focused on calvarial defect models, which have the

advantages of minimal load-bearing and a large surface area. This study aims to solve the somewhat more challenging problem of repairing segmental bony defects such as those of the mandible and long bones. Four groups of decellularized bone tubes with cortical perforations were implanted subcutaneously in a rabbit model: empty bone tubes, bone tubes containing fibrin glue alone, bone tubes containing fibrin glue and freshly isolated autologous adipose-derived stem cells (ASCs), and bone tubes containing fibrin glue and predifferentiated autologous ASCs. Results showed a foreign body response characterized by fibrous capsule formation with minimal angiogenesis and no evidence of osteoblastic activity. Substantial changes are needed if this model is to become viable.

Fotuhi, P., Y. H. Song, et al. (2007). "Electrophysiological consequence of adipose-derived stem cell transplantation in infarcted porcine myocardium." Europace **9**(12): 1218-21.

AIMS: Aim of this study was to investigate the effect of intracoronary administration of freshly isolated adipose-derived mononuclear cells (ADMCs) on myocardial vulnerability to arrhythmia induction after infarction. METHODS AND RESULTS: A transmural myocardial infarction in an experimental porcine model was induced by occlusion of the mid-left anterior descending artery with an angioplasty balloon for 3 h. Upon reperfusion, a cellular suspension with freshly isolated ADMCs (1.5 x 10(6) cells/kg BW) or vehicle alone was injected into the infarct artery. All animals underwent a programmed ventricular stimulation at 8 weeks follow-up for possible induction of ventricular arrhythmias using a train of 8 S1 stimuli. Cell injections did not cause acute ventricular arrhythmia, bradycardia, or conduction block. The cycle length of the ventricular arrhythmia was compared at 1 and 10 s following its induction. Despite comparable infarct size in both groups, we found that the cycle length of the induced ventricular arrhythmia in the ADMC-treated group was significantly longer compared with control animals ($P < 0.05$). We also found that extra-stimuli were required for arrhythmia induction in the ADMC-treated group compared with control animals. CONCLUSION: Freshly isolated autologous stem cell therapy is not proarrhythmic in pigs.

Fraser, J., I. Wulur, et al. (2007). "Differences in stem and progenitor cell yield in different subcutaneous adipose tissue depots." Cytotherapy **9**(5): 459-67.

BACKGROUND: Human adipose tissue has been shown to contain multipotent cells with properties similar to mesenchymal stromal cells. While there have been many studies of the biology of these cells, no study has yet evaluated issues associated with tissue harvest. METHODS: Adipose tissue was obtained from the subcutaneous space of the abdomen and hips of 10 donors using both syringe and pump-assisted liposuction. Tissue was digested with collagenase and then assayed for the presence of different stem and progenitor cell types using clonogenic culture assays, including fibroblast colony-forming unit (CFU-F) and alkaline phosphatase-positive colony-forming unit (CFU-AP). Paired analysis of samples obtained from the same individual was used to compare harvest method and site. RESULTS: Syringe suction provided significantly greater recovery of adipocytes and a non-significant trend towards improved recovery of cells in the adipocyte-depleted fraction. There was considerable donor-to-donor variation in stem cell recovery. However, paired analysis of tissue obtained from different subcutaneous sites in the same donor showed that tissue harvested from the hip yielded 2.3-fold more CFU-F/unit volume and a 7-fold higher frequency of CFU-AP than that obtained from the abdomen. These differences were statistically significant. DISCUSSION: Harvest site influences the stem and progenitor cell content of subcutaneous adipose tissue.

Freyberg, S., Y. H. Song, et al. (2009). "Thrombin peptide (TP508) promotes adipose tissue-derived stem cell proliferation via PI3 kinase/Akt pathway." J Vasc Res **46**(2): 98-102.

A synthetic peptide representing the receptor-binding domain of human thrombin (TP508) promotes angiogenesis and accelerates wound healing in animal models. However, the mechanisms underlying the therapeutic effects of TP508 have not been clearly defined. In this study, we set out to determine whether TP508 could stimulate stem cell proliferation. Adipose tissue-derived stem cells (ASCs) were incubated with TP508 (5 microg/ml) and cell proliferation was determined by bromodeoxyuridine (BrdU) incorporation. Our data showed that TP508 treatment significantly stimulated BrdU incorporation in ASCs ($p < 0.01$). The increased BrdU incorporation induced by TP508 was abolished by the PI3 kinase (PI3K) inhibitor LY294002 at 50 microM. Western blot analysis of ASCs revealed increased phosphorylation of Akt in response to TP508 when compared to unstimulated controls. These results indicate that TP508 exerts proliferative effects on ASCs via the PI3K/Akt pathway.

Gaetani, P., M. L. Torre, et al. (2008). "Adipose-derived stem cell therapy for intervertebral disc regeneration: an in vitro reconstructed tissue in alginate capsules." Tissue Eng Part A **14**(8): 1415-23.

The degenerative pathologies of the intervertebral disc have a remarkable social impact in the industrialized countries and can provide serious disabilities in the population. The current treatment consists of conservative treatments (such as symptomatic pharmacological therapies and physiokinetic therapy) and surgical treatments (intervertebral fusion, total disc replacement, nucleus pulposus (NP) replacement, or surgical exeresis). Recent advances in cell therapy foresee the possibility of regenerating the damaged disc; the autologous disc tissue can be withdrawn, in vitro regenerated, and re-implanted. The aim of this work was to verify whether autologous adipose-derived adult stem cells can improve the quality of an in vitro reconstructed nucleus pulposus tissue. A three-dimensional (3D) co-culture of NP cells and adipose tissue non-adipocyte fraction cells (nAFs) was assessed in a previously developed alginate 3D culture system following the good manufacturing practice guidelines to ensure patient safety for clinical studies. Morphological investigation of cultured and co-cultured cells was performed using transmission electron microscopy and immunofluorescence for collagen type I, aggrecan, CD90, CD34, and vimentin. Results indicate that co-culture of NP and nAFs improves the quality of the in vitro reconstructed tissue in term of extracellular matrix production and 3D cell organization. Technological resources are available for NP cell encapsulation intended for regenerating the intervertebral disc.

Garcia-Olmo, D., M. Garcia-Arranz, et al. (2005). "A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation." Dis Colon Rectum **48**(7): 1416-23.

PURPOSE: The effective management of fistulas in patients with Crohn's disease presents an extremely challenging problem. Mesenchymal adult stem cells extracted from certain tissues, such as adipose tissue, can differentiate into various cell types. Therefore, we have tried to use such cells to stimulate healing of Crohn's fistulas. METHODS: We designed a prospective Phase I clinical trial, involving five patients with Crohn's disease, to test the feasibility and safety of autologous stem cells transplantation in the treatment of fistulas. We also studied the expression of various cell markers and the growth rates of the lipoaspirate-derived cells that were used for transplantation. RESULTS: One patient was excluded because of bacterial contamination of cultured cells. We inoculated nine fistulas in four patients with autologous adipose tissue-derived stem cells at Passage 3 or earlier. Eight inoculated fistulas were followed weekly for at least eight weeks. In six fistulas, the external opening was covered with epithelium at the end of Week 8, and, thus, these fistulas were considered healed (75 percent). In the other two fistulas, there was only incomplete closure of the external opening, with a decrease in output flow (not healed; 25 percent). No adverse effects were observed in any patient at the end of the follow-up period (minimum follow-up,12 months; maximum follow-up, 30 months; follow-up average, 22 months). CONCLUSIONS: To our knowledge, this is the first report of a clinical trial of cell therapy using autologous stem cells obtained from a lipoaspirate. Our results indicate that our protocol is feasible and safe for the treatment of fistulas in Crohn's disease. The number of patients included and the uncontrolled nature of Phase I clinical trials do not allow demonstration of the effectiveness of the treatment. However, the results of the present study encourage to perform further studies in Phase II.

Garcia-Olmo, D., D. Herreros, et al. (2009). "Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion." Int J Colorectal Dis **24**(1): 27-30.

BACKGROUND: Expanded adipose-derived stem cells (ASC) have been shown to be effective in treating Crohn's patients with enterocutaneous fistulas. It is possible that unexpanded cells corresponding to the stromal vascular fraction (SVF) may also be effective. MATERIALS AND METHODS: A subpopulation of patients from a previous proof-of-concept phase I study with enterocutaneous fistulas received autologous expanded ASCs. The same selection criteria for inclusion were applied to patients who underwent SVF implantation to treat enterocutaneous fistulas. After tract curettage, cell suspensions (either SVF cells from lipoaspirate or expanded ASCs) were injected into the tract walls, and the fistulous tract was sealed with fibrin adhesive (with or without cells). RESULTS: In the series that received ASCs, four fistulas could be evaluated, and cure was achieved in three out of four cases. In the series that received SVF cells, four fistulas were evaluated, with cure achieved in one out of four cases. CONCLUSIONS: Although a comparison of case series cannot be considered firm evidence, a therapeutic protocol that uses expansion prior to implantation does seem to be more effective than one that uses SVF cells directly from a lipoaspirate sample.

Haimi, S., G. Gorianc, et al. (2009). "Characterization of zinc-releasing three-dimensional bioactive glass scaffolds and their effect on human adipose stem cell proliferation and osteogenic differentiation." Acta Biomater **5**(8): 3122-31.

While the addition of zinc ions to bioactive ceramics has been shown to enhance the proliferation and osteogenic differentiation of osteoblast-like cells, contradictory results have been found. Therefore, the effect of zinc-releasing ceramics on cell proliferation and differentiation into osteogenic lineages requires further clarification. The aim of this study was to evaluate the effects of zinc addition on the degradation profile of three-dimensional bioactive glass scaffold, and on the proliferation and osteogenesis of human adipose stem cells (hASCs) in these scaffolds. Bioactive glass scaffolds containing Na(2)O, K(2)O, MgO, CaO, B(2)O(3), TiO(2), P(2)O(5) and SiO(2) were prepared. The degradation was evaluated by weight loss measurement, scanning electron microscopy and elemental analysis. The degradation profile of bioactive glass was shown to slow down with the addition of zinc. Qualitative live/dead staining showed that zinc addition to bioactive glass inhibits cell spreading and proliferation of hASCs. However, zinc addition had no significant effect on DNA content, alkaline phosphatase activity and osteopontin concentration of hASCs when measured quantitatively. Our results suggest that the possible stimulatory effect of addition of zinc on hASC proliferation and osteogenesis was not detected because addition of zinc slowed down the degradation rate of the studied bioactive glass scaffolds.

Hanson, A. D., M. E. Wall, et al. (2007). "Effects of oxygen plasma treatment on adipose-derived human mesenchymal stem cell adherence to poly(L-lactic acid) scaffolds." J Biomater Sci Polym Ed **18**(11): 1387-400.

Plasma treatment of substrate surfaces can be utilized to improve adhesion of cells to tissue-engineered scaffolds. The purpose of this study was to enhance cell adhesion to non-woven poly(L-lactic acid) (PLLA) scaffolds using oxygen plasma treatment to increase surface hydroxyl groups and thereby enhance substrate hydrophilicity. It was hypothesized that oxygen plasma treatment would increase the number of adipose-derived human mesenchymal stem cells (hMSCs) that adhered to melt-blown, non-woven PLLA scaffolds without affecting cell viability. The number of cells that adhered to the oxygen plasma-treated (10 min at 100 W) or untreated PLLA scaffolds was assessed at 2, 4, 8, 12, 24 and 48 h post-seeding via DNA analysis. Cell viability and morphology were also assessed at 2, 4, 8, 12 and 24 h post-seeding via a live/dead assay and hematoxylin staining, respectively. Oxygen plasma treatment decreased the contact angle of water from 75.6 degrees to 58.2 degrees , indicating an increase in the surface hydrophilicity of PLLA. The results of the DNA analysis indicated that there was an increased number of hMSCs on oxygen plasma treated scaffolds for two of the three donors. In addition, oxygen plasma treatment promoted a more even distribution of hMSCs throughout the scaffold and enhanced cell spreading at earlier time points without altering cell viability. This early induction of cell spreading and the uniform distribution of cells, in turn, may increase future proliferation and differentiation of hMSCs under conditions that simulate the microenvironment in vivo.

Hattori, H., M. Sato, et al. (2004). "Osteogenic potential of human adipose tissue-derived stromal cells as an alternative stem cell source." Cells Tissues Organs **178**(1): 2-12.

Adult bone marrow contains mesenchymal stem cells (bone marrow-derived mesenchymal stem cells; BMSCs) which contribute to the generation of mesenchymal tissue such as bone, cartilage, muscle and adipose. However, using bone marrow as a source of stem cells has the limitation of a low cell number. An alternate source of adult stem cells that could be obtained in large quantities, under local anesthesia, with minimal discomfort would be advantageous. Human adipose tissue obtained by liposuction was processed to obtain a fibroblast-like population of cells or adipose tissue-derived stromal cells (ATSCs). In this study, we compared the osteogenic differentiation of ATSCs with that of BMSCs. Both cell types were cultured in atelocollagen honeycomb-shaped scaffolds with a membrane seal (ACHMS scaffold) for three-dimensional culturing in a specific osteogenic induction medium. Optimal osteogenic differentiation in both cell types, as determined by alkaline phosphatase cytochemistry, secretion of osteocalcin, mineral (calcium phosphate) deposition and scanning electron microscopy, was obtained with the same three-dimensional culture. Furthermore, osteoblastic lining in vivo was examined using ATSC-seeded or BMSC-seeded scaffolds in nude mice. The present results show that ATSCs have a similar ability to differentiate into osteoblasts to that of BMSCs.

Hebert, T. L., X. Wu, et al. (2009). "Culture effects of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) on cryopreserved human adipose-derived stromal/stem cell proliferation and adipogenesis." J Tissue Eng Regen Med **3**(7): 553-61.

Previous studies have demonstrated that EGF and bFGF maintain the stem cell properties of proliferating human adipose-derived stromal/stem cells (hASCs) in vitro. While the expansion and cryogenic preservation of isolated hASCs are routine, these manipulations can impact their proliferative and differentiation potential. This study examined

cryogenically preserved hASCs (n = 4 donors), with respect to these functions, after culture with basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) at varying concentrations (0-10 ng/ml). Relative to the control, cells supplemented with EGF and bFGF significantly increased proliferation by up to three-fold over 7-8 days. Furthermore, cryopreserved hASCs expanded in the presence of EGF and bFGF displayed increased oil red O staining following adipogenic induction. This was accompanied by significantly increased levels of several adipogenesis-related mRNAs: aP2, C/EBPalpha, lipoprotein lipase (LPL), PPARgamma and PPARgamma co-activator-1 (PGC1). Adipocytes derived from EGF- and bFGF-cultured hASCs exhibited more robust functionality based on insulin-stimulated glucose uptake and atrial natriuretic peptide (ANP)-stimulated lipolysis. These findings indicate that bFGF and EGF can be used as culture supplements to optimize the proliferative capacity of cryopreserved human ASCs and their adipogenic differentiation potential.

Heydarkhan-Hagvall, S., K. Schenke-Layland, et al. (2008). "Human adipose stem cells: a potential cell source for cardiovascular tissue engineering." Cells Tissues Organs **187**(4): 263-74.

BACKGROUND/AIMS: A crucial step in providing clinically relevant applications of cardiovascular tissue engineering involves the identification of a suitable cell source. The objective of this study was to identify the exogenous and endogenous parameters that are critical for the differentiation of human adipose stem cells (hASCs) into cardiovascular cells. METHODS: hASCs were isolated from human lipoaspirate samples, analyzed, and subjected to two differentiation protocols. RESULTS: As shown by fluorescence-activated cell sorter (FACS) analysis, a population of hASCs expressed stem cell markers including CXCR4, CD34, c-kit, and ABCG2. Further, FACS and immunofluorescence analysis of hASCs, cultured for 2 weeks in DMEM-20%-FBS, showed the expression of smooth muscle cell (SMC)-specific markers including SM alpha-actin, basic calponin, h-caldesmon and SM myosin. hASCs, cultured for 2 weeks in endothelial cell growth medium-2 (EGM-2), formed a network of branched tube-like structures positive for CD31, CD144, and von Willebrand factor. The frequency of endothelial cell (EC) marker-expressing cells was passage number-dependent. Moreover, hASCs attached and formed a confluent layer on top of electrospun collagen-elastin scaffolds. Scanning electron microscopy and DAPI staining confirmed the integration of hASCs with the fibers and formation of a cell-matrix network. CONCLUSION: Our results indicate that hASCs are a potential cell source for cardiovascular tissue engineering; however, the differentiation capacity of hASCs into SMCs and ECs is passage number- and culture condition-dependent.

Ishimura, D., N. Yamamoto, et al. (2008). "Differentiation of adipose-derived stromal vascular fraction culture cells into chondrocytes using the method of cell sorting with a mesenchymal stem cell marker." Tohoku J Exp Med **216**(2): 149-56.

The incidence of arthritic diseases is rapidly increasing in most advanced countries. Articular cartilage, which is the most important tissue in the joint, consists of chondrocytes and abundant extracellular matrix, including aggrecan, and shows poor self-repair. We studied the potential of stem cells in mouse subcutaneous adipose tissue as a source of cells to regenerate cartilage tissue. Analysis of adipose-derived stromal vascular fraction culture cells (ADSVFs) using mesenchymal stem cell markers showed that CD90-positive cells accounted for 93.8%, CD105-positive cells for 68.5%, and p75 neurotrophin receptor (p75NTR, CD271)-positive cells for 36.1%. These results indicate that cells positive for mesenchymal stem cell markers are present in ADSVFs. The CD105-positive or -negative cells were isolated from ADSVFs by magnetic cell separation (MACS), and the efficiency of differentiation into chondrocytes was compared with using three methods of pellet method, gel-coating method, and gel-embedding sheet method. Using the CD105-positive cells and the gel-embedding sheet method, aggrecan mRNA was detected about three times higher than pellet and gel-coating methods. The above data suggest that ADSVFs could be differentiated into chondrocyte-like cells in the gel-embedding sheet method and could be useful in regenerative medicine to treat cartilage defects or cartilage degenerative disease. The use of cells sorted by mesenchymal stem cell markers from adipose tissue would gain position in the repair of cartilage tissue.

Jeon, E. S., Y. J. Kang, et al. (2005). "Role of MEK-ERK pathway in sphingosylphosphorylcholine-induced cell death in human adipose tissue-derived mesenchymal stem cells." Biochim Biophys Acta **1734**(1): 25-33.

Sphingosylphosphorylcholine (SPC) is a bioactive lipid molecule involved in a variety of cellular responses. In the present study, we demonstrated that treatment of human adipose tissue-derived mesenchymal stem cells (hATSCs) with D-erythro-SPC resulted in apoptosis-like cell death, as demonstrated by decreased cell viability, DNA strand breaks, the increase of sub-G1 fraction, cytochrome c

release into cytosol, and activation of caspase-3. In contrast, the exposure of hATSCs to L-threo-SPC did not induce the cell death, suggesting that the SPC-induced cell death was selective for the D-erythro-stereoisomer of SPC. The D-erythro-SPC-induced cell death was prevented by DEVD-CHO, a caspase-3 specific inhibitor, and Z-VAD-FMK, a general caspase inhibitor, suggesting that the SPC-induced cell death of hATSCs occurs through the cytochrome c- and caspase-3-dependent pathways. In addition, D-erythro-SPC treatment stimulated the activation of mitogen-activated protein kinases, such as ERK and c-Jun NH2-terminal protein kinase (JNK), and the D-erythro-SPC-induced cell death was completely prevented by pretreatment with the MEK inhibitor, U0126, but not by pretreatment with the JNK inhibitor, SP600125, and the p38 MAPK inhibitor, SB202190, suggesting a specific involvement of ERK in the D-erythro-SPC-induced cell death. Pretreatment with U0126 attenuated the D-erythro-SPC-induced release of cytochrome c. From these results, we suggest that ERK is involved in the SPC-induced cell death of hATSC through stimulation of the cytochrome c/caspase-3-dependent pathway.

Jurgens, W. J., M. J. Oedayrajsingh-Varma, et al. (2008). "Effect of tissue-harvesting site on yield of stem cells derived from adipose tissue: implications for cell-based therapies." Cell Tissue Res **332**(3): 415-26.

The stromal vascular fraction (SVF) of adipose tissue contains an abundant population of multipotent adipose-tissue-derived stem cells (ASCs) that possess the capacity to differentiate into cells of the mesodermal lineage in vitro. For cell-based therapies, an advantageous approach would be to harvest these SVF cells and give them back to the patient within a single surgical procedure, thereby avoiding lengthy and costly in vitro culturing steps. However, this requires SVF-isolates to contain sufficient ASCs capable of differentiating into the desired cell lineage. We have investigated whether the yield and function of ASCs are affected by the anatomical sites most frequently used for harvesting adipose tissue: the abdomen and hip/thigh region. The frequency of ASCs in the SVF of adipose tissue from the abdomen and hip/thigh region was determined in limiting dilution and colony-forming unit (CFU) assays. The capacity of these ASCs to differentiate into the chondrogenic and osteogenic pathways was investigated by quantitative real-time polymerase chain reaction and (immuno)histochemistry. A significant difference (P = 0.0009) was seen in ASC frequency but not in the absolute number of nucleated cells between adipose tissue harvested from the abdomen (5.1 +/- 1.1%, mean +/- SEM) and hip/thigh region (1.2 +/- 0.7%). However, within the CFUs derived from both tissues, the frequency of CFUs having osteogenic differentiation potential was the same. When cultured, homogeneous cell populations were obtained with similar growth kinetics and phenotype. No differences were detected in differentiation capacity between ASCs from both tissue-harvesting sites. We conclude that the yield of ASCs, but not the total amount of nucleated cells per volume or the ASC proliferation and differentiation capacities, are dependent on the tissue-harvesting site. The abdomen seems to be preferable to the hip/thigh region for harvesting adipose tissue, in particular when considering SVF cells for stem-cell-based therapies in one-step surgical procedures for skeletal tissue engineering.

Kim, J. H., M. R. Lee, et al. (2008). "IFATS collection: Selenium induces improvement of stem cell behaviors in human adipose-tissue stromal cells via SAPK/JNK and stemness acting signals." Stem Cells **26**(10): 2724-34.

In the present study, the potential of selenium to enhance stem cell behavior through improvement of human adipose tissue-derived stromal cells (ATSCs) and the associated molecular mechanism was evaluated. Selenium-induced improvement in stem cell behavior of human ATSCs caused expression of several genes, indicating downregulated mature cell marker proteins coupled with increased cell growth and telomerase activities after the overexpression of Rex1, Nanog, OCT4, SOX2, KLF4, and c-Myc. Also, selenium-treated ATSCs significantly downregulated p53 and p21 tumor suppressor gene products. Selenium induced active growth and growth enhanced by the activation of signal proteins in ATSCs via the inhibition of reactive oxygen species-mediated phospho-stress-activated protein kinase/c-Jun N-terminal protein kinase activation. The selenium-induced activation of extracellular regulated kinases 1/2 and Akt in ATSCs resulted in a subsequent induction of the expression of stemness transcription factors, particularly Rex1, Nanog, and Oct4, along with definitive demethylation on regulatory regions of Rex-1, Nanog, and Oct4. The results of our small interfering RNA knockdown experiment showed that Rex1 plays a major role in the proliferation of selenium-induced ATSCs. Selenium-treated ATSCs also exhibited more profound differentiation into mesodermal and neural lineages. We performed a direct comparison of gene expression profiles in control ATSCs and selenium-treated ATSCs and delineated specific members of important growth factor, signaling, cell adhesion, and transcription factor families. The observations of improved life span and multipotency of selenium-

treated ATSCs clearly indicate that selenium-treated ATSCs represent an extraordinarily useful candidate cell source for tissue regeneration. Disclosure of potential conflicts of interest is found at the end of this article.

Kim, W. S., B. S. Park, et al. (2009). "Antiwrinkle effect of adipose-derived stem cell: activation of dermal fibroblast by secretory factors." J Dermatol Sci **53**(2): 96-102.

BACKGROUND: Adipose-derived stem cells (ADSC) have wound-healing and antioxidant effects on human skin via secretion of growth factors and activation of dermal fibroblasts. OBJECTIVE: Paracrine mechanism reducing ultraviolet-B (UVB)-induced wrinkles by ADSC is investigated in this study. METHODS AND RESULTS: Wrinkles were induced by an eight-week UVB irradiation, and were significantly improved by the subcutaneous injection of ADSC in hairless mice. In a replica analysis, parameters involving wrinkles were improved with mid-level and high doses of ADSC (1x10(4) and 1x10(5) cells). Dermal thickness and collagen contents in the dermis also were increased in the ADSC-injected groups. To characterize the paracrine mechanism involving the antiwrinkle effect of ADSC, a conditioned medium of ADSC (ADSC-CM) was directly incubated in human dermal fibroblasts (HDF). UVB irradiation reduced the proliferation of HDF, but this was reversed by the pretreatment of ADSC-CM in a dose-dependent manner. In a cell cycle analysis, ADSC-CM decreased the UVB-induced apoptotic cell death, which was demonstrated by the reduced sub-G1 phase of HDF. In addition, the ADSC-CM increased the protein expression of collagen type I and decreased the protein level of matrix metalloproteinase 1 in HDF, which may account for the increased collagen contents in the dermis. CONCLUSIONS: Collectively, these results indicate that the ADSC and its secretory factors are effective for UVB-induced wrinkles, and the antiwrinkle effect is mainly mediated by reducing UVB-induced apoptosis and stimulating collagen synthesis of HDF.

Kim, Y. J., J. T. Kim, et al. (2008). "ICAT participates in proliferation and osteogenic differentiation of human adipose tissue-derived mesenchymal stem cell." Life Sci **83**(25-26): 851-8.

AIMS: The Wnt/beta-catenin pathway plays a critical part in several cell physiology events associated with embryonic development and adult homeostasis, including determination, proliferation, migration, and differentiation. However, the role of Wnt signaling in osteoblastogenesis from mesenchymal stem cells (MSC) remains a controversial matter. Therefore, in the present study, we investigated how ICAT (inhibitor of beta-catenin and TCF-4), a negative regulator of the Wnt signaling pathway, influenced differentiation and proliferation of human adipose tissue-derived stromal cells (hASC). MAIN METHODS: To mediate ICAT overexpression in hASC, we used a lentiviral gene transfer technique. We further determined the role of ICAT by RNAi technique. KEY FINDINGS: ICAT-transduced hASC exhibited lower TCF promoter activity and cellular growth capacity than control cells, but ICAT overexpression did not affect hASC attachment efficiency. ICAT overexpression also increased osteogenic differentiation. Conversely, introduction of an ICAT siRNA oligonucleotide increased TCF promoter activity and cellular proliferation, but it inhibited osteogenic differentiation. SIGNIFICANCE: Taken together, these findings indicated that ICAT participated in regulating hASC proliferation and differentiation by modulating Wnt signaling.

Kingham, P. J., D. F. Kalbermatten, et al. (2007). "Adipose-derived stem cells differentiate into a Schwann cell phenotype and promote neurite outgrowth in vitro." Exp Neurol **207**(2): 267-74.

Experimentally, peripheral nerve repair can be enhanced by Schwann cell transplantation but the clinical application is limited by donor site morbidity and the inability to generate a sufficient number of cells quickly. We have investigated whether adult stem cells, isolated from adipose tissue, can be differentiated into functional Schwann cells. Rat visceral fat was enzymatically digested to yield rapidly proliferating fibroblast-like cells, a proportion of which expressed the mesenchymal stem cell marker, stro-1, and nestin, a neural progenitor protein. Cells treated with a mixture of glial growth factors (GGF-2, bFGF, PDGF and forskolin) adopted a spindle-like morphology similar to Schwann cells. Immunocytochemical staining and western blotting indicated that the treated cells expressed the glial markers, GFAP, S100 and p75, indicative of differentiation. When co-cultured with NG108-15 motor neuron-like cells, the differentiated stem cells enhanced the number of NG108-15 cells expressing neurites, the number of neurites per cell and the mean length of the longest neurite extended. Schwann cells evoked a similar response whilst undifferentiated stem cells had no effect. These results indicate adipose tissue contains a pool of regenerative stem cells which can be differentiated to a Schwann cell phenotype and may be of benefit for treatment of peripheral nerve injuries.

Kingham, P. J., C. Mantovani, et al. (2009). "Notch independent signalling mediates Schwann cell-like

differentiation of adipose derived stem cells." Neurosci Lett **467**(2): 164-8.

Adipose derived stem cells (ASC) differentiate into a Schwann cell (SC)-like phenotype but the signalling pathways mediating this are unknown. We hypothesised that notch might be involved, given its important role in regulating SC development. Rat ASC were differentiated using bFGF, PDGF, GGF-2 and forskolin. RT-PCR analysis showed that mRNA for notch-1 and notch-2 receptors and the notch responsive gene, hes-1, were expressed throughout the differentiation process whereas jagged-1 a notch ligand, and the hey-1 gene were markedly down-regulated. In contrast delta-1 was up-regulated with differentiation and was strongly expressed by rat primary SC. Treatment of ASC with N-[N-(3,5-difluorophenacetyl-l-alanyl)]-S-phenylglycine t-butyl ester (DAPT), a gamma-secretase inhibitor which blocks notch signalling, had no effect on up-regulation of SC proteins S100 or GFAP during differentiation. Furthermore, when co-cultured with NG108-15 neurons, differentiated ASC cultures treated in the absence or presence of DAPT enhanced neurite outgrowth to similar levels. Differentiated ASC expressed PMP-22 but P0 was only present when co-cultured with dorsal root ganglia neurons. DAPT did not affect the expression of these myelin proteins. Thus, ASC express components of the notch signalling pathway but our studies suggest notch is unlikely to play a role in the neurotrophic activity and myelination capability of ASC differentiated into SC-like cells.

Knippenberg, M., M. N. Helder, et al. (2005). "Adipose tissue-derived mesenchymal stem cells acquire bone cell-like responsiveness to fluid shear stress on osteogenic stimulation." Tissue Eng **11**(11-12): 1780-8.

To engineer bone tissue, mechanosensitive cells are needed that are able to perform bone cell-specific functions, such as (re)modeling of bone tissue. In vivo, local bone mass and architecture are affected by mechanical loading, which is thought to provoke a cellular response via loading-induced flow of interstitial fluid. Adipose tissue is an easily accessible source of mesenchymal stem cells for bone tissue engineering, and is available in abundant amounts compared with bone marrow. We studied whether adipose tissue-derived mesenchymal stem cells (AT-MSCs) are responsive to mechanical loading by pulsating fluid flow (PFF) on osteogenic stimulation in vitro. We found that ATMSCs show a bone cell-like response to fluid shear stress as a result of PFF after the stimulation of osteogenic differentiation by 1,25-dihydroxyvitamin D3. PFF increased nitric oxide production, as well as upregulated cyclooxygenase-2, but not cyclooxygenase-1, gene expression in osteogenically stimulated AT-MSCs. These data suggest that AT-MSCs acquire bone cell-like responsiveness to pulsating fluid shear stress on 1,25-dihydroxyvitamin D3-induced osteogenic differentiation. ATMSCs might be able to perform bone cell-specific functions during bone (re)modeling in vivo and, therefore, provide a promising new tool for bone tissue engineering.

Lee, E. Y., Y. Xia, et al. (2009). "Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF." Wound Repair Regen **17**(4): 540-7.

Adipose-derived stem cells (ADSCs) have been shown to induce wound-healing effects. Because inflammation near the wound area induces oxygen deficiency, it is interesting to elucidate the effect of hypoxia on the function of ADSCs. In this work, we asked: (1) does hypoxia alter the wound-healing function of ADSCs? and (2) what are the major factors responsible for the alteration in the wound-healing function? Effect of hypoxia on the proliferation of ADSCs was first examined that hypoxia (2% O(2)) enhanced the proliferation of ADSCs in either the presence of serum or in the absence of serum. The conditioned medium of ADSCs harvested under hypoxia (hypoCM) significantly promoted collagen synthesis and the migration of human dermal fibroblasts, compared with that in normoxia (norCM). In the animal studies, hypoCM significantly reduced the wound area compared with norCM. Furthermore, mRNA and protein measurements showed that hypoxia up-regulated growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Inhibition of VEGF and bFGF using neutralizing antibodies reversed the migration of the wounded human dermal fibroblasts and the healing of wounds in animal experiment. Collectively, these results suggest that hypoxia increases the proliferation of ADSCs and enhances the wound-healing function of ADSCs, at least partly, by up-regulating the secretion of VEGF and bFGF.

Lee, S. T., K. Chu, et al. (2009). "Slowed progression in models of Huntington disease by adipose stem cell transplantation." Ann Neurol **66**(5): 671-81.

OBJECTIVE: Adipose-derived stem cells (ASCs) are readily accessible and secrete multiple growth factors. Here, we show that ASC transplantation rescues the striatal pathology of Huntington disease (HD) models. METHODS: ASCs were isolated from human subcutaneous adipose

tissue. In a quinolinic acid (QA)-induced rat model of striatal degeneration, human ASCs (1 million cells) were transplanted into the ipsilateral striatal border immediately after the QA injection. In 60-day-old R6/2 mice transgenic for HD, ASCs (0.5 million cells) were transplanted into each bilateral striata. In in vitro experiments, we treated mutant huntingtin gene-transfected cerebral neurons with ASC-conditioned media. RESULTS: In the QA model, human ASCs reduced apomorphine-induced rotation behavior, lesion volume, and striatal apoptosis. In R6/2 transgenic mice, transplantation of ASCs improved Rota-Rod performance and limb clasping, increased survival, attenuated the loss of striatal neurons, and reduced the huntingtin aggregates. ASC-transplanted R6/2 mice expressed elevated levels of peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha) and reactive oxygen defense enzymes and showed activation of the Akt/cAMP-response element-binding proteins. ASC-conditioned media decreased the level of N-terminal fragments of mutant huntingtin and associated apoptosis, and increased PGC-1alpha expression. INTERPRETATION: Collectively, ASC transplantation slowed striatal degeneration and behavioral deterioration of HD models, possibly via secreted factors.

Lee, W. C., T. M. Maul, et al. (2007). "Effects of uniaxial cyclic strain on adipose-derived stem cell morphology, proliferation, and differentiation." Biomech Model Mechanobiol **6**(4): 265-73.

Cells and tissues in vivo are subjected to various forms of mechanical forces that are essential to their normal development and functions. The arterial blood vessel wall is continuously exposed to mechanical stresses such as pressure, strain, and shear due to the pulsatile nature of blood flow. Vascular smooth muscle cells (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 not only regulated by chemical factors such as transforming growth factor-beta(1) (TGF-beta(1)), but also by mechanical factors such as uniaxial strain. The purpose of our study was to explore the effects of TGF-beta(1) alone or in combination with uniaxial cyclic strain on adipose-derived stem cell (ASC) morphology, proliferation, and differentiation. Low passage ASCs were stimulated with 10% strain at 1 Hz for 7 days, with or without TGF-beta(1). Cyclic strain inhibited proliferation, and caused alignment of the cells and of the F-actin cytoskeleton perpendicular to the direction of strain. Strain alone resulted in a decrease in the expression of early SMC markers alpha-SMA and h (1)-calponin. While the response of SMCs and other progenitor cells such as bone marrow stromal cells to mechanical forces has been extensively studied, the roles of these forces on ASCs remain unexplored. This work advances our understanding of the mechanical regulation of ASCs.

Lei, L., W. Liao, et al. (2007). "Biological character of human adipose-derived adult stem cells and influence of donor age on cell replication in culture." Sci China C Life Sci **50**(3): 320-8.

To investigate the biological character of human adipose-derived adult stem cells (hADAS cells) when cultured in vitro and the relationship between hADAS cell's replication activity and the donor's age factor, and to assess the stem cells as a new source for tissue engineering. hADAS cells are isolated from human adipose tissue of different age groups (from adolescents to olds: <20 years old, 21-40 years old, 41-60 years old and >61 years old groups). The protein markers (CD29, CD34, CD44, CD45, CD49d, HLA-DR, CD106) of hADAS cells were detected by flow cytometry (FCM) to identify the stem cell, and the cell cycle was examined for P20 hADAS cells to evaluate the safety of the subculture in vitro. The generative activity of hADAS cells in different age groups was also examined by MTT method. The formula "TD = t x log2/logNt - logN0" was used to get the time doubling (TD) of the cells. The results showed that the cells kept heredity stabilization by chromosome analysis for at least 20 passages. The TD of these cells increased progressively by ageing, and the TD of the <20 years old group was lower than that of the >61 years old group (statistical analysis of variance (ANOVA), $P=0.002$, $P<0.05$). These findings suggested that a higher level of hADAS cells replication activity was found in the younger donators, and they represent novel and valuable seed cells for studies of tissue engineering.

Liang, L., T. Ma, et al. (2009). "Therapeutic potential and related signal pathway of adipose-derived stem cell transplantation for rat liver injury." Hepatol Res **39**(8): 822-32.

Aim: Liver transplantation is the only currently effective therapy for end-stage chronic liver disease and severe acute liver failure, but its use is limited by high cost and a shortage of allografts. Here we explored the effectiveness of transplanting adipose-derived stem cells (ADSCs) into rats with experimentally induced liver injury. Methods: ADSCs obtained from rats were hepatogenic induced in vitro with MAPK pathways inhibitors preconditioning. In vivo, ADSCs were transplanted into rats via different routes and serum liver function markers from post-

operative rats were tested. Results: When grown in adipogenic induction medium, ADSCs were able to differentiate into adipocytes. In hepatogenic induction medium, ADSCs were able to differentiate into hepatocyte-like cells, with appropriate changes in morphology and appropriately elevated expression of hepatocyte-specific markers. ERK1/2 phosphorylation activity was also significantly upregulated during the hepatogenic differentiation process, and was blocked by the ERK/MAPK pathway-specific inhibitor PD98059. In a rat liver injury model, intravenously injected ADSCs successfully engrafted into recipient livers. We found that injection via the hepatic portal vein was more efficient than via the dorsal vein of the penis. ADSC transplantation into damaged livers significantly decreased the level of serum liver enzymes such as alanine aminotransferase and aspartate aminotransferase, and improved serum albumin level. Both the number of engrafted cells and the improvement of liver function reached a peak two weeks after transplantation. Conclusion: Transplanted ADSCs appear to be therapeutically effective in the rat liver injury model, which may ultimately provide a therapeutic alternative to liver transplantation in human patients.

Lin, Y. C., C. A. Brayfield, et al. (2009). "Peptide modification of polyethersulfone surfaces to improve adipose-derived stem cell adhesion." Acta Biomater **5**(5): 1416-24.

Polyethersulfone (PES) is a nondegradable, biocompatible, synthetic polymer that is commonly utilized as a membrane material for applications such as hemodialysis, ultrafiltration and bioreactor technology. Various studies have shown surface modification to be a valuable tool in the development of nondegradable materials which promote cell adhesion. Cells of interest include adipose-derived stem cells (ASCs). ASCs are multipotent mesenchymal stem cells that are useful for various regenerative medicine applications. In this study, we hypothesized that PES surfaces modified with a peptide sequence based from fibronectin, such as Arg-Gly-Asp (RGD), Arg-Gly-Asp-Ser and Gly-Arg-Gly-Asp-Ser, would increase ASC adhesion compared to unmodified PES surfaces. The synthetic peptides were covalently bonded to amine-modified PES surfaces using 1-ethyl-3-(dimethylaminopropyl) carbodiimide. The surfaces were characterized using a ninhydrin assay and contact angle measurements. The ninhydrin assay confirmed the presence of amine groups on the surface of peptide-treated PES disks. Advancing water contact angles were analyzed to detect changes in the hydrophilicity of the polymer surfaces, and results indicated our PES membranes had excellent hydrophilicity. The attachment and proliferation of human ASCs was assessed and RGD-treated surfaces resulted in a higher number of attached ASCs after 6 and 48 h, as compared to unmodified PES surfaces. Additionally, varying concentrations of the RGD peptide sequence concentration were examined. These results indicate that PES membranes modified with the RGD peptide sequence can be utilized for enhanced ASC attachment in biomedical applications.

Mitchell, J. B., K. McIntosh, et al. (2006). "Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers." Stem Cells **24**(2): 376-85.

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 (SVF) cells relative to serial-passaged adipose-derived stem cells (ASCs). The initial SVF cells contained colony-forming unit fibroblasts at a frequency of 1:32. Colony-forming unit adipocytes and osteoblasts were present in the SVF cells at comparable frequencies (1:28 and 1:16, respectively). 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 SVF cells and increased significantly with successive passages. The stem cell-associated marker CD34 was at peak levels in the SVF cells and/or early-passage ASCs and remained present, although at reduced levels, throughout the culture period. Aldehyde dehydrogenase and the multidrug-resistance transport protein (ABCG2), both of which have been used to identify and characterize hematopoietic stem cells, are expressed by SVF cells and ASCs at detectable levels. Endothelial cell-associated markers (CD31, CD144 or VE-cadherin, vascular endothelial growth factor receptor 2, von Willebrand factor) were expressed on SVF cells 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, compared with the heterogeneity of the crude SVF.

Ning, H., G. Liu, et al. (2009). "Identification of an aberrant cell line among human adipose tissue-derived stem cell isolates." Differentiation **77**(2): 172-80.

Adipose tissue-derived stem cells (ADSC) are isolated from the stromal vascular fraction (SVF)

of adipose tissue and considered an excellent cell source for regenerative medicine. During the isolation and propagation of several human ADSC cell lines, we observed the emergence of an unusual cell line designated HADSC-6. Although initially fibroblast-like as typical ADSC are, HADSC-6 cells became homogeneously cuboid in shape, had very little cytoplasm, and formed aggregates with capsule-like boundary. Proliferation assay showed that HADSC-6 grew much faster than typical HADSC cell lines, such as HADSC-20. Immunocytochemistry showed that HADSC-6 did not express endothelial markers CD31 and vWF, and matrigel tube formation assay showed that it was unable to form endothelial-like tube structures. However, LDL uptake, a reliable endothelial marker, was positively identified. Chromosomal analysis showed that HADSC-6 cells were hypertriploid, and soft agar colony formation assay showed that they were able to proliferate and form large colonies in an anchorage-independent manner. However, tumorigenicity test showed that HADSC-6 was unable to form tumors in athymic mice. RT-PCR analysis showed that both HADSC-6 and HADSC-20 expressed VEGF-A, VEGF-B, VEGF-D, and VEGFR1 but not VEGFR2 or VEGFR3. VEGF-C, however, was expressed at a high level in HADSC-20 but undetectable in HADSC-6. In the IGF system, IGF-1 was abundantly expressed in HADSC-20 but marginally detectable in HADSC-6, and IGF-1R was abundantly expressed in HADSC-6 but not detectable in HADSC-20. In the FGF system, bFGF was abundantly expressed in HADSC-20 but marginally detectable in HADSC-6, and FGFR1 was abundantly expressed in both. Taken together, these results suggested that HADSC-6 cells were spontaneously transformed from the endothelium; therefore, they were further compared to previously published data of four naturally occurring human angiosarcoma cell lines. The results showed that the established angiosarcoma cell lines exhibit considerable variations among themselves and HADSC-6 displayed most of these variable characteristics.

Oedayrajsingh-Varma, M. J., S. M. van Ham, et al. (2006). "Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure." Cytotherapy **8**(2): 166-77.

BACKGROUND: Adipose tissue contains a stromal vascular fraction that can be easily isolated and provides a rich source of adipose tissue-derived mesenchymal stem cells (ASC). These ASC are a potential source of cells for tissue engineering. We studied whether the yield and growth characteristics of ASC were affected by the type of surgical procedure used for adipose tissue harvesting, i.e. resection, tumescent liposuction and ultrasound-assisted liposuction. METHODS: Frequencies of ASC in the stromal vascular fraction were assessed in limiting dilution assays. The phenotypical marker profile of ASC was determined, using flow cytometry, and growth kinetics were investigated in culture. ASC were cultured under chondrogenic and osteogenic conditions to confirm their differentiation potential. RESULTS: The number of viable cells in the stromal vascular fraction was affected by neither the type of surgical procedure nor the anatomical site of the body from where the adipose tissue was harvested. After all three surgical procedures, cultured ASC did express a CD34+ CD31- CD105+ CD166+ CD45- CD90+ ASC phenotype. However, ultrasound-assisted liposuction resulted in a lower frequency of proliferating ASC, as well as a longer population doubling time of ASC, compared with resection. ASC demonstrated chondrogenic and osteogenic differentiation potential. DISCUSSION: We conclude that yield and growth characteristics of ASC 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.

Otto, T. C. and M. D. Lane (2005). "Adipose development: from stem cell to adipocyte." Crit Rev Biochem Mol Biol **40**(4): 229-42.

Cell culture models have been developed to study commitment and subsequent differentiation of preadipocytes into adipocytes. Bone morphogenetic protein 4 commits mesenchymal stem cells to the adipose lineage. Other factors, including Wnt signaling, cell density, and cell shape, play a role in lineage commitment. Following commitment to the adipose lineage, growth-arrested preadipocytes can differentiate to adipocytes by treatment with insulin-like growth factor 1, glucocorticoid and an agent that increases cAMP level. This process is characterized by a rapid and transient increase in CCAAT/enhancer binding protein (C/EBP) beta and synchronous re-entry into the cell cycle. Acquisition of DNA-binding by C/EBPbeta occurs after the transcription factor becomes phosphorylated. The cells enter a growth-arrested state and begin terminal differentiation. C/EBPalpha, peroxisome proliferator-activated receptor gamma, and adipocyte determination, and differentiation-dependent factor 1 coordinate the expression of genes that create and maintain the adipocyte phenotype.

Park, I. S., M. Han, et al. (2009). "The correlation between human adipose-derived stem cells

differentiation and cell adhesion mechanism." Biomaterials **30**(36): 6835-43.

In recent years, research in the areas of stem cells has dramatically increased, including studies of cellular adhesion to a substrate. We sought to determine the adhesive properties of human adipose-derived stem cells (hASCs) for extracellular matrix proteins. The adhesion of hASCs to collagens and laminin was completely inhibited by a monoclonal antibody, Mab 2253, which binds to the beta1 integrin subunit. These data indicate that hASC adhesion to collagens and laminin was exclusively mediated by an integrin. Cell adhesion on fibronectin (Fn) was inhibited by the heparin-binding peptide (HBP) in the presence of Mab 2253, but not by either Mab 2253 or HBP alone. These results indicate that both the beta1 subunit and the heparan sulfate proteoglycan participated in the cell adhesion to Fn. Microscopic views showed extensive spreading of hASCs cultured on Fn, whereas the cells maintained a round shape when cultured on a heparin-binding domain (HBD) substrate. hASCs differentiated into adipocytes, which stained positive for lipid vacuoles by Oil Red-O analysis, more readily on HBD substrate than on FN substrate. These results suggest that hASCs have an adhesion mechanism for the HBD of Fn and hASC morphology is controlled by the adhesion mechanism and strongly correlated with adipogenic differentiation.

Prichard, H. L., W. M. Reichert, et al. (2007). "Adult adipose-derived stem cell attachment to biomaterials." Biomaterials **28**(6): 936-46.

Attachment of adipose-derived stem cells (ASCs) to biomaterials prior to implantation is a possible strategy for mediating inflammation and wound healing. In this study, the ASC percent coverage was measured on common medical grade biosensor materials subjected to different surface treatments. Cell coverage on silicone elastomer (poly-dimethylsiloxane) was below 20% for all surface treatments. Polyimide (Kapton), polyurethane (Pellethane) and tissue culture polystyrene all exhibited >50% coverage for surfaces treated with fibronectin (Fn), fibronectin plus avidin/biotin (dual ligand), and oxygen plasma plus fibronectin treatments (FnO2). The fibronectin treatment performed as well or better on polyimide, polyurethane, and tissue culture polystyrene compared to the dual ligand and fibronectin oxygen plasma-treated surfaces. Cell detachment with increasing shear stresses was <25% for each attachment method on both polyimide and polyurethane. The effects of attachment methods on the basic cell functions of proliferation, metabolism, ATP concentration, and caspase-3 activity were analyzed yielding proliferation profiles that were very similar among all of the materials. No significant differences in metabolism, intracellular ATP, or intracellular caspase-3 activity were observed for any of the attachment methods on either polyimide or polyurethane.

Radtke, C., B. Schmitz, et al. (2009). "Peripheral glial cell differentiation from neurospheres derived from adipose mesenchymal stem cells." Int J Dev Neurosci **27**(8): 817-23.

Mesenchymal stem cells derived from bone marrow and adipose tissue are being considered for use in neural repair because they can differentiate after appropriate induction in culture into neurons and glia. The question we asked was if neurospheres could be harvested from adipose-derived stem cells and if they then could differentiate in culture to peripheral glial-like cells. Here, we demonstrate that adipose-derived mesenchymal stem cells can form nestin-positive non-adherent neurosphere cellular aggregates when cultured with basic fibroblast growth factor and epidermal growth factor. Dissociation of these neurospheres and removal of mitogens results in expression of the characteristic Schwann cell markers S100 and p75 nerve growth factor receptor and GFAP. The simultaneous expression of these glia markers are characteristic features of Schwann cells and olfactory ensheathing cells which have unique properties regarding remyelination and enhancement of axonal regeneration. When co-cultured with dorsal root ganglion neurons, the peripheral glial-like cells derived from adipose mesenchymal stem cells aligned with neuritis and stimulated neuritic outgrowth. These results indicate that neurospheres can be generated from adipose-derived mesenchymal stem cells, and upon mitogen withdrawal can differentiate into peripheral glial cells with neurotrophic effects.

Ramos, T. V., T. Wang, et al. (2009). "Adipose stem cell side population in the mouse." J Tissue Eng Regen Med **3**(6): 430-41.

Adipose tissue has become a reliable source of adult stem cells, which appear to possess a yet-undetermined degree of plasticity. With the difficulties associated with harvesting adult bone marrow stem cells, adipose tissue may represent a valuable and easily acquired source of stem cells. Stem cells have been identified using the DNA binding dye Hoechst 33342 and flow cytometry in various tissues known as the side population (SP). The present study shows, for the first time, the presence of side population stem cells in adult adipose tissues. Flow cytometric identification and isolation of this subpopulation of stem cells revealed that in the mouse there are 2.5% of adipose SP cells within the stromal

vascular fraction of adipose tissue. In culture, mouse adipose SP cells showed the capacity to undergo in vitro differentiation into osteogenic, chondrogenic and adipogenic lineages. In NOD/SCID mice, freshly sorted mouse adipose SP cells were able to engraft and assist in wound healing. This animal model study showed that adipose SP cells were able to regenerate epithelial layers and connective tissue with minor scar formation. The ability of this novel cell population within adipose tissue to undergo directional differentiation in vitro and to regenerate skin in vivo has potential impact for uses in surgical dermal applications.

Riekstina, U., I. Cakstina, et al. (2009). "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis." Stem Cell Rev **5**(4): 378-86.

Mesenchymal stem cells (MSCs) have been isolated from a variety of human tissues, e.g., bone marrow, adipose tissue, dermis, hair follicles, heart, liver, spleen, dental pulp. Due to their immunomodulatory and regenerative potential MSCs have shown promising results in preclinical and clinical studies for a variety of conditions, such as graft versus host disease (GvHD), Crohn's disease, osteogenesis imperfecta, cartilage damage and myocardial infarction. MSC cultures are composed of heterogeneous cell populations. Complications in defining MSC arise from the fact that different laboratories have employed different tissue sources, extraction, and cultivation methods. Although cell-surface antigens of MSCs have been extensively explored, there is no conclusive evidence that unique stem cells markers are associated with these adult cells. Therefore the aim of this study was to examine expression of embryonic stem cell markers Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4 in adult mesenchymal stem cell populations derived from bone marrow, adipose tissue, dermis and heart. Furthermore, we tested whether human mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions. We found that bone marrow MSCs express embryonic stem cell markers Oct4, Nanog, alkaline phosphatase and SSEA-4, adipose tissue and dermis MSCs express Oct4, Nanog, SOX2, alkaline phosphatase and SSEA-4, whereas heart MSCs express Oct4, Nanog, SOX2 and SSEA-4. Our results also indicate that human adult mesenchymal stem cells preserve tissue-specific differences under in vitro culture conditions during early passages, as shown by distinct germ layer and embryonic stem cell marker expression patterns. Studies are now needed to determine the functional role of embryonic stem cell markers Oct4, Nanog and SOX2 in adult human MSCs.

Ross, A. S., R. Tsang, et al. (2008). "Expression of p107 and p130 during human adipose-derived stem cell adipogenesis." Biochem Biophys Res Commun **366**(4): 927-31.

Within the first 24h of hormonally stimulated adipocyte differentiation, murine 3T3-L1 preadipocytes undergo a mitotic expansion phase prior to terminal differentiation. During this time, the cell cycle regulatory proteins, p130 and p107 undergo dramatic differential expression and the transient increase in expression of p107 appears to be required for terminal differentiation. Recently, human adipose-derived human stem cells (hASC) of mesenchymal origin have been used as a model of human adipocyte differentiation and we sought to determine if differentiating hASC undergo clonal expansion and if the regulated expression of p130/p107 was similar to that observed during 3T3-L1 adipogenesis. Results indicate that differentiating hASC, unlike 3T3-L1 cells do not undergo clonal expansion and p130 expression gradually diminishes across differentiation. However, p107 expression is transiently increased during hASC differentiation in a manner analogous to 3T3-L1 cells suggesting a similar role for p107 in terminal differentiation in human adipocytes.

Safford, K. M. and H. E. Rice (2005). "Stem cell therapy for neurologic disorders: therapeutic potential of adipose-derived stem cells." Curr Drug Targets **6**(1): 57-62.

There is growing evidence to suggest that reservoirs of stem cells may reside in several types of adult tissue. These cells may retain the potential to transdifferentiate from one phenotype to another, presenting exciting possibilities for cellular therapies. Recent discoveries in the area of neural differentiation are particularly exciting given the limited capacity of neural tissue for intrinsic repair and regeneration. Adult adipose tissue is a rich source of mesenchymal stem cells, providing an abundant and accessible source of adult stem cells. These cells have been termed adipose derived stem cells (ASC). The characterization of these ASCs has defined a population similar to marrow-derived and skeletal muscle-derived stem cells. The success seen in differentiating ASC into various mesenchymal lineages has generated interest in using ASC for neuronal differentiation. Initial in vitro studies characterized the morphology and protein expression of ASC after exposure to neural induction agents. Additional in vitro data suggests the possibility that ASCs are capable of neuronal activity. Progress in the in vitro characterization of ASCs has led to in vivo

modeling to determine the survival, migration, and engraftment of transplanted ASCs. While work to define the mechanisms behind the transdifferentiation of ASCs continues, their application to neurological diseases and injuries should also progress. The subject of this review is the capacity of adipose derived stem cells (ASC) for neural transdifferentiation and their application to the treatment of various neurologic disorders.

Santiago, L. Y., R. W. Nowak, et al. (2006). "Peptide-surface modification of poly(caprolactone) with laminin-derived sequences for adipose-derived stem cell applications." Biomaterials **27**(15): 2962-9.

Human adipose tissue has been recognized as a source of adult stem cells for tissue engineering applications such as bone, cartilage, and soft tissue repair. For the success of these tissue-engineering approaches, a cell delivery vehicle such as a hydrogel or scaffold is required to position the stem cells at the site of need. Surface modification techniques have been instrumental in the development of scaffolds that promote cell-surface interactions. In this study, poly(caprolactone) (PCL), surfaces were modified in order to promote the attachment and proliferation of adipose-derived stem cells (ASCs). RGD, YIGSR, and IKVAV peptide sequences derived from the extracellular matrix protein laminin were each covalently attached to an aminated polymer surface using carbodiimide chemistry. The surface was characterized using scanning electron microscopy (SEM), goniometry and X-ray photoelectron spectroscopy (XPS). The attachment and proliferation of ASCs was assessed on the different peptide-treated surfaces. XPS analysis confirmed the presence of the peptide sequences on the surface of the polymer as indicated by the increase in the nitrogen/carbon ratio on the surface of the polymer. Among all peptide sequences tested, IKVAV-treated surfaces had a significantly greater number of ASCs bound 2 and 3 days after cell seeding. SEM confirmed differences in the morphology of the cells attached to the three peptide-treated surfaces. These results indicate that IKVAV is a suitable peptide sequence for use in surface modification techniques aimed at improving the attachment of ASCs to a tissue-engineered scaffold.

Schipper, B. M., K. G. Marra, et al. (2008). "Regional anatomic and age effects on cell function of human adipose-derived stem cells." Ann Plast Surg **60**(5): 538-44.

Adipose tissue has been shown to contain adult mesenchymal stem cells that have therapeutic applications in regenerative medicine. There is evidence that the ability of adipose precursor cells to grow and differentiate varies among fat depots and changes with age. Defining these variations in cell function and molecular mechanisms of adipogenesis will facilitate the development of cell-based therapies. We compared cells harvested from 5 different subcutaneous (SC) adipose depots in 12 female patients classified into 3 age ranges (25-30, 40-45, and 55-60 years old). Capacity for differentiation of isolated adipose-derived stem cells (ASCs) with and without ciglitazone, a strong peroxisome proliferatoractivated receptors (PPAR)-gamma agonist, was assessed in vitro. ASCs were also characterized by lipolytic function, proliferation, and sensitivity to apoptosis. Additionally, PPAR-gamma-2 protein expression was determined. We observed a difference in the apoptotic susceptibility of ASCs from various SC depots, with the superficial abdominal depot (above Scarpas layer) significantly more resistant to apoptosis when compared with the 4 other depots. We have also demonstrated that a PPAR-gamma agonist aids in the induction of differentiation in cells from all depots and ages. Although sensitivity to apoptosis was linked to anatomic depot, differences in cell proliferation were related primarily to age. Stimulated free glycerol release has been shown to be highest in the arm depot. The arm depot has also consistently shown expression of PPAR-gamma-2 with and without a PPAR-gamma agonist. Younger patients have increased PPAR-gamma-2 expression in all depots, whereas the older patients have consistent elevated expression only in the arm and thigh depots. We have shown there is variability in function of ASCs that have been harvested from different SC depots. Additionally, we have shown age-related changes in function. These data will help select patients and cell harvest sites most suitable for tissue engineering therapies.

Sonoda, E., S. Aoki, et al. (2008). "A new organotypic culture of adipose tissue fragments maintains viable mature adipocytes for a long term, together with development of immature adipocytes and mesenchymal stem cell-like cells." Endocrinology **149**(10): 4794-8.

Adipose tissue that consists of mature and immature adipocytes is suggested to contain mesenchymal stem cells (MSCs), but a culture system for analyzing their cell types within the tissue has not been established. Here we show that three-dimensional collagen gel culture of rat sc adipose tissue fragments maintained viable mature adipocytes for a long term, producing immature adipocytes and MSC-like cells from the fragments, using immunohistochemistry, ELISA, and real time RT-PCR. Bromodeoxyuridine uptake of mature adipocytes was detected. Adiponectin and leptin, and

adipocyte-specific genes of adiponectin, leptin, and PPAR-gamma were detected in culture assembly, whereas the lipogenesis factor insulin (20 mU/ml) and inflammation-related agent TNF-alpha (2 nm) increased and decreased, respectively, all of their displays. Both spindle-shaped cell types with oil red O-positive lipid droplets and those with expression of MSC markers (CD105 and CD44) developed around the fragments. The data indicate that adipose tissue-organotypic culture retains unilocular structure, proliferative ability, and some functions of mature adipocytes, generating both immature adipocytes and CD105+/CD44+ MSC-like cells. This suggests that our method will open up a new way for studying both multiple cell types within adipose tissue and the cell-based mechanisms of obesity and metabolic syndrome.

Trivedi, H. L., A. V. Vanikar, et al. (2008). "Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin." Transplant Proc **40**(4): 1135-9.

Type 1 diabetes mellitus (DM) is an autoimmune disorder with disturbed glucose/insulin metabolism, which has no medical treatment other than life-long insulin therapy, despite which 30% of subjects develop organ failure. Herein we have reported the use of human adipose-tissue-derived, insulin-making mesenchymal stem cells (h-AD-MSC) transfused with unfractionated cultured bone marrow (CBM) in 5 insulinopenic DM patients. PATIENTS AND METHODS: Five (M:F, 2:3) insulinopenic DM patients of 0.6 to 10 years' duration, ages 14 to 28 years under treatment insulin (Human with 14-70 U/d) showed postprandial blood sugars between 156 to 470 mg%, glycosylated hemoglobin 6.8% to 9.9% and c-peptide levels of 0.02 to 0.2 ng/mL. They underwent intraportal administration of xenogeneic-free h-AD-MSC (mean dose = 1.5 mL; cell counts, 2.1 x 10(3)/muL). The CD45-/90+/73(+) cells (29.8/16.8%) showed c-peptide levels of 3.08 ng/mL, insulin level of 1578 micro IU/mL. The aliquot was supplemented with CBM (mean dose 94 mL with cell counts: 18.7 x 10(3)/microL) containing CD45-/34+ elements of 0.93%. The Institutional Review Board approved the study protocol and consent forms. RESULTS: All patients were successfully infused CBM plus h-AD-MSC without any untoward effects and showed 30% to 50% decreased insulin requirements with 4- to 26-fold increased serum c-peptide levels, with a mean follow-up of 2.9 months. CONCLUSION: This report describes safe and effective treatment of insulinopenic diabetics using insulin-producing h-AD-MSC plus CBM without xenogeneic materials.

Vindigni, V., L. Michelotto, et al. (2009). "Isolation method for a stem cell population with neural potential from skin and adipose tissue." Neurol Res.

OBJECTIVE: In recent years, research on stem cells has been focused on the development of personalized cell-based therapies. Owing to their homing properties, adult human stem cells are a promising source of autologous cells to be used as therapeutic vehicles. Multiple potential sources for clinically useful stem and progenitor cells have been identified, including autologous and allogenic embryonic, fetal and adult somatic cells from neural, adipose and mesenchymal tissue. In the present report, we describe a simple protocol to obtain an enriched culture of adult stem cells organized in neurospheres from two post-natal tissues: skin and adipose tissue. METHODS: Adult stem cells isolated from skin and adipose tissue derived from the same adult donor were amplified under varying conditions related to the coating of the chamber slide and the presence of serum and/or growth factors, such as with EGF and FGF2. Neurospheres were then expanded and evaluated in terms of proliferation and gene expression. RESULTS: Adipose and skin derived neurospheres were comparable in size, quantity of cells and genes expressed. Cells from both types of tissue grew optimally without slide coating, in the presence of serum and with the combined addition of FGF2 and EGF. DISCUSSION: We describe a method for isolating and improving a population of multipotent adult precursor cells from the two most accessible adult tissue sources: skin and adipose tissue. This autologous adult stem cell population could be used for cell replacement or cell therapies.

Wang, L., J. Deng, et al. (2009). "Adipose-derived stem cells are an effective cell candidate for treatment of heart failure: an MR imaging study of rat hearts." Am J Physiol Heart Circ Physiol **297**(3): H1020-31.

This study assessed the potential therapeutic efficacy of adipose-derived stem cells (ASCs) on infarcted hearts. Myocardial infarction was induced in rat hearts by occlusion of the left anterior descending artery (LAD). One week after LAD occlusion, the rats were divided into three groups and subjected to transplantation of ASCs or transplantation of cell culture medium (CCM) or remained untreated. During a 1-mo recovery period, magnetic resonance imaging showed that the ASC-treated hearts had a significantly greater left ventricular (LV) ejection fraction and LV wall thickening than did the CCM-treated and untreated hearts. The capillary density in infarct border zone was significantly higher in the ASC-treated hearts than in the CCM-treated and untreated hearts. However, only 0.5% of the ASCs recovered from the ASC-treated hearts were stained positive for

cardiac-specific fibril proteins. It was also found that ASCs under a normal culture condition secreted three cardiac protective growth factors: vascular endothelial growth factor, hepatocyte growth factor, and insulin-like growth factor-1. Results of this study suggest that ASCs were able to improve cardiac function of infarcted rat hearts. Paracrine effect may be the mechanism underlying the improved cardiac function and increased capillary density.

Winter, A., S. Breit, et al. (2003). "Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells." Arthritis Rheum **48**(2): 418-29.

OBJECTIVE: To compare the chondrogenic potential of human bone marrow-derived mesenchymal stem cells (BMSC) and adipose tissue-derived stromal cells (ATSC), because the availability of an unlimited cell source replacing human chondrocytes could be strongly beneficial for cell therapy, tissue engineering, in vitro drug screening, and development of new therapeutic options to enhance the regenerative capacity of human cartilage. METHODS: Quantitative gene expression of common cartilage and cell interaction molecules was analyzed using complementary DNA array technology and reverse transcription-polymerase chain reaction during optimization of cell differentiation, in order to achieve a molecular phenotype similar to that of chondrocytes in cartilage. RESULTS: The multilineage potential of BMSC and ATSC was similar according to cell morphology and histology, but minor differences in marker gene expression occurred in diverse differentiation pathways. Although chondrogenic differentiation of BMSC and ATSC was indistinguishable in monolayer and remained partial, only BMSC responded (with improved chondrogenesis) to a shift to high-density 3-dimensional cell culture, and reached a gene expression profile highly homologous to that of osteoarthritic (OA) cartilage. CONCLUSION: Hypertrophy of chondrocytes and high matrix-remodeling activity in differentiated BMSC spheroids and in OA cartilage may be the basis for the strong similarities in gene expression profiles between these samples. Differentiated stem cell spheroids represent an attractive tool for use in drug development and identification of drug targets in OA cartilage-like tissue outside the human body. However, optimization of differentiation protocols to achieve the phenotype of healthy chondrocytes is desired for cell therapy and tissue engineering approaches.

Wolbank, S., A. Peterbauer, et al. (2007). "Labelling of human adipose-derived stem cells for non-invasive in vivo cell tracking." Cell Tissue Bank **8**(3): 163-77.

Human adipose-derived stem cells (ASC) can be expanded in an undifferentiated state or differentiated along the osteogenic, chondrogenic, adipogenic, myogenic, endothelial and neurogenic lineage. To test their in vivo and in situ regenerative potential, their fate needs to be traced after application in suitable defect models. Non-invasive imaging systems allow for real time tracking of labelled cells in the living animal. We have evaluated a bioluminescence cell tracking approach to visualise ASC labelled with luciferase in the living animal. Two procedures have been tested to efficiently label human stem cells with a reporter gene (luciferase, green fluorescent protein), namely lipofection with Lipofectamine 2000 and electroporation with a Nucleofector device. With both lipofection and nucleofection protocols, we have reached transfection efficiencies up to 60%. Reporter gene expression was detectable for 3 weeks in vitro and did not interfere with the phenotype and the stem cell properties of the cells. By means of a highly sensitive CCD camera, we were able to achieve real time imaging of cell fate for at least 20 days after application (intravenous, intramuscular, intraperitoneal, subcutaneous) in nude mice. Moreover, we were able to influence cell mobility by choosing different modes of application such as enclosure in fibrin matrix. The optical imaging system with transient transfection is an elegant cell-tracking concept to follow survival and fate of human stem cells in small animals.

Xu, Y., L. Liu, et al. (2008). "Myelin-forming ability of Schwann cell-like cells induced from rat adipose-derived stem cells in vitro." Brain Res **1239**: 49-55.

Although Schwann cell (SC) transplantation can enhance peripheral and central nerve repair experimentally, it is difficult to generate sufficient SC quickly for clinical application. So alternative cell systems for SC are desired. SC-like cells induced from adipose-derived stem cells (ADSC) may be one of the ideal alternative cell systems for SC. However, myelin-forming ability, which is the most important characteristics and function of SC, has not been investigated in SC-like cells from ADSC up to now. In this experiment, ADSC were harvested from rat inguinal fat pad. Rat ADSC were fibroblast-like in shape, almost all the cells expressed mesodermal marker fibronectin, and only few cells expressed neural stem cell marker nestin. A mixture of glial growth factors (Heregulin, bFGF, PDGF and forskolin) could induce rat ADSC into SC-like cells. SC-like cells were spindle-like in shape and expressed glial markers GFAP and S100, similar to genuine SC.

When intracellular cAMP was increased, SC-like cells could express myelin protein p0. More importantly, when co-cultured with rat pheochromocytoma cell line (PC12 cells), SC-like cells could induce the differentiation of PC12 cells rapidly and form myelin structures with PC12 cells in vitro. Our data further demonstrated that SC-like cells from ADSC were able to form myelins and these cells may benefit the treatment of peripheral and central nerve injuries.

Xu, Y., Z. Liu, et al. (2008). "Neurospheres from rat adipose-derived stem cells could be induced into functional Schwann cell-like cells in vitro." BMC Neurosci **9**: 21.

BACKGROUND: Schwann cells (SC) which are myelin-forming cells in peripheral nervous system are very useful for the treatment of diseases of peripheral nervous system and central nervous system. However, it is difficult to obtain sufficient large number of SC for clinical use, so alternative cell systems are desired. RESULTS: Using a procedure similar to the one used for propagation of neural stem cells, we could induce rat adipose-derived stem cells (ADSC) into floating neurospheres. In addition to being able to differentiate into neuronal- and glial-like cells, neurospheres could be induced to differentiate into SC-like cells. SC-like cells were bi- or tri-polar in shape and immunopositive for nestin and SC markers p75, GFAP and S-100, identical to genuine SC. We also found that SC-like cells could induce the differentiation of SH-SY5Y neuroblastoma cells efficiently, perhaps through secretion of soluble substances. We showed further that SC-like cells could form myelin structures with PC12 cell neurites in vitro. CONCLUSION: These findings indicated that ADSC could differentiate into SC-like cells in terms of morphology, phenotype and functional capacities. SC-like cells induced from ADSC may be useful for the treatment of neurological diseases.

Yoshimura, K., K. Sato, et al. (2008). "Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells." Aesthetic Plast Surg **32**(1): 48-55; discussion 56-7.

BACKGROUND: Lipoinjection is a promising treatment but has some problems, such as unpredictability and a low rate of graft survival due to partial necrosis. METHODS: To overcome the problems with lipoinjection, the authors developed a novel strategy known as cell-assisted lipotransfer (CAL). In CAL, autologous adipose-derived stem (stromal) cells (ASCs) are used in combination with lipoinjection. A stromal vascular fraction (SVF) containing ASCs is freshly isolated from half of the aspirated fat and recombined with the other half. This process converts relatively ASC-poor aspirated fat to ASC-rich fat. This report presents the findings for 40 patients who underwent CAL for cosmetic breast augmentation. RESULTS: Final breast volume showed augmentation by 100 to 200 ml after a mean fat amount of 270 ml was injected. Postoperative atrophy of injected fat was minimal and did not change substantially after 2 months. Cyst formation or microcalcification was detected in four patients. Almost all the patients were satisfied with the soft and natural-appearing augmentation. CONCLUSIONS: The preliminary results suggest that CAL is effective and safe for soft tissue augmentation and superior to conventional lipoinjection. Additional study is necessary to evaluate the efficacy of this technique further.

Yoshimura, K., K. Sato, et al. (2008). "Cell-assisted lipotransfer for facial lipoatrophy: efficacy of clinical use of adipose-derived stem cells." Dermatol Surg **34**(9): 1178-85.

BACKGROUND: Lipoinjection is a promising treatment, but its efficacy in recontouring facial lipoatrophy remains to be established. OBJECTIVE: The objective was to evaluate the efficacy and adverse effects of lipoinjection and supplementation of adipose-derived stem/stromal cells (ASCs) to adipose grafts. METHODS: To overcome drawbacks of autologous lipoinjection, we have developed a novel strategy called cell-assisted lipotransfer (CAL). In CAL, stromal vascular fraction containing ASCs was freshly isolated from half of an aspirated fat sample and attached to the other half of aspirated fat sample with the fat acting as a scaffold. This process converts relatively ASC-poor aspirated fat into ASC-rich fat. We performed conventional lipoinjection (non-CAL; n=3) or CAL (n=3) on six patients with facial lipoatrophy due to lupus profundus or Parry-Romberg syndrome. RESULTS: All patients obtained improvement in facial contour, but the CAL group had a better clinical improvement score than did the non-CAL patients, although the difference did not reach statistical significance (p=.11). Adipose necrosis was found in one non-CAL case who took perioperative oral corticosteroids. CONCLUSION: Our results suggest that CAL is both effective and safe and potentially superior to conventional lipoinjection for facial recontouring. The authors have indicated no significant interest with commercial supporters.

Yu, J. M., E. S. Jun, et al. (2008). "Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo." Stem Cells Dev **17**(3): 463-73.

Mesenchymal stem cells (MSCs) have generated a great deal of interest in clinical situations, due principally to their potential use in regenerative

medicine and tissue engineering applications. However, the therapeutic application of MSCs remains limited, unless the favorable effects of MSCs for tumor growth in vivo and the long-term safety of the clinical applications of MSCs can be understood more thoroughly. In this study, MSCs derived from human adipose tissues (hASCs) together with tumor cells were transplanted subcutaneously or intracranially into BALB/c nude mice to observe tumor outgrowth. The results indicated that hASCs with H460 or U87MG cells promoted tumor growth in nude mice. Our histopathological analyses indicated that the co-injection of tumor cells with hASCs exerted no influence on the formation of intratumoral vessels. Co-culture of tumor cells with hASCs or the addition of conditioned medium (CM) from hASCs effected an increase in the proliferation of H460 or U87MG cells. Co-injection of hASCs with tumor cells effected an increase in tumor cell viability in vivo, and also induced a reduction in apoptotic cell death. CM from hASCs inhibited hydrogen peroxide-induced cell death in H460 or U87MG cells. These findings indicated that MSCs could favor tumor growth in vivo. Thus, it is necessary to conduct a study concerning the long-term safety of this technique before MSCs can be used as therapeutic tools in regenerative medicine and tissue engineering.

Yukawa, H., H. Noguchi, et al. (2009). "Cell transplantation of adipose tissue-derived stem cells in combination with heparin attenuated acute liver failure in mice." Cell Transplant **18**(5): 611-8.

The effect of adipose tissue-derived stem cells (ASCs) in combination with heparin transplantation on acute liver failure mice with carbon tetrachloride (CCl(4)) injection was investigated. CCl(4) is a well-known hepatotoxin and induces hepatic necrosis. Heparin did not affect the viability of ASCs for at least 24 h. The injection of heparin into the caudal tail vein decreased slightly the activities of the alanine aminotransferase (ALT), asparate aminotransferase (AST), and lactate dehydrogenase (LDH) in plasma. In the transplantation of ASCs (1 x 10(6) cells) group, there was a trend toward decreased activities of all markers. However, four out of six mice died of the lung infarction. In the transplantation of ASCs in combination with heparin group, there was also a trend toward decreased activities of all markers. In addition, all mice survived for at least the duration of the study period. In conclusion, the transplantation of ASCs in combination with heparin was thus found to effectively treat acute liver failure.

Zhou, H. R., E. K. Kim, et al. (2007). "Obesity-associated mouse adipose stem cell secretion of monocyte chemotactic protein-1." Am J Physiol Endocrinol Metab **293**(5): E1153-8.

Studies showed that monocyte chemotactic protein-1 (MCP-1) concentrations are increased in obesity. In our current study, we demonstrate that plasma MCP-1 level in leptin-deficient ob/ob mice is significantly higher than in lean mice. Furthermore, we determined that basal adipose tissue MCP-1 mRNA levels are significantly higher in ob/ob mice compared with lean mice. To determine the mechanisms underlying obesity-associated increases in plasma and adipose tissue MCP-1 levels, we determined adipose tissue cell type sources of MCP-1 production. Our data show that adipose tissue stem cells (CD34(+)), macrophages (F4/80(+)), and stromal vascular fraction (SVF) cells express significantly higher levels of MCP-1 compared with adipocytes under both basal and lipopolysaccharide (LPS)-stimulated conditions. Furthermore, basal and LPS-induced MCP-1 secretion levels were the same for both adipose F4/80(+) and CD34(+) cells, whereas adipose CD34(+) cells have twofold higher cell numbers (30% of total SVF cells) compared with F4/80(+) macrophages (15%). Our data also show that CD34(+) cells from visceral adipose tissue depots secrete significantly higher levels of MCP-1 ex vivo when compared with CD34(+) cells from subcutaneous adipose tissue depots. Taken together, our data suggest that adipose CD34(+) stem cells may play an important role in obesity-associated increases in plasma MCP-1 levels.

Zhu, Y., T. Liu, et al. (2008). "Adipose-derived stem cell: a better stem cell than BMSC." Cell Biochem Funct **26**(6): 664-75.

To further study the proliferation and multi-differentiation potentials of adipose-derived stem cells (ADSCs), the cells were isolated with improved methods and their growth curves were achieved with cck-8. Surface protein expression was analyzed by flow cytometry to characterize the cell phenotype. The multi-lineage potential of ADSCs was testified by differentiating cells with adipogenic, chondrogenic, osteogenic, and myogenic inducers. The results showed that about 5 x 10(5) stem cells could be obtained from 400 to 600 mg adipose tissue. The ADSCs can be continuously cultured in vitro for up to 1 month without passage and they have several logarithmic growth phases during the culture period. Also, the flow cytometry analysis showed that ADSCs expressed high levels of stem cell-related antigens (CD13, CD29, CD44, CD105, and CD166), while did not express hematopoiesis-related antigens CD34 and CD45, and human leukocyte antigen HLA-DR was also negative. Moreover, stem cell-related transcription factors, Nanog, Oct-4, Sox-2, and Rex-1

were positively expressed in ADSCs. The expression of alkaline phosphatase (ALP) was detected in the early osteogenic induction and the calcified nodules were observed by von Kossa staining. Intracellular lipid droplets could be observed by Oil Red staining. Differentiated cardiomyocytes were observed by connexin43 fluorescent staining. In order to obtain more stem cells, we can subculture ADSCs every 14 days instead of the normal 5 days. ADSCs still keep strong proliferation ability, maintain their phenotypes, and have stronger multi-differentiation potential after 25 passages.

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a. Mesenchymal stem cells (MSC) have been characterized as multipotent cells which are able to differentiate into several mesodermal and nonmesodermal lineage cells and this feature along with their extensive growth and comprehensive immunomodulatory properties establish them as a promising tool for therapeutic applications, including cell-based tissue engineering and treatment of immune-mediated disorders. Although bone marrow (BM) is the most common MSC source, cells with similar

characteristics have been shown to be present in several other adult tissues. Adipose tissue (AT), large quantities of which can be easily obtained, represents an attractive alternative to BM in isolating adipose tissue-derived MSC (AT-MSC). BM-MSCs and AT-MSCs share some immunomodulatory properties as they are both not inherently immunogenic and suppress the proliferation of alloantigen- or mitogen-stimulated T-cells. Our purpose was to comparatively examine under appropriate in vitro conditions, phenotypes, morphology and some functional properties of BM-MSCs and AT-MSCs, such as differentiation potential and especially the ability to suppress the immunoglobulin production by mitogen-stimulated B-cells. While the morphological, immunophenotypical, colony-forming and adipogenic characteristics of both types of cells were almost identical, AT-MSCs showed less potential for osteogenic differentiation than BM-MSCs. We found that AT-MSCs not only inhibited the Ig-production but also suppressed this B-cell function to a much greater extent compared to BM-MSC. This finding supports the potential role of AT-MSCs as an alternative to BM-MSCs for clinical purposes.

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