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THE ROLE OF FAT GRAFTING AND ADIPOSE-DERIVED STEM CELLS
IN BREAST RECONSTRUCTION

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy in the College of Medicine at the
University of Kentucky

By

Krishna Subhash Vyas

Lexington, Kentucky

Director: Dr. Henry C. Vasconez, Professor of Surgery

Lexington, Kentucky

2016

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ABSTRACT OF DISSERTATION

THE ROLE OF FAT GRAFTING AND ADIPOSE-DERIVED STEM CELLS IN BREAST RECONSTRUCTION

Fat grafting is a common surgical procedure that involves the transfer of fat from one area of the body to another in order to improve contour deformities, such as in breast reconstruction. Advantages of the technique include using autologous tissue rather than a foreign body and the added benefit of having liposuction to remove fat from an undesirable location. Although adipose tissue could be the ideal soft tissue filler, fat grafting is plagued by tremendous variability in long-term retention, with volume survival rates of 20-80%, resulting in suboptimal outcomes and repetitive procedures.

The mechanisms contributing to long-term fat graft survival and resorption are not well understood. The discovery of multipotent mesenchymal adipose tissue-derived stem cells (ASCs) in subcutaneous adipose tissue has encouraged the study of their role in fat graft survival. ASCs are observed to survive after grafting, and in fact play a major role in adipocyte survival, regeneration and differentiation through adipogenesis and paracrine effects. In fact, lipoaspirate supplemented with ASCs has been shown to improve angiogenesis and long-term graft retention through the release of factors. Many adipose graft enrichment strategies encompassing growth factors, platelet-rich plasma, stem cells, gene therapy and tissue engineering have been attempted to augment and improve the viability of fat grafts. Therefore, a systematic review was undertaken to optimize safety and outcomes related to these enrichment strategies.

Recently, concerns have been raised from several regulatory bodies, including the FDA, regarding safety of fat grafting in the setting of breast reconstruction. ASCs within lipoaspirate have been postulated to create an inflammatory tumor microenvironment, to encourage angiogenesis, and to potentially contribute to tumorigenesis. Therefore, a review of both local data and a systematic review was undertaken to determine oncological outcomes of fat grafting to the breast.

Finally, we test the hypothesis that ASCs derived from obese donors exhibit compromised ASC functionality, leading to reduced fat graft retention when compared to non-obese subjects. Collectively, the studies that comprise this dissertation generate and critically appraise evidence for the safety, efficacy and outcomes of fat grafting in the setting of breast reconstruction.

Keywords: adipose tissue; adipose-derived stem cell; plastic and reconstructive surgery; lipotransfer, fat grafting, obesity

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06-01-2016

THE ROLE OF FAT GRAFTING AND ADIPOSE-DERIVED STEM CELLS
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Chapter 1: Introduction

Autologous fat grafting is a common surgical procedure that involves the transfer of fat from one area of the body to another in order to improve contour deformities. Fat grafting refers to the procedure in which fat is harvested by liposuction from a part of the body where it is unwanted and injected into an area where it can be used to add volume or correct a contour deformity. Advantages of the technique include using autologous fatty tissue rather than a foreign body and the added benefit of having liposuction to remove fat from an undesirable location.

There is tremendous clinical interest in the utilization of fat grafting for soft tissue reconstruction, with thousands of cases performed each year in the treatment of volume loss due to trauma, scars, wounds, fistulas, disease, congenital defects, or the natural process of aging (7). In the setting of breast reconstruction, for example, fat grafting provides significant aesthetic improvement in breast reconstruction (8), is a viable option for improving the quality of irradiated breast skin (9), and can help to alleviate post-mastectomy pain syndrome (10). Ideally, fat grafting could become an alternative to breast implants or complex microvascular tissue flap surgery in breast reconstruction (8, 11-13). Although fat could become the ideal soft tissue filler, it is plagued by its tremendous variability in long-term graft retention, with volume survival rates of 20-80%, resulting in suboptimal outcomes and repetitive procedures (14).

The mechanisms contributing to long-term fat graft survival and resorption are not well understood. The recent discovery of multipotent mesenchymal adipose tissue-derived stem cells (ASCs) in subcutaneous adipose tissue has encouraged the study of their role in enhancing fat grafts for applications in tissue engineering, wound healing, soft tissue augmentation and fat graft survival. Avascular fat grafts initially rely on diffusion for survival until revascularization occurs. Revascularization of the free fat graft is essential to graft survival and is influenced by ASCs, which are more resistant to hypoxic conditions when compared to adipocytes. ASCs are observed to survive after grafting, and in fact play a major role in adipocyte survival and differentiation. These ASCs survive for up to 3 days and regenerate adipose tissue through adipogenesis and paracrine effects. ASCs also promote revascularization of ischemic free fat grafts through the release of proangiogenic paracrine growth factors, including vascular endothelial growth factor (VEGF). In fact, lipoaspirate supplemented with ASCs has been shown to improve angiogenesis and long-term graft retention through the release of these factors. Several adipose graft enrichment strategies encompassing growth factors, platelet-rich plasma, adipose-derived and bone marrow stem cells, gene therapy and tissue engineering have been attempted to augment and improve the viability of fat grafts.

While there is tremendous interest in the use of fat grafting for aesthetic and reconstructive breast surgery, concerns have been raised from several regulatory bodies, including the Food and Drug Administration and professional organizations, regarding efficacy and safety in the setting of breast reconstruction. ASCs have been postulated to

create an inflammatory tumor microenvironment, to encourage angiogenesis, and to possibly differentiate into carcinoma associated fibroblasts, potentially contributing to tumorigenesis. However, concerns regarding the practice of fat grafting are not limited solely to the propagation of oncological transformation.

In 1987, the American Society of Plastic Surgeons (ASPS) issued a report condemning the practice of fat grafting to breast tissue due to the lack of long-term clinical trials and the possibility that scarring and calcification resulting from the procedure could interfere with breast cancer diagnosis. Research in the field has resulted in a reversal of the previously recommended moratorium with an investigation by the ASPS Task Force revealing no evidence of fat grafting impeding cancer detection or any indication that the procedure stimulates cancer recurrence. In light of the recent FDA regulatory changes regarding the processing of fat, a systematic review was undertaken to determine the safety, efficacy, satisfaction, and oncological outcomes of fat grafting to the breast.

Due to the significant variability in fat grafting outcomes and tremendous interest of ASCs graft retention, the final study attempts to describe the impact of a specific donor physiological condition, obesity, on ASC functionality. Obesity ($\text{BMI} > 30 \text{ kg/m}^2$) is a growing epidemic in the United States, affecting more than 33% of adults. Obesity is characterized by chronic low-grade systemic inflammation; however, the effects of obesity on the intrinsic cellular properties of ASCs are largely unknown. The objective of this final study is to characterize the effects of obesity on ASC functionality and fat graft retention in breast reconstruction patients. ASCs isolated and cultured from the

lipoaspirate of lean and obese women undergoing fat grafting for breast reconstruction were compared by ASC yield, viability, growth kinetics, capacity for multi-lineage differentiation into adipogenic and osteogenic lineages, and growth factor expression *in vitro*. Understanding how obesity affects ASC function may help to elucidate why lean patients anecdotally have better fat graft retention compared to obese patients.

Collectively, the objectives and specific aims of this dissertation are to generate and critically appraise evidence for the safety, efficacy and outcomes of fat grafting in the setting of breast reconstruction. Chapter 2 of this dissertation begins with a general introduction to adipose and breast tissue and the role of ASCs. Chapter 3 discusses the FDA regulatory issues regarding fat grafting. Chapter 4 identifies and appraises systematic evidence for autologous fat grafting to the breast, which includes a review of the safety, efficacy, satisfaction, and oncological outcomes. Chapter 5 continues this theme with a local review of the same variables. Chapter 6 is a review article on the impact of ASCs in fat grafting. Chapter 7 is a systematic review of the effects of obesity on ASC function. Chapter 8 is an experimental study exploring the effects of obesity on ASC functionality and its relationship to fat graft retention in breast reconstruction patients. Chapter 9 is a systematic review of adipose graft enrichment strategies. Finally, Chapter 10 summarizes the evidence and discusses the clinical implications and limitations, and recommends specific direction for future research.

Chapter 2: Adipose and Breast Tissue and

Adipose-Derived Stem Cells

Chapter 2 provides a primer and discussion of the literature on adipose and breast tissue and the role of adipose-derived stem cells. Adipose tissue is not only the primary site of storage for excess energy, but is also a metabolically active and dynamic endocrine organ that is capable of synthesizing many biologically active compounds. This tissue is not only composed of adipocytes, but also of other cell types that make up the stromal vascular fraction. In order to understand fat grafting in breast reconstruction, it is important to understand adipose tissue, breast tissue and the role of adipose-derived stem cells. The following subsections describe the function of adipose tissue, the isolation and identification of adipose-derived stem cells, the development and regenerative potential of these cells, and ongoing clinical trials involving them. Finally, the cell types composing breast tissue is briefly discussed.

2.1 Adipose: Cell Types Composing the Tissue

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Adipose tissue is a loose connective tissue with the primary function of storing lipids that can be harvested for energy. This function is performed by adipocytes, which comprise the vast majority of cells in adipose tissue. However, multiple other cell types can be found in adipose tissue; these cells are grouped under the category of the stromal vascular fraction (SVF). Cells of the SVF include preadipocytes, fibroblasts, vascular endothelial and smooth muscle cells, mesenchymal stem cells, endothelial progenitor cells, and immune cells such as anti-inflammatory M2 macrophages and T regulatory cells. An understanding of the cell types in adipose tissue is crucial for many relevant clinical applications, such as approaches to dealing with obesity and also potential therapeutic uses of adipose stem cells for various disease processes.

Function of Adipocytes

“Adipose tissue” often refers to white adipose tissue, which generally comprises around 20 percent (with great variation) of human body mass. In many mammals, including human infants, brown adipose tissue is also present for thermogenic function in the absence of shivering. Human adults also have remnants of brown adipose tissue, generally in the neck or upper chest region, but white adipose tissue is the most prevalent and clinically relevant.

White adipocytes. The majority of white adipose tissue is located either subcutaneously or viscerally, although small deposits can be found in a variety of locations, from within the bone marrow to around the epicardium, within joints and in craniofacial pads.

Subcutaneous adipose tissue is located in the hypodermis throughout the body and especially in regions such as the hips, abdomen, or thighs. Visceral adipose tissue is packed in between organs of the abdomen, and is thought to be the most clinically relevant for disease processes such as obesity and type 2 diabetes mellitus. The primary cell type in white adipose tissue is the white adipocyte.

White adipocytes have a distinctive histological appearance of a single (unilocular) large lipid droplet surrounded by a thin layer of cytoplasm with a flattened peripheral nucleus. Multiple hormone and other receptors are present on the surface of white adipocytes; this, coupled with endogenous adipocyte hormone production, gives white adipose tissue tremendous endocrine function in addition to its storage capacities. In particular, white adipocytes play key endocrine roles in energy metabolism and sex hormone levels.

One way that energy metabolism is regulated is through white adipocytes' synthesis and secretion of leptin, a peptide hormone that inhibits appetite in the hypothalamus.

Circulating levels of leptin are proportional to the amount of white adipose tissue in the body, and leptin resistance has been implicated in obesity. Energy metabolism is also regulated by the presence of insulin receptors on white adipocytes, which inhibit lipolysis in the presence of sufficient glucose in the bloodstream.

Sex hormone levels in the body are influenced by white adipocytes' ability to synthesize estradiol, via their production of the enzyme aromatase that converts androgens into estrogen. However, the primary function of the white adipocyte overall is to store lipid. Lipid content of adipose tissue overall increases with age, due to hypertrophy of white adipocytes. Although excess energy intake can result in the formation of new adipocytes, weight loss results in merely shrinkage of existing adipocytes rather than a decrease in number.

Brown adipocytes

Brown adipocytes have a greater ratio of cytoplasm to lipid content, and multiple smaller lipid droplets (multilocular) when compared to white adipocytes. The cytoplasm contains multiple mitochondria, which lend to the brown color of the cell and work to generate heat via lipid oxidation. Unlike white adipocytes, brown adipocytes express uncoupling-protein 1 (UCP-1), which drives the generation of heat by dissipating the mitochondrial proton gradient (leading to direct heat production rather than ATP production and storage). Brown adipose tissue in general also exhibits greater vascularization, due to a

greater need for oxygen by the mitochondria. Recent research has looked into the possible expression of UCP-1 by white adipose tissue as a method of combating obesity.

Function of Stromal Vascular Fraction

Preadipocytes

Preadipocytes are fibroblast-like cells derived from mesenchymal stem cells.

Preadipocytes are committed to the adipocyte lineage and are regularly present in adipose tissue in small quantities, where they serve both to replenish dying adipocytes (adipocyte turnover is around 10 percent per year) and to increase existing adipocyte numbers when energy stores are plentiful. Preadipocytes often reside in close proximity to the vasculature of adipose tissue and express the transcription factor PPAR, which has been identified as essential to adipogenesis. Preadipocytes require a specific, high-lipid microenvironment in order to differentiate into adipocytes; however, in the case of obesity, preadipocyte numbers actually decrease, perhaps as a compensatory mechanism to prevent excess irreversible adipocyte formation.

One of the factors that allow preadipocytes to maintain stable reservoirs of adipogenesis is their expression of telomere reverse transcriptase, which prevents the shortening of telomeres and subsequent DNA degradation over generations of replication. Differing populations of preadipocytes give rise to brown and white adipocytes, and within white adipocytes, there are regional differences as well. For example, visceral preadipocytes take much longer than their subcutaneous counterparts to differentiate and mature into adipocytes; this may explain the hypertrophy and greater lipid accumulation of visceral adipocytes.

A greater amount of lipids in each adipocyte in turn influences the adipokines (signaling proteins from adipose tissue) that are secreted, which can have profound clinical effects. For example, visceral adipocytes secrete much less of the adipokine adiponectin, and this has been shown to decrease insulin sensitivity and ramp up pro-inflammatory processes in visceral adipose tissue compared to subcutaneous. Research on how to alter preadipocyte gene expression, and thereby change adipocyte characteristics, has been relevant both for obesity and also in potential approaches to treating lipodystrophic disorders.

Mesenchymal stem cells

From mesodermal origin, mesenchymal stem cells (MSCs) are present in many different connective tissues, such as within the bone marrow. In the microenvironment of adipose tissue, MSCs generally differentiate into preadipocytes. However, MSCs can still be induced to develop into osteogenic, chondrogenic, myogenic, and other lineages, and have been heralded for their great research potential. Harvested via liposuction, in vitro studies of processed lipoaspirate (PLA) have yielded MSCs that are being studied for their use in autologous stem cell transplant. Human adipose tissue shows great potential for potential stem cell use due to its availability, quantity, and ease of obtainment.

Endothelial progenitor cells

Separate from mesenchymal stem cells, endothelial progenitor cells (EPCs) have been identified that give rise to adipose tissue vasculature. These EPCs are free-circulating and bone marrow derived, and usually present in the SVF in small quantities. They contain

angiogenic and/or hematopoietic cell markers. It has been postulated that in obesity, there are greater numbers of EPCs trapped in the adipose tissue rather than free to circulate, and thus angiogenic ability overall is reduced in obese patients. EPCs have also been the subject of much research recently involving potential transplantation to rebuild vessels damaged by atherosclerosis and stenosis.

Immune cells

Both T-regulatory immune cells and macrophages are resident to the SVF. The T-regulatory cells (T-regs) are immune suppressive cells formed from the activation of T cells in the absence of costimulatory signals, and their presence in adipose tissue has shown to be induced by markers generated from MSCs in adipose. T-regs in turn help promote the presence of macrophages. The macrophages present in adipose tissue are interesting in that they possess markers for both pro- and anti-inflammatory processes. On a surface level, adipose tissue macrophages (ATMs) express markers and receptors similar to M2-type macrophages, which promote tissue repair. ATMs, like M2 macrophages, can also be induced to secrete anti-inflammatory proteins like IL-10 and IL-1 receptor antagonist.

However, these same ATMs also secrete inflammatory proteins such as TNF- α , IL-1, and IL-6 in quantities high enough to offset any anti-inflammatory activity by both the ATMs and T-regs. It is postulated that the ATM protein secretion is under the regulation of adipocytes, which also secrete these inflammatory proteins themselves, and both of these processes contribute to the low-grade inflammatory state often present in obesity.

Other cells

Vascular and extracellular matrix cells that are present throughout the body are also found in adipose tissue. Similar to vasculature throughout the body, the vessels of adipose tissue consist of endothelial and smooth muscle cells. These endothelial cells include both ordinary endothelial cells like those found elsewhere and also specialized endothelial cells that appear to be able to induce preadipocyte generation. Fibroblasts also help secrete collagen and other extracellular matrix components that help form the structure of adipose tissue.

Overall, many different cells in adipose tissue have vast potential for further research investigation. Especially with the rise of obesity, there has been a much greater international interest in learning more about the components of adipose tissue and how they can be altered. In particular, MSCs and preadipocytes show promise for future use both in treating obesity and a plethora of other disorders.

Both adipocytes and components of the stromal vascular function are integral parts of adipose tissue, and understanding their functions will be a critical cornerstone of future learning.

Further Readings

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2.2 Adipose: Tissue Function

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The parenchyma of adipose tissue consists of adipocytes suspended in a connective tissue matrix, which functions as both a crucial endocrine organ as well as a site for metabolic activity. Two types of adipose tissue have been identified: brown and white. Brown adipose tissue (BAT) in humans is present at birth and provides non-shivering heat generation, while white adipose tissue (WAT) is present in adults and is a highly metabolic, endocrine organ. Pathology can occur both from adipose tissue deficiency as well as excess.

Brown Adipose Tissue (BAT)

Non-shivering thermogenesis. Brown adipocytes utilize oxygen and lipids as substrates to produce heat. The functional thermogenic unit consists of a brown adipocyte maintained within a structural network of connective tissue with access to a rich blood supply and innervation. The vascular network serves the BAT by both delivering substrate and

signaling molecules to the organ as well as carrying away the heat product to the body. Therefore, access to an ample vascular network is necessary in order to achieve maximal generation and distribution of the BAT-generated heat. Heat generation is achieved by a mitochondrial protein known as uncoupling protein-1 (UCP1) or thermogenin. It allows for combustion of fatty acids in the respiratory chain without the production of ATP; instead, heat is the form of energy that is released.

Signal transmission. The ventromedial (VLM) hypothalamic nucleus of the brain coordinates information regarding body energy reserves and body temperature. When a thermogenic demand is sensed by the VLM, the information is relayed via the sympathetic nervous system. The neurotransmitter norepinephrine (NE) is released and binds β -3 adrenergic receptors in the BAT to trigger an intracellular cascade that eventually leads to the generation of heat and an increased body temperature.

Thermogenic demand. Pre-adipocytes represent a rapidly accessible stem cell population that can replicate and differentiate into mature BAT under situations of increased thermogenic demand.

White Adipose Tissue (WAT)

Steroid hormone metabolism. Adipose tissue serves a crucial role in processing steroid hormones produced in the adrenal glands and gonads. This processing is referred to as “tissue-specific pre-receptor steroid hormone metabolism” and is necessary for full activation or inactivation of the circulating steroid hormones. The enzymes required to perform this process are extensive and include cytochrome P450-dependent aromatase,

3 β -hydroxysteroid dehydrogenase (HSD), 3 α HSD, 11 β HSD1, 17 β HSD, 17 α -hydroxylase, 5 α -reductase, and UDP-glucuronosyltransferase 2B15.

Aromatase is an especially important adipose enzyme in that it converts androgens into estrogens. In postmenopausal women, gonadal synthesis of estrogens becomes diminished and adipose tissue accounts for all of the circulating estrogen.

Reservoir for energy storage. Despite the large capacity of adipose tissue to secrete proteins and metabolize steroid hormones, the major secretory product of WAT is fatty acids. Adipocytes store triglycerides internally as a lipid droplet through an enzymatic process. First, triacylglycerides absorbed from the diet or synthesized in the liver reach their storage site (adipose tissue) and are converted into fatty acids via the enzyme lipoprotein lipase (LPL). They are then combined with the metabolic glucose product glycerol phosphate to reform triacylglyceride inside the adipocyte. When the cell receives signals that the body requires use of the free fatty acids for energy, they are then broken down via an enzyme called hormone sensitive lipase (HSL) that breaks apart the stored triglycerides to release free fatty acids. HSL responds to hormones such as catecholamines and glucagon to increase the free fatty acid concentration in the plasma so that it can be utilized for energy. Therefore, the sympathetic nervous system is a primary modulator of triacylglycerol breakdown.

Endocrine functions. Adipose tissue as an organ consists of several different tissue types including adipocytes, connective tissue, nerves, stromovascular cells, and immune cells. These tissues function in synchrony to express and secrete several hormonal and non-hormonal products including leptin, angiotensinogen, adiponectin, acylation-stimulating

protein, retinol-binding protein, tumor necrosis factor alpha (TNF α), interleukin-6, plasminogen activator inhibitor-1, adiponectin, complement components, and resistin. Several of these mediators are discussed below.

Secreted proteins

- **Leptin** is a polypeptide (16-kDa) containing 167 amino acids and has a structural configuration similar to that of cytokines. Although leptin can be synthesized in several sites of the body including the stomach, placenta, and mammary glands, the predominant site of its synthesis is in WAT. The primary role of leptin is to serve as a messenger to the body that the level of energy is at a sufficient state. Therefore, adipose tissue mass and nutritional status are the main mediators of leptin and directly correlate to circulating levels. These levels rapidly decline with caloric restriction and weight loss. Leptin can also be modulated by other chemical mediators: it is increased by insulin, steroids, and TNF α , and is decreased by β 3-adrenergic activity, androgens, free fatty acids, growth hormone (GH), and peroxisome proliferator-activated receptor- γ agonists. Genetically modified mice that have a recessive knockout of the leptin gene are referred to as ob/ob mice and are profoundly obese. The lack of circulating leptin leads to an absent detection of energy sufficiency and causes the mice to eat to excess. Therefore these mice are often applied as research models for type 2 diabetes.
- **TNF α** is a transmembrane protein (26-kDa) that becomes biologically active after cleavage. Levels of TNF α positively correlate with obesity and insulin resistance.

Induction of insulin resistance can be achieved in vitro and in vivo via chronic TNF α exposure.

- **Interleukin-6 (IL-6)** is similar to TNF α in that it is a cytokine produced by adipose tissue that also is associated with obesity and insulin resistance, and circulating levels have been shown to decrease with weight loss. It also serves as a predictor of type 2 diabetes as well as cardiovascular disease.

Adipose tissue excess (obesity). Associations of obesity are referred to as metabolic syndrome and are characterized by insulin resistance, hyperglycemia, dyslipidemia, hypertension, and prothrombotic and proinflammatory states.

Adipose tissue deficiency (lipodystrophy). A deficiency of adipose is also associated with characteristics of metabolic syndrome.

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2.3 Adipose: Stem and Progenitor Cells in Adults

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Adipose tissues play major roles in storage and active regulation of metabolism. In addition to these functions, adipose tissue has properties that give it potential application for tissue regeneration or transfer. Adipose may be a source of unique, pluripotent (possessing the ability to form into cells originating from any of the three germ layers: ectoderm, mesoderm, endoderm) stem cells. The utilization of stem cells and cytokines can lead to tissue repair and the regeneration of damaged tissues. Other multipotent (possessing the ability to differentiate into multiple but limited cell types) progenitor cells can be drawn in an undifferentiated state. Progenitor cells are considered to have already committed to differentiation on a specific cellular pathway. There may be fewer political, legal, and ethical issues with adipose stem and progenitor cells as compared to embryonic stem cell use.

Multi-Lineage Potential

Stem cells must have the ability to continually divide (self-renewal), maintain viability long term, and have the potential to differentiate. Stem cells extracted from bone marrow (mesenchymal stem cells) have shown multi-lineage potential through extensive study and have been suggested as alternatives to embryonic stem cells in mesodermal defect repair and disease management. However, issues with pain, morbidity, and low cell number during extraction impede the practical use of bone marrow stem cells.

Like stems cells derived from bone marrow, adipose tissue is of mesodermal origin. Adipose-derived stem cells (ADSCs) can differentiate in vitro (isolated studies in experimental biology) toward osteogenic (bone), adipogenic (fat), myogenic (muscle), and chondrogenic (connective tissue) lineages when treated with established lineage-specific factors. Studies have shown that ADSCs show lineage-specific genes to distinctive cell lines such as osteocytes or myocytes when stimulated to develop into different cells.

ADSCs can differentiate into adipocytes, chondrocytes, and osteoblasts, a feature known as multipotency. A single ADSC is also capable of cloning itself and then further differentiating into multiple lineages, a capacity known as clonogenicity. For example, human ADSCs show in vitro evidence of differentiation along myocyte lineage pathways. When cultured with myocyte lineage factors, adipocytes fuse and express protein markers of skeletal myocyte lineage. This suggests that these cells have the potential to repair damaged skeletal muscle. ADSCs can also differentiate into osteoblast-like cells by depositing calcium phosphate mineral into their extracellular matrix and expressing

osteogenic genes and proteins. Evidence suggests that these cells have the potential to accelerate repair at fracture sites.

The range of differentiation that ADSCs possess extends beyond bone, muscle, and connective tissue. There may be possibility of repairing gastrointestinal and urinary tract smooth muscle defects with ADSCs. Factors can also differentiate these cells along the cardiac myocyte pathway and may be a source of regeneration for cardiac tissues damaged from infarction or ischemic injury. Preliminary studies have also implicated ADSCs in the regeneration of the central and peripheral nervous system following traumatic injury.

Adhesion proteins associated with hematopoietic stem cells can form on ADSCs, which can also secrete cytokines (substances secreted by cells of the immune system) and promote differentiation along the B-cell, T-cell, and myeloid (white blood cell) lineages. This possible application can extend to conditions that weaken patients' immune systems such as those patients undergoing high-dose chemotherapy or suffering from inborn errors of metabolisms.

Harvesting Adipose-Derived Stem Cells

Adipose tissue is an exciting resource for tissue regeneration and soft tissue repair because it houses one of the richest reservoirs of stem cells in the human body. Thus, stem cells collected from adipose tissue do not need to be cultured in order to obtain a therapeutically vital number of cells. Well-nourished humans store excess calories in adipose tissue that increase cell volume and expansion of the number of differentiated

adipose cells, suggesting that adipose tissue progenitor cells exist within adult fat tissue. ADSCs can also be harvested easily with little harm to the patient through the process of liposuction, making adipose stem and progenitor cells much more accessible than bone marrow cells. ADSCs cultured in vitro have shown consistent profiles of cell-surface proteins, which include adhesion proteins, receptor molecules, surface enzymes, extra-cellular matrix proteins and glycoproteins, skeletal proteins, hematopoietic (involved in blood formation) cell markers, complement regulatory proteins, and histocompatibility antigens (immune system components).

The immunophenotype of ADSCs resemble other adult stem cells from bone marrow and skeletal muscle. Methods of harvesting the tissue have dramatic effects on the ability of the cells to proliferate and differentiate in culture. Several studies report a negative correlation between patient age and the yield of donor cells and proliferation. Many concerns remain for the standardization and optimization of methods for cell isolation, culture, and application.

Future Development

ADSCs may prove to be an ideal option for tissue engineering in regenerative medicine since they are self-renewable, plentiful, and easily accessible through minimally invasive procedures. Studies suggest that ADSCs can be used in the treatment of type 1 diabetes mellitus, obesity, cardiovascular disease, lipodystrophy, and neurodegenerative diseases. For example, ADSCs have the ability to be carriers for gene delivery vehicles through transduction, the process by which foreign DNA is introduced into another cell through a viral vector. In surgical application, ADSCs can aid with neovascularization of free fat

grafts (transplanted adipose tissue). Much more preclinical research and development must be dedicated to ADSCs before they can be used in treatment. Optimizing methods to harvest and preserve viable adipose tissue is of vital importance, but patient safety must be the priority.

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2.4 Adipose: Current Research on Isolation or Production of Therapeutic Cells

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Adipose-derived stem cells, or ASCs, are a unique population of stem cells isolated from adipose tissue. These multipotent stem cells present an alternative to the widely used embryonic and hematopoietic stem cell lineages for laboratory and clinical applications in regenerative medicine. Adipose tissue is abundant in the body and exists in several forms, including bone marrow, mammary tissue, and mechanical, brown (multilocular), and white (unilocular) adipose tissues.

Traditional methods of gathering stem cells include the controversial isolation of embryonic stem cells and the painful process of procuring stem cells from bone marrow. In contrast to these methods, an efficient manner of isolating and producing reliable stem cell populations from abundant, easily accessible adipose tissue presents an appealing alternative for clinical therapeutic applications. Thus far, adipose stem cells have been shown capable of multipotent mesodermal differentiation, as well as potential endodermal and ectodermal lineages *in vitro*.

Evidence for Adipocyte Precursor Cells

Progressive osseous heteroplasia (POH), a pathologic condition that leads to heterotopic bone formation within subcutaneous adipose and, eventually, muscle tissue, demonstrates the presence of adipocytes, osteoblasts, and chondrocytes upon histologic examination of the resultant lesions. Pathologic evidence from this rare, autosomal-dominant, inherited genetic defect suggests that ADCs are at least capable of differentiation into the aforementioned mesodermal lineages. Along with POH, lupus and Paget disease are also known to present with calcification of subcutaneous adipose, providing further evidence for the presence of multipotent ASCs within adipose tissue. Additionally, research using ligand-induced adipogenesis for the chemotherapeutic treatment of liposarcomas suggests that these cancers may derive from a stem cell progenitor; stimulation by both long-chain fatty acids and synthetic steroid compounds induces adipocyte formation from liposarcoma-derived cells. Furthermore, radioactive tracing to measure adipocyte turnover rates in obese patients indicates a lifespan of six to 15 months for these fully differentiated adult adipocytes, a value that seems to indicate the presence of a controlled replacement mechanism of mature cells by resident stem cell–adipocyte precursors. Evaluation of individuals who have undergone rapid weight loss through either metabolic or procedural means also supports this concept: Not only do existing adipocytes increase in volume but new adipocytes emerge in a homeostatic process to maintain a relatively constant level of adipose tissue within the organism.

Origin of ASCs

Adipose tissue is abundant in the body and exists in several forms, including bone marrow, mammary tissue, and mechanical, brown, and white adipose tissues. It is speculated that ASCs arrive in adipose tissue via distribution of circulating fibroblasts derived from the bone marrow that then colonize the respective adipose tissues throughout the body. Transplanted bone marrow-derived fibroblasts similar to the ones already discussed have been shown capable of differentiating into adipocytes upon proper chemical stimulation and in the presence of a lipid-rich diet; however, the exact origin and distribution of ASC populations remains unknown. Regarding the relative abundance of stem cell populations within the body's various adipose depots, current evidence suggests that richer concentrations of progenitor cells exist in the arm and abdomen relative to samples taken from the thigh or breast. Further studies, along with effective isolation techniques, are still needed to optimize processes for procuring ASCs in the most efficient manner from donors.

Cell Isolation From Adipose Tissue

Older methods of isolating cells from adipose tissue involved thoroughly rinsing minced animal fat pads, digestion with collagenases, and centrifugation to separate the desired stromal vascular fraction (SVF) that contained the processed lipoaspirate cells within a heterogeneous mixture. Finally, the plastic-adherent cells within the isolated SVF were selectively purified on the surface of tissue-culture flasks to enrich the concentration of adipocyte precursors.

Researchers are developing more efficient isolation methods that use advances in liposuction and reconstructive plastic surgery; in this process, plastic surgeons use a cannula to infuse subcutaneous adipose tissue with an anesthetic-containing saline solution. The procedure produces aspirations of adipose-tissue fragments containing viable adipocyte precursors within the SVF. Following collection of tissue samples, centrifugation at 1,200 G optimizes the ASC fraction recovered from the liposuction aspirate. However, surgical procedures involving ultrasound-assisted liposuction have shown adverse effects on the quantity of viable cells abstracted via the procedure. Once isolated, ASCs double in vitro within two to four days. Since massive quantities of tissue must be handled to isolate significant quantities of desired cells, methods such as rotating, temperature-controlled collagenase incubators, and bag-within-a-bag sieves are being tested to assist in procuring the desired cellular fraction from liposuction aspirate samples. Thanks to the development of these novel techniques for efficiently isolating ASCs, larger-scale commercial isolation methods are in development and becoming a realistic possibility for clinical application.

Purification and Identification

To identify a stem cell, researchers evaluate the presence and absence of various surface marker proteins, or antigens, which are important for immune recognition by leukocytes. Studies analyzing the immunophenotype of cells abstracted from liposuction aspirate find much consistency in the surface markers of these adipose-derived cells, indicating that there is, in fact, a unique population of adipocyte precursors present within the aspirate. Among the antigens used to identify stem cell populations, ASCs have been identified as

positive for CD29, CD34, CD54, CD90, CD105, CD166, and human leukocyte antigen (HLA)-ABC markers; they are negative for CD31, CD45, CD106, CD146, and HLA-DR markers. Additionally, ASCs may be purified from the heterogeneous subcutaneous adipose and vascular fraction SC+VF by exploiting their plastic-adherent characteristic and their multipotent differentiation potential.

Multipotency of ASCs

It is now well known that adipose-derived stem cells can differentiate into adipocytes, chondrocytes, and osteoblasts. Not only are they capable of this multipotency, but their clonogenicity has been established as well. That is, a single ASC has been shown capable of cloning itself and then further differentiating into multiple lineages, eliminating the possibility of multiple precursors producing the respective observed lineages. Beyond the mesodermal tripotency seen in conditions of pathologic calcification, researchers have more recently successfully induced in vivo ASC differentiation into neurogenic ectodermal cells consistent with neurons, oligodendrocytes, and Schwann cells. Other confirmatory studies have elicited ASC commitment to hepatogenic, pancreaticogenic, myogenic, hematopoietic supporting, and endodermal lineages by targeting various chemical inductive factors to the cells.

Both endogenous and synthetic chemicals have successfully induced differentiation into determinate cell lineages. Cardiomyocytes have been induced using the iron transporter, transferrin, interleukins 3 and 6, as well as vascular endothelial growth factor.

Endothelial cells, on the other hand, result from ASC exposure to basic fibroblast growth factor, and epidermal growth factor. Differentiation into many cell lineages indicates that

ASCs contain vast potential, perhaps far wider than that initially suggested by pathological evidence; it is even theorized that these cells are pluripotent, much like embryonic stem cells.

Clinical Trials With Therapeutic ASCs

Compared to hematopoietic stem cells, ASCs exhibit greater long-term genetic stability and are more immunoprivileged (i.e., evidence suggests they are effective at preventing severe graft-versus-host disease). Therefore, presently, these cells seem potentially safer and more effective than their adult stem cell counterparts. Currently, dozens of clinical trials are underway to evaluate their efficacy in various regenerative treatments.

Immunosuppressive studies have shown that ASCs suppress T-cell-mediated immunity and inflammation while activating regulatory T cells, which downregulate inflammatory mediators and reduce the tissue response of inflammatory cells. Other clinical trials are investigating applications in cardiovascular and hepatic disease, type 1 and 2 diabetes, amyotrophic lateral sclerosis, multiple sclerosis, immunosuppression, limb ischemia, and bone reconstruction.

Regenerative capacities for lumpectomy patients and perianal fistulas are also being investigated. Current theories on the mechanism of action for ASCs include the paracrine secretion of signaling molecules like cytokines or growth factors that would guide the differentiation of local cells to the necessary type to speed recovery; in the treatment of ischemia, ASCs may help remove toxins by producing antioxidants and free-radical scavengers to aid in tissue recovery.

While many clinical trials are underway to address the safety and efficacy of ASCs, much remains to be seen regarding the potential these versatile cells have in therapeutic and regenerative settings. The vast array of possible treatments being explored will undoubtedly continue to revolutionize the management of disease.

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2.5 Adipose: Development and Regeneration Potential

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Human adipose tissue serves as an important endocrine and metabolic organ. Adipose tissue is a complex tissue composed mainly of mature adipocytes surrounded by a connective tissue matrix, in addition to stromovascular cells, nerve tissue, and immune cells. Together, these components play an important role in insulating the body, storing energy as lipids, and producing and metabolizing hormones. By secreting factors such as leptin, resistin, estrogen, and cytokines, adipocytes act as the major component of a highly active endocrine organ that targets various tissues in the body to maintain homeostasis. A lack or complete absence of adipose tissue (lipodystrophy) or overproduction of adipose tissue (obesity) can result in metabolic complications such as type 2 diabetes, insulin resistance, hepatic steatosis, and hypertriglyceridemia. The plastic nature of adipose tissue, with its regenerative properties and ability to expand and contract in response to shifts in energy balance, is most evident in the obesity epidemic. For many years, scientists and clinicians have investigated the development of adipose tissue at the cellular level; however, with modern biotechnological advances in stem cell

research, the study of adipogenesis has been focused on the discovery and function of adipose-derived stem cells (ASCs). Capable of differentiating into cells of nonmesodermal and mesodermal origin, ASCs serve as an important target for adipose tissue engineering and regenerative medicine.

Adipose Tissue: A Source of Multipotent Stem Cells

Stem cells are cell populations that possess multilineage potential, self-renewing capacity, and long-term viability. Originating from the stroma of bone marrow, mesenchymal stem cells (MSCs) have been widely studied as an example of adult stem cells that are capable of differentiating into chondrocytes, osteoblasts, adipocytes, and myoblasts in vivo and in vitro. While MSCs are promising candidates for disease management and mesenchymal defect repair, the use of MSCs in the clinic has been limited due to complications associated with morbidity, pain, and low cell count/tissue volume upon harvest.

In recent years, researchers have focused attention to adipose tissue as an alternative source of adult stem cells. Much like bone marrow, adipose tissue is derived from mesenchymal origin and contains an easily isolated stroma. ASCs exhibit stable proliferation and growth kinetics in culture and in the presence of specific inducing factors can differentiate into chondrogenic, osteogenic, adipogenic, and myogenic lineages. Due to the ubiquitous nature of human adipose tissue, large quantities of ASCs can be easily obtained with little patient discomfort or donor site morbidity.

The multipotent nature of ASCs is evident in various human pathologies. In children with progressive osseous heteroplasia (POH), an autosomal dominant genetic defect that causes ectopic bone formation within subcutaneous adipose depots, chondrocytes and osteoblasts can be found within colonies of adipocytes. Histologic analysis implies a tripotent capacity of ASCs to differentiate into cells of chondrogenic, osteogenic, and adipogenic origin. Obesity also presents additional evidence supporting the presence of stem cells in adipose tissue. Adipocytes have a turnover rate ranging between 6 and 15 months. While various behavioral, genetic, and epigenetic factors can contribute to obesity, in vivo studies have demonstrated the existence of stem cell populations that replace mature adipocytes throughout the lifetime of humans.

Adipose-Derived Stem Cell Isolation

Humans contain five major types of adipose tissue: bone marrow, mammary, mechanical, brown, and white. Each type of adipose depot serves a unique biological function and contains a distinct stem cell profile. White adipose tissue contains higher amounts of multipotent stem cells compared to brown adipose tissue, with subcutaneous depots providing higher yields of ASCs compared to visceral fat. Within subcutaneous white adipose tissue, greater numbers of stem cells have been harvested from arm regions compared to the abdomen, thigh, and breast. ASCs recovered from superficial abdominal regions were found to be the most resistant to apoptosis. Furthermore, ASCs from younger donors have demonstrated greater cell adhesion and proliferation compared to older donors. It has yet to be determined which adipose tissue depot serves as the optimal location for stem cell recovery.

Samples of subcutaneous adipose tissue are often obtained from subjects under local anesthesia. Current methods for isolating ASCs depend on collagenase digestion of tissue followed by centrifugation to isolate primary adipocytes from the stromal vascular fraction (SVF). ASCs display morphology similar to fibroblasts, which makes phenotypic identification difficult, and do not exhibit the intercellular lipid droplets that are found in adipocytes. Isolated ASCs are grown in monolayer culture utilizing specific cell culture techniques.

Regeneration, Repair, and Tissue Engineering

Traditionally, rehabilitation of injured or diseased organs and tissues has required tissue replacement through the use of autologous tissues since the body rejects tissue transplants with foreign antigen. With advances in modern biotechnology, researchers have placed an emphasis on developing tissue-engineered substitutes that are better suited in restoring, maintaining, and improving tissue function. The technology of tissue engineering involves an interdisciplinary field of physicians, engineers, and scientists who utilize adult stem cells to be directly implanted into the host or expanded in culture. In the latter technique, stem cells are differentiated and combined with tissue-engineered scaffolds and growth factors to develop tissue and organ systems. Tissue engineering can be used as a tool for transplantation, rehabilitation, and reconstructive surgery.

ASCs have the potential to regenerate and repair different types of tissues through a variety of mechanisms. ASCs can provide a beneficial impact on diseased or injured tissues/organs by producing and secreting soluble factors. Some of the growth factors and cytokines secreted by ASCs include hepatocyte growth factor (HGF), insulin-like growth

factor (IGF-1), vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF- α), fibroblast growth factor (FGF), adiponectin, transforming growth factor- β (TGF- β) and other angiogenic, anti-apoptotic, and anti-inflammatory factors. Certain soluble factors can also promote tissue repair and wound healing by recruiting endogenous stem cells. This newly formed stem cell population acts in a paracrine manner that can be stimulated to differentiate along the lineage pathway required for tissue repair.

ASCs can act as a viable source of free-radical scavengers, antioxidant chemicals, and chaperone/heat shock proteins. In injured regions such as ischemic sites, ASCs act in such a manner to clear the local environment of toxic substances, thereby improving recovery of surviving cells. Recent studies have demonstrated the capacity for bone marrow-derived MSCs to deliver mitochondria to injured cells and rescue aerobic metabolism. Comparable studies on ASCs may uncover a similar potential to contribute mitochondria.

Therapeutic benefits of ASCs also differ between autologous (derived from the same individual's body) and allogenic (derived from genetically dissimilar individual) transplantation. While autologous ASCs can be beneficial from histocompatibility, infectious, and regulatory perspectives, it is rare for patients to provide their own therapeutic cells. Researchers have determined that a human's ASCs that are passaged in cell culture, compared to freshly isolated cells, have reduced surface histocompatibility antigen expression and suppressed immune reactivity when cultured together with allogenic cells. While this implies that passaged ASCs may not produce a cytotoxic T-cell response when transplanted in vivo, comprehensive testing is required before clinical

implementation. If proven correct, the use of allogenic ASCs in regenerative medicine holds the potential to lower costs of cell therapies, to improve availability of stem cells, and to reduce complications associated with organ and tissue failure.

The Future of ASCs

Deriving stem cells from adipose tissue has proven to be an efficacious, safe, and simple process with little donor site morbidity. Furthermore, stem cell yields from adipose tissue are far greater than most stem cell reservoirs in the human body. While they may be suitable candidates in regenerative medicine, various limitations still remain. One of the major concerns with the use of ASCs is that very few in vivo clinical trials have been conducted compared to the large number of in vitro preclinical studies. In addition, many scientific questions remain unclear. Firstly, the specific transcription factors and key molecular events that allocate ASCs to a particular lineage have not been identified. Secondly, evidence implies that the ability for ASCs to differentiate may depend on the anatomic source and the donor's age and gender. Furthermore, methods for large-scale manufacturing with appropriate quality control and quality assurance have yet to be developed. To fulfill expectations and to determine if ASC-based therapies can be successfully implemented in treatment, further investigation is required.

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2.6 Adipose: Existing or Potential Regenerative Medicine Strategies

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A stem cell is a cell that has the ability to self-renew and differentiate into one or more types of cells. Therefore, stem cells hold great promise for regeneration and repair of tissues. Recent study has focused on their use for the treatment of Parkinson disease, Alzheimer disease, cancer, myocardial infarction injuries, breast reconstruction, diabetes mellitus, autoimmune diseases, and much more. Unlike the embryologic stem cells that aroused much controversy, adipose-derived stem cells (ASCs) are derived from adults and are noncontroversial. Furthermore, research suggests ASCs are a better stem cell source than the conventional mesenchymal stem cells—the bone marrow stem cells (BMSCs).

Shift From BMSCs to the ASCs

Historically, BMSCs were the most frequently used mesenchymal stem cell pool. However, ASCs are more advantageous in several respects. The pool of ASCs is larger than that of BMSCs. They can be collected by liposuction with local anesthesia, whereas

bone marrow acquisition is more invasive, requires general anesthesia, and carries a greater risk for mortality. Furthermore, clinical data show that ASCs have a higher proliferation rate than BMSCs. ASCs can grow to 90 percent confluence within three days compared to BMSCs, which can take a week to reach the same mark.

Potential of ASCs

ASCs are multipotent and mesenchymal in origin. Initially, ASCs were studied for differentiation into chondrogenic, myogenic, and osteogenic cell types. However, further research showed transdifferentiation capacity extending beyond the traditional mesenchymal lineage. ASCs are now known to be capable of skeletal myogenesis, cardiac myogenesis, neurogenesis, and angiogenesis.

ASC use in regenerative therapy involves redirection from normal reparative function to generation of new tissue in areas that are diseased or received trauma. In addition to their proliferative capacity, ASCs also decrease inflammation and release growth factors, allowing focused healing. Their anti-inflammatory nature suggests potential for treating autoimmune and inflammatory diseases, such as rheumatoid arthritis, inflammatory bowel disease, and graft-versus-host disease.

Clinical Applications and Published Clinical Trials

ASC therapy is gaining popularity. Most studies report no adverse effects and the majority of outcomes were beneficial. However, rigorous trials are lacking and most publications are case reports and noncontrolled studies.

The clinical applications of ASCs discussed in this article are spinal cord injury; diabetes mellitus; breast reconstruction and augmentation; facial lipoatrophy; rheumatoid arthritis; multiple sclerosis; hematologic and immunologic disorders; complex perianal or enterocutaneous fistulas and tracheomediastinal fistula; bone tissue repair; cardiovascular disease; cancer; and musculoskeletal regeneration.

Spinal Cord Injury

Stem cells have been studied intensely for spinal cord injury because the damaged axons and neurotransmitter-producing neurons cannot be regenerated by the human body. As a result, individuals with spinal cord injury suffer loss of sensory and motor function below the site of injury. Scientists started working with stem cells with the hope that they would promote new regeneration of neurons to promote healing. Indeed, in 2011, eight patients with spinal cord injury who were treated with intravenous infusions of autologous ASCs were shown to have improved motor function after 12 weeks.

Type 1 Diabetes Mellitus

Type 1 diabetes mellitus occurs because of autoimmune attack on pancreatic cells. The number of beta islet cells is reduced as a result, leading to decrease in insulin and C-peptide production. ASCs were studied for their ability to regenerate pancreatic beta islet cells. In a study of ASC therapy administered to five patients with diabetes, results showed a 30% to 50% decrease in insulin requirements and increase in serum C-peptide levels during a follow-up period of 2.9 months. No adverse effects were noted.

Breast Reconstruction and Augmentation and Facial Lipoatrophy

It should come as no surprise that ASCs have the potential for adipose tissue regeneration. In 2008, ASCs were used successfully for breast augmentation. Normally, the body resorbs injections of unprocessed adipose tissues. However, when patients were injected with a mixture of ASCs and unprocessed adipose tissue, they retained the volume over the next 12 months. Similar success was shown in facial lipoatrophy.

Autoimmune Diseases

ASCs have potential for treatment of autoimmune diseases. In 2010, there was a case report of ASC use in a 67-year-old woman with rheumatoid arthritis. She was treated with autologous ASCs isolated from liposuction; subsequently, she reported reduced joint pain and stiffness. Additionally, authors measured the levels of rheumatoid factor as a more objective measurement and noted a decrease after treatment. The patient had no side effects.

ASCs have also been used for the treatment of multiple sclerosis, another autoimmune disease. Three patients with multiple sclerosis received intravenous infusions of ASCs, allogeneic CD34+ cells, and mesenchymal cells. Patients reported significant improvement of symptoms.

Hematologic and Immunologic Disorders

Researchers have also studied ASCs for the treatment of graft-versus-host disease, idiopathic thrombocytopenic purpura, and pure red-cell aplasia. Patients were given intravenous infusion of allogeneic ASCs. Treatment was successful in graft-versus-host disease and pure red-cell aplasia; in idiopathic thrombocytopenic purpura, remission was achieved. However, the effect of ASCs on alloreactivity in patients who have undergone solid-organ transplantation is not yet known.

Fistulas

Potential use of ASCs for fistulas has been demonstrated in treatment of perianal, enterocutaneous, and tracheomediastinal fistulas. To study the effect of ASCs on perianal and enterocutaneous fistulas, the fistulas of the patients were injected with autologous ASCs mixed with proteinaceous fibrin glue. Results of phase 1 and 2 clinical trials showed four times the healing compared to the control group. Again, no adverse effects were reported.

To study the effect of ASCs on a patient with lung cancer-induced tracheomediastinal fistula, the patient's fistula was injected with autologous ASCs mixed with fibrin glue. Epithelialization of the fistula was observed three months later and was completely closed one year after treatment. This case is particularly encouraging, as fistula progression into blood vessels occurs frequently and is often fatal. No side effect was noted.

Bone Tissue Repair

In 2004, there was a case report on a seven-year-old girl who had a calvarial defect from a severe head injury. The first attempt at treatment, fixation of calvarial fragments via titanium miniplates, was unsuccessful. She was then treated with a mixture of autologous ASCs and autologous bone from the iliac crest. Three months after the surgery, computed tomography scan confirmed successful calvarial bone formation.

Cardiovascular Diseases and Cancer

Not all studies with ASCs have shown positive results. Study of ASCs for treatment of acute myocardial infarction and cancer are two examples where results have been inconsistent.

Musculoskeletal Regeneration (Clinical Study on Animal Models)

Musculoskeletal regeneration is an area of intense research because there is a limited pool of muscle progenitor cells, called satellite cells. Therefore, ASCs were used as a potential therapy for muscular disorders. In 2006, intravenous injection of allogeneic ASCs was shown to restore muscle function in murine muscular dystrophy. Successful use of ASCs in intervertebral disc regeneration has been also reported. In addition, topical administration of adipose stem cells on rabbits' tendons accelerated tendon repair rate and tensile strength was increased, supporting the transdifferentiation potential of the ASCs in vivo and in vitro.

Conclusion

Though their effectiveness is still unproven, treatment with ASCs in regenerative medicine appears promising. However, their benefit in the treatment of cancer is particularly weak and presents a major concern, since ASCs secrete cytokines that may affect cancer metastases. More research is needed for conclusive evidence, and further work will be required to determine the safety of ASCs.

Likewise, standard protocols for ASC studies do not exist yet. The ideal procedure for acquiring ASCs, the optimal number of stem cells that should be used for each procedure, and the safe number of stem cells that can be injected into different organs will have to be determined.

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2.7 Breast: Cell Types Composing the Tissue

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This article provides a brief introduction to the cell types that compose human breast tissue. It focuses on the anatomy of the human breast, the major types of tissues and cells in breast tissue, and the role stem cells play in normal breast tissue and pathologies.

The human breast is located on the upper ventral region on the body and is a distinguishing feature of mammals. The breast is part of the skin and, therefore, part of the integumentary system. Breast tissue is composed of three main types of tissue: glandular tissue, connective tissue, and adipose tissue. The breast contains no muscle tissue. These components have various functions in the breast, which will be discussed further. The breast contains many lobes, which are composed of lobules. The lobules are composed of the aforementioned adipose tissue. These lobules give the breast its size and shape. All of these tissues are made up of various cell types that have different functions. The importance of stem cells in breast tissue is enormous. Breast cancer is the second leading cause of death for women in the United States, and it is a great concern among

health care professionals. Therefore, understanding how stem cells operate is critical for how some breast pathologies are treated. The use of stem cells in regenerative medicine has become more realistic in the past several years.

Anatomy of Breast Tissue

The base of the breast is attached to the thoracic cavity by the pectoralis muscles. The space located between the pectoralis muscle and the breast is referred to as the retromammary space. This space is responsible for providing mobility to the breast. The next layer of tissue is a layer of fat called adipose tissue, which forms the majority of the breast. Then the suspensory Cooper's ligaments extend from the superficial fascia, which is located on top of the layer of adipose tissue, into the breast to provide support. The lobules composed of adipose tissue form into a cone shape, which converge into the nipple. The areola, a dark pigmented area, surrounds the nipple. There are four major types of tissue in the human breast: epithelium, stroma, adipose tissue, and glandular tissue. The epithelium lines the surfaces and cavities throughout the body. The stroma is the connective tissue in the body. Adipose tissue makes up the majority of breast tissue and is composed of adipocytes (fat cells), preadipocytes, fibroblasts, endothelial cells, and various immune cells. There are two main types of adipose tissue: white adipose tissue and brown adipose tissue. The breast is also composed of glandular tissue. The breast is an apocrine gland that contains many sebaceous glands, which secrete sebum to waterproof the skin. Another apocrine function of the breast is to produce milk for infants.

Introduction to Stem Cells

A stem cell is an undifferentiated cell that is capable of giving rise to more cells of the same type indefinitely, and from which other types of cells can arise through differentiation. There are two main types of stem cells: pluripotent stem cells and multipotent stem cells. Pluripotent stem cells have the ability to differentiate into any of the three types of germ layers: endoderm, mesoderm, and ectoderm. Multipotent stem cells have the ability to differentiate into multiple, yet limited cell types. An example of this is a blood stem cell that can differentiate into lymphocytes, monocytes, or neutrophils but cannot differentiate into bone cells or heart cells.

Cell Types in Breast Tissue

There are three major epithelial cell types in breast tissue. The epithelial cell types are basal cells, luminal cells, and myoepithelial cells. The epithelium is composed of luminal cells and myoepithelial cells. These major cell types express different patterns of keratin that helps distinguish between them.

Luminal cells

These inner cells are surrounded by myoepithelial cells, which help expel secretions and assist in the movement of fluid. The luminal cells consist of differentiated cells and also many types of cells that are in between luminal cells and stem cells.

Basal cells

These outer cells consist mostly of differentiated cells with several mammary stem cells.

Myoepithelial cells

These cells are sandwiched between the stroma and luminal cells in breast tissue. They are frequently found in the glandular epithelium and are involved in expelling secretions from the exocrine glands.

Adipose-Derived Stem Cells (ASCs)

As discussed earlier, adipose tissue is composed of adipocytes, or fat cells. ASCs have remarkable plasticity and have the ability to differentiate into several different cell types, including adipocytes, cardiomyocytes, chondrocytes, epidermal cells, endothelial cells, hepatocytes, myocytes, osteoblasts, cells similar to glial cells, and cells similar to neurons.

These characteristics make ASCs a very useful type of stem cell. Furthermore, ASCs have shown the ability to differentiate into cells with angiogenic characteristics since they are derived from the stromal vascular fraction. The stromal vascular fraction of adipose tissue is the important portion because it contains a significant amount of preadipocytes, mesenchymal stem cells, endothelial progenitor cells, and immune cells such as T-cells, B-cells, mast cells, and macrophages. Also, ASCs have been identified as cells that secrete growth factors such as insulin-like growth factor (IGF), hepatocyte growth factor (HGF), pro-angiogenic growth factor, and vascular endothelial growth factor (VEGF). These characteristics of ASCs make them extremely valuable tools for regenerative medicine.

Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells have the ability to differentiate into osteoblasts, chondrocytes, myocytes, neurons, and adipocytes. MSCs are derived from mesoderm, which forms connective tissue in the body. However, the MSCs in breast tissue are derived from breast adipose tissue. These MSCs work to stimulate the growth of tumor cells in breast cancer, and can make the disease metastasize. MSCs play a role in basal-type cancer by degrading the extracellular matrix and facilitating the invasion of basal-like cancer cells. Further research on the role of MSCs in breast cancer progression should be performed.

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Chapter 3: Regulatory Issues Regarding Fat Grafting

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Autologous fat grafting or lipotransfer is a dynamic and widely accepted modality to improve functional and aesthetic form for volume loss or deformity due to aging, facial and body lipodystrophies, congenital anomalies, breast augmentation and reconstruction, wound healing, and other soft tissue deficiencies. Fat grafting has been praised as the ideal soft tissue filler due to its abundant supply, low donor site morbidity, versatility, and biocompatibility. A recent survey of American Society of Plastic Surgery (ASPS) members reported that 85% of facial aesthetic surgeons used the technique in face-lift procedures.¹ Adipose-derived stem cells (ASCs) within adipose tissue have also demonstrated efficacy in wound healing and tissue engineering.² Although fat grafting has been utilized for over a century, its popularity has markedly increased in recent times. Notable deviations and abuses from accepted practice have also become more common.

In December 2014, the Food and Drug Administration (FDA) released three guidance documents that would change the regulation of human cells, tissues, and cellular- or tissue-based products (HCT/Ps) from adipose tissue, effectively restricting fat grafting

procedures to a study environment and requiring registration and report manufacturers (in this case, surgeons). These documents addressed same surgical procedure exception,³ minimal manipulation of HCT/Ps,⁴ and HCT/Ps derived from adipose tissue.⁵ In the case of fat grafting in breast reconstruction and the use of stromal vascular fraction (SVF) and adipose-derived stem cells (ASCs), HCT/Ps from adipose tissue would be considered as biologic drugs, and surgeons would need to apply for premarket FDA approval and a license.

While the draft guidance is not yet finalized, it does provide the latest report to date of the FDA's perspective on the use of fat injections in breast reconstruction and the use of ASCs. A team of ASPS experts has reviewed the guidance documents and drafted a response to elucidate why these FDA interpretations are misguided.

HCT/Ps

The FDA regulates human cells and tissues to control the spread of communicable diseases. HCT/Ps are defined as products containing human cells or tissues intended for implantation, transplantation, infusion, or transfer into a human recipient. Notably, organs and blood products are regulated through other mechanisms. There are two types of HCT/P depending on the risk category: 361 (lower-risk) and 351 (higher-risk) HCT/Ps.

361 HCT/Ps

A subset of category 361 includes procedures that take place in the same operative session, which are exempt from FDA regulation, but are guided by laws established by state medical boards and professional societies. 361 HCT/Ps require registration and product listing with the FDA. Products fall under this category with the following conditions. This lower-risk category:

- is minimally manipulated (for cells and nonstructural tissue, processing does not alter the relevant biologic characteristics; for structural tissue, processing does not alter the original characteristics of the tissue related to the tissue's utility for reconstruction, repair, or replacement).
- is intended for homologous use (that is, the HCT/P performs the same function in the recipient and the donor).
- does not combine cells, tissues, drugs, or devices.
- does not have systemic effect or does not depend on metabolic activity of other living cells for its primary function (or if metabolic, is autologous and for use in a first- or second-degree relative).

351 HCT/Ps

351 HCT/Ps include drugs and biologics that are under the jurisdiction of FDA regulation and require a biologic license from the FDA.

Minimal Manipulation

Defining "minimal manipulation" is an important consideration because regulatory pathways differ; if the product is more than "minimally manipulated," then the product must go through the therapeutic "351 HCT/P" category of FDA regulation. Exceptions to the rule include removal of an HCT/P from an individual and implant of the HCT/P to the same individual during the same surgical procedure (such as an autologous skin graft harvested and utilized for burn reconstruction), or licensed products.

The primary function of HCT/Ps in the donor is used to determine whether the function is structural or nonstructural, and therefore how HCT/Ps should be regulated. The structural components of adipose tissue include adipocytes and the extracellular matrix.

Decellularized fat tissue-derived extracellular matrix is considered more than minimally manipulated because processing alters the ability of the tissue to provide structural function. Manipulation of cells or tissues includes enzymatic digestion, separation of cell populations, cell culture and expansion, introduction of drugs or growth factors, and cryopreservation. Processing steps such as rinsing, cleansing, or sizing of the tissue are exempt.

ASPS has requested that the FDA expand the scope of exemptions to include centrifugation of lipoaspirate in preparation for fat grafting, morselized cartilage for grafting, and dilation of a vessel graft using a solution containing a pharmacologic agent, for example, in free tissue transfer. ASPS has also drafted a response to redefine adipose

tissue as serving both structural and nonstructural functions, including endocrine, immune, and regenerative functions, such as in the treatment of radiation dermatitis.

According to the current guidance statements, ASCs derived from the SVF for reconstruction and repair in the same operative session constitute a biologic drug and must be approved by the FDA in advance and tested in clinical trials before routine use in patients. The classification of ASCs as drugs by the FDA would add costs, resources, and time for surgeons who perform therapies with ASCs.

Homologous Use

Another issue is the definition of "homologous use." Although fat has many functions, including endocrine, metabolic, and immunologic, the FDA considers fat solely as a structural tissue that functions to cushion and support other subcutaneous tissues and defines the breast as having a sole function of lactation. Therefore, decellularizing adipose tissue eliminates its structural capacity, makes it more than minimally manipulated, and represents a nonhomologous use when applied to the breast. Furthermore, any surgeon who wishes to utilize SVF and isolate cells from a structural tissue must now submit an Investigational New Drug Application (IND) to the FDA and have an approved Institutional Review Board (IRB) application.

ASPS Response

The ASPS has proposed replacing the concept of "main donor function" to "intended use." The FDA defines skin with relationship to its *external* function (as a barrier to retain moisture and protection from the external environment); however, acellular dermal

matrix (ADM) is used *internally* in breast reconstruction. The structural function is preserved in ADM and should represent homologous use.

ASPS has proposed that the definition of adipose tissue be expanded from solely structural to both structural and nonstructural, depending on intended use. The breast should be considered as a structural tissue with important functions in both lactation and sexual function. ASPS has proposed that fat grafting for breast reconstruction should be considered for homologous structural use that is only minimally manipulated.

Furthermore, isolation and digestion of SVF from adipose tissue does not alter the relevant biologic characteristics of individual cells and therefore should be considered minimal manipulation.

ASPS is also working to secure a meeting with the FDA's Center for Biologics Evaluation and Research and has addressed the draft guidance documents. The General Registry of Autologous Fat Transfer (GRAFT) of the Plastic Surgery Foundation (PSF) is a quality improvement initiative to establish a nationwide web-based registry of fat grafting for aesthetic and reconstructive breast surgery. Data collection will begin in October 2015 and will include patient demographics, procedural variables, complications, and the incidence of new or recurrent breast cancers. This data will be used to clarify the safety of fat grafting and to establish best practice guidelines.

Plastic surgery leadership, including ASPS and PSF among others, is working to preserve the future of fat grafting and of our specialty.

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Chapter 4: Autologous Fat Grafting to the Breast: A Systematic Review of Safety, Efficacy, Satisfaction, and Oncological Outcomes

Summary

Background: There is tremendous interest in the use of autologous fat grafting (AFG) for aesthetic and reconstructive breast surgery. However, concerns have been raised regarding its safety and efficacy. The primary objective of this systematic review was to determine the safety, efficacy, satisfaction, and oncological outcomes of AFG to the breast.

Methods: A systematic review was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Electronic databases searched included PubMed, MEDLINE, EMBASE, SCOPUS, Cochrane Library, and clinical trial registries on autologous fat grafting to breast tissue. Title and abstract screening and full text assessment undertaken separately by two independent researchers. Data was extracted and organized in a database. Random effects models were used to estimate pooled complication rates, breast cancer recurrence rates, and patient satisfaction. Meta-regression was performed using a random effects model to determine factors that predicted the outcomes of interest.

Results: A total of 100 articles involving 7,817 patients with a mean follow-up period of 25.8 months (1-156 months) were included. No randomized controlled studies were found. 2.77 percent of the patients undergoing fat grafting to healthy breast tissue experienced major complications requiring a surgical intervention or hospitalization. Fat necrosis was the commonest reported complication (48.16%), biopsy of a subsequent

breast lump was required in 140 cases. Two patients with breast cancer (0.11%) after AFG for cosmetic purposes were reported. 137 patients with breast cancer (2.26%) after AFG for reconstructive purposes were reported. Average breast volume gain ranged from 27.1% to 106% relative to the grafted fat volume.

Conclusions: AFG is a potentially useful aesthetic and reconstructive tool with a relatively low complication rate. The majority of patients and clinicians are satisfied or very satisfied with the results. Long term clinical and radiological follow-up is required.

Background

The technique of autologous lipotransfer for cosmetic indications has long been employed in the field of plastic and reconstructive surgery. Gustav Neuber was amongst the first to introduce the concept of fat transplantation in 1893 through his work in the treatment of facial defects with lipofilling [1]. This technique was first adapted for the breast in 1895 by Czerny for the reconstruction of post-mastectomy defects through the transplant of an autologous lipoma [2]. In 1987, Bircoll and Novack further improved upon the practice of fat grafting through the use of autologous lipoaspirate for transplantation [3]. The procedure is now being used quite frequently for numerous indications. However, this evolution and rise in employment of the technique through the years has spawned concerns for safety by practitioners in the field. One such issue is the concern that autologous adipose stem cells are tumorigenic. Studies have shown, in both animal and human models, that adipose cells exert a positive regulatory effect on the proliferation of tumor cell lines [4, 5]. It was suggested by Freese et al., in their systematic review of the oncological impact of adipose derived stem cells, that these

adipose cells create an inflammatory tumor microenvironment, encourage angiogenesis, and possibly differentiate into carcinoma associated fibroblasts [6]. Concerns regarding the practice of fat grafting were initially not limited solely to the propagation of oncological transformation. In 1987, the American Society of Plastic Surgeons (ASPS) issued a report condemning the practice of fat grafting to breast tissue due to the lack of long-term clinical trials and the possibility that scarring and calcification resulting from the procedure could interfere with breast cancer diagnosis [7]. Continued research in the field has resulted in a reversal of the previously recommended moratorium with an investigation by the ASPS Task Force revealing no evidence of fat grafting impeding cancer detection or any indication that the procedure stimulates cancer recurrence [8]. However, though this report by the ASPS departed from the previous stance that fat grafting should be avoided, the task force was unable to make a confident recommendation due to a paucity of available data. Instead, the authors stated that the technique is a “promising and clinically relevant research topic” with the need for further study and investigation.

Due to the requirement for further insight into this procedure, our study was undertaken to systematically review the literature in order to investigate autologous fat grafting (AFG) for both reconstructive and cosmetic breast procedures. In order to further elucidate the efficacy and safety of AFG indicated for both cosmetic breast augmentation and reconstruction post-mastectomy/breast conserving therapy the rate of complication occurrence, oncological occurrence, and patient satisfaction resulting from AFG was analyzed.

Methods

A Literature search was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Electronic databases searched included PubMed, MEDLINE, Cochrane Library, and clinical trial registries on autologous fat grafting to breast tissue. Title and abstract screening and full text assessment was undertaken separately by independent researchers. Data was extracted by two researchers and organized in a database. Random effects models were used to estimate pooled complication rates, breast cancer recurrence rates, and patient satisfaction. Meta-regression was performed using a random effects model to determine factors that predicted the outcomes of interest.

Variables of Interest

The outcomes of interest in this study were complication events (grouped into those requiring hospitalization or surgery and those not requiring hospitalization or surgery), breast cancer recurrence, and patient satisfaction. Satisfaction was determined from articles explicitly noting subjective patient reports of “good” or “excellent.” Numerical satisfaction surveys were not included in the analysis. Independent variables extracted for the analysis were procedure indication (cosmetic or oncological), follow-up time (months), and mean volume of autologous fat injected (mL).

Statistical Analysis

The data analysis was divided into two phases. First, random effects models (selected due to the heterogeneity of study populations) were used to estimate pooled complication rates, breast cancer recurrence rates, and patient satisfaction rates. A total rate and individual rates for either cosmetic or oncological subgroups were calculated. Studies with a sample size of 1 were excluded from the meta-analysis (n=21). Second, meta-regression with a random effects model was performed for our outcomes of interest. Predictor variables included in the model were the independent variables extracted and any relevant other outcomes, such as complication rates as factors predicting patient satisfaction. Data were analyzed using Comprehensive Meta-Analysis version 3.0 (Biostat Inc., Englewood, New Jersey, USA).

Results

Out of 100 articles, 7,817 patients with a mean follow-up period of 25.8 months (1-156 months) were analyzed. A mean volume of 166.0 mL of autologous fat per breast was injected, and average breast volume gain ranged from 27.1% to 106% relative to the grafted fat volume.

Through meta-analysis, the pooled rate of complications requiring surgical intervention or hospitalization was 2.62% (n=52 studies, $p<0.001$). The rate of complications not requiring hospitalization determined was 8.40% (n=60 studies, $p<0.001$). Fat necrosis was the commonest reported complication (48.16%), and biopsy of a subsequent breast lump was required in 140 cases. The pooled rate of breast cancer complication was

2.54% ($p<0.001$), with an estimated rate in the cosmetic and oncological cohorts of 1.70% and 3.04%, respectively ($n=64$ studies, $p<0.0001$). Two patients with breast cancer (0.11%) after AFG for cosmetic purposes were reported. 137 patients with breast cancer (2.26%) after AFG for reconstructive purposes were reported. Patient satisfaction rate determined by meta-analysis was 88.93% for the cosmetic cohort, 80.88% for the oncological cohort, and 84.72% overall ($n=21$ studies, $p<0.001$).

For the meta-regression results, multiple factors were significantly associated with an effect on complications requiring hospitalization ($n=35$ studies). Mean volume injected predicted a slightly higher rate ($b=0.005$, $p=0.010$), while post-operative cancer recurrence ($b=-0.094$, $p=0.006$) and cosmetic indication ($b=-0.579$, $p=0.033$) predicted a significantly lower complication rate. None of these factors were significantly associated with complications not requiring hospitalization or surgical intervention. In the analysis for patient satisfaction rate ($n=16$), cosmetic indication was significantly associated with a lower satisfaction rate ($b=-1.515$, $p=0.044$). Mean volume injected and complications, both requiring and not requiring hospitalization, were not associated with satisfaction rate. Post-operative cancer recurrence ($n=35$ studies) was negatively associated with cosmetic indication ($b=-1.673$, $p=0.001$) and complications requiring hospitalization ($b=-0.109$, $p<0.001$), while number of follow-up months was a predictor for breast cancer recurrence ($b=0.025$, $p=0.025$). Mean volume of fat injected and complications not requiring hospitalization were not associated with cancer recurrence.

Discussion

Many options exist for breast reconstruction after breast cancer therapy and cosmetic breast augmentation. One of the most popular options for elective breast augmentation in the past has been implant-based technique. In 2015, the ASPS reported that 279,143 breast augmentations were carried out with the use of these implants. Though a popular and decidedly common procedure, this technique is not without its own risks and complications. In a report published by the FDA in 2011, twenty to forty percent of patients receiving primary augmentation and forty to seventy percent of patients receiving breast reconstruction via implant procedures required reoperation upon the breast in the first eight to ten years after the initial operation [9]. In one study of Allergan silicone implants, rupture was experienced in 10.1% of cases in breast augmentation and in 27.2% of reconstructions post cancer treatment. Additionally, significant rates of side effects such as capsular contracture were also reported by the study. Upon analysis of the data obtained by our review, the overall complication rates for breast augmentation and breast reconstruction with fat grafting appear to compare favorably with complication incidences experienced with breast implantation. Our study determined the rate of serious complications requiring surgery or hospitalization to be 2.62% and the rate of minor complications to be 8.40%. This result suggests superiority to other reconstruction methods when compared to a study by Alderman et al. investigating breast reconstruction complication rates of TRAM and implant based procedures. This study found that complications occurred in 45.4% of their study population with 31.6% of patients experiencing major complications [10]. When compared to this study and the reoperation rates for silicone implant placement cited by the FDA, the use of fat grafting in breast

augmentation and reconstruction appears to not only be an equivalently safe alternative technique, but possibly a superior method for these procedures in terms of surgical complications.

Beyond the surgical and perioperative complications of fat grafting, a major focus of concern by the medical community has been upon the oncological safety of grafting adipose tissue to the breast. Numerous studies have indicated a link between adipose derived stem cells and oncological promotion. One such study found that adipose tissue contained multiple progenitor cells that encouraged metastasis and cancer growth [11]. Many clinical trials have also been undertaken to investigate this link of autologous lipotransfer to oncological stimulation in breast tissue. One such study carried out by Petit et al. investigated the rate of breast cancer recurrence in 59 patients with intraepithelial neoplasia after undergoing reconstruction with lipofilling [12]. This study determined that patients undergoing fat grafting experienced an 18% five-year locoregional recurrence rate compared to a 3% recurrence rate in those patients not undergoing a lipofilling procedure. While this result did not bode well for the prospects of fat grafting in breast procedures, other studies have indicated more encouraging findings. In a study by Kronowitz et al. of 719 breasts reconstructed with lipofilling and 670 without following segmental or total mastectomy indicated for breast cancer treatment, locoregional recurrence was observed in 1.3% of the cases undergoing lipofilling and 2.4% of breasts reconstructed without fat grafting [13]. This equated to a five-year locoregional recurrence rate of 1.6% and 4.1% for the lipofilling and non-lipofilling reconstruction cohorts, respectively. The data compiled by our analysis

indicated a similar oncologic safety profile with a pooled breast cancer complication rate of 2.54%, 1.70% in the cosmetic augmentation cohort and 3.04% in the oncological reconstruction cohort. In contrast to the study by Petit et al., these rates are indicative of a very low occurrence of malignant stimulation by the transplanted fat grafted to both healthy and previously cancerous tissue. Based upon these results, it appears that fat grafting can be employed for breast reconstruction and augmentation purposes without placing the patient at an overtly elevated risk for the development or recurrence of breast malignancy.

In addition to exhibiting an acceptable complication and oncological incidence rate, fat grafting to breast was also shown to result in excellent patient satisfaction based upon our analysis. Of the patients receiving lipofilling for reconstructive and cosmetic purposes, satisfaction rates of 80.8% and 88.93% were observed respectively. The pooled satisfaction rate amongst all patient cohorts was determined to be 84.72% overall (n=21 studies, $p < 0.001$). This level of satisfaction compares favorably to the level of contentment achieved through the use of implant based procedures. One study of 1529 women receiving breast implants for both cosmetic and reconstructive purposes by Handel et al. revealed an average satisfaction level of 4.4 out of 5 resulting from breast augmentation and 4.0 out of 5 for those undergoing reconstruction with implants [14]. Another study of 450 patients receiving saline implants carried out by Cunningham et al. revealed 93% of patients were satisfied or very satisfied with the result of their breast augmentation [15]. Based upon these comparisons, fat grafting appears to be nearly as

efficacious in achieving patient satisfaction following breast augmentation and reconstruction as implant based techniques.

Conclusions

Based upon the results of the analysis, it appears that autologous fat grafting for the purposes of breast augmentation and reconstruction is both efficacious and safe.

Oncological and surgical complication rates determined by this study indicate favorable results when compared to studies of other reconstructive methods. Additionally, patient satisfaction appears to be non-inferior when compared to implant based techniques.

Currently, it appears the technique of autologous fat grafting is a viable tool for breast procedures both in the cosmetic and reconstructive setting.

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Table 4-1: Fat grafting for cosmetic augmentation.

Author, Date	n	Mean volume injected/breast (mL)	Surgeries required	Minor Complications	Major Complications	Cancer Occurrence Reported	Follow-up time (months)
Cheng Li et al. 2014[16]	105	205 (120 to 250)	1.28	3	0	0	18 (6-72)
Derder et al. 2014[17]	10	285 (200-500)	2 (1-4)	1	0	0	68 (60-77)
Klit et al. 2014[18]	11	210 (95-370)	1 (1-3)	0	0	0	13 (7-63)
Peltoniemi et al. 2013[19]	18	292 (180-438)	1	3	0	0	18-24
Auclair et al. 2013[20]	197	NR	1	2	1	0	5 (1 month to 3.25 years)
Bulgin et al. 2013[21]	1	185	1	0	0	0	12
Khoury et al. 2012[22]	71	282 (90-600)	1	12	1	0	44
Kim et al. 2012 [23]	1	NR	NR	0	1	0	42
Del Vecchio 2012[24]	12	625	1	0	0	0	12
Del Vecchio and Bucky 2011[25]	25	220-550	1	0	0	0	6
Kamakura et al. 2011[26]	20	244.9 right breast, 235.1 left	NR	2	0	0	9

Veber et al. 2011[27]	31	200.8 +/-64.8	NR	8	0	0	16
Lee et al. 2011[28]	1	250	NR	0	1	0	9
Wang et al. 2011[29]	48	50-170	1-4	13	0	0	NR
Yang and lee 2010[30]	1	972	4	1	0	0	53
Delay et al. 2010 [31]	1	161.8	5	0	0	0	12
Talbot et al. 2010[32]	1	100	1	0	1	0	1
Yoshimura et al. 2010[33]	15	R: 268+/-29, L: 259 +/- 39	1	0	0	0	12
Überreiter et al. 2010[34]	52	184 (120-293)	2 in 85% cases	2	0	0	6
Herold et al. 2010[35]	10	(150-293)	1	0	0	0	6
Mu et al. 2009[36]	157	90-360	NR	0	9	0	15
Illouz and sterodimas 2009[37]	439	145 (25-180)	3 (1-5)	129	13	0	12
Lazzarettie et al. 2009[38]	1	NR	NR	0	1	0	120
Hyakusoku et al. 2009[39]	12	NR	NR	2	12	0	39

Delay et al. 2009[40]	136	NR	NR	10	1	0	12
Wang et al. 2008[41]	33	50-60	3 (1-5)	1	0	0	NR
Zocchi and Zuliani 2008[42]	181	375 (160-745)	NR	19	0	0	12
Pinsolle et al. 2008[43]	8	96 (25-200)	2.1 (1-5)	0	1	0	NR
Zheng et al. 2008[44]	66	101 (60-120) into subq 73 (60-90) into subglandular tissue	1.83	9	2	0	37(13-61)
Yoshimura et al. 2008[45]	40	total: 270 mean R: 277+/- 39, L: 268 +/- 48	1	2	0	0	6
Carvajal and Patino 2008[46]	20	NR	NR	0	0	0	34.5
Coleman and Sabeiro 2007[47]	15	278.6	NR	4	0	2	67
Pulagam et al. 2006[48]	1	NR	NR	1	0	0	156
Kwak et al. 2004[49]	1	NR	NR	0	1	0	NR
Valdatta et al. 2001[50]	1	NR	NR	0	1	0	NR

Castello et al. 1999[51]	1	NR	NR	0	1	0	10
Maillard 1994[52]	1	NR	NR	0	1	0	36
Horl et al. 1989[53]	1	NR	NR	0	1	0	1
Matsudo and Toledo 1988[54]	21	NR	NR	8	0	0	18
Bircoll and Novack 1987[3]	1	100	1	0	0	0	36
Bircoll 1987[55]	1	90	1	0	0	0	30

Major complications described as those requiring hospitalization or additional surgical treatment.

Table 4-2. Fat grafting for reconstruction post surgical breast cancer intervention.

Author, Date	n	Mean volume Injected/breast (mL)	Surgeries required	Minor Complications	Major Complications	Cancer Occurrence	Follow-up time (months)
Kaoutzanis et al. 2016[56]	108	50 (15-180)	1.3 (1-4)	1	0	0	20.2 (6.3-57.4)
Kim et al. 2014[57]	102	49.3 (10-183)	1-3	18	0	1	NR
Silva-Vergara et al. 2016[58]	195	160 (20-480)	1.6	23	3	10	31
Pinell-White et al. 2015[59]	46	29 (10-90)	3.57	NR	NR	3	50.4
Weichman et al. 2013[60]	100	147.8 (22-564)	1.12	0	1	0	18 (12-41)
Al-Kalla et al 2014[61]	1	70, 141, 20 mL in successive procedures	3	0	0	0	6
Biazus et al. 2015[62]	20	121 (68-228)	1	6	0	0	20.75 (13-29)
Brenelli et al. 2014[63]	59	52.3 first, (32.8, 52.8 for second and third defects)	1.3	3	0	3	34.4 +/-15.3
Uda et al. 2014[64]	14	256 (150-400)	2.3	0	2	0	21 (6-54)

Bonomi et al. 2013[65]	31	247 (80-455)	1.4 (1-2)	3	0	1	21 (6-36)
Chaput et al. 2013 [66]	1	NR	NR	NR	NR	1	4
Ihrai et al. 2013[67]	64	38 (10-80) 1st session, 60 for 2nd and third	1.57 (1-5)	1	0	2	46.4 SD 21.4
Le Brun et al. 2013 [68]	42	80	1.3	NR	NR	0	24.5 (12-192)
Petit et al. 2013[12]	59	NR	NR	NR	NR	6	38
Beck et al. 2012 [69]	10	76.5	1	NR	NR	0	36
Cigna et al. 2012[70]	20	NR	NR	1	0	0	6
Doren et al 2012[71]	278	50 (5-200)	2	45	0	9	28 (0.56-168)
Mestak and zimovjanov a 2012[72]	14	NR	1.4 (1-3)	NR	NR	0	3 to 12
Parikh et al. 2012[73]	37	42.8+/- 26.0	2.2	39	0	1	12 minimum

Perez-cano et al. 2012[74]	67	1st session 91 (23-163), 6 months 81 (22-143). Median defect volume was 100 (35-150)	1.35	10	0	0	12
Petit et al. 2012[75]	321 AFG group (642 control)	NR	NR	NR	NR	8	26
Petit et al. 2011[76]	513	107.3 (5-400)	1.3	17	1	29	19.2 (1-107)
Rietjens et al. 2011[77]	155 oncological patients	48 (6-183)	1.2	7	0	1	18.3 (6-49)
Rigotti et al. 2010[78]	137	NR	NR	NR	NR	14	91.2 (37.2-229.2)
Sinna et al. 2010[79]	200	176 (35-405)	1.22	2	6	0	14.5 (4-52)
Delaporte et al. 2009[80]	15	600	3 (2-5)	NR	NR	0	28 (7-49)
Delay et al. 2009[40]	734	NR	NR	29	0	0	12 clinical, 120 for oncology

Fitoussi et al. 2009[81]	1	NR	NR	NR	NR	NR	12
Iannace et al. 2009[82]	15	100	4.1	NR	NR	0	12
Illouz and Sterodimas 2009[37]	381	145 (25-180)/ session. Total mean =540 (25-900)	3 (1-5)	NR	NR	0	12
Kanchwala et al. 2009[83]	110	31 (18-48)	2	NR	NR	0	21 (6-84)
Panettiere et al. 2009 [84]	20	24.5 (8-50)	3.4 (1-7)	0	0	0	17.6 +/-6.7
Amar et al. 2008[85]	15	62.3 (30-100)	1	5	0	0	9
Cotrufo et al. 2008[86]	42	NR	NR	1	0	0	7
Delay et al. 2008[87]	42	154 (43-227)	1.3	NR	NR	6	20.5
Missana et al. 2007 [88]	69	prosthesis 107, LD and prosthesis 147.2, LD 142.5, TRAM 142.1, conservative treatment 75	1.1	NR	NR	0	11.7 (1-38.4)
Pierrefeu-lagrange et al. 2006 [89]	30	164.7 (34-290)	1.1	NR	NR	0	NR

Pulagam et al. 2006[48]	1	NR	1	1	0	0	NR
Spear et al. 2005[90]	37	116	1.1	4	0	0	15 (3 weeks - 7 years)
Sarfati et al. 2011[91]	28	115 (70-275)	3 (2-3) prior to implant placement	3	1	0	17 (4 to 34)
Losken et al. 2011[92]	107	40 (5-150)	1.32	12	0	0	8 (1 - 2.5 years)
Serra-Renom et al. 2010[93]	65	150+/-25 1st stage, 150+/-30	2 fat grafts (6 cases required 3rd stage 75 mL)	0	0	0	1 year
de Blacam et al. 2011[94]	49	67	1.63	3	4	0	2.4 years (5 months-4.1 years)
Salgarello et al. 2011[95]	42	120 (25 to 231) for platelet enriched, 115 (21-169)	1.88 fat alone, 1.82 platelet group Total: 1.86	8	0	0	9 months

Choi et al. 2013[96]	90	Group A (40 breasts): 151 (111-216) Group B (42): 51 (12-72) Group C (41) 93 (75- 108)		0	0	0	140 days
Costantini et al. 2013[97]	24	114.8+/-55	2.2	8	0	1	1 year
Hoppe et al. 2013[98]	28	159+/-61	4 to 6 (4.15)	6	1	1	2.6 years (6 months to 3.7 years)
Howes et al. 2014[99]	1	400	1	0	0	0	1 year
Khoury et al. 2015[100]	488	225 (100 to 400)	3.2	5	25	6	2.5 years (6 months to 7 years)
Kronowitz et al. 2016[13]	719	1-100 (688 70.3%), 101-200 (210 21.5%), 201-300 (55 5.6%), 301-400 18 (1.8%), 401-500 (2 0.2%), >500 (5 0.3%)	1:761 (74.3%), 2: 181 (7.7%),3: 36 (3.5%)	NR	NR	26	60
Laporta et al. 2015[101]	20	211 (169-260)	Mean 1.1	0	0	0	23 (12-34)
Longo et al. 2014[102]	21	137 (90-175) non irradiated per session, 108 (40-160) irradiated per session	3 non irradiated , 5.4 irradiated	0	0	0	34.8 (23-52) non, 17.2 (6-3) radiation

Mestak et al. 2013[103]	1	840 total to right, 790 left (1: 260, 210/2:280,280/3:300,300)	3	0	0	0	4
Garcia et al. 2016[104]	37	50 (20-80)	1	11	0	0	12
Riggio et al. 2013[105]	60	47.13, 38.28 2nd sess, 55.12 3rd	1.37	NR	NR	6	92.2 (5-132)
Schultz et al. 2012[106]	43	40 (6-200)	1.55 (1-3)	2	0	0	6 months or longer
Seth et al. 2012[107]	69	20 to 50 (absolute range 20 -200)	1.1 (1-4)	1	0	0	24.8+/-5.9 fg group
Tissiani and Alonso 2016[108]	19	NR	NR	1	3	0	16 month control, 36 SG
Mestak et al. 2016 [109]	32	NR	NR	NR	NR	2	27 (14-88)

NR: not reported. Major complications described as those requiring hospitalization or additional surgical treatment.

Chapter 5: Autologous Fat Grafting to the Breast: An Institutional Review of Safety and Oncological Outcomes

Summary:

Background: Autologous fat grafting has been widely used for more than two decades during breast reconstruction for postmastectomy patients. However, few studies evaluate clinical outcomes in this patient population. The purpose of this study was to assess complications, radiographic changes and locoregional cancer recurrence outcomes in patients undergoing autologous fat grafting after breast reconstruction in postmastectomy patients.

Methods: We retrospectively reviewed the records of consecutive postmastectomy patients who underwent autologous fat grafting after breast reconstruction at a university center over a 10-year period. Patients with at least 3 months of follow-up were included. Medical records were reviewed for demographics, operative details, complications, incidence of palpable masses, and/or suspicious breast imaging findings, and locoregional cancer recurrence. Descriptive statistics were generated.

Results: The records of 124 patients undergoing lipofilling procedures from January 2006 to January 2016 were reviewed. Their ages ranged from 23 to 79 years (mean, 45.77 years). Fat grafts were harvested, processed, and injected using the Coleman technique. The mean number of fat grafting procedures was 1.50 (range, 1-6) per

breast, receiving 97.2 mL of autologous adipose on average during each session. The most common indication for reconstruction in this study was found to be for aesthetic correction following mastectomy (90.6 % of patients). Fat grafting was most commonly used as an adjunctive therapy following initial implant (54.9% of patients) and flap (43.4% of patients) reconstruction. The time from first oncological surgery to fat grafting occurred after a mean of 27.51 months and median of 14 months. Following the completion of fat grafting, patients were followed by a plastic surgeon for an average of 1.054 months. Twenty-six complications were found to have occurred, resulting in a complication rate of 21.3% in this population of patients. The most commonly reported complications were liponecrosis (19.2% of complications) and infection (15.3% of complications). During oncologic follow up, six patients were reported to have experienced breast cancer recurrence following autologous fat grafting for reconstruction resulting in a recurrence rate of 4.8%. Additionally, of the 59 patients with reported radiologic follow up, seven patients exhibited radiological abnormalities in the post-operative period (11.9%).

Conclusions: In this population of breast cancer patients who had mastectomy with reconstruction, fat transfer was not associated with a higher risk of cancer recurrence. Based on these preliminary findings, autologous fat grafting appears to be a relatively safe procedure for refinement of the reconstructed breast in postmastectomy patients.

Background:

Mastectomy and breast-conserving surgery are important tools employed in the treatment of breast cancer. While they are life saving measures for many patients, these procedures often result in poor aesthetic outcomes. Many methods exist for post-surgical breast reconstruction to correct defects of oncologic therapy. One such method is that of autologous fat grafting (AFG). The technique of AFG was first reportedly utilized in post-mastectomy breast reconstruction by Czerny in 1895 through the use of an autologous lipoma for aesthetic repair [1]. In 1987, Bricoll and Novack revolutionized the technique through the use of lipoaspiration for the acquisition of the autologous adipose tissue for radical post-mastectomy reconstruction [2]. Since this advancement, the practice has increased in popularity and employment in the field of reconstructive surgery. To this end, AFG often serves as an adjunctive procedure for the refinement of breast implant and flap-based reconstructions to improve residual contour and volume deficits [3]. Adipose tissue itself is ideal for such means as it is soft, formable, autologous, readily available, and easily harvested [4]. Furthermore, there exists a high level of satisfaction after AFG among both patients and providers (93.4% and 90.1%, respectively according to a review by Groen et al.) in part due to the relatively straightforward surgical process, low complication rate, and aesthetic outcomes associated with the procedure [5]. All of these factors have contributed to the widespread use of AFG. In fact, a 2013 survey of members of the American Society of Plastic Surgeons found that 62% of all respondents commonly used AFG for reconstructive breast surgery [6].

Despite its popularity, AFG has not always been accepted as an acceptable treatment option for breast reconstruction. In 1987, the American Society of Plastic and Reconstructive Surgeons (ASPRS) advised against AFG due to concerns of potential adverse effects on breast imaging, specifically in screening for breast cancer and recurrence [7].

Since this original ruling, several studies have shown that AFG is a reasonably safe option for refinement of breast reconstruction in this patient population. Complication rates, for example, remain on average between 0.9% and 8% per patient and include relatively benign occurrences such as donor and recipient site infection, hematoma, and palpable masses [8-10]. In 2012, the same organization issued a new position statement, indicating that AFG in postmastectomy breast reconstruction “yields aesthetic improvement and significant patient satisfaction,” with “relatively low” complication rates [11]. Despite this new stance, questions still remain about the overall safety of the technique and impact upon radiological surveillance.

The purpose of this study was to address these concerns and the efficacy of the procedure through the analysis of clinical complications, radiographic imaging results and cancer recurrence in postmastectomy patients who underwent AFG after breast reconstruction.

Methods:

We retrospectively reviewed the records of consecutive postmastectomy patients who underwent autologous fat grafting after breast reconstruction at a university center over a 10-year period (January 2006 to January 2016). Postmastectomy patients who underwent autologous fat grafting after breast reconstruction with 3 months of follow-up were identified using the Current Procedural Terminology (CPT) codes 15770, 19361, 19364, 19366, 19367, 19368, 19369, 19380, 20926, S2066, S2067, and S2068. Medical records (operative reports for oncology and plastic surgery notes, and follow-up notes from clinic) were reviewed for demographic characteristics, breast cancer diagnosis and reconstructive and fat grafting operative details. The indications, location and volume for grafting were also recorded. Data on oncologic follow-up, fat grafting complications, incidence of palpable masses, and/or suspicious breast imaging findings, and locoregional cancer recurrence were recorded.

The clinical and demographic characteristics included age (years) at time of breast reconstruction, indication (cancer or cosmetic), location of cancer (right, left, bilateral), treatment (immediate, mastectomy, or breast conservative treatment), histopathology (ductal intraepithelial neoplasia, lobular intraepithelial neoplasia, invasive ductal carcinoma, invasive lobular carcinoma, other), histologic Grade, pT, pN, receptors (estrogen, progesterone, Her-2, triple negative), adjuvant therapy (lymphadenectomy, radiotherapy, chemotherapy, hormonal therapy), and follow up to oncologist (months).

The oncologic follow-up and lipofilling operative data included the procedure, type of breast reconstruction (implant, type of flap, etc), date of AFG case, number of fat grafting sessions, volume injected in mL (specified left or right and if more than one time), follow up to plastic surgeon after AFG case (months), time from oncologic surgery to AFG (months), and if an inpatient or outpatient.

The lipofilling complications included presence and type of complication, (liponecrosis/oil cyst, prosthesis rupture, hematoma, infection, donor site complication), breast cancer recurrence and radiographic changes.

Lipofilling technique:

Autologous fat grafting was performed under general anesthesia. Fat was prepared and grafted according to the standard Coleman technique. After harvest from the abdomen or flanks, the fat was centrifuged with the resulting oil and serous components decanted. The processed fat was transferred to 3-mL syringes and injected using blunt infiltration cannulas, placing a small aliquot of fat with each withdrawal of the cannula. All 4 surgeons' technique remained consistent throughout the period of the study.

Results:

For the purposes of this study, the records of 124 patients undergoing lipofilling procedures from January 2006 to January 2016 were reviewed. The median age of the patients at the time of first lipofilling procedure was 45.77 (range 23 to 79 years).

Patients included in this study population received a mean number of 1.5 fat grafting sessions per breast, receiving 97.2 mL of autologous adipose on average during each session. The most common indication for reconstruction in this study was found to be for aesthetic correction following mastectomy (90.6 % of patients). Fat grafting was most commonly used as an adjunctive therapy following initial implant (54.9% of patients) and flap (43.4% of patients) reconstruction. The time from first oncological surgery to fat grafting occurred after a mean of 27.51 months (median=14 months). Additional clinical and lipofilling data are indicated in Tables 1 and 2, respectively.

Following the completion of fat grafting, patients were followed by a plastic surgeon for an average of 1.054 months. Twenty-six complications were found to have occurred, resulting in a complication rate of 21.3% in this population of patients. The most commonly reported complications were liponecrosis (19.2% of complications) and infection (15.3% of complications). During oncologic follow up, ten patients were reported to have experienced breast cancer recurrence following autologous fat grafting for reconstruction resulting in a recurrence rate of 10.5%. Additionally, of the 59 patients with reported radiologic follow up, seven patients exhibited radiological abnormalities in the post-operative period (11.9%).

Table 5-1 Clinical and Demographic Characteristics		
	n	%
Location		
Left	52	47.3
Right	45	40.9
Bilateral	13	11.8
Indication		
Cancer	118	92.9
Cosmetic	9	7.1
Treatment		
Mastectomy	60	50.8
Bilateral mastectomy	47	39.8
Lumpectomy	4	3.4
Other / not specified	7	5.9
Histopathology		
Ductal intraepithelial neoplasia	28	30.8
Lobular intraepithelial neoplasia	1	1.1
Invasive ductal carcinoma	48	52.7
Invasive lobular carcinoma	5	5.5
Other	9	9.9
Histologic Grade		
I	10	23.2
II	11	25.6
III	22	51.2

pT		
1	35	57.4
2	23	37.7
3	1	1.6
4	2	3.3
pN		
Positive lymph nodes	30	37.0
Negative lymph nodes	51	63.0
Receptors		
Estrogen	56	65.1
Progesterone	42	48.8
Her-2	18	20.9
Triple Negative	12	14.0
Adjuvant therapy		
Lymphadenectomy	2	2.3
Radiotherapy	30	34.5
Chemotherapy	65	74.7
Hormonal therapy	28	32.3

Table 5-2 Oncologic follow-up and lipofilling data		
	n	%
Type of Breast Reconstruction		
Implant	67	54.9
Flap	53	43.4
Other	2	1.6
Number of fat grafting session (mean)	1.50	
Total volume Injected (mean, mL)	97.2	
Follow-up to plastic surgeon after FG case (mean, months)	1.054	
Time from oncologic surgery to FG (mean, months)	27.51 (median=14 months)	
Inpatient	10	8.1
Outpatient	114	91.9

Table 5-3 Lipofilling complications		
	n	%
Complication		
Yes	26	21.3
No	96	78.7
If yes, type		
Liponecrosis	5	4.1
Oil Cyst	1	0.08
Prosthesis rupture	2	1.6
Hematoma	1	0.08
Infection	4	3.3
Donor site complication	4	3.3
Breast cancer recurrence		
Yes	10 (6 specifically after AFG)	10.5
No	85	89.5
Radiographic changes		
Yes	7	11.9
No	52	88.1

Discussion:

In recent years, fat grafting has become an oft-used tool for reconstructive procedures indicated in the repair of defects secondary to oncological breast surgery. Since the reversal of the previous admonishment of the practice by the ASPS, the technique greatly increased in frequency of use [11]. The procedure is often employed, as was the case in this study, as an adjunctive aesthetic procedure following implant or flap repair to correct residual defects. Due to the popularity and utility of this procedure, several studies have investigated the safety of this procedure in terms of surgical complications. In a systematic review of AFG in post mastectomy breast reconstruction by Agha et al., it was determined that the overall complication rate observed following AFG procedures was 7.3% [12]. Furthermore, 62% of the complications in the 35 studies reviewed were found to be due to the relatively benign condition of liponecrosis. Compared to the complication rate determined by Agha et al., the complication rate exhibited by the cohort of this study was significantly higher at 21.3%. Despite this inferior complication rate, the results of our study are encouraging as a great deal of the complications reported were not significant. In fact, 23.1% of all complications were due to liponecrosis or oil cysts discovered in the post-operative period. Therefore, this procedure can still be considered to have a relatively favorable safety profile based upon the data.

In addition to surgical complications, many questions have arisen concerning the oncological safety of lipofilling. Adipose tissue has previously been discovered to

promote angiogenesis and create an inflammatory microenvironment, two actions that are feared to promote tumorigenesis [13]. A recent study of adipose tissue harvested from healthy human donors found that adipose derived stem cells stimulated the metastasis of a tumor cell in a mouse host when compared to injection of tumor cells only [14]. Due to these concerns and discoveries, multiple investigations have been directed towards the oncological effects of fat grafting to breast tissue. In the review by Agha et al., the incidence of new or recurrent breast cancer was determined to be 4.4% [12]. A recent study of 195 patients undergoing AFG for breast reconstruction indicated a similar rate of oncologic occurrence of 5.1% [15]. The occurrence rate determined in our patient population was found to be 10.5%. The oncological occurrence determined in this study may be elevated due to the cohort investigated. Of the patients included in this investigation 34.9% were afflicted with HER-2 positive or triple negative breast cancers, both poor prognostic indicators. Additionally, 22 patients were found to have grade III carcinomas. Additionally, in one large study focusing upon the oncological recurrence of breast cancer in 1027 patients with stage I and II disease, the locoregional recurrence rate was found to be 8% [16]. This recurrence rate is similar to that determined in our study, indicating an acceptable oncological outcome for lipofilling in post-mastectomy patients.

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Chapter 6: Do Stem Cells Have an Effect When We Fat Graft?

Fat Versus Fiction

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Rinker BD, Vyas KS. Do Stem Cells Have an Effect When We Fat Graft? *Ann Plast Surg.* 2016 Jun;76 Suppl 4:S359-63.

Summary: Fat grafting has become a widely accepted modality of soft tissue restoration and has found applications in many areas of aesthetic and reconstructive plastic surgery. Numerous claims have been made regarding the regenerative effects of fat grafting on the recipient bed. The purpose of this paper is to survey the available literature to answer the question of whether fat grafting has a positive effect on the surrounding tissues. It has been convincingly demonstrated that fat grafts contain viable adipose-derived stem cells (ASCs). The fate of these cells is determined by the microenvironment of the recipient bed, but animal studies have shown that a large fraction of ASCs survive engraftment. Numerous clinical studies have demonstrated the positive effects of fat grafting on recipient tissues. Improvement in validated scar scores as well as scar stiffness measurements have been documented after fat grafting of burn scars. Fat grafting has also been convincingly demonstrated to improve the quality of irradiated tissues, as measured by validated clinical scales and staged histology. It is ultimately unclear whether ASCs are responsible for these effects, but the circumstantial evidence is weighty. Fat grafting

is effective for volumizing and improving skin quality in the setting of radiation, burns, and other scars. The observed effects are likely due to ASCs, but the evidence does not support the routine use of ASC-enriched fat grafts.

Introduction: Autologous fat grafting has become a widely accepted method used by plastic surgeons to correct soft tissue defects due to its simplicity, versatility, low donor site morbidity, and biocompatibility. Fat grafts have been used as soft tissue filler to improve function and aesthetic form after trauma (1), thermal or radiation damage (2), the correction of congenital anomalies (3) or aging (4). In the aesthetic surgery arena, they have been used for body and facial contouring (5,6) and breast augmentation. However, the applicability of autologous fat grafting has been limited by variability in long-term graft retention, with estimates of the survival rate by volume ranging from 20% to 80% (7–10).

The recent discovery of multipotent mesenchymal adipose tissue-derived stem cells (ASCs) in subcutaneous adipose tissue has encouraged the study of their role in enhancing fat grafts for applications in soft tissue augmentation, tissue engineering, and wound healing (11). The objective of this review is to evaluate the role of ASCs in clinical applications of fat grafting, to answer the question, “Do stem cells have an effect when we perform fat grafting?” Applying the deductive nomological model of logicians, this question can be addressed by reducing it to its 3 component parts, which are: (1) Does fat grafting transfer viable stem cells? (2) Does fat grafting induce tissue

regeneration? (3) Are the stem cells responsible for the observed regenerative effects?

Does Fat Grafting Transfer Viable Stem Cells?

In 2000, Halvorsen et al (12) described the osteogenic differentiation of ASCs, and in 2001, Zuk et al (13) demonstrated adipose tissue as an easily accessible and abundant source of adult mesenchymal stem cells with multilineage potential. Although ASCs share many of the characteristics of other mesenchymal stem cells (14), including self-renewal and multipotency, they can be obtained much more easily and with higher cellular yield. The ASCs are isolated from the stromal vascular fraction (SVF) of collagenase-digested adipose tissue. Adipose harvested through direct excision or with the Coleman technique with centrifugation has been shown to yield the most SVF cells and ASCs (15). The SVF is a heterogeneous population of cells and rich source of ASCs, preadipocytes, vascular progenitors, fibroblasts, pericytes, macrophages, mast cells, and lymphocytes (16). Culture of SVF on a tissue culture surface with growth media yields an adherent and more homogeneous population of ASCs that can be expanded in vitro and differentiated into cells of ectodermal and endodermal lineages, including adipocytes, osteoblasts, chondrocytes, endothelial cells, neuron-like cells, myocytes, and cardiomyocytes (12, 17–19).

Factors influencing ASC populations include donor age (20), sex, harvesting and processing technique, harvest site (21), and other variables; this contributes to heterogeneity among results and influences complicates direct comparison of studies. The studies of ASCs in fat grafting must take into consideration patient variability, volume of

lipoaspirate and recipient site, and the number of SVF and ASCs in the lipoaspirate, which may account for the differences observed among studies. Some studies conclude that the lower abdomen or inner thigh are the preferable donor sites for adipose tissue harvesting due to the relative ease of access and high yield of ASCs (22), although the current literature does not support a significant difference in the volume or weight of grafted fat among donor sites (23). A recent systemic review of the literature revealed no significant difference in outcomes of grafted fat obtained from different donor sites, donor site preparations, harvesting technique, harvesting cannula size, centrifugation speed, or when tumescent technique was used. Clinical studies favored fat processed by centrifugation compared to sedimentation and higher retention was observed with slower reinjection in less mobile areas (24).

Fat grafting has been used clinically for over 100 years, but the underlying cellular and molecular mechanisms of the engraftment process remain to be elucidated. Avascular fat grafts rely on diffusion for survival until revascularization occurs; this results in cell death with subsequent volume loss, fibrosis, and the development of oil cysts. The cell survival theory (25) and host replacement theory were the predominant hypotheses of the engraftment process until Eto et al (26) described the fate of adipocytes based on 3 zones defined by the local microenvironment and distance from the surface. Within the peripheral/regenerating zone, adipocytes and ASCs within 300 μ M from the surface survive. Although viability may be preserved in the intermediate/ inflammatory zone, adipocytes in the central/necrotic zone die within the first 24 hours due to lack of nutritional diffusion, severe ischemia, necrosis, and graft resorption. The ASCs are more

resistant to hypoxic conditions (27), however, and survive for up to 3 days to regenerate the adipose tissue through adipogenesis, angiogenesis, and paracrine effects. More recent models suggest that recipient adipocytes may be more significant in replacing the necrotic adipocytes (28). A study using green fluorescent protein mice reveal that regenerated adipocytes mostly originate from ASCs within the grafted tissue, although only ASCs located adjacent to the necrotic adipocytes differentiated directly into adipocytes (29). In contrast, a recent transcriptional analysis of ASCs within grafted fat transplants demonstrated no changes in adipogenic differentiation, challenging the belief that ASCs contribute to regenerating adipocytes (30).

Revascularization of the free fat graft is essential to graft survival and is influenced by ASCs. The ASCs differentiate into endothelial cells in vitro and were once thought to directly contribute to vasculogenesis after fat transfer (31). Studies of transgenic mice demonstrate that the neovasculature of fat grafts is derived from the recipient animal, refuting the direct contribution of ASCs in revascularization (28). The current predominant hypothesis is that ASCs promote revascularization of ischemic free fat grafts through the release of proangiogenic paracrine growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, hepatocyte growth factor, and insulin-like growth factor-1 (32,33). Lipoaspirate supplemented with ASCs has been shown to improve angiogenesis and long-term graft retention through the release of these factors (34,35). Thus, ASCs are observed to survive after grafting, and in fact play a major role in adipocyte survival and differentiation.

Does Fat Grafting Induce Tissue Regeneration?

Fat grafting was pioneered by Neuber and Czerny, working separately in Germany in the 1890s (36,37). It was used enthusiastically by Gillies, who observed that the subcutaneous application of “fat parcels” improved the quality of scars. Autologous fat grafting fell from favor until the 1990s, when Coleman³⁸ developed the technology of structured fat grafting and observed positive effects on the skin, even in the presence of severe atrophy and scarring.

Wound repair is a process of inflammation, angiogenesis, tissue development, and remodeling. Fat grafting has been used as a treatment modality for complex wounds, burns, and scars due to its influence on the wound healing process, including stimulation of angiogenesis (39), release of cytokines and growth factors such as transforming growth factor- β , granulocyte colony-stimulating factor, and VEGF, recruitment of endogenous stem cells to the wound bed, and by direct differentiation into cells. Fat grafting has been shown to reduce inflammation and fibrosis, decrease hypertrophic scarring, and decrease healing time (40–42). Several applications of autologous fat grafting provide direct evidence for the regenerative effects of the technique.

Scars

Adult stem cells are important for the mobilization and coordination of cells during the healing process (43). The discovery of ASCs and refinement of the fat grafting and cell-based therapy processes permitted the introduction of large numbers of ASCs into

targeted treatment areas to improve healing. Local injection of ASCs in a pig model has been shown to decrease scar size and pliability and improve color through the inhibition of TGF- β against fibroblasts, decrease activity of mast cells, and increase expression of matrix metalloproteinases that all contribute to scar remodeling (44). Pallua et al (45) reported decreased pain, improved scar color, quality, pigmentation, stiffness, and pliability in patients with facial scars treated with lipofilling.

Radiation-Induced Injury

Fat grafting can reverse chronic skin changes secondary to radiation, and these effects are likely mediated by ASCs. In an irradiated mouse model, human fat grafts were injected into the subcutaneous plane of the scalp, resulting in attenuated dermal thickness and collagen deposition and increased vascularity in the irradiated tissue compared to the non-irradiated tissue (46). In 2007, Rigotti et al (47) demonstrated improved tissue wound healing with neovessel formation and significant clinical improvement in breast cancer patients undergoing repeated transplantation of purified autologous lipoaspirates for radiation-induced wounds. In 2009, Panetti et al (48) investigated the effects of fat grafting on the functional and aesthetic aspects of irradiated reconstructed breasts compared to control. A significant improvement in all clinical symptoms and aesthetic scores was achieved in the fat grafting group compared with the control group.

Burns

Fat grafting has also been successful in the management of thermal injury (49) and burn scars (41). Bruno et al (50) treated half of a scar area with processed lipoaspirate and

collected biopsies before treatment and at 3 and 6 months after the treatment.

Immunohistochemical evaluation revealed a marked improvement in vascularity, dermal papillae, and increased collagen organization and elastic fibers with lipofilling. Klinger et al (51) reported on lipoaspirate treatment in 3 adult patients with hemifacial hypertrophic scars and keloids from severe burns. The scar was examined through punch biopsy before and after treatment and at 6-month follow-up revealed new collagen deposition, local hypervascularity, and dermal hyperplasia with a significant improvement in skin texture and thickness. Several other clinical studies have reported similar success with the use of lipoaspirate in the management of burn scars for not only filling soft tissue voids (52) but also for the resolution of pain and functional improvement. In a study by Klinger et al (42) of 20 patients with retractile and painful burn scars restricting normal daily activity and joint mobility, autologous fat graft led to a significant improvement in joint mobility and scar appearance. Baptista et al (53) described the benefits of autologous fat grafting for the management of painful burn scars and noted improvement of symptoms in the majority of patients with no reported complications. Patients with severe burns may require serial transplantation to overcome underlying structural defects of the fibrotic tissue.

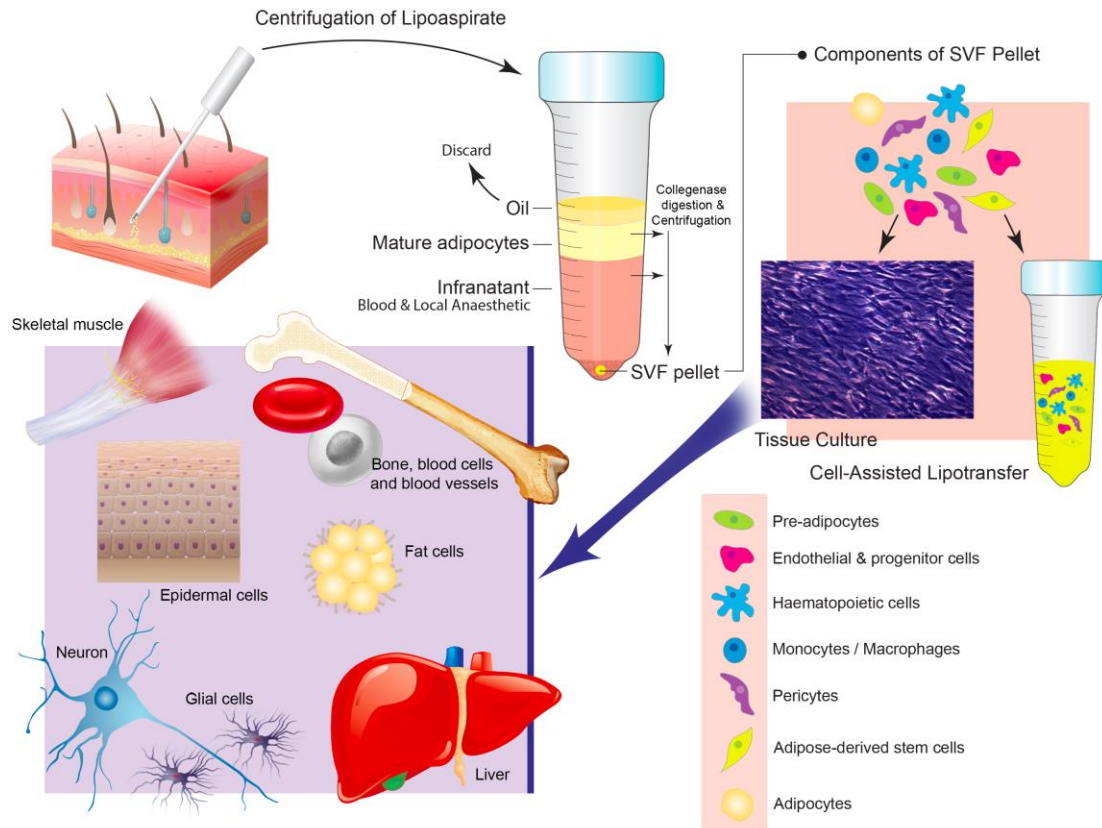


Figure 6-1. SVF in cell assisted lipotransfer or tissue culture. Liposuction of subcutaneous adipose tissue followed by centrifugation yields lipoaspirate. The oil layer is discarded to reduce the incidence of oil cysts. The SVF, which contains cells such as pre-adipocytes and adipose-derived stem cells, can be obtained through collagenase digestion and centrifugation of lipoaspirate. The SVF can be utilized in cell-assisted lipotransfer or can be cultured in vitro. Adipose-derived stem cells are multipotent and demonstrate plasticity in culture; these cells can be induced to differentiate into adipocytes, myocytes, osteoblasts, chondrocytes, endothelial cells, hepatocytes, hematopoietic cells, and other cell types by exposure to growth factors.

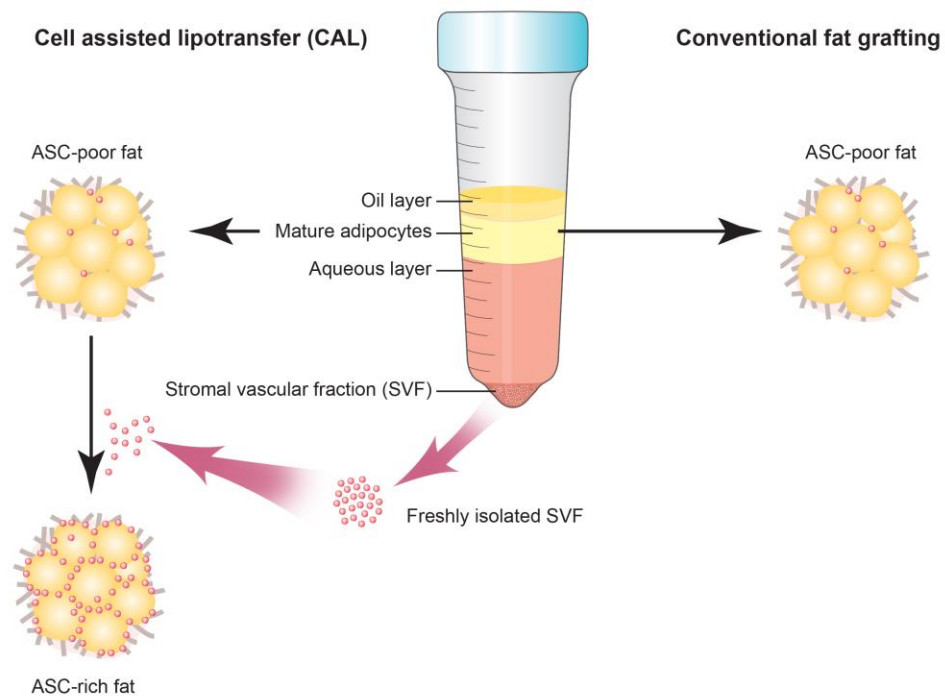


Figure 6-2. Conventional lipotransfer compared to cell-assisted transfer. In conventional lipotransfer, preinjection processing is limited to cleansing and centrifugation. In cell-assisted lipotransfer, a portion of the lipoaspirate is subjected to enzymatic digestion and centrifugation to isolate the stromal vascular fraction, which contains adipose-derived stem cells and other cells thought to improve graft retention. These cells are added to lipoaspirate to yield a stem cell enriched lipograft.

Are the Stem Cells Responsible for the Observed Regenerative Effects?

It has not been definitively proven that ASCs are the causative agent for the observed regenerative effects after fat grafting, rather than the trauma of injection or inflammatory mediators. However, the circumstantial evidence for ASCs is compelling. The ASCs have been shown to support tissue regeneration by differentiating to replace defective cells or through the release of paracrine factors that directly accelerate tissue repair via host-derived cells (54). For example, ASCs secrete several growth factors in response to hypoxia, including the proangiogenic factor VEGF and anti-inflammatory cytokine interleukin (IL)-10 (54). The growth factors brain-derived growth factor, glial-derived growth factor, and nerve growth factor have been shown to augment nerve regeneration. The secretion of growth factors, such as VEGF, hepatocyte growth factor, insulin-like growth factor-1, granulocyte colony-stimulating factor, IL-6, and IL-8 can affect processes such as angiogenesis, wound healing, and/or immunomodulation. These paracrine effects of ASCs are likely responsible for the observed tissue regeneration after fat grafting for complex wounds, burns, scars, and radiation-induced skin injury.

Additional circumstantial evidence for ASCs as the regenerative agent in fat grafting is provided by the observed effects of cell-assisted lipotransfer (CAL) (Fig. 1). Enrichment of fat grafts with concentrated ASCs through CAL significantly enhances volume retention (55) and neovascularization and reduces the adverse effects of lipoinjection, such as fibrosis and cyst formation (31). The CAL been heralded by some as superior to conventional lipoinjection (31, 56, 57). The specific mechanisms contributing to improved fat graft volume retention with CAL are not well understood, but it is debated

that ASCs can differentiate directly into adipocytes and contribute to tissue regeneration and secrete antiapoptotic factors to reduce the loss of adipocytes (Fig. 2). A more evidence based hypothesis affirms the role of ASCs in the release of angiogenic growth factors that promote improved graft vascularization in the hypoxic graft environment (30). This process has been successful in cosmetic breast augmentation and soft tissue reconstruction (56, 58–61), facial contouring (62,63) and facial lipoatrophy (56,57,64).

In a clinical trial of patients with craniofacial microsomia, CAL enhanced fat volume retention for facial recontouring compared with conventional lipoinjection (65). Kølbe et al (66) conducted the first clinical study to assess the effect of lipofilling with fat grafts enriched with ex vivo expanded ASCs. This triple-blinded placebo-controlled clinical trial of 10 healthy adults demonstrated improved volume retention in fat grafts enriched with ex-vivo expanded ASCs compared to conventional fat grafts. However, not all clinical studies have reported improved graft survival rates with CAL. In 2 studies of women undergoing breast augmentation with CAL, there was no significant advantage of CAL over conventional techniques when measured by magnetic resonance imaging at 6 months postoperatively (59,67).

Conclusions

Autologous free fat grafting is a safe and dynamic procedure used by plastic surgeons to improve functional and aesthetic form for volume loss or deformity for a variety of indications, including trauma, burn or radiation damage, scars and complex wounds, congenital anomalies, aging, body and facial contouring, and breast augmentation and

reconstruction. Current practice for autologous fat grafting for soft tissue reconstruction or augmentation has been limited by variability in long-term graft retention, resulting in suboptimal outcomes and repetitive procedures. Evidence-based research suggests that the SVF and ASCs may improve fat graft survival, largely through angiogenic properties, although superiority of cell-assisted lipotransfer over conventional lipotransfer has not been unequivocally demonstrated. The tissue regenerative properties of fat grafts in the setting of radiation, burns, and scars are likely due to ASCs. High-quality clinical trials to demonstrate safety and efficacy are necessary to guide the development of protocols for clinical practice.

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Chapter 7: Profile of Adipose Derived Stem Cells in Obese and Lean Environments:

A Review

Summary

Introduction: Due to the demand of stem cells for applications in regenerative medicine, new methods of isolating these cells are highly sought. Adipose tissue is a readily available and non controversial source of multipotent stem cells with low risk to potential donors. However, donor Body Mass Index (BMI) over 25 has been associated with altered cellular microenvironment and thus has implications for stem cell efficacy in recipients. This systematic review explores the existing literature on adipose-derived stem cells (ASCs) and the effect of donor obesity on cellular function.

Methods: The search term “obesity on adipose derived stem cells” was used to search Pubmed database. 243 papers were retrieved. Of those, 4 were meta-analyses and 31 were reviews.

Results: There is agreement on reduced ASC function in response to obesity in terms of angiogenic differentiation, proliferation, migration, viability and on an altered and inflammatory transcriptome. Osteogenic differentiation and cell yield do not show reasonable agreement. Finally, weight loss partially rescues some of the aforementioned features.

Conclusions: Generally, obesity reduces ASC stem cell qualities and may have an effect on their therapeutic value. As weight loss and some biomolecules have been shown to rescue these qualities, further research should be conducted on methods to return obese derived ASCs to baseline.

Introduction

The growing field of modern regenerative medicine requires the development of a reliable source of stem cells for tissue engineering and other medical applications. Stem cells are characterized by the ability to self-regenerate and also to form multiple types of terminally differentiated cells. While embryonic stem cells are totipotent in their differentiation potential, adult stem cells have been found to be multipotent and much less controversial in their origin. In fact, adipose tissue, which is commonly discarded after procedures such as liposuction, is an easily accessible and cost effective source of adult stem cells. These adipose-derived stem cells (ASCs) can then be used in multiple therapeutic avenues, including healing bone defects, organ repair, vascular regeneration and increasing fat tissue survival rate [1, 2].

The exploration of adipose derived stem cells (ASCs) as a source of material in regenerative medicine has raised concerns as to the optimal donor environment. Studies have shown previously that age adversely affects ASC proliferation and differentiation potential [3-7]. In addition, gender and anatomic location of extraction play a role in differentiation capability [6-8]. However, it is important to note that the viability of adipocytes does not differ between obese and lean individuals in fat taken from the flank,

inner thigh and lower abdomen [9]. ASCs from fat taken from the omentum also showed no difference in proliferation as compared with those taken from subcutaneous fat in one study [7]. However, Cleveland-Donovan et al.¹⁰ suggests that subcutaneous ASCs have a greater capacity for proliferation, differentiation and survival as compared with omental ASCs due to impairment of the AKT pathway. In addition, according to three studies, body mass index (BMI) does not influence the concentration of processed lipoaspirate cells [2, 11, 12]. However, Yu et al [13] suggest a positive correlation between BMI and yield while van Harmelen et al [14] and Aust et al [15] suggest a negative correlation. The variable results could partially be explained by the error prone, high subject variability and protocol dependent nature of quantifying ASCs, as noted by Bakker et al. [16]. Clinically, the totality of these results suggests age is a strong prognostic factor of ASC graft usefulness while donor site and gender may also play a role. However, the effect of BMI on graft utility is clearly an area of contention, especially with respect to lipoaspirate yield.

Given the fact that many ASCs are extracted from the excess adipose tissue of overweight (BMI > 25) or obese (BMI > 30) donors, the potential influence of this microenvironment has become an area of clinical relevance. Healthy adipose tissue is increasingly seen not only as an energy reservoir, but also as an endocrine organ with a role in normal metabolic regulation. On the other hand, adipocytes in obese patients have been proven 'dysfunctional' due to their induction of a hypoxic and mildly inflammatory cellular microenvironment [17, 18].

The WHO estimates over one billion overweight (BMI > 25) and 300 million obese (BMI > 30) individuals worldwide. In addition, the prevalence of obesity is increasing, with over 20% of the United States already falling under that category [19]. With a significant part of the prospective donor population ASCs experiencing the environment produced by obesity, substantial efforts have been made to characterize that environment's effects.

Altered Environment

An important first step in understanding obesity's effects on ASCs is characterizing the obesity microenvironment. Obesity is one of the conditions, along with diabetes and aging, that induces cellular stress in the form of low-level inflammation and storage of excess lipids [17]. Generally, this is through adipocyte hypertrophy, coinciding with decreased angiogenesis and lipogenesis [20]. Interestingly, ASCs have been found to not only promote the generation of inflammatory macrophages, but also to directly differentiate into these cells [21]. Therefore, the presence of inflammatory cells and cytokines in the adipose tissue of obese patients may be in large part due to site-specific mesenchymal ASCs [22, 23]. In addition, obesity increases the level of local and systemic circulating inflammatory cytokines which encourages migration of macrophages into the subcutaneous adipose tissue, adding another source to the inflammatory cells [24]. One of the upregulated cytokines potentially thought to increase this localization of inflammatory cells is osteopontin [25]. NFkB is shown to be heavily upregulated in obese ASCs responding to hypoxia, providing another mechanism for the increased inflammatory response [26].

Hypoxia has been noted in the white adipose tissue of obese mice, indicated by a 1.7 fold increase in lactate and an increase in bound pimonidazole, which forms adducts with thiol in hypoxic environments [18]. The hypoxia is partly due to a decrease in perfusion.

Expectedly, as HIF1A is thought to be a key mediator in the hypoxia response, affecting cell survival and metabolism, it is decreased in obese ASCs [27].

One study showed that even after bariatric surgery-induced weight loss, the ASCs isolated from a formerly obese individual's adipose tissue still showed negative effects on mitochondrial function. These formerly obese ASCs also remained more pro-adipogenic despite normal BMI, potentially through epigenetic mechanisms, which may explain the reason why weight loss is difficult to maintain for many obese patients [20].

Altered Transcriptome Profile

Superficially, many of the surface markers on obese-derived vs lean-derived ASCs are similar, such as CD29, CD44, CD90 and CD105 [28]. Upon further investigation, obesity-induced inflammation can in fact cause lasting functional differences in ASCs from obese donors. One study showed increased mRNA expression of pro-inflammatory cytokines such as TNF-alpha and IL-6 by obese ASCs, which further caused no clinical improvement when used in murine models of multiple sclerosis [29]. Additionally, a study showed strong upregulation of NFkB in response to hypoxia [26].

These results were bolstered by another study in domestic pigs fed an atherogenic diet vs normally fed controls for 16 weeks. At the end of the study, ASCs in the obese pigs expressed higher amounts of TNF-alpha, which further correlated in vitro with enhanced

adipogenic and osteogenic differentiation [30]. Lee et al [31] also showed the overexpression of chemokines MCP-1 and MIP-1 alpha, and adhesion molecules fibronectin that respectively attract and retain macrophages to tissue. Further, Nair et al. showed an upregulation of various inflammatory genes, including IL-8 and CD53 in ASCs isolated from obese Pima Indian individuals [32]. Finally, several microRNAs implicated in differentiation and cell senescence are upregulated in obese ASCs [33]. It is important to note that IL-6, mentioned previously, and IL-8 in high levels are also a cause of cell senescence [34]. Thus, the preponderance of evidence points to ASCs not only being influenced by, but contributing to the low grade inflammatory environment observed in obesity.

Differentiation Potential

ASC, as multipotent cells, have the potential to revolutionize reconstructive surgery by increasing availability of high demand tissue types. For example, the reconstruction of craniomaxillofacial defects, whether inherited or acquired, is often limited by the scarcity of available bone tissue. In one study, ASCs derived from lean human subjects showed an increased propensity towards osteogenic differentiation as compared to those derived from obese individuals, both in vitro and in vivo [35]. When the obese ASCs were supplemented with estradiol, they regained their osteogenic propensity. This contradicts the previously mentioned Zhu et al study using porcine models of obesity, which showed increased osteogenic differentiation in obese-derived ASCs that correlated with increased TNF-alpha expression [30]. The finding also contradicts Yang et al., which showed an

increase in osteogenic differentiation potency with increasing BMI in human derived ASCs [12].

Obese-derived ASCs also showed reduced overall differentiation and angiogenic differentiation potential compared to their lean-derived counterparts, limiting their efficacy as regenerative tools. [14, 36, 37]. Roldan et al showed that this reduction in differentiation capacity was associated with dysregulation of Sonic Hedgehog, Wnt and Notch signaling pathways [33]. Isakson et al. [38] also showed dysregulation of Wnt and increased TNF- α led to a proinflammatory, macrophage-like phenotype which limited the normal differentiation capacity of ASCs. Studies also show that obese-derived ASCs exhibit decreased expression of genes essential to embryogenesis, wound repair and angiogenesis such as the HOX and T-box genes [28].

Migration Potential

In order to effectively aid wound healing and other regenerative processes, ASCs must be able to migrate to injured tissue with the aid of chemotactic factors such as SDF-1, CXCL16 and CXCR4 [39]. Reliably reproducible chemotaxis would potentially even allow the intravenous delivery of ASCs to distant injury sites within the body [40]. Oñate et al showed that obese-derived ASCs had significantly lower expression of SDF1 as compared to lean controls, indicating that obesity may affect the delivery of ASCs to injured tissue [28]. In contrast, a study showed that when responding to stimuli like MCP1 and HMGB1, obese human derived ASCs doubled their migration capacity. However, it was also found that under basal conditions that population has a reduced

migration capacity as there is no dose dependent response in migration to chemotactic factors like TNF-A, SDF-1 or IL-8 [37].

Proliferation Potential

The preponderance of studies show ASCs from obese populations display impaired proliferation potential and decreased cell survival [26, 27, 41]. One study however showed no significant correlation between BMI and proliferation [2]. Perez et al found a significantly increased population doubling time in obese derived ASCs as opposed to lean ASCs in mice and humans [27]. The study also found an increased fraction of pre-apoptotic cells in obese ASC populations. This could potentially be due to the reduced telomerase activity and upregulation of p21, increasing DNA instability and apoptosis, respectively [27].

Obese individuals do not all have the same microenvironment in their tissues; but the presence of metabolic syndrome is indicative of a certain subset of cells. Oñate et al reported that only ASCs from patients with metabolic syndrome had a significantly higher population doubling time than ASCs from normal weight or metabolically healthy obese patients [36]. Another study by the same group was able to differentiate increasing degrees of impairment between healthy lean, healthy obese and metabolic syndrome obese individuals [28]. Metabolic derangement also limits the lifespan of ASCs derived from obese subjects; in a porcine model of obesity, ASCs showed increased senescence compared to ASCs from lean pigs [30].

Weight Loss

As mentioned previously, ASCs isolated from previously obese individuals have been reported to be pro-adipogenic and maintain negative mitochondrial function. Thus, as previously obese individuals may be an important source of ASCs, it is important to characterize ASCs isolated from this environment. Some studies have shown a significant decrease in inflammatory cytokines [42] and inflammatory cell infiltration [43] in subcutaneous adipose tissue following weight loss. However, effects on ASCs themselves have been less encouraging. Mitterberger et al [44] found no change in ASCs isolated from previously obese populations. Indeed, ASCs from the previously obese group achieved an adipocytic phenotype faster than those isolated from lean patients [44, 45]. However, Petrangeli et al. [26] noted obese ASCs recover differentiation potential features after hypoxia and Perez et al. noted that ASCs from formerly obese mice partially recovered viability [46]. A study also showed ASCs from previously obese patients had the same osteogenic potential as those from controls [45]. In Perez et al., formerly obese ASCs showed partial recoveries to non-obese baseline in proliferation, cell morphology and migration potential but not live cell fraction or angiogenic potential [46]. More generally, in a study examining the effects of bariatric surgery in women, ASCs from previously obese individuals showed the most lipid accumulation and secreted more MCP-1, an inflammatory cytokine [47]. The data would suggest that though inflammation is generally reduced following weight loss, ASC differentiation potential and transcriptome profile do not completely return to baseline.

Discussion

Metabolic health seems to play a larger role in predicting the performance of ASCs than a strict BMI cutoff. The presence of this metabolic derangement due to obesity results in an inflammatory cellular microenvironment, which reduces the proliferation potential of ASCs. In terms of angiogenic differentiation potential, obesity appears to play a negative role while in osteogenic potential there is less of a clear relation. This may be an important distinction for ASCs used in scaffolds and other bone grafts. Additionally, studies show that obesity may decrease ASC therapeutic value by altering the cell transcriptome, impairing migration, slowing doubling time and hastening senescence.

Many of these studies rely heavily on animal models of diet-based obesity for a set amount of weeks. Further research should attempt to more accurately model human obesity, which is also dependent on stress level, genetics and amount of exercise. This would more accurately predict the effect of donor obesity on ASCs and thus, the efficacy of using these cells in regenerative medicine. Further research should also further elucidate methods by which obese-derived ASCs may be returned to their healthy state, as some studies found using estradiol, TNF-alpha and weight loss. Based on the current body of knowledge, it can be assumed that ASCs derived from obese, metabolically unhealthy donors do not exhibit the same properties and are potentially less therapeutically valuable as those derived from lean, healthy donors. This should be taken into account when the ASCs are used for clinical purposes.

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Chapter 8: The Effect of Obesity on Adipose Stem Cell Functionality and Fat Graft Retention

ABBREVIATIONS:

ADQ: adiponectin

ARS: Alizarin red staining

ASC: Adipose tissue-derived stem cell

BMI: Body mass index

BMSC: Bone marrow-derived stem cell

CFU: Colony-forming unit

DMEM: Dulbecco's modified Eagle's medium

FACs: Fluorescence-activated cell sorting

FBS: Fetal bovine serum

HIF-1 α : hypoxia inducible factor

LPL: lipoprotein lipase

MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)

ORO: Oil-red-O

P/S: penicillin/streptomycin

RT-PCR: real time polymerase chain reaction

RUNX-2: runt-related-transcription factor 2

SVF: stromal vascular fraction

TIE2: tyrosine kinase 2

VEGF: vascular endothelial growth factor

Summary

Purpose: Fat grafting has become a widely accepted modality of soft tissue restoration and has found applications in many areas of plastic surgery, such as in breast reconstruction; however, current practice is plagued by tremendous variability in long-term retention, resulting in suboptimal outcomes and repetitive procedures. Anecdotally, we have experienced reduced graft retention in obese patients when compared to lean patients. Adipose-derived stem cells (ASCs) within adipose tissue are thought to contribute to fat graft survival; this has led to questions about donor conditions, such as obesity, on ASC function. Our study will determine the impact of obesity on ASC function as it relates to fat graft retention.

Methods: ASCs were isolated and cultured from the lipoaspirate of female patients who were undergoing autologous fat grafting at the time of secondary breast reconstruction and grouped into lean (BMI<30) and obese (BMI>30). ASC yield, viability, growth kinetics, and capacity for multi-lineage differentiation into adipogenic and osteogenic lineages were compared *in vitro*. Adipogenic differentiation was assessed at 1 week of differentiation with Oil Red O staining and photometric quantification. Early osteogenic differentiation was assessed at 1 week of differentiation with alkaline phosphatase staining and quantification. Late osteogenic differentiation was assessed at 2 weeks of differentiation with Alizarin Red stain in with photometric quantification and quantification of calcium content. Clinical correlation with serial photographs and questionnaires from the patients and surgeons were performed to assess retention over time.

Results: ASCs derived from obese donors exhibited a significantly decreased ability to

proliferate when compared to ASCs derived from non-obese subjects. Obese patients were more likely to have graft resorption at the 3 month ($p=0.046$), 6 month ($p=0.003$) and 12 month ($p=0.002$) visits when compared to non-obese patients. Fat graft retention also positively correlated to ASC proliferation ($r=0.49$, $p=0.03$).

Conclusion: Altered ASC behavior of the obese subcutaneous adipose depot may contribute to reduced long-term graft retention in obese patients due to diminished proliferation potential. In addition to other ASC properties, the compromised ability of ASCs to proliferate in obese subjects should be considered in applications of cell-based technologies and therapies such as tissue engineering, stem cell banking and fat grafting.

Introduction

Autologous fat grafting is a dynamic and widely accepted modality of soft tissue restoration in plastic and reconstructive surgery that is used to improve functional and aesthetic form (1-3) due to its easy accessibility, abundance, and benefits of non-immunogenicity. Fat grafting refers to the procedure in which fat is harvested by liposuction from a part of the body where it is unwanted and injected into an area where it can be used to add volume or correct a contour deformity. Advantages of the technique include using autologous fatty tissue rather than a foreign body (such as a filler or implant) and the added benefit of having liposuction to remove fat from an undesirable location. The concept of fat grafting has been of interest since the inception of whole-fat grafts in 1893 and became more popular with the advent of liposuction in the 1980s (4), which enabled the recovery of significant volumes of fat that could be reintroduced into patients as grafts (5). Since then, hundreds of reports have highlighted the benefits of fat

transfer for the management of soft tissue defects and contour deformities from trauma (4), disease (5), congenital defects (6), wound and scars (7), breast reconstruction (8, 9), and other reconstructive and aesthetic purposes.

In the year 2013 alone, over 95,000 reconstructive breast procedures were performed and the number of patients seeking breast reconstruction is greatly increasing (1, 10-13).

From 1998 to 2008, the Nationwide Inpatient Sample database registered 178,603 mastectomies and 51,410 immediate breast reconstructions. Breast reconstruction following mastectomy has benefits on body image, sexuality, self-esteem and quality of life. Along with implants, fat grafting is a widely utilized modality in breast augmentation and reconstruction (**Figure 8-1**). In a recent national survey of plastic surgeons, 88% who performed fat grafting to the breast reported using it in breast reconstruction. Autologous fat grafting can modify the size, shape and contour of the breasts (10, 11), improve the quality of irradiated breast skin (12), and alleviate post-mastectomy pain syndrome (13). Fat grafting is a valuable adjunct to other methods of tissue augmentation and reconstruction, such as implants or complex microvascular tissue flap procedures (14-17). Ideally, fat grafting could become an alternative to implants in breast reconstruction (11-13).

Despite the long history of fat grafting, the adoption of autologous fat as the ideal soft tissue filler is plagued by tremendous variability in long-term graft retention, resulting in unpredictable results, suboptimal outcomes and repetitive procedures (14-17). In fact, only about 20-80% of grafted fat remains. The mechanisms contributing to long-term fat

graft survival and resorption are not well understood. Previous research has attempted to identify factors that may contribute to variability in fat grafting outcomes, including the role of adipose-derived stem cells (ASCs), which survive after grafting and promote the regeneration of adipocytes (**Figure 8-2**). Several ongoing clinical trials are exploring the potential of enriching fat grafts with ASCs to promote long-term graft survival, particularly to the breast. While there is significant interest to understand the role of ASCs in fat grafting, little is known about the impact of obesity on ASC functionality. Understanding how obesity affects ASC function may help us to understand why anecdotally that lean patients have better fat graft retention compared to obese patients.

Obesity (BMI > 30 kg/m²) is a growing epidemic in the United States, affecting more than 33% of adults. The effects of obesity on the intrinsic cellular properties of ASCs are still largely unknown, and no study has correlated these variables to fat graft retention (15-24). We aim to determine the effect of obesity on ASC function (yield, proliferation, and differentiation) and to correlate *in-vitro* ASC function with fat graft retention. We hypothesize that obesity is associated with compromised ASC function *and reduced fat graft retention*. *Our results may help to define* which patients will benefit most from fat grafting procedures, leading to more predictable fat grafting outcomes and refinement of future cell-based therapies and regenerative applications utilizing ASCs.

Methods

Donor Selection and Informed Consent

All protocols were reviewed and approved by the University of Kentucky Institutional

Review Board prior to tissue collection and all subjects provided informed consent. Patients who had a scheduled consultation for secondary breast reconstruction with fat grafting with a University of Kentucky plastic surgeon and who were identified by study personnel as candidates for the study were informed of the opportunity to donate otherwise discarded lipoaspirate and to participate in the study. Patients were informed that participation in the study was completely voluntary and that refusal to participate in the study would not affect medical care. Demographic data on each subject was limited to age, ethnicity, sex, height, weight, body mass index, medical history and comorbidities, history of breast cancer, chemotherapy or radiation, operative technique, fat harvest volume and fat graft volume.

Donor Demographics

Lipoaspirate was obtained with informed consent from subjects undergoing elective liposuction (n=19). Inclusion criteria comprised female patients ranging in age from 18-65 years with a BMI <30 (non-obese) or BMI >30 (obese) who have scheduled autologous fat grafting using subcutaneous abdominal tissue for secondary breast reconstruction between January 1, 2012 and January 1, 2016. These criteria were selected to minimize variables such as sex, age and depot site that may contribute to lipoaspirate variability. Furthermore, harvesting and processing techniques were standardized. Due to potential impact on adipose tissue function, we excluded patients with a history of diabetes mellitus, those on insulin or immunosuppressive drugs such as corticosteroids, with autoimmune disease, connective tissue disease, hematologic abnormalities, prior or scheduled chemotherapy and/or radiotherapy, current smokers, pregnant women, and those with abnormalities of adipose tissue (e.g., lipodystrophy).

Lipoaspirate Harvesting

Lipoaspirate was harvested by liposuction performed under general anesthesia and sterile conditions. In all cases, subcutaneous adipose tissue was harvested from the lower abdominal region utilizing the Coleman technique with a standard harvest cannula on a 5-ml syringe under manual suction. The harvested fat was centrifuged in the operating room (G force-1,006g) to obtain approximately 75 cc of purified fat from each patient (**Figure 8-3**). The tissue was sent directly to the research laboratory for stem cell isolation following centrifugation.

ASC Isolation and Culture

ASCs were processed from fresh human subcutaneous adipose lipoaspirate according to previously published methods with minor modifications (19). Briefly, lipoaspirate was rinsed with warm sterile phosphate-buffered solution to remove erythrocytes, followed by removal of the oil and saline layers. The remaining fat layer was digested with Dulbecco's Modified Eagle Medium–Low Glucose (DMEM, Life Technologies, Grand Island, NY) containing 0.15% collagenase type I (Worthington Biochemical, Lakewood, NJ) for 60 minutes at 37°C on a shaker. After centrifugation (300g) for 5 minutes at room temperature, the supernatant was aspirated and discarded. The cellular pellet was resuspended in DMEM with 10% fetal bovine serum (FBS, Walkersville, MD), 1% Antibiotic/Antimycotic and 1% Penicillin-Streptomycin, then filtered through a 100- and 40- μ m strainer and centrifuged at 300g for another 5 min. The supernatant was again discarded. The cell pellet was then re-suspended in red cell lysis buffer and centrifuged before re-suspending the SVF in complete medium and cultured in plates that were maintained in a humid atmosphere of 5% carbon dioxide/95% air at 37°C. Cultures were

washed every day with warm PBS and maintained in stromal medium until 90% confluent. Primary isolated ASCs derived from each individual donor were compared before the first passage. ASCs were evaluated under phase contrast microscopy (Olympus CKX41) after primary isolation. The adherent population was then harvested by digestion with trypsin (0.05%)/EDTA (1 mM) at 37°C for five minutes, washed in stromal medium and replated at 5×10^3 ASCs per sq cm (Passage 1) or cryopreserved for future use.

Cell Yield and Viability

The trypan blue exclusion assay (Sigma, SA, 200-786-7) was used to count the number of viable cells. A fresh solution of 10 uL trypan blue (0.05%) in water was mixed with 10 uL of each cell suspension for 5 minutes. Non-viable cells stain blue. Non-viable and viable cells were counted in a hemocytometer using light microscopy (Olympus CKX41).

Growth Kinetics and Proliferation Assay

To measure proliferation, cells were seeded in at a density of 5,000 cells/well and then cultured in ASC plating medium for 72 hours at 37°C and 5% CO₂. Proliferation was measured using the MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay according to manufacturer's instructions and fluorescence intensity was measured using a micro-plate reader. Cell number was calculated and the percentage increase in cell proliferation was calculated based on existing and seeding cell count number.

Colony Forming Unit Assay

To examine differences in ASC growth, colony forming unit (CFU) assays were

performed. ASCs were plated in 6 well plates and cultured in a 37°C incubator with humidified 5% CO₂. After 14 days of culture, the media was removed and the plates were washed with 3 mL PBS. 3% crystal violet in 100% methanol was added and the plates were incubated for 10 minutes at room temperature. The plates were washed with dH₂O until the background was clear. The stained colonies were examined under a microscope and the numbers of colonies greater than 2 mm in diameter were counted. Percent CFU was defined as the number of colonies per plate divided by the number of cells plated x 100.

Adipogenic Differentiation Assay

Adipogenic differentiation was assessed at 1 and 2 weeks with Oil Red O (ORO) staining and photometric quantification. Briefly, cells were seeded in triplicate into standard tissue culture plates at 3×10^4 cells/cm² per well and maintained at 37°C in a 5% carbon dioxide incubator under 95% humidity, and adipogenic differentiation medium (ADM) (consisting of Dulbecco's Modified Eagle's (DMEM)-Ham's F-12 medium, 10% fetal bovine serum (FBS), 15 mM HEPES at pH 7.4, 33 µM biotin, 17 µM pantothenate, 100 nM human recombinant insulin, 1 µM dexamethasone, 0.5 mM isobutylmethylxanthine, 5 µM rosiglitazone, 1% penicillin/streptomycin, 1% antibiotic/antimycotic) was added after cell attachment for three days before being converted to adipocyte maintenance medium (identical to adipogenic differentiation medium but without isobutylmethylxanthine and rosiglitazone) for the remaining nine days. The medium was replaced every other day for up to 14 days before fixation. Cultures were photographed under phase contrast microscopy to monitor morphological changes. Adipogenic differentiation was identified by the formation of lipid droplets in the cytoplasm. The

percentage of differentiated ASCs producing lipid droplets was assessed by percentage of intracytoplasmic incorporation of Oil Red O (ORO) into monolayers at day 7 and day 14 of adipogenesis using phase contrast microscopy at 10x and 40x magnification and then extracted with isopropanol and quantified by absorbance spectrophotometry at 520 nm.

Osteogenic Differentiation Assay

To measure osteogenic differentiation, the extent of extracellular calcium deposition was assessed by Alizarin Red S stain with photometric quantification. Briefly, cells were seeded in triplicate into standard tissue culture plates at 3×10^4 cells/cm² per well and maintained at 37°C in a 5% carbon dioxide incubator under 95% humidity until at least 80% confluence. Confluent cultures of ASCs were subjected to Osteogenic Differentiation Medium (DMEM/Hams F-12, 10% FBS, 1% antibiotic/antimycotic, 1% penicillin/streptomycin, 10 mM β -glycerophosphate, 50 μ g/ml sodium 2-phosphate ascorbate, 10^{-8} M dexamethasone) and maintained in culture for 14 days with medium changes every third day. The cultures were rinsed three times with 150 mM NaCl, fixed in 70% ethanol, and stained with Alizarin Red in order to assay extracellular mineralization. Briefly, cells were fixed with 4% paraformaldehyde at room temperature for 10 minutes. After washing two times with deionized water, 1 ml of alizarin red S solution (2%, pH 4.2) was added to each well. Each well was then washed with deionized water four times. The plate was dried at room temperature. The amount of matrix mineralization was determined by dissolving the cell-bound Alizarin Red S in 20% methanol and 10% acetic acid with gentle shaking for 15 minutes at room temperature and quantifying spectrophotometrically at 415 nm. Stains were performed in triplicate

and all measurements were normalized to the total protein content of a well seeded at equal density.

RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was harvested and processed by means of RNeasy Mini Kit (Qiagen; Valencia, CA) according to the manufacturer's instructions. cDNA was synthesized from extracted RNA with TaqMan Reverse Transcription Reagents Kit (Invitrogen). An ABI Prism 7900HT Sequence Detection System (Applied Biosystems; Foster City, CA, USA) was used to perform quantitative real-time polymerase chain reaction (qRT-PCR) with Power SYBR Green PCR Master Mix (Applied Biosystems) as the reporter. qRT-PCR analysis was conducted to detect gene expression levels of the adipogenic differentiation marker lipoprotein lipase (*LPL*) and adiponectin (*ADQ*) was determined in a subset of samples. Each sample was run in duplicate and the mean values of the duplicates were used to calculate the transcript level. Gene expression values were normalized to 18S ribosomal RNA as internal control in the corresponding samples and the quantity of 18S did not vary between the two groups ($p=0.31$). Gene expression of early osteogenic marker runt-related-transcription factor 2 (*RUNX-2*) was analyzed and assessed in a manner identical to that described above by qRT-PCR in a subset of samples. The mRNA expression of vascular endothelial growth factor (*VEGF*), hypoxia inducible factor (*HIF*)- 1α , and endothelium-specific receptor tyrosine kinase 2 (*TIE2*) under normoxic conditions were determined with real-time RT-PCR. Specific primers for the genes examined are based on their PrimerBank sequences (**Table 1**).

Clinical Evaluation

Patient photographs were taken serially at 2 weeks, 3, 6 and 12 months to evaluate fat graft retention clinically. The serial two-dimensional photographs and clinical evaluation were used to objectively evaluate breast contour based on percent fullness. Patients in both groups were monitored for the occurrence of adverse events of any type, logoregional cancer recurrences, fat necrosis, oil cysts, infection or skin necrosis. The patient and two reconstructive breast surgeons were asked to rate the fullness of the fat graft at each of the aforementioned time periods, specific issues such as nodularity, irregular shape/contour, dimpling, over or underfilling), complications (pain or infection) and time to resolution of these issues or complications). Patients were asked to rate their satisfaction of the procedure with questions such as whether the results were as expected, whether they would have this procedure again, and whether they would recommend this procedure to a family member or friend. During each clinic visit or through telephone follow-up, the patient reported by what quantity the fat graft had decreased from baseline.

Statistical Analysis

Data are shown as mean, median and percentage. Statistical analyses were performed using GraphPad Prism software (GraphPad Prism Software v5.01 (GraphPad Software, San Diego, CA). Numerical data are presented as means \pm SDs. Statistical comparisons between the lean and obese groups were made using student's t-tests. Pearson r and p-values were obtained through correlation analysis. Regression analysis with least fit ordinary squares for proliferation and differentiation and the R^2 coefficients were evaluated. Results were reported as a positive or negative correlation depending on the relationship of R^2 to 0 (weakest correlation) or 1 (strongest correlation). The statistical

significance was set at $p < 0.05$.

Results

This prospective study included 19 women who underwent fat grafting for secondary breast reconstruction. The two groups were significantly different in body mass index (mean lean=26.4, mean obese=33.7; $p < 0.0001$). Analysis of the satisfaction assessment questionnaire revealed that both groups were satisfied with the aesthetic result of the fat grafting procedure. In both groups, the majority of patients would undergo the fat grafting procedure again and would recommend the fat grafting procedure to a friend. At 12-month follow-up, the obese group had less fat graft retention than lean group as determined by self ($p = 0.04$) and surgeon ($p = 0.01$) questionnaires and clinical evaluations (**Figure 8-4**). The interrater reliability for the raters was found to be Kappa = 0.68, 95% CI (0.50, 0.89). Obese patients were more likely to have graft resorption at each follow-up at the final (12 month) outcome when compared to lean patients (**Figure 8-5**). There was no difference in the rates of complications such as fat necrosis or oil cysts between the two groups ($p = 0.32$). No patients reported locoregional cancer recurrence following AFG during the follow-up period.

Obesity does not impact cell yield

Obesity had no impact on cell yield (**Figure 8-6**). The mean yield achieved was $364 \pm 126 \times 10^3$ cells/mL of lipoaspirate. No significant difference was found when comparing the mean cell yield ratios between the lean (0.331 [0.07-0.575]) and obese (0.420 [0.233-0.679]) groups ($p > 0.05$).

Obesity impacts ASC kinetics

Non-obese ASCs displayed significantly faster growth kinetics (**Figure 8-7**) and proliferation (**Figure 8-8**) than those from obese environments. Obese subjects (0.3834 ± 0.0265) demonstrated reduced proliferative potential when compared to lean subjects (0.2665 ± 0.04195); $p < 0.003$ as measured by MTT assays.

Obesity reduced CFU potential *in vitro*

Lean ASCs formed a significantly higher percentage of colonies (33.2 ± 2.93) than obese ASCs (23.2 ± 2.67), $p = 0.04$ (**Figure 8-9**).

Obese ASCs demonstrated reduced adipogenic differentiation potential *in vitro*

After one week of adipogenic differentiation, Oil Red O staining was performed. Less lipid droplet formation was observed in obese ASCs compared to lean ASCs (**Figure 8-10 and 8-11**, $p = 0.04$). However, the expression levels of *LPL* and *ADQ* were not significantly different between the lean and obese cohorts (**Figure 8-12** and **Table 8-1**).

Obese ASCs demonstrated reduced osteogenic differentiation potential *in vitro*

To measure osteogenic differentiation, the extent of extracellular calcium deposition was assessed by Alizarin Red S stain at 14 and 21 days. Obesity reduced early and late osteogenic differentiation potential (**Figure 8-13**, $p = 0.04$). However, the expression levels of the early osteogenic gene *RUNX2* were not significantly different between the lean and obese cohorts (**Figure 8-12**).

Correlation with retention

Fat graft retention varied between lean and obese phenotypes ($p < 0.01$) at each visit and at the 12-month follow-up (**Figure 8-4 and 8-5**). Correlation analysis of age, satisfaction, proliferation, colony formation, and adipogenic differentiation between lean and obese donors was performed. No statistically significant difference in graft retention for age ($p = 0.8$) or satisfaction ($p = 0.2$) was identified. There was a positive trend for retention and satisfaction (**Figure 8-14**). There was a statistically significant relationship between proliferation and retention ($p = 0.03$, $r = 0.4996$). There was an upward trend toward colony formation and retention, but this did not reach statistical significance ($p = 0.12$, $r = 0.4363$). There was no relationship between adipogenic differentiation and retention as assessed by oil red O stain and retention ($p = 0.51$, $r = -0.109$) (**Figure 8-15**).

Discussion

ASCs are prominent tools in regenerative medicine due to their abundance, ease of isolation and multipotent capacity. Due to the dynamic use of fat grafting across clinical applications, much effort has been taken to understand the impact of patient characteristics on fat graft viability. In this study, we demonstrate that an obese environment modulates *in vitro* properties of ASCs. There is accumulating evidence of decreased proliferation and differentiation potential with increased age, radiotherapy, and diabetes, although this is not consistent among all studies. In this study, ASCs derived from obese donors exhibited compromised properties. In this study, cell yield did not differ between the lean and obese groups. However, non-obese ASCs displayed significantly faster growth kinetics and proliferation compared to obese ASCs and had

increased proliferation when measured by the MTT assays. Clonogenic ability as measured by colony forming potential was also significantly reduced in obese subjects. When measuring adipogenic differentiation of ASCs by Oil Red O staining with spectrophotometric quantification, the obese cohort had a significantly lower rate of adipogenesis when compared to the non-obese group. Adiponectin, a protein hormone and anti-inflammatory cytokine that modulates glucose and lipid metabolism, was not statistically significant between the lean and obese cohorts, but inversely correlated to BMI and had a weak upward trend toward increased retention. Obesity also reduced early and late osteogenic differentiation potential as measured by alizarin red staining. RUNX2, a marker for early osteogenic differentiation, demonstrated a weak downward trend with BMI, suggesting impaired osteogenic potential for the obese. ASC proliferation potential and fat graft retention were significantly reduced in the obese. Therefore, it is possible that the ability of ASCs to proliferate may impact graft retention. Although our adipogenic staining revealed a statistically significant difference between the lean and obese cohorts, there was no statistically significant difference when related to retention. Adiponectin, a protein hormone and anti-inflammatory cytokine that modulates glucose and lipid metabolism, was inversely correlated to BMI. RUNX2, a marker of early osteogenic differentiation, showed a weak downward trend with BMI and retention. Hypoxia-inducible factor-1 (HIF1a) is a transcription factor that accumulates during hypoxia and increases the mRNA expression of several variety of genes that stimulate angiogenesis and other pathways. HIF1a showed a slight trend upwards with increased retention. The major inducer of angiogenesis is vascular endothelial growth factor (VEGF) and ASCs secrete this cytokine that contributes to their angiogenic

properties. There was a downward trend for VEGF products with increasing BMI and no trend with retention. The TEK tyrosine kinase (TIE2) is an angiopoietin 1 receptor that is involved in the pathway for angiogenesis and vascular maturation. The data show an upward trend of TIE2 with increased BMI and increased retention.

Our results are in agreement with that of groups who previously reported that ASCs from obese individuals were compromised differentiation potential (20). Perez et al. reported reduced proliferative abilities of obese ASCs and also changes in telomerase activity and DNA telomere length, suggesting decreased self-renewal capacity. In addition, metabolic analysis demonstrated impaired mitochondrial content and function in obese ASCs (21). This same group has also demonstrated impaired differentiation and migration properties in ASCs derived from obese patients (22, 23). Perez reported decreased cell proliferation, viability, and adipogenic capacity in obese mice (24). Roldan noted that human omental ASCs derived from morbidly obese individuals were capable of initiating the adipogenic differentiation process, but maturation was impaired after two weeks; the group also observed decreased cell proliferation, premature senescence, and increased cytokine secretion (25). The plasticity of preadipocytes and inverse relationship between lipid storage and proinflammatory capacity have also been noted (26). Van Harmelen et al found that the adipogenic differentiation capacity of subcutaneous mammary adipose tissue is decreased in obese women (27). Furthermore, Onate et al. showed diminished adipogenic and angiogenic differentiation (28) and an upregulation of inflammatory genes in ASCs derived from obese patients (29). ASCs are currently being used in cell-assisted lipotransfer techniques in breast reconstruction. Notably, if ASCs derived from ex-obese patients have adipogenic memory of the obesity phenotype, then it reasonable to

assume that an altered inflammatory memory also exists, thus potentially worsening tumor progression. Obesity is a chronic inflammatory state and therefore future studies should evaluate inflammatory genes that may contribute to diminished ASC function. While there is much variability in outcomes among studies, this could be partially explained by differences in harvesting technique or *in vitro* methodologies.

Understanding ASCs after massive weight loss has tremendous implications for regenerative medicine. ASCs promote tissue repair and regeneration by indirectly producing soluble factors or by directly differentiating into ASCs. For example, if ASCs are isolated from ex-obese patients, the regenerative potential may be modified and distinct from those derived from non-obese patients. Understanding the impact of obesity and other patient characteristics is scarce, but is crucial for surgeons when considering the amount of lipoaspirate to inject, to improve patient selection, and to counsel patient expectations with regards to outcomes and the need for repeat procedures.

Limitations

This study has many limitations; first, the *in vitro* methodologies may not correspond to what occurs *in vivo*. The cohort of patients included in the study may not be representative of the general population, which may limit the generalizability.

Furthermore, none of our patients were morbidly obese, diabetic, or with metabolic. Future research should include subgroup analysis of these populations and consider the influence of massive weight loss on ASC memory and function. Furthermore, it will be important to assess other properties of the SVF, such as macrophages and T cells, to better understand their role in fat graft survival both *in vitro* and *in vivo*.

This study is limited by absence of a standardized metric to evaluate graft stability. Current evaluation of fat grafts is limited to clinical evaluation, ultrasound (US), magnetic resonance imaging (MRI), computerized tomography (CT), and three-dimensional (3-D) imaging. Although most of the currently published clinical outcome studies rely on patient and surgeon reported outcomes, ideally, graft volume retention should be quantified with a more objective and validated assessment tool such as with 3-D imaging or MRI to monitor fat graft outcomes over time. However, MRI confers both cost and risk to the patient and 3-D imaging is not a readily available methodology for volumetry. Two-dimensional clinical photographs and clinician assessment with questionnaires are standard methods for assessing fat graft retention. The kappa coefficient showed substantial agreement among raters; the evaluation of aesthetic results was overall positive, suggesting that contour irregularities were improved by the fat grafting procedure. Lim et al. utilized 2D photographic analysis in their 2012 study assessing patients with craniofacial microsomia and Treacher Collins Syndrome (30). Therefore, we utilized serial photographs, clinical evaluations and a questionnaire to assess fat graft volume over time.

Conclusions

ASCs derived from obese donors (BMI>30) have impaired cell function. This suggests ASCs in adipose tissue of obese patients have impaired capacity for spontaneous or therapeutic repair than ASCs from non-obese metabolically normal individuals. Although many factors contribute to fat graft retention, altered adipose cell behavior of the obese subcutaneous adipose depot may contribute to reduced long-term graft retention in obese patients. Of all measured variables, ASC proliferation was most related to retention.

Nonetheless, the results discussed here should be considered for all applications involving ASCs, including in cell-based therapies, tissue engineering and stem cell banking.

Disclosure

The authors have no conflicts of interest to disclose.

Acknowledgments

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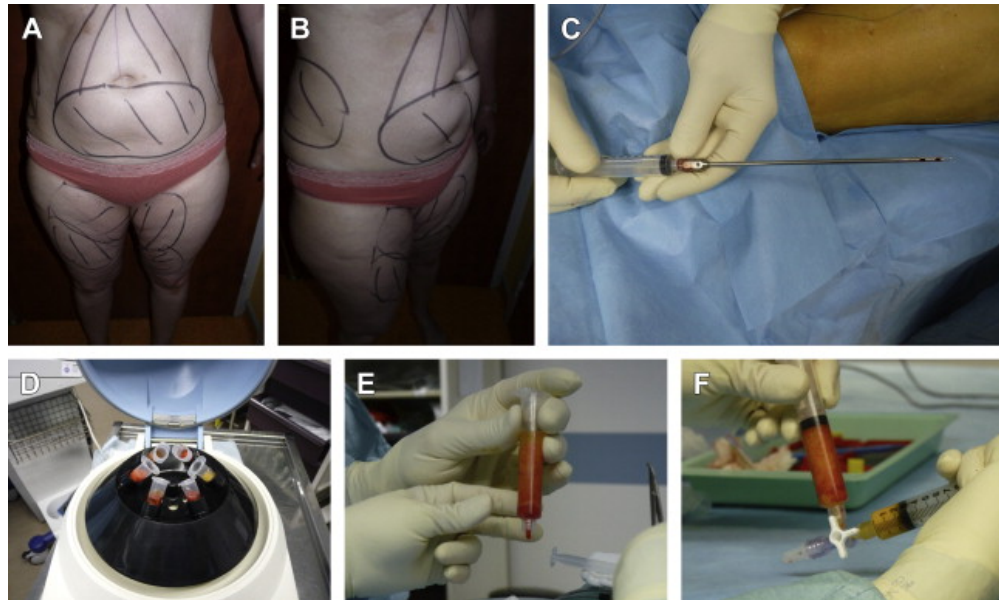


Figure 8-1. Fat harvesting and preparation. (A) Markings on the donor site. (B) Markings on the donor site, oblique view. (C) Harvesting with the cannula fitted directly on to the 10-mL Luer Lock syringe. (D) Centrifugation of the syringes in batches of 6 for 20 minutes. (E) Centrifugation separates the fat into 3 layers. Only the middle layer of purified fat is retained. (F) Transfer from one syringe to another, using a 3-way tap to obtain 10-mL syringes containing pure fat.

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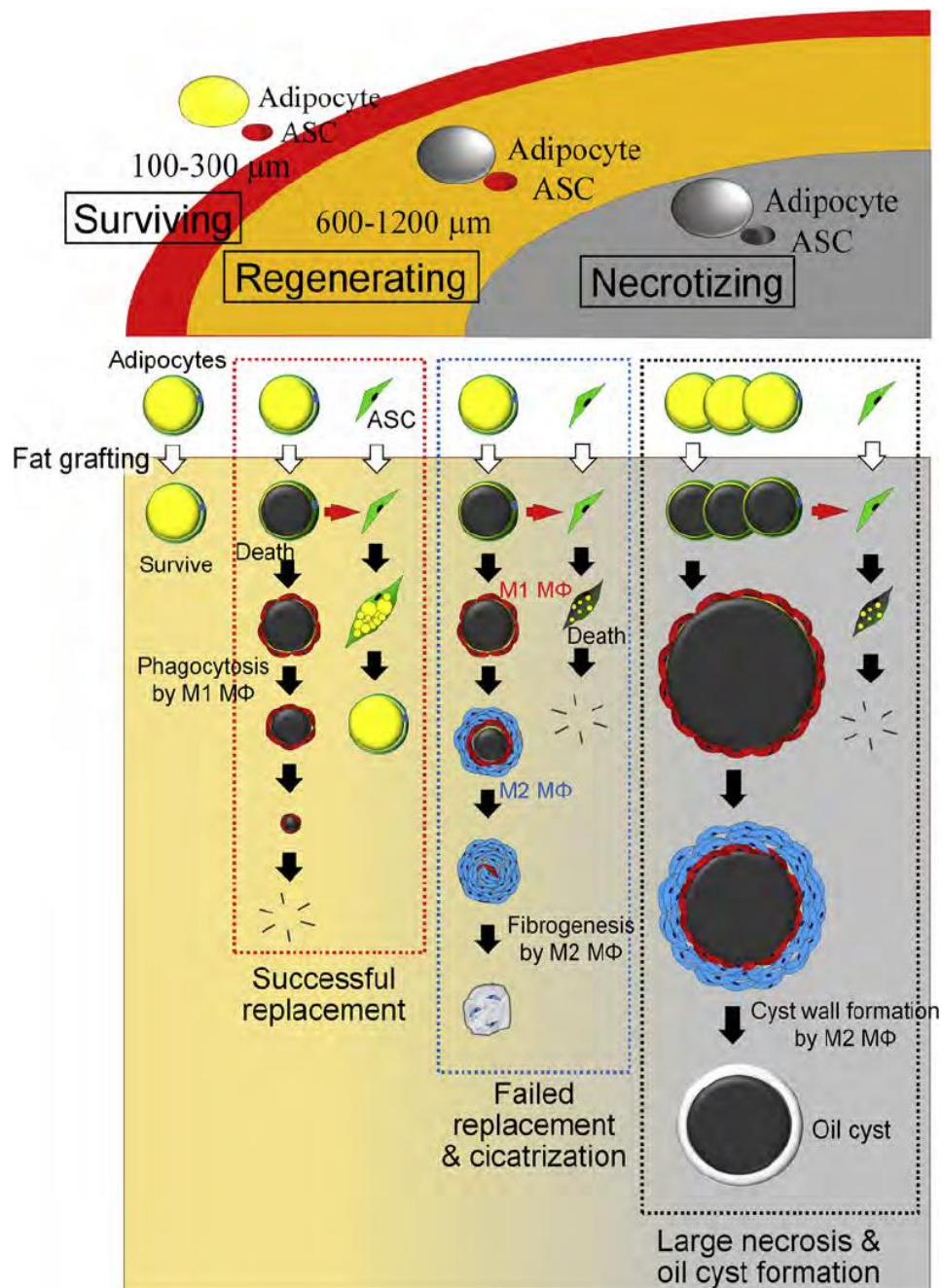


Figure 8-2. The fate of nonvascularized fat grafting is controversial. Long-term retention of fat graft occurs both due to adipocyte survival within transplanted fat and by ASC adipogenesis. Eto *et al.* found that transplanted fat grafts can be divided into three zones. The outer 300 μm forms the peripheral zone and survives through plasma imbibition. The central zone represents the innermost zone of the graft where all adipocytes and progenitor cells die. In the intermediate zone, adipocytes still die, but ASCs are able to survive and regenerate, contributing to long-term tissue remodeling and fat retention. Therefore, ASCs are better able to endure ischemic insult and therefore play a major role in fat graft survival.

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Mashiko T, Yoshimura K. How does fat survive and remodel after grafting? Clin Plast Surg. 2015 Apr;42(2):181-90. doi: 10.1016/j.cps.2014.12.008. Epub 2015 Jan 29. Review. PubMed PMID: 25827562.

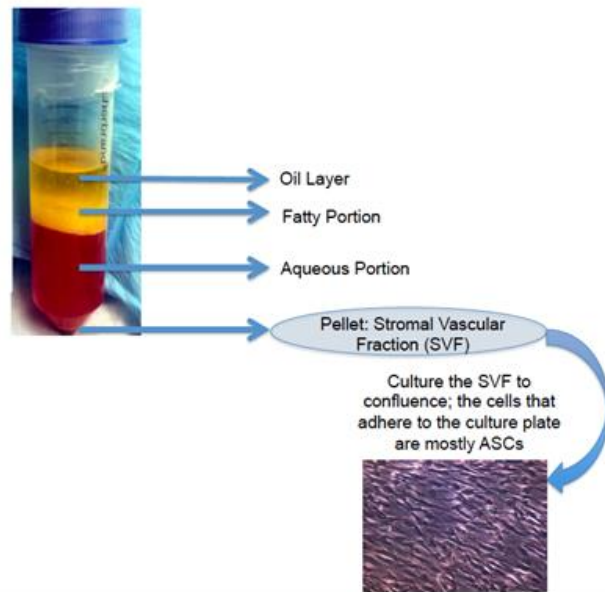


Figure 8-3. Lipoaspirate processing. Lipoaspirate (whole fat tissue derived from liposuction) is obtained through a liposuction cannula and centrifuged. The upper (oil) and lower (serous) layers are decanted. The middle fatty layer is injected into the patient in the process of fat grafting. A portion of the lipoaspirate is saved and is subjected to washes, collagenase digestion, and centrifugation. The stromal vascular fraction (SVF) is separated from the mature adipocytes. The SVF is plated and grown to confluence.

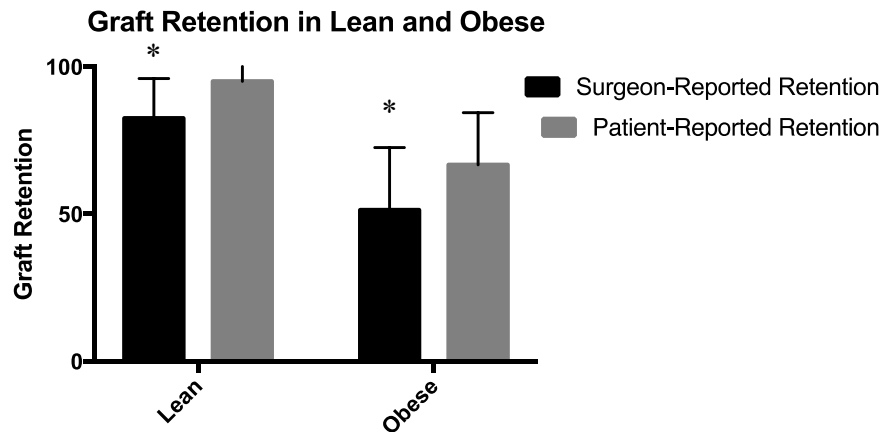


Figure 8-4. Surgeon and Patient-Reported Graft Retention Outcomes. At 12-month follow-up, the obese group had less fat graft retention than lean group as determined by self ($p=0.04$) and surgeon ($p=0.01$) questionnaires and clinical evaluations. The interrater reliability for the raters was found to be Kappa = 0.68, 95% CI (0.50, 0.89).

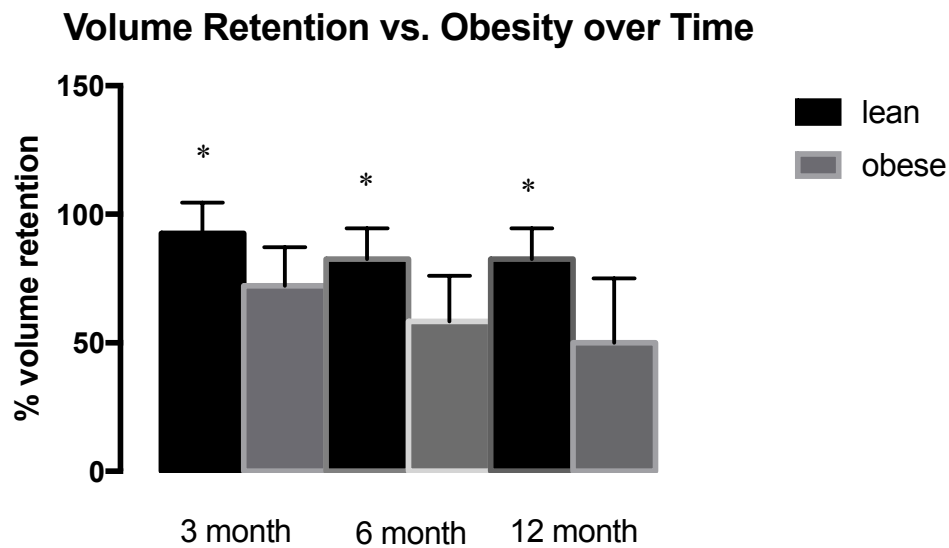


Figure 8-5. Volume retention vs. obesity over time. Obese patients were more likely to have graft resorption at the 3 month (t-test, $p=0.046$), 6 month (t-test, $p=0.003$) and 12 month (t-test $p=0.002$) visits when compared to lean patients.

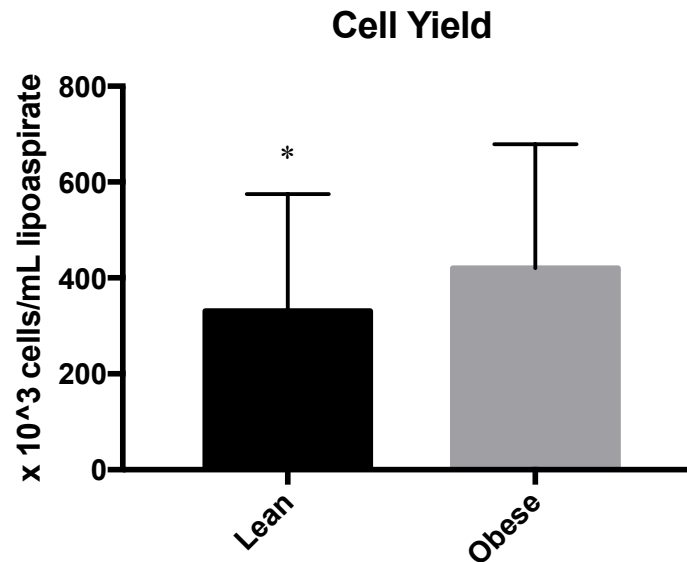


Figure 8-6. Cell yield did not differ between the lean and obese groups. The mean yield achieved was $364 \pm 126 \times 10^3$ cells/mL of lipoaspirate. No significant difference was found when comparing the mean cell yield ratios between the lean (0.331 [0.07-0.575]) and obese (0.420 [0.233-0.679]) groups ($p > 0.05$).

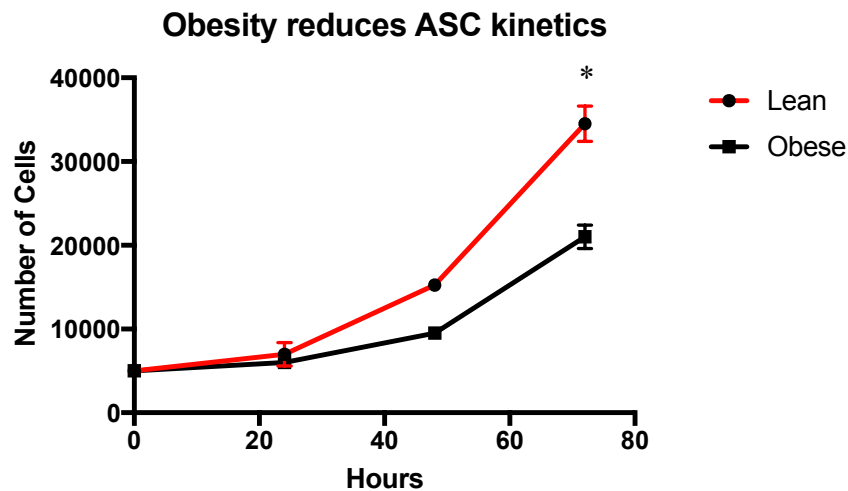


Figure 8-7. Obesity reduces ASC kinetics. Proliferation was measured by cell counting. Data represent mean \pm SEM. Non-obese ASCs displayed significantly faster growth kinetics and proliferation than those from obese environments.

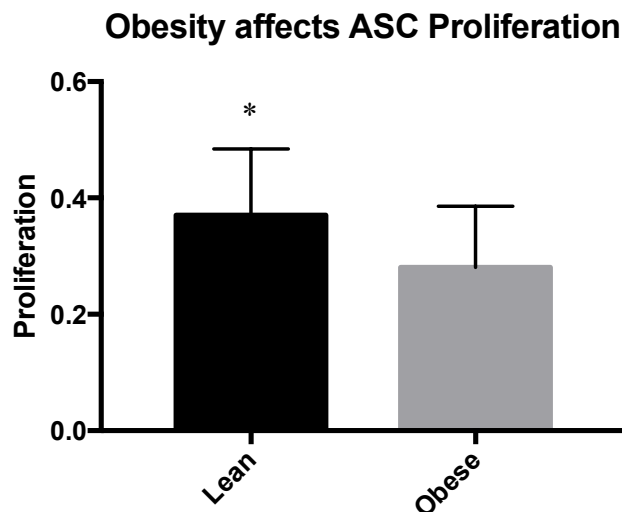


Figure 8-8. Obesity affects ASC proliferation as measured by MTT assays. Cell growth was measured by MTT assays. Obese subjects (0.3834 ± 0.0265) demonstrated reduced proliferative potential when compared to lean subjects (0.2665 ± 0.04195); $p < 0.003$. Values are reported as mean \pm SEM.

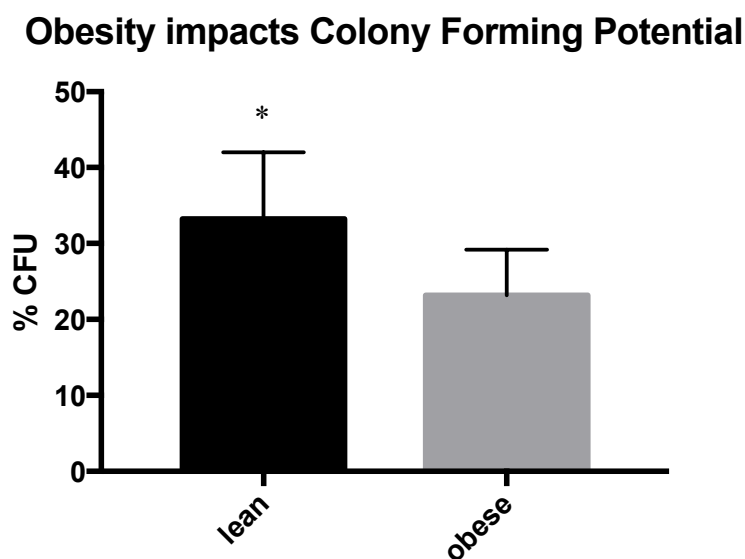


Figure 8-9. Clonogenic ability as measured by colony forming potential is reduced in the obese population. ASCs were plated in 6 well plates and cultured for 14 days, washed with PBS, and stained with 3% crystal violet in 100% methanol for 10 minutes. The plates were washed with dH₂O until the background was clear. The stained colonies were examined under an inverted microscope and the numbers of colonies greater than 2 mm in diameter were counted. %CFU was defined as the number of colonies per plate divided by the number of cells plated \times 100. When grouped, the lean group formed a significantly higher percentage of colonies (33.2 ± 2.93) compared to the obese (23.2 ± 2.67); $p = 0.04$. Values are reported as mean \pm SEM.

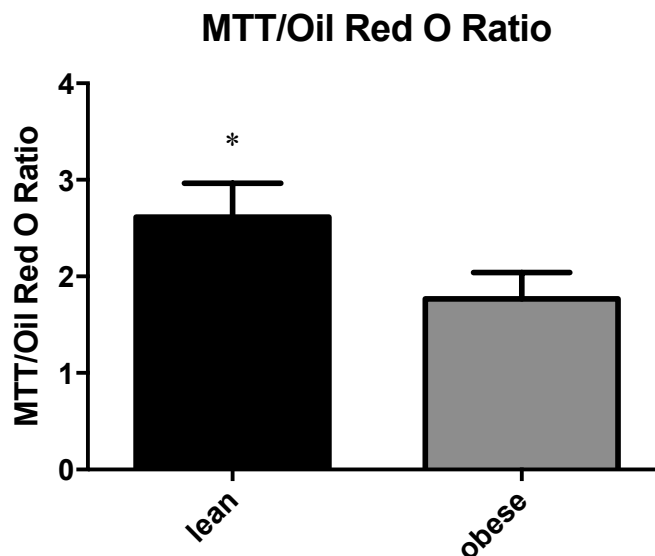


Figure 8-10. Adipogenic differentiation of ASCs was assessed by *in vitro* staining with Oil Red O and measured by spectrophotometric quantification. Even accounting for differences in cell proliferation, the obese cohort had a significantly lower rate of adipogenesis compared to the lean cohort; $p=0.04$.

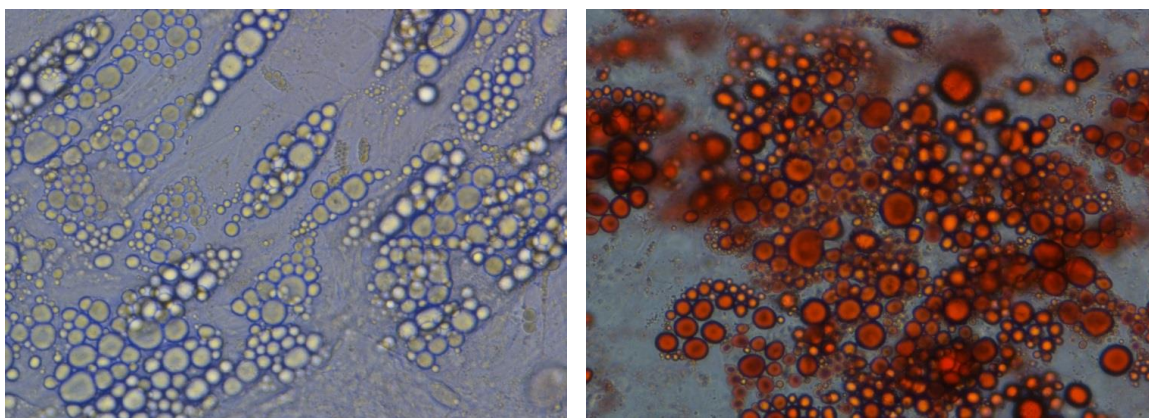


Figure 8-11. Unstained (left) and stained (right, with Oil Red O) lipid droplets during adipogenesis at 40X magnification.

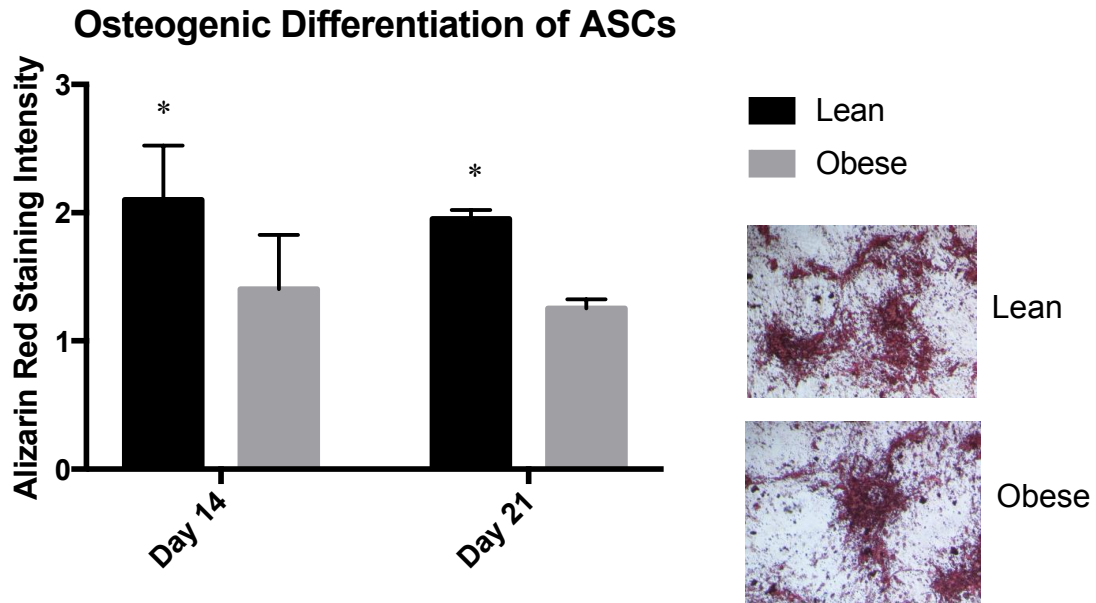


Figure 8-12. Obesity reduces early and late osteogenic differentiation potential as measured by alizarin red staining. Higher alizarin red staining intensity represents more osteogenic differentiation. More osteogenic differentiation was observed in both early (day 14) and late (day 21) osteogenesis in the lean group compared to the obese group.

Gene	Primer forward sequence (5'-3')	Primer reverse sequence (3'-5')
LPL	GCTCGTGCTGACTCTGGCCG	TCTTCTTTGGTCGGCGGCGG
ADQ	GGCCGTGATGGCAGAGAT	TTTCACCGATGTCTCCCTTAGG
RUNX2	TGGTTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
HIF-1a	CAGAGCAGGAAAAGGAGTCA	AGTAGCTGCATGATCGTCTG
VEGF	CTCTACCTCCACCATGCCAAG	AGACATCCATGAACTTCACCACTTC
TIE2	CGCATAACCAGTGAAACAGC ATTGC	CCCTGTGCTCAGACAGAAATGAGA
18S	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA

Table 8-1. Primers used for qRT-PCR. 25 nmole DNA Oligo from IDT

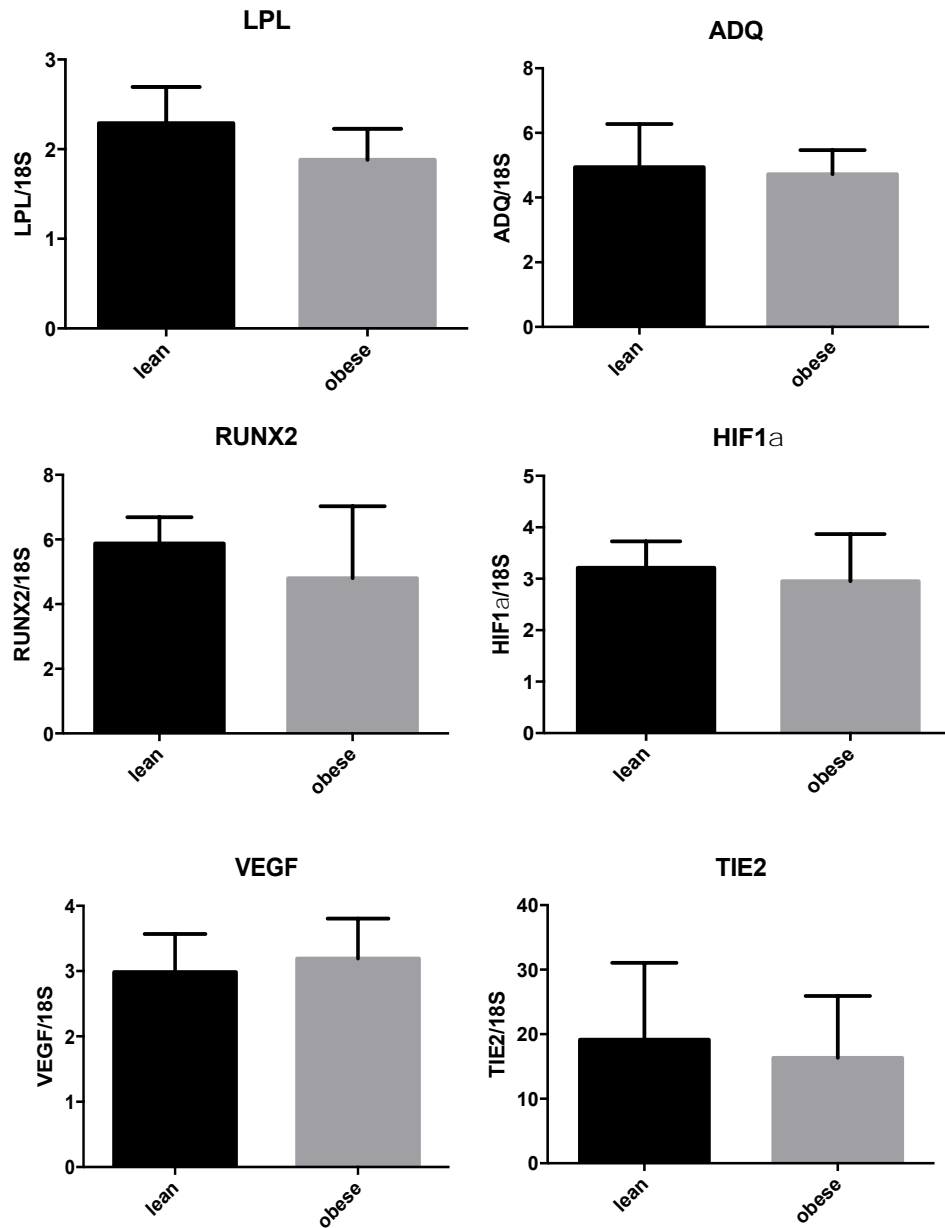


Figure 8-13. Comparison of mRNA expression of LPL, ADQ, RUNX2, HIF1a, VEGF and TIE2 in the ASCs of lean and obese donors. When grouped, there was no statistically significant difference between these factors assessed by unpaired t-test; LPL, $p=0.5$; ADQ, $p=0.9$; RUNX2, $p=0.6$; HIF1a, $p=0.8$; VEGF, $p=0.8$ and TIE2, $p=0.9$.

Factor	r coefficient BMI	p- value	r coefficient Retention	p- value
<i>LPL</i>	0.0268	0.9159	0.2368	0.3291
<i>ADQ</i>	-0.2799	0.2605	0.0455	0.8532
<i>RUNX2</i>	-0.05828	0.8183	-0.05828	0.8183
<i>HIF1a</i>	0.01722	0.9459	0.1574	0.5198
<i>VEGF</i>	-0.1076	0.6708	0.01962	0.9365
<i>TIE2</i>	0.433	0.0727	0.2944	0.2212

Table 8-2. Correlation analysis of factors LPL, ADQ, RUNX2, HIF1a, VEGF and TIE2 between lean and obese donors. There was trend toward lower levels of ADQ and VEGF and higher levels of TIE2 with increased BMI, although these did not reach statistical significance. There was a trend toward higher levels of LPL, HIF1a, and TIE2 with increased retention, although these did not reach statistical significance.

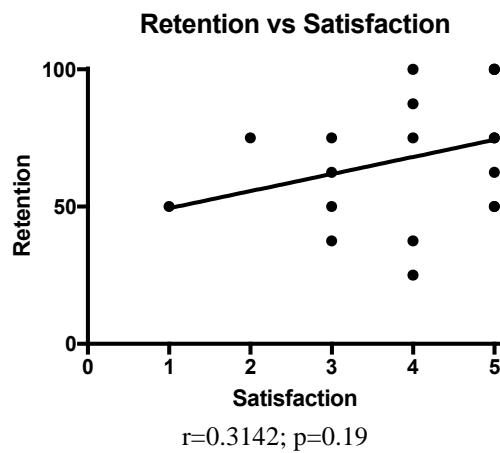
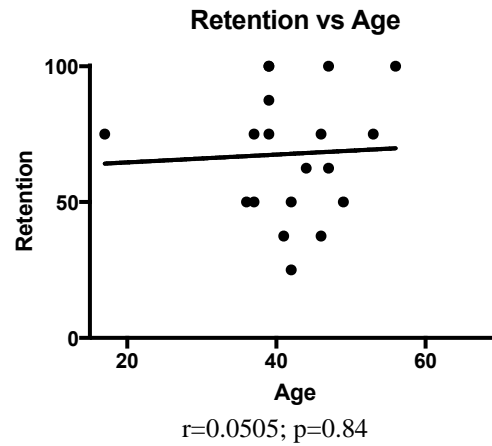


Figure 8-14. Correlation analysis of age and satisfaction between lean and obese donors. There was no statistically significant difference in graft retention for age ($p=0.8$) or satisfaction ($p=0.2$). There was a positive trend for retention and satisfaction.

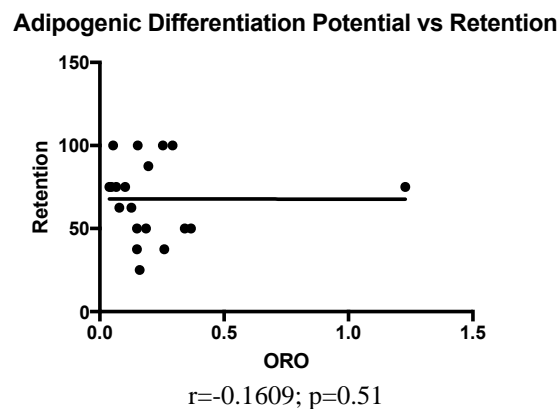
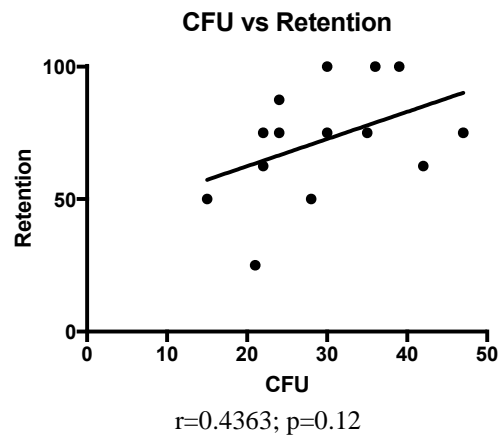
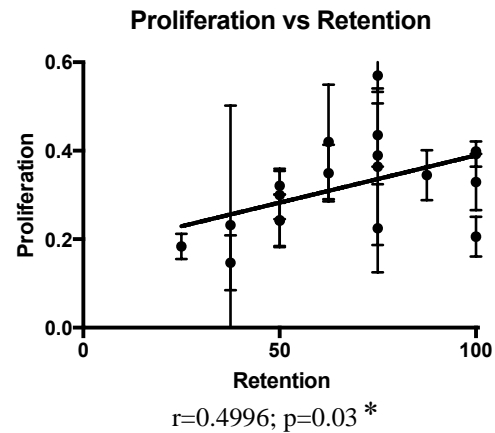


Figure 8-15. Correlation analysis of proliferation, colony formation and adipogenic differentiation between lean and obese donors. There was a statistically significant relationship between proliferation and retention ($p=0.03$, $r=0.4996$). There was an upward trend toward colony formation and retention, but this did not reach statistical significance ($p=0.12$, $r=0.4363$). There was no relationship between adipogenic differentiation as assessed by oil red O stain and retention ($p=0.51$, $r=-0.109$).

Chapter 9: Adipose Graft Enrichment Strategies: A Systematic Review

Summary

Purpose: Autologous fat grafting is a dynamic modality used in reconstructive surgery as an adjunct to improve functional and aesthetic form. However, current practices in fat grafting for soft tissue augmentation are plagued by tremendous variability in long-term graft retention, resulting in suboptimal outcomes and repetitive procedures. This systematic review identifies and critically appraises the evidence for various enrichment strategies that can be used to augment and improve the viability of fat grafts.

Methods: A comprehensive literature search of the Medline and PubMed databases was conducted for animal and human studies published through October 2015 with multiple search terms related to adipose graft enrichment agents encompassing growth factors, platelet-rich plasma, adipose-derived and bone marrow stem cells, gene therapy, tissue engineering, and other strategies. Data on level of evidence, techniques, complications, and outcomes were collected.

Results: A total of 1064 articles were identified of which 132 met inclusion criteria. The majority of enrichment strategies demonstrated positive benefit for fat graft survival, particularly with growth factors and adipose-derived stem cell enrichment. Platelet-rich plasma and adipose-derived stem cells had the strongest evidence to support efficacy in human studies and may demonstrate a dose-dependent effect.

Conclusions: Improved understanding of enrichment strategies contributing to fat graft survival can help to optimize safety and outcomes. Controlled clinical studies are lacking, and future studies should examine factors influencing graft survival through double-blinded, randomized controlled clinical trials in order to obtain consistent outcomes and to establish recommendations.

Introduction

The concept of fat grafting was initially pioneered by Neuber in 1893, and transitioned into popularity almost a century later with the advent of liposuction. Lipoplasty enabled the recovery of significant volumes of fat that could be reintroduced to patients as grafts [1]. Since then, autologous fat grafting has evolved to become a dynamic modality used in reconstructive surgery as an adjunct to improve functional and aesthetic form. There is tremendous clinical interest in the utilization of fat grafting for soft tissue reconstruction and augmentation with thousands of cases performed each year in the treatment of patients with volume loss due to trauma, disease, congenital defects, or the natural process of aging. However, current practice is plagued by tremendous variability in long-term graft retention, with some studies estimating a volume survival rate of 20-80%, resulting in suboptimal outcomes and repetitive procedures [2-4]. Furthermore, the Food and Drug Administration (FDA) released guidelines changing the regulation of human cells, tissues, and cellular-or-tissue-based-products in December 2014. These products would be considered as biologic drugs by the FDA and surgeons would need to apply for premarket FDA approval and licensure prior to their use. The American Society of Plastic

Surgery has drafted a response to explicate why these FDA regulations are misguided [5].

This systematic review critically appraises the level of evidence for adipose graft enrichment agents, including growth factors, platelet-rich plasma, adipose derived and bone marrow stem cells, gene therapy, tissue engineering, and other strategies.

Methods

A systematic literature review of the PubMed database was performed using the following search algorithm:

(Fat AND ("transplantation" OR "transplantation" OR "grafting" OR "transplantation" OR "grafting" AND ((fat AND ("transplantation"[Subheading] OR "transplantation"[All Fields] OR "transplantation"[MeSH Terms] OR "transplantation"[All Fields] OR "organ transplantation"[MeSH Terms] OR ("organ"[All Fields] AND "transplantation"[All Fields]) OR "organ transplantation"[All Fields])) OR lipofilling[All Fields] OR liposculpture[All Fields] OR lipografting[All Fields]) AND (("intercellular signaling peptides and proteins"[MeSH Terms] OR ("intercellular"[All Fields] AND "signaling"[All Fields] AND "peptides"[All Fields] AND "proteins"[All Fields]) OR "intercellular signaling peptides and proteins"[All Fields] OR ("growth"[All Fields] AND "factors"[All Fields]) OR "growth factors"[All Fields]) OR ("vascular endothelial growth factor a"[MeSH Terms] OR "vascular endothelial growth factor a"[All Fields] OR "vegf"[All Fields]) OR FGF[All Fields] OR IGF[All Fields] OR EGF[All Fields] OR PDGF[All Fields] OR EPO[All Fields] OR ("platelet-rich plasma"[MeSH Terms] OR ("platelet-rich"[All Fields] AND "plasma"[All Fields]) OR "platelet-rich plasma"[All Fields] OR ("platelet"[All Fields] AND "rich"[All Fields] AND "plasma"[All Fields]))

OR "platelet rich plasma"[All Fields]) OR ("Pharmacol Res Perspect"[Journal] OR "prp" OR "genetic therapy" OR ("genetic" AND "therapy"[All Fields]) OR "genetic therapy"[All Fields] OR ("gene"[All Fields] AND "therapy"[All Fields]) OR "gene therapy"[All Fields]) OR (("adipose tissue"[MeSH Terms] OR ("adipose"[All Fields] AND "tissue"[All Fields]) OR "adipose tissue"[All Fields]) AND ("engineering"[MeSH Terms] OR "engineering"[All Fields])) OR (("adipose tissue"[MeSH Terms] OR ("adipose"[All Fields] AND "tissue"[All Fields]) OR "adipose tissue"[All Fields]) AND derived[All Fields] AND ("stem cells"[MeSH Terms] OR ("stem"[All Fields] AND "cells"[All Fields]) OR "stem cells"[All Fields])))). A total of 1064 studies were identified on this preliminary search (**Figure 1**). Relevant articles were selected through assessment of titles and abstract as well as the reference lists of related articles. Pertinent articles were selected and reviewed. 932 studies were unrelated to fat grafting enrichment. Among the remaining articles, 109 were animal studies and 23 were human studies. With regards to human studies, there were no reports on Vascular Endothelial Growth Factors (VEGF), Fibroblast Growth Factor (FGF), Insulin-Like Growth Factor (IGF), or Epidermal Growth Factor (EGF) in enrichment strategies. There was one study on Platelet-Derived Growth Factor (PDGF), one study on erythropoietin (EPO), 11 studies on Platelet-Derived Growth Factor (PRP), and ten studies Adipose Tissue-Derived Stem Cells (ASCs). With regards to animal or *in vitro* studies, there were 12 reports on VEGF, 11 for FGF, one for IGF, one for EGF, one for PDGF, one for EPO, six for PRP, and 33 for ASC. Other enrichment strategies characterized 43 studies. Neutral, negative, or positive results from these studies are summarized in **Figures 2 and 3**.

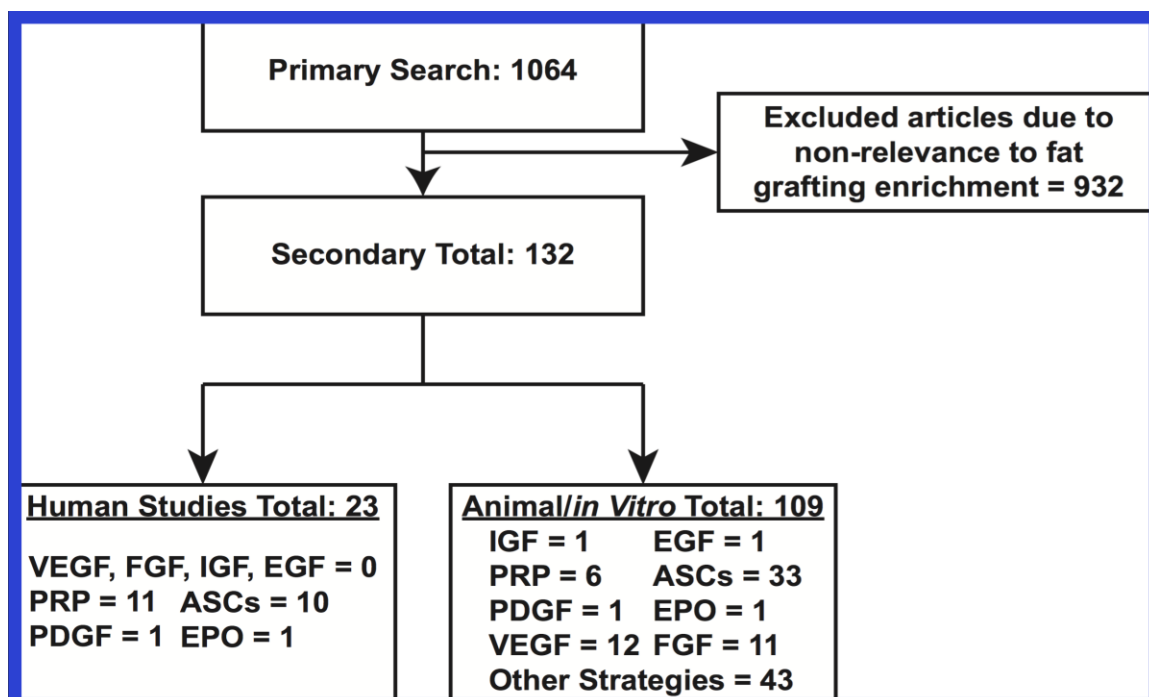


Figure 9-1. Search Strategy

A comprehensive literature search of the Medline and PubMed databases was conducted for animal and human studies published through October 2015 with multiple search terms related to adipose graft enrichment agents encompassing growth factors, platelet-rich plasma, adipose-derived and bone marrow stem cells, gene therapy, tissue engineering, and other strategies. Data on level of evidence, techniques, complications, and outcomes were collected.

Legend: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Insulin-like Growth Factor (IGF), Epidermal Growth Factor (EGF), Adipose-derived Stem Cells (ASCs), Platelet Derived Growth Factor (PDGF), Erythropoietin (EPO), Platelet-Rich Plasma (PRP)

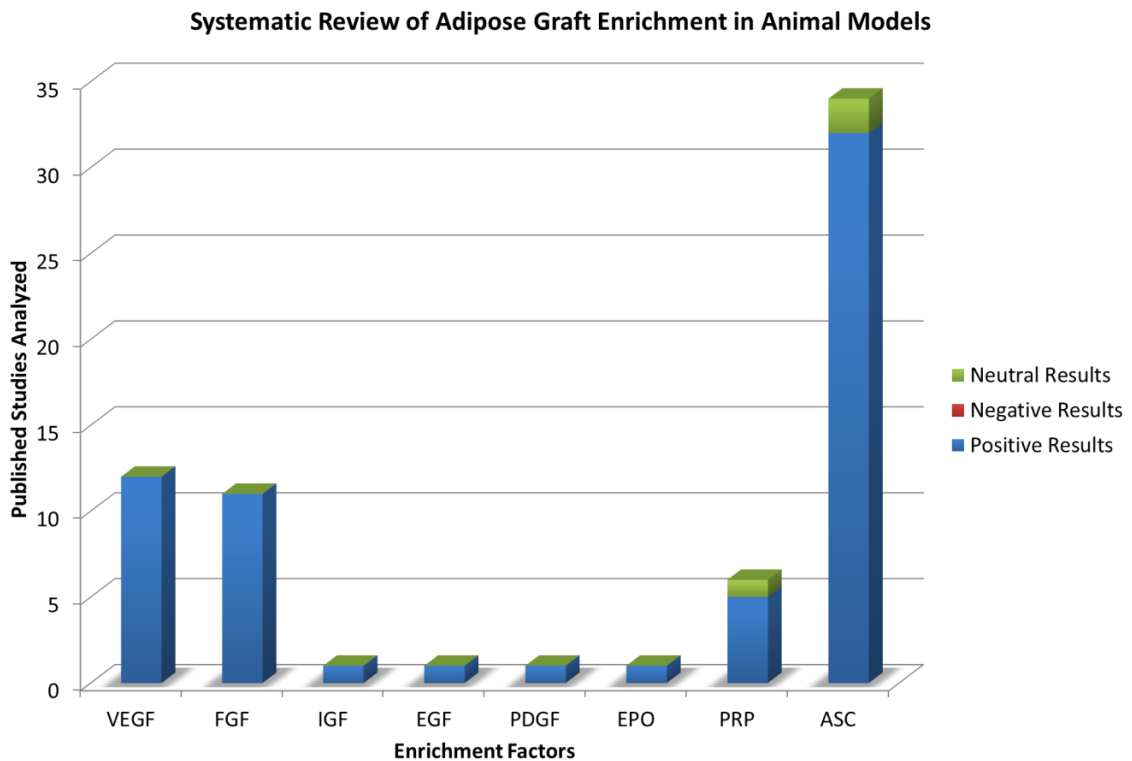


Figure 9-2. Systematic Review of Adipose Graft Enrichment in Animal Models. The majority of enrichment strategies demonstrated positive benefit for fat graft survival, particularly with growth factors and adipose-derived stem cell enrichment.

*Not included in graphs: 43 studies related to other enrichment factors and methods

Legend: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Insulin-like Growth Factor (IGF), Epidermal Growth Factor (EGF), Adipose-derived Stem Cells (ASCs), Platelet Derived Growth Factor (PDGF), Erythropoietin (EPO), Platelet-Rich Plasma (PRP)

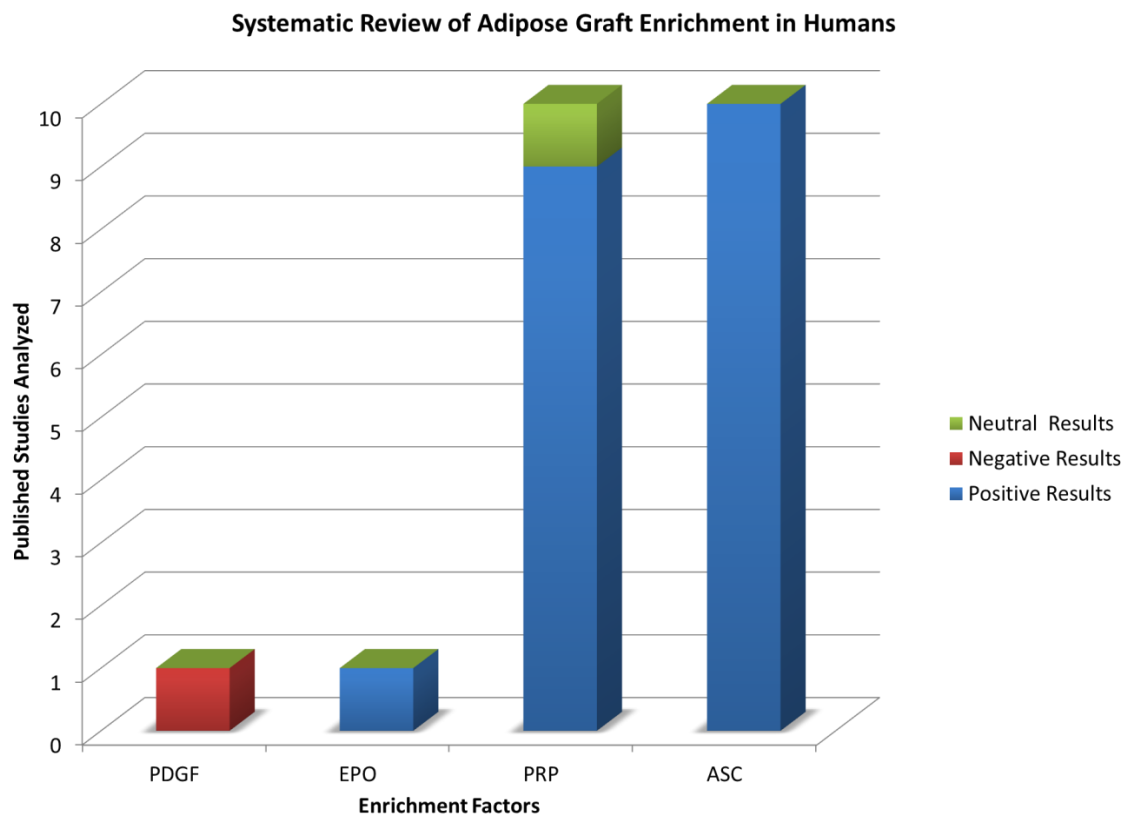


Figure 3. Systematic Review of Adipose Graft Enrichment in Humans. Platelet-rich plasma and adipose-derived stem cells had the strongest evidence to support efficacy in human studies and may demonstrate a dose-dependent effect.

*Not included in graphs: 43 studies related to other enrichment factors and methods

Legend: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Insulin-like Growth Factor (IGF), Epidermal Growth Factor (EGF), Adipose-derived Stem Cells (ASCs), Platelet Derived Growth Factor (PDGF), Erythropoietin (EPO), Platelet-Rich Plasma (PRP)

Factors that influence fat graft survival

Autologous fat grafting has become a dynamic modality used in reconstructive surgery as an adjunct to improve functional and aesthetic form. However, the factors leading to successful long-term outcomes in fat grafting are not fully understood. Until fully understood, producing consistent results will continue to be a challenge. Kato *et al.* used animal models to show that a process of dynamic tissue remodeling occurs over a period of three months following fat grafting [6-9]. During this time, varying degrees of adipogenesis and macrophage mediated replacement of fat with scarring and oil cyst formation occurs. Several groups have attempted to identify which factors contribute to the variability in outcomes in autologous fat grafting. These factors may influence the process of dynamic tissue remodeling. One of the initial aspects reviewed was fat donor site and volume of fat used for grafting.

In a recent retrospective study, Small et al. looked at patients who underwent autologous fat injection to reconstructed breasts and used three-dimensional imaging to find that the choice of donor site (anterior abdomen versus lateral thigh), radiated versus non-radiated breast tissue, and volume of fat injected did not play a significant role in volumetric retention of the fat graft at 20 weeks post-revision [10], although previously published studies described that patients receiving higher volumes of injected fat had slower volume loss and greater total volume retention [11].

Pressure and shear have previously been described as important variables that should be taken into consideration during the harvesting and preparation of fat grafts. In order to

determine the role of pressure and shear on ultimate fat graft viability, Lee *et al.* performed liposuction on fresh human panniculectomy specimens, subjected the lipoaspirates to negative pressure, positive pressure, or shear stress and injected them into nude mice and examined the samples at four weeks [12]. Only the samples subjected to shear stress via fast injection versus slow injection showed a significant decrease in graft weight and unfavorable changes in histology. The authors concluded that shear stress plays a more important role in fat graft viability than pressure. In contrast, Cheriyan *et al.* showed that low-pressure abdominal lipoaspiration resulted in greater cell viability at seven days post-operation than high-pressure abdominal lipoaspiration. They hypothesize that this difference in findings may be attributable to differences in harvesting and processing between the two studies, given that multiple factors play a role in the success of autologous fat grafting [13].

A less appreciated yet promising tool in fat grafting is the graft-to-capacity (GC) ratio, which looks at the volume of fat injected relative to the volume of the recipient site. Using 3D imaging, del Vecchio *et al.* looked at 30 cases of women who underwent breast augmentation with autologous fat grafting and calculated an average GC ratio of 117 percent +/- 22 percent [14]. They found that there was an inverse relationship between the GC ratio and the percentage volume maintenance of fat at 12 months post-augmentation. If the ratio was more than one standard deviation above the mean, there was lower percentage volume maintenance of fat. This can occur if the volume of fat injected is excessive, if the volume of the recipient site is not adequate to accommodate the volume injected, or a combination of both. In contrast, if the ratio was more than one

standard deviation below the mean, there was higher percentage volume maintenance of fat. One effective method employed in the study was to use tissue expansion to increase the capacity, or volume of the recipient site, to accommodate the desired amount of fat to be grafted while still maintaining an appropriate GC ratio. It is interesting to note that the correlation coefficient between the GC ratio and percentage volume maintenance was 0.62, suggesting that other factors also contribute to the long term success of autologous fat grafts. For example, a variety of harvesting techniques were used which could have had an effect on percentage volume maintenance, such as shear stress as described above by Lee *et al.* [12, 14]. Another factor that must be taken into account is the intrinsic compliance of the recipient site tissue. The breast tissue of a multiparous healthy woman may accommodate a greater volume of fat than the breast tissue of a woman with a history of radiation treatment to the breast [14]. While many factors contribute to fat graft survival, this systematic review focuses on evidence for enrichment strategies.

Growth Factors in Fat Graft Enrichment

Growth factors comprise a set of proteins or steroid hormones whose function is to stimulate the growth of specific tissues and to facilitate cellular division and differentiation (**Figure 4**). Generally, growth factors serve as intercellular signaling molecules that bind with particular cell surface receptors to impart their influence. Brief descriptions of various growth factors are articulated below, as well as their potential impacts on the enrichment and survivability of fat grafts.

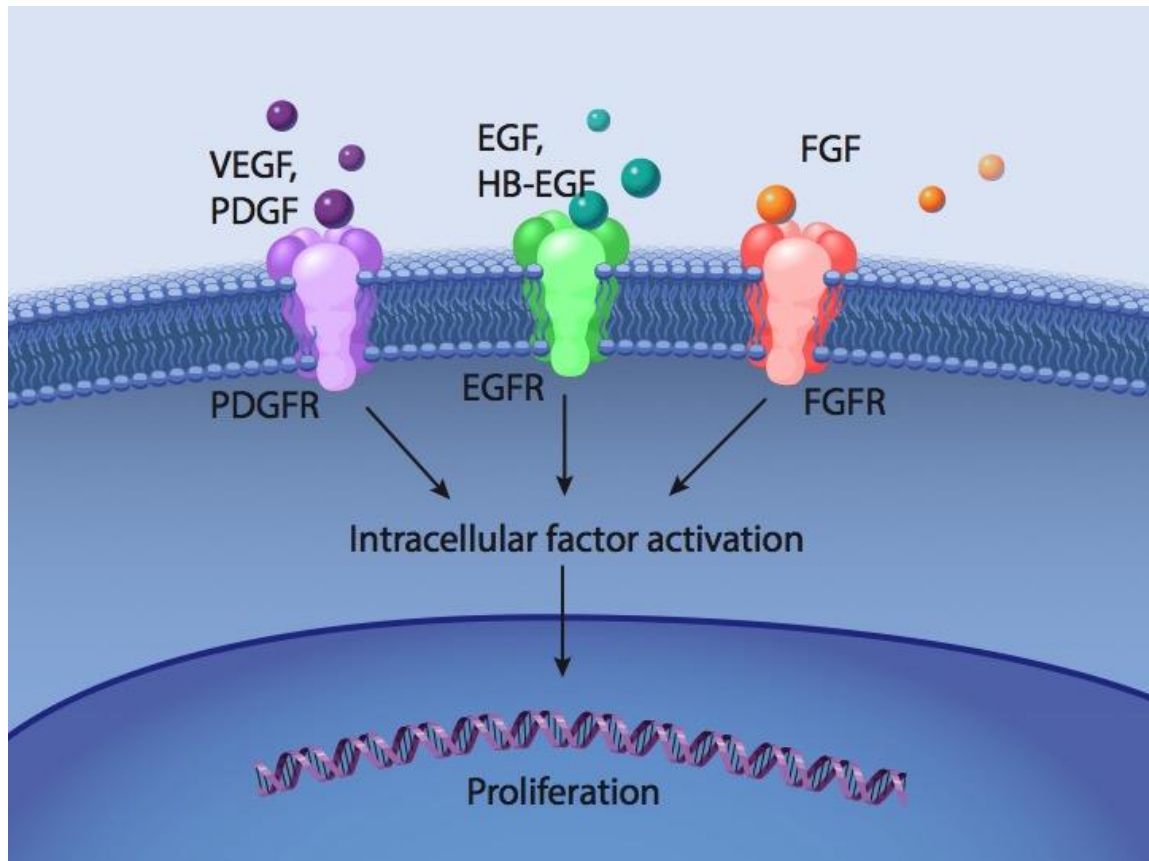


Figure 9-4. Growth Factor Signaling. Growth factors comprise a set of proteins or steroid hormones whose function is to stimulate the growth of specific tissues and to facilitate cellular division and differentiation. Generally, growth factors serve as intercellular signaling molecules that bind with particular cell surface receptors to impart their influence.

Legend: Vascular Endothelial Growth Factor (VEGF), Platelet Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF) and respective receptors.

Vascular Endothelial Growth Factors (VEGF) are involved with angiogenesis and enhanced vascularization. While no studies have been performed to examine the effects of VEGF enrichment of fat in humans, VEGF enrichment has universally demonstrated positive effects in rodent models (**Table 1**). Numerous methods, including VEGF-loaded microspheres and nanospheres, transfected Adipose-Derived Stem Cells (ASCs), Bone Marrow Derived Stem Cells (BMSCS), and gene therapy, have demonstrated significantly improved fat graft neovascularization, enhanced survival, and quality of adipose tissue.

Kakudo *et al.* described techniques for adipose-derived regenerative cell (ADRC)-enriched fat grafting. ADRCs comprise a heterogeneous assortment of cells (e.g., vascular smooth muscle cells, adult stem cells, leukocytes, endothelial cells, endothelial progenitor cells) that are resident in adipose tissue subjected to collagenase digestion [15, 16]. Recent studies suggest that fat grafts enriched with cultured ADRCs might result in augmented graft viability [17, 18]. Lu *et al.* suggested that ADRC fat grafts transfected with VEGF exhibited the highest density of capillaries and graft longevity [17].

Fibroblast Growth Factors (FGF) comprise a group of heparin-binding proteins that are involved with the propagation, differentiation and survival of a broad range of cells and tissues, embryonic development, angiogenesis, wound healing, and endocrine signaling. β -FGF is involved in the process of vasculogenesis and angiogenesis. While no studies have been performed to examine the effects of FGF enrichment of fat in humans,

enrichment has demonstrated universally positive effects in animal models, including enhanced graft volume vascularization, viability, and retention compared to control (**Table 2**). Hong *et al.* investigated the effects of transfer media that consisted of β -FGF and insulin to the viability of fat grafts that were introduced to rabbit models. Enhanced quality and viability of transplanted adipose tissue was noted [30]. It was determined that β -FGF has the capacity to directly (as a mitogen for mesenchyme-derived cells) and indirectly (serving as an angiogenic factor) stimulate pre-adipocytes to augment the mass of fat grafts [31].

Insulin-Like Growth Factors (IGF) comprise proteins that are closely akin to insulin and engaged in an intricate system that facilitates cell communication. These growth factors also promote cell proliferation and inhibit apoptosis. IGF-1 has the capacity for initiating preadipocyte differentiation. A study by Boney *et al.* suggests that IGF-binding proteins may have a critical role in modulating the activity of IGF-1 in adipogenesis [42]. Cervelli *et al.* described the use of activated autologous platelet-rich plasma (PRP), derived from blood centrifugation, as a strategy for releasing multiple growth factors, including IGF-1, which are thought to be significant in facilitating fat graft survival [43-45]. No studies have been performed to examine the effects of IGF enrichment of fat in humans. Yuksel *et al.* delivered sustained doses of basic FGF, as well as insulin and IGF-1 to rats using poly(lactic-coglycolic acid)-polyethylene glycol microspheres. It was discovered that each of these growth factors served to increase the fat graft mass and volume in contrast to control blank microspheres (**Table 3**) [31, 46].

Epidermal Growth Factor (EGF) is a low-molecular-weight polypeptide that is found in a number of human tissues with functionality encompassing angiogenesis, keratinocyte and endothelial chemotaxis, mitogenesis of epithelial and mesenchymal cells, and fibroblasts, and control of collagenase secretion [47]. No human studies have been conducted to assess the affects of EGF, but a study by Park *et al.* demonstrated increased survival rate and neovascularization in fat grafts placed within rabbits ears. *In vitro*, EGF has augmented propagation and differentiation of ASCs (**Table 4**). [48-51].

Platelet-Derived Growth Factor (PDGF) is a dimeric glycoprotein that is a potent mitogen for fibroblasts and angiogenesis that can form capillary tubes in adipose tissue-derived stem cells [52]. It is created and stored in platelet α -granules and released subsequent to platelet activation, and is also generated by a number of other cell species, including endothelial cells, macrophages, and smooth muscle cells. Above a certain dosage threshold, PDGF exhibits an inhibitory effect on human preadipocyte differentiation *in vitro* [53]. Although an animal study by Craft *et al.* demonstrated encouraging results, a double blind clinical trial by Fontdevila *et al.* demonstrated no statistical advantage to using PDGF over controls (**Tables 5 and 6**). This study involved autologous human fat grafting to a patients face and carried a level of evidence of 2b [54, 55]. Craft *et al.* bound PDGF to 20-160 μm gelatin microspheres and demonstrated its capability for the preservation of adipose weight and structural integrity in mouse model xenografts [55].

Erythropoietin (EPO) is a glycoprotein hormone and a cytokine that mediates the generation of red blood cells (erythropoiesis) [56]. It is involved in responding to neuronal injuries [57]. In wound healing processes it promotes cellular propagation and differentiation, has cytoprotective properties, and is pro-angiogenic and functions to inhibit apoptosis [58]. Although no *in vivo* human trials have been conducted, one *in vitro* human study by Sabbatini using a three-week EPO treatment regimen demonstrated sustained revascularization and reduced inflammation (**Tables 7 and 8**). [59] Furthermore, Hamed *et al.* observed that EPO-treated human fat grafts within mice had increased volume and weight, exhibited elevated microvascular density and angiogenic factor expression, and decreased inflammation [60].

Platelet Rich Plasma (PRP)

Platelet-Rich Plasma (PRP) consists of blood plasma enriched with approximately a five-fold concentration of platelets in comparison to ambient plasma, and is typically prepared via whole blood centrifugation. It contains numerous growth factors including IGF-1, EGF, VEGF (A and C), PDGF (-AA, -AB, and -BB) and transforming growth factor (TGF- β 1 and - β 2), which are released by PRP via α -granules on their activation by blood resident collagen [61]. These growth factors promote proliferation of endothelial cells, angiogenesis, and proliferation of adipocyte progenitor cells [47, 61]. PRP has been used to help augment wound healing, bone regeneration, and autologous fat grafting [62].

PRP enrichment demonstrates positive effects in both human and animal models, including enhanced graft volume vascularization and retention compared to control.

However, several studies yielded no benefits over controls (**Tables 9 and 10**). Li *et al.* used animal models to demonstrate that fat grafts treated with PRP prior to transplantation into nude mice had a significantly lower necrosis area ratio and higher number of micro-vessels than control [63]. Sadati *et al.* explored the utilization of PRP to augment autologous fat graft volume retention over a 30-month timeline involving 2033 grafts. Most of the 580 patients involved in the study demonstrated greater graft volume retention and survival with PRP over extended durations, in contrast to control subjects [64]. Gentile *et al.* showed that patients treated with PRP-enriched autologous fat grafting for breast reconstruction had a significantly greater percentage maintenance of contour and volume compared to the control group at one year follow-up [65]. On the contrary, Salgarello retrospectively reviewed 42 women receiving breast fat grafting; this analysis of clinical outcomes per surgeons and patients, rate of liponecrosis, and necessity of further fat grafting offered little support for PRP enhancement [66].

Adipose Tissue-Derived Stem Cells (ASCs)

Adipose Tissue-Derived Stem Cells (ASCs) are distinguished by their immunosuppressive attributes and low immunogenicity; hence, they may be applied to impart graft tolerance while averting autoimmunity [82]. Both human and animal models have almost universally exhibited positive results using ASCs, including minimal atrophy and increased vascularization (see tables 12 and 13). Kølke *et al.* undertook a triple-blind placebo-controlled trial that included 13 subjects to elucidate the survival of fat grafts that were enriched with ASCs (20×10^6 ASCs per mL of fat - 2000 times the nominal physiological level) as compared to non-enriched fat grafts. The results revealed that the

ASC-enriched fat grafts exhibited considerably higher residual volumes (>80% after four months) than the controls. The ASC enriched fat grafts contained higher volumes of adipose and new connective tissue and less necrotic tissue [83]. Sterodimas *et al.* established that patients receiving ASC-enriched fat grafts achieved results in fewer sessions compared to controls and had significantly higher satisfaction after 6 months, although by 18 months the groups were not significantly different [84]. In patients with craniofacial microsomia, ASC-enriched fat grafts demonstrated an 88% retention volume compared to 54% for controls [85].

Gene Therapy

Angiogenesis plays a key role in maintaining the long-term viability of autologous fat grafts. Neovascularization is vital for graft survival beyond 48 hours [62]. VEGF is one of the most important growth factors involved in this process. As discussed previously, PRP provides multiple growth factors, including VEGF, which may improve fat graft survival. However, PRP is not the only source of VEGF. A new modality being explored is the use of adenovirus vectors containing the VEGF gene. The goal would be to increase the levels of VEGF to promote neovascularization and enhance graft survival. Yi *et al.* showed that when adenovirus vectors containing the VEGF gene were mixed with adipose tissue and subsequently transplanted into mice, there was greater capillary density, and less cyst formation and fibrosis when compared to the control groups at 15 weeks post-transplantation [28]. Using a similar approach, Lu *et al.* used adenovirus vector containing the VEGF gene to transfect adipose-derived stem cells, which were

then mixed with human adipose tissue and transplanted into mice. At six months post-transplantation, there was a significant increase in graft survival, capillary density and significantly less fat necrosis and fibrosis relative to control [17]. These adenovirus vectors can be used to introduce other genes for growth factors that could augment fat graft viability too. However, due to the integrating nature of many viral vectors, more research is moving towards non-viral transfecting systems. One promising example is minicircle DNA vectors, which do not integrate into genomic DNA, have low levels of immunogenicity due to intracellular degradation of the bacterial components of the plasmid, and offer high intracellular expression levels of genes. Studies are needed with fat grafting to determine effects of this modality, but it appears to be very promising [126-129].

Adipose Tissue Engineering

Adipose Tissue Engineering may facilitate the enrichment and subsequent survival of fat grafts. Wang *et al.* combined human adipose-derived stem cells (hASCs) with a decellularized human adipose tissue extracellular matrix (hDAM), including VEGF, collagen, and sulfated glycosaminoglycan. These subcutaneously implanted engineered grafts in rat models sustained their volume at eight weeks. They did not initiate an immune response, and proceeded to undergo remodeling as evidenced by adipose tissue formation, host cell infiltration, and neovascularization [100]. Lequeux *et al.* investigated the seeding of autologous ASCs onto collagen substrates to enhance fat-enriched hypodermal tissue in a porcine wound model. Subsequent to culturing for ten days, the ASC scaffolds and controls were implanted in adult porcine models. It was shown that

the vascularized ASC scaffolds possessed an augmented layered connective/extracellular tissue matrix within the subcutaneous tissue in comparison with controls [130].

Other Studies

The enhancement of fat grafts is not exclusive to the methods represented above. Rather, autologous fat grafting represents a rapidly emerging technique and investigations into many enhancement methods exist. These include but are not restricted to the inclusion of traditional eastern remedies such as *salvia miltiorrhiza*, employment of biological scaffolds, and a variety of harvesting and injection techniques (**Table 13**).

Discussion

Autologous fat grafting is a widely utilized procedure by plastic surgeons to augment functional and aesthetic form. However, unpredictability of volume retention and other complications leaves providers with the inability to determine the optimal technique. Multiple donor sites can be utilized in autologous fat grafting without a significant effect on outcomes. However, careful planning should be undertaken when determining the volume of fat to be grafted in relation to the volume of the recipient site, which can be assessed using the GC ratio. The authors suggest that there may be a positive correlation between a lower GC ratio and long-term percentage volume maintenance, as demonstrated by del Vecchio *et al.* [14] In addition, the intrinsic compliance of the recipient tissue must be taken under consideration when determining the optimal amount of fat to be injected for the desired outcome. If the volume of the recipient site cannot meet the patient's expectations, then the surgeon should consider employing tissue

expansion to optimize safety, long-term percentage volume maintenance, and patient satisfaction.

Inconsistent study designs and methodologies create an obstacle in determining an optimal fat grafting technique. The authors suggest that 3D imaging should be used as the gold standard to monitor volume retention in future studies, which would allow for more accurate comparison of results among different studies. In addition, there have been conflicting results among studies on the effects of aspiration pressure. Lee *et al.* concluded that harvest pressure did not affect graft viability while Cheriyan *et al.* showed that lower harvest pressure led to greater fat graft viability. [12,13] Performing large double-blind randomized controlled studies need to be performed should allow for a consensus to be reached. However, both studies demonstrated that slow fat injection, resulting in lower sheer stress than fast fat injection, resulted in greater fat graft viability. Currently, the authors propose that low-pressure aspiration and slow fat injection should be employed to increase fat graft viability.

In addition to harvesting techniques and donor/recipient site considerations, studies have indicated that varying adjunct strategies augment fat graft survival. A process of dynamic tissue remodeling occurs after fat grafting, particularly in the first three months post-operatively [6]. During this time, different degrees of adipogenesis, liponecrosis, scarring, oil cyst formation, and differentiation and proliferation of ASC transpires. Of central importance to this process is angiogenesis as it provides long-term graft viability. Several growth factors contribute to angiogenesis, in addition to other vital processes

involved in incorporation of the graft into the recipient site, including VEGF, IGF, EGF, PDGF, FGF, and EPO. Enrichment of fat grafts through VEGF activated autologous stromal vascular fraction and adipose-derived regenerative cells may assist with the improvement of fat graft viability. FGF has been shown to increase fat graft mass, volume, quality and viability, but their effects on these fat grafts were only examined in animals. β -FGF has the capacity to directly and indirectly stimulate pre-adipocytes to augment the mass of fat grafts and support metabolic requirements for enhancing their vascular structure. Although universally backed by affirmative results, the large number of animals studies provides a wanting level of evidence. The authors recommend performing an FDA approved double-blind randomized controlled studies in humans to determine the utility of VEGF and FGF for adipose graft enhancement in humans. According to new regulations, all fat grafting procedures should obtain FDA approval prior to initiation.

IGF has the capacity to initiate preadipocyte differentiation and modulate activity in adipogenesis. The authors found only one study examining IGF in animals [31]. The results, although beneficial, raised a few questions. Yuksel *et al.* utilized microspheres with no IGF only control. Furthermore, the deficient level of evidence indicates the need for further studies in animals and humans with and without microspheres to determine the appropriate usage of IGF.

EGF has been shown to increase the proliferation of hASCs and adipogenesis-related mRNAs as well as hasten the propagation and hASC differentiation. However, only one

animal study has been conducted and the authors recommend further studies before a conclusion can be made [51].

PDGF exhibits an inhibitory effect on human preadipocyte differentiation *in vitro* and demonstrated preservation of adipose weight and structural integrity in mouse model xenografts. Fontdevila *et al.* indicated the PDGF alone provided negative results, but Craft *et al.* suggested PDGF with microsphere administration provided some benefit. [54, 55] These studies reveal that further research should pursue the use of PDGF use with microspheres.

The EPO-treated fat grafts had increased volume and weight, exhibited elevated microvascular density and angiogenic factor expression, and decreased inflammation and apoptosis in a dose-linear fashion. However, there is only one *in vitro* study with human tissue involving EPO [59]. A randomized control study is recommended before EPO may be used in a clinical setting.

The use of PRP, which contains multiple growth factors, can be incorporated with fat grafts to increase survival and long-term viability [73, 65]. However, optimal platelet concentration and preparation methods must be determined and standardized so that results of subsequent larger-scale studies can be compared. [65,66,78] The authors find this discrepancy worrisome and further studies involving breast tissue are necessary to settle the discrepancy between several PRP human studies.

Considerable advances have also been established in stem cell technologies, gene therapy, and adipose tissue engineering. ASC-enriched fat grafts exhibited considerably higher residual volumes of adipose and new connective tissue compared to controls. Rodent studies involving adenovirus vector-containing VEGF genes mixed with adipose tissue demonstrated greater capillary density, and less cyst formation and fibrosis when compared to the control groups. In a similar rodent study, VEGF transfected ASCs demonstrated significantly increased graft survival and capillary density, and significantly less fat necrosis and fibrosis relative to the control groups at six months following transplantation. Engineered grafts implanted in rat models undergo adipose tissue formation, host cell infiltration, and neovascularization without rejection. Many human studies with varying levels of evidence have demonstrated good functionality in humans. The decreased immune activity in fat grafts, decreased atrophy, decreased number of procedures and the good levels of evidence indicate that ASC-enriched fat grafts have great potential for future use in human fat grafts. Further use of non-integrating DNA technology like minicircle vectors may also augment current strategies [126].

Future Directions

Improved understanding of variables contributing to adipose graft survival will optimize lipofilling procedures, making them safer and more effective. Safety should remain a priority in experimental therapies such as ASCs and gene therapy. Novel methods in gene therapy and the use of stem cells are currently under investigation to improve fat graft viability and long-term survival (**Figure 5, 6**). Controlled clinical studies are lacking, and

future studies should examine factors influencing graft survival through double-blinded, randomized control clinical trials in order to obtain consistent outcomes and to establish recommendations.

Discussion	Future Directions
VEGF	
Enrichment of fat grafts through VEGF activated autologous stromal vascular fraction and ASCs may assist with the improvement of fat graft viability.	The authors recommend performing an FDA approved double-blind randomized controlled studies in humans to determine the utility of VEGF for adipose graft enhancement in humans.
FGF	
FGF has been shown to increase fat graft mass, volume, quality and viability, but their effects on these fat grafts have only been examined in animals. β -FGF has the capacity to directly and indirectly stimulate pre-adipocytes to augment the mass of fat grafts and support metabolic requirements for enhancing their vascular structure. Although universally backed by affirmative results, the large number of animal studies provides a wanting level of evidence.	The authors recommend performing an FDA approved double-blind randomized controlled studies in humans to determine the utility of FGF for adipose graft enhancement in humans.
IGF	
IGF has the capacity to initiate preadipocyte differentiation and modulate activity in adipogenesis. The authors found only one study examining IGF in animals [1]. The results, although beneficial, raised a few questions. Yuksel <i>et al.</i> utilized microspheres with no IGF as a control.	The deficient level of evidence indicates the need for further studies in animals and humans with and without microspheres to determine the efficacy of IGF.
EGF	
EGF has been shown to increase the proliferation of ASCs and adipogenesis-related mRNAs as well as hasten the propagation and ASC differentiation.	Only one animal study has been conducted and the authors recommend further studies before a conclusion can be made [2].
PDGF	
PDGF exhibits an inhibitory effect on human preadipocyte differentiation <i>in vitro</i> and demonstrated preservation of adipose weight and structural integrity in mouse model xenografts. Fontdevila <i>et al.</i> reported that PDGF alone yielded negative results, but Craft <i>et al.</i> suggested PDGF with microsphere administration provided some benefit. [3, 4]	These studies reveal that further research should pursue the use of PDGF with and without microspheres.
EPO	
The EPO-treated fat grafts had increased volume and weight, exhibited elevated microvascular density and angiogenic factor expression, and decreased inflammation and apoptosis in a dose-linear fashion. However, there is only one <i>in vitro</i> study with human tissue involving EPO [5].	A randomized control study is recommended before EPO may be used in a clinical setting.
PRP	
The use of PRP, which contains multiple growth factors, can be incorporated with fat grafts to increase survival and long-term viability [6,7]. However, optimal platelet concentration and preparation methods must be determined and standardized should be applied so that results of subsequent larger-scale studies can be compared [6,8,9].	Further studies involving breast tissue are necessary to settle the discrepancy between several PRP human studies.
ASC	
ASC-enriched fat grafts exhibited higher residual volumes of adipose and new connective tissue compared to controls. Rodent studies involving adenovirus vector-containing VEGF genes mixed with adipose tissue demonstrated greater capillary density, and less cyst formation and fibrosis when compared to the control groups. In a rodent study, VEGF transfected ASCs demonstrated significantly increased graft survival and capillary density, and less fat necrosis and fibrosis relative to the control groups at six months following transplantation [10].	Many human studies with varying levels of evidence have demonstrated good functionality in humans. The decreased immune activity in fat grafts, decreased atrophy and decreased number of procedures and the high levels of evidence indicate that ASC-enriched fat grafts have great potential for therapeutic use [10].

Figure 9-5. Discussion and Future Direction

Future Directions

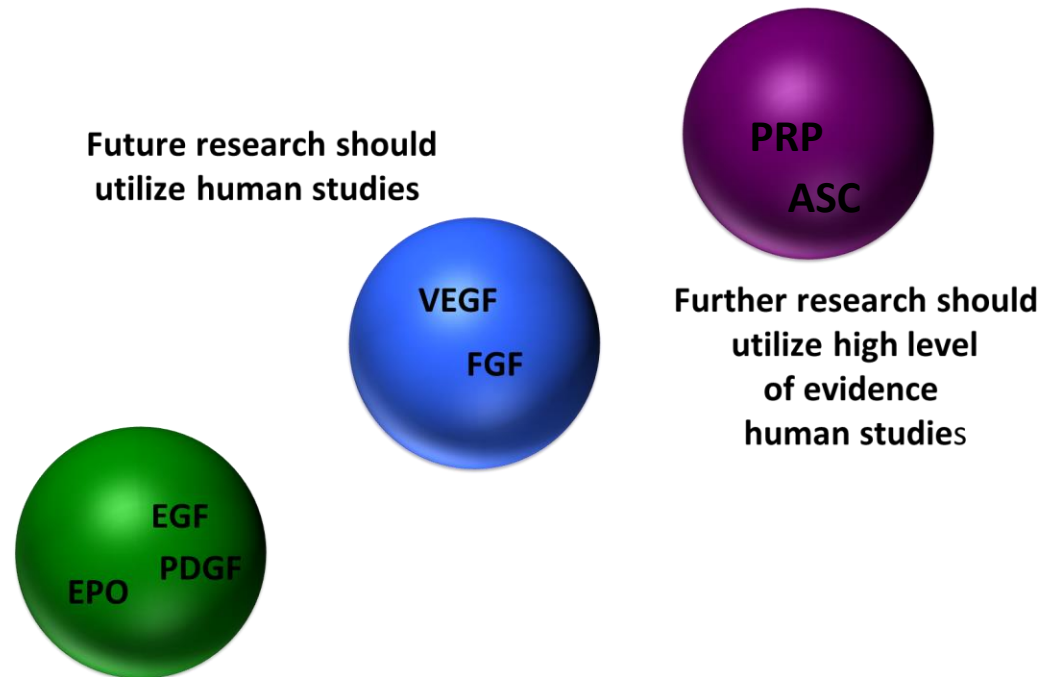


Figure 9-6. Future Directions for adipose graft enrichment strategies in humans.

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Table 9-1. VEGF Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Jun-Jiang, 2015	25812001 Vascular endothelial growth factor 165-transfected adipose-derived mesenchymal stem cells promote vascularization-assisted fat transplantation [19]	In vitro	N/A	N/A	5	Group 1: VEGF165 recombinant gene incorporated into the adenovirus pAdEasy-1 system and transfected into ASCs Group 2: Blank adenovirus pAdEasy-1 system transfected into ASCs Control: No virus transfected into ASCs	NA	The VEGF165 adenoviral vector induced greater proliferation of ASCs compared to the blank virus and control groups	+
Ding, 2014	25180953 Effect of Calcium Alginate (CA) Microsphere Loaded with Vascular Endothelial Growth Factor on Adipose Tissue Transplantation [20]	Mice	Subcutaneous abdominal fat from a healthy, young woman	Dorsum	5	Group 1: adipocytes + VEGF-enriched CA microspheres Group 2: adipocytes + free VEGF Control: adipocytes + empty CA microspheres Control: adipocytes Group 1-4 were implanted subcutaneously into the dorsum of each mouse (n=96)	3, 6, and 12 weeks	Mass and microvascular density of grafts in the group with CA microspheres loaded with VEGF were statistically higher than that of other groups in a time-dependent manner	+
Li, 2014	24740717 Improvement in autologous human fat transplant survival with SVF plus VEGF-PLA nano-sustained release microspheres [21]	Nude mice	Byproduct of skin grafting surgery on 30 yr old human female	3 random sites	5	Group 1: fat + SVF + VEGF-PLA (polylactic acid) nano-sustained release microspheres Group 2: fat + SVF Control: fat + DMEM Groups 1-3 were injected subcutaneously into 3 random sites on each mouse (n=18)	2 months	Mean wet weight of fat was higher in SVF+VEGF-PLA versus SVF cells alone and control graft. SVF+VEGF-PLA were composed of adipose tissue, had greater capillary density and VEGF expression, more capillaries, less fat necrosis and less fibrosis compared to the control	+

Zhang, 2014	24559057 Effect of chitosan nanospheres loaded with VEGF on adipose tissue transplantation: a preliminary report [22]	Nude mice	Lipoaspirate from subcutaneous abdominal fat of a healthy young woman	Dorsum	5	Group 1: adipocytes + chitosan nanospheres with VEGF Group 2: adipocytes + free VEGF Control: adipocytes + empty chitosan nanospheres Control: adipocytes only Chitosan nanospheres loaded with VEGF were mixed with adipocytes and injected subcutaneously into the dorsa of mice at four points, with each mouse carrying four different grafts	3, 6, and 12 weeks	The weight and vascularization of treated-grafts were significantly higher than controls in a time-dependent manner	+
Tervala, 2014	24150116 Analysis of fat graft metabolic adaptation and vascularization using positron emission tomography- computed tomographic imaging [23]	Mice	Mouse epididymal region	Forehead	5	Group 1: fat + VEGF-A (n=9) Control: fat (n=9) Metabolic activity and fat graft volume were measured with PET and CT scans. Histologic analysis was also performed	4 and 12 weeks	VEGF-A therapy enhanced the survival and capillary density of the transferred fat after surgery. Both groups showed higher glucose metabolism in the transplanted fat than in the fat before transplantation	+
Chang, 2014	23429223 Improvement of the survival of autologous free- fat transplants in rats using vascular endothelial growth factor 165-transfected bone mesenchymal stem cells (BMSCs) [24]	Sprague- Dawley Rat	Rat inguinal fat	Back	5	Each rat (n=36) received all 3 fat mixtures at randomly selected sites: Group 1: BMSCs with VEGF165 gene transfected Group 2: BMSCs without transfection Control: low glucose DMEM	30, 90 and 180 days	Group 1 had a significantly higher survival rate and higher capillary density. Both groups 1 and 2 had significantly less fat fibrosis and necrosis than the control	+

Chung, 2012	22772067 VEGF microsphere technology to enhance vascularization in fat grafting [25]	Nude mice	Human lipoaspirate	Flank	5	Group 1: VEGF-loaded PLGA were injected in a lipoaspirate scaffold into mice microspheres (n=6) Control: empty microspheres (n=6) Control: lipoaspirate (n=6)	3 and 6 weeks	VEGF-loaded PLGA injections had a higher mass, volume and vascularization compared to injections without VEGF	+
Topcu, 2012	22351845 Increasing the viability of fat grafts by vascular endothelial growth factor [26]	Wistar rat	Inguinal adipofascial flap	Dorsal inter- scapular region	5	Group 1: VEGF prior to fat graft (n=6) Group 2: VEGF with the graft (n=6) Control: empty microsphere with graft (n=6) Control: fat graft only (n=6)	90 days	Graft viability of groups 1-3 was significantly higher than control. Groups 1 and 2 had higher relative adipocyte index compared to control and group 3. Rats preconditioned with VEGF had a significantly higher microvascular density than other experimental groups	+
Lu, 2009	20009828 Improvement of the survival of human autologous fat transplantation by using VEGF- transfected adipose-derived stem cells [17]	Nude mice	Lipoaspirate from human thigh	Four locations on each mouse	5	Each mouse (n=72) was injected at one of four locations with the following: Group 1: VEGF- transfected ADSCs Group 2: ADSCs Group 3: 10 mmol/L insulin Control: DMEM	6 months	Group 1 showed the highest survival percent (74.1 +/- 12.6), with less fat necrosis and fibrosis and significantly higher capillary density compared to control	+
Lei, 2008	18230293 Effect of rhVEGF gene transfection on survival of grafts after autologous free granular fat transplantation in rats [27]	Sprague- Dawley rat	Inguinal fat pads	Back	5	Group 1: fat + plasmid cDNA with gene encoding rhVEGF (n=16) Control: fat + blank plasmid (n=16) Control: fat + normal saline (n=16)	Day 7, 15 and 30	Weights of the two control groups were significantly lower than the group injected with the rhVEGF gene. The expression of VEGF and micro-vessels was significantly higher in the group injected with the rhVEGF gene	+

Yi, 2007	17293285 VEGF gene therapy for the survival of transplanted fat tissue in nude mice [28]	Nude mice	Human breast	Scalp	5	Group 1: human breast fat treated with adenovirus-mediated VEGF (Ad-VEGF) (n=10) Group 2: adenovirus-mediated green fluorescent protein gene (GFP) (n=10) Control: normal saline (n=10)	15 weeks	Fat survival volume, weight, and capillary density were significantly higher in the group treated with Ad-VEGF, compared to groups 2 and 3. There was less cyst formation and fibrosis in the group treated with Ad-VEGF. Mice treated with Ad-VEGF also had significantly higher VEGF protein levels after the fat injection, compared to groups 2 and 3	+
Nishimura, 2000	10942136 Microvascular angiogenesis and apoptosis in the survival of free fat grafts [29]	Rats	Inguinal fat pads	Back	5	Free fat grafts enhanced with VEGF and grafted tissue was examined for necrosis and signs of vascularization (n=25)	Day 7, 30, 90 and 180	Revascularization of the graft around day 7	+

Table 9-2. FGF Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Jiang, 2015	25695105 Improvement of the survival of human autologous fat transplantation by adipose-derived stem-cells-assisted lipotransfer combined with bFGF [32]	Immuno-compromised nude mice	Human fat tissue	Back	5	Human fat tissues were mixed with ASCs, ASCs plus 100 U of bFGF, or medium as the control.	12 weeks	Mixtures with ASCs significantly increased the weight and volume of fat grafts, ASCs and ASCs with bFGF showed less fibrosis but more microvasculature density. Higher survival and vascularization in bFGF treated implants.	+
Nakamura, 2011	21210502 Increased survival of free fat grafts and vascularization in rats with local delivery of fragmin/protamine microparticles containing FGF-2 [33]	Fisher 344 rat	Inguinal region	Dorsal side pockets	5	Group 1: fat combined with fragmin/protamine micro-particles containing FGF-2 (n=48) Group 2: grafts containing only FGF-2, only fragmin-protamine micro-particles, or only PBS	30 and 120 days	Control fat grafts were significantly resorbed after 30 days, while the group with fragmin/protamine micro-particles containing FGF-2 experienced minimal resorption and higher graft volume retention.	+
Hong, 2010	19556175 Enhancing the viability of fat grafts using new transfer medium containing insulin and beta-fibroblast growth factor in autologous fat transplantation [30]	New Zealand White rabbits	Inguinal region	Dorsum	5	Group 1: modified DMEM (n=6) Group 2: modified DMEM with insulin (n=6) Group 3: modified DMEM with insulin and beta-FGF (n=6) Control: saline	1, 3, 6 and 12 months	Groups 2 and 3 showed significantly greater viability of fat grafts than the saline control. There were more mature adipocytes and reduced cyst formation and fibrosis in groups 2 and 3	+
Kuramochi, 2008	18837800 Matrix metalloproteinase 2 improves the transplanted adipocyte survival in mice [34]	Mice	Inguinal region	Randomly chosen different areas	5	Transplants were combined with bFGF in the presence of MMP-2 (group 1) or with MMP-2 inhibitor (group 2)	4 weeks	Transplant with MMP-2 had faster fat accumulation, higher PPAR gamma mRNA levels, and faster glucose uptake.	+

Marra, 2008	18349632 FGF-2 enhances vascularization for adipose tissue engineering [35]	Nude mice	Human abdomen	Back	5	Control: preadipocytes without FGF-2 (n=5) Group 2: preadipocytes with FGF-2 loaded microspheres (n=5)	14 days	Vascularization was significantly enhanced with FGF- 2 loaded microspheres	+
Tamura, 2007	17917834 Adipose tissue formation in response to basic fibroblast growth factor [36]	Beagle dogs	Subcutaneous fat	Vocal cords	5	Recurrent laryngeal nerve paralysis was prepared in 12 beagle dogs. Fat was injected into the ipsilateral and contralateral vocal cord for experimental and control groups. Group 1: Mixture of fat with bFGF, gelatin microspheres, and collagen sponge Control: Autologous fat	8 weeks and 24 weeks	In vocal cords injected with bFGF and fat mixture, immature adipocytes were found at 8 weeks after injection. Vocal cords with untreated fat showed a significant decrease in volume over time	+
Yazawa, 2006	17014671 Influence of vascularized transplant bed on fat grafting [37]	Rabbit	Posterior neck	Dorsal subdermal layer	5	Silicone substrate was coated with gelatin containing FGF, then transplanted into the dorsal subdermal layer in a rabbit. After 2-4 weeks, autologous fat was transplanted into the capsule (n=13)	4 weeks	In groups pretreated with growth factors, proliferation of blood vessels was observed, while overall necrosis was observed in untreated controls	+
Kimura, 2003	12695078 Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor [38]	Mice	Human adipose tissue from breast	Back	5	A collagen sponge acted as a cell scaffold and contained preadipocytes and gelatin microspheres (containing bFGF). Group 1: Sponge with preadipocytes and microspheres with bFGF (n=6) Group 2: Sponge with preadipocytes and free bFGF (n=6) Group 3: Sponge + bFBF microsphere (n=6) Group 4: Sponge + preadipocytes (n=6) Group 5: Preadipocytes + microspheres of bFGF (n=6)	6 weeks	Group 1 presented the most viable fat growth	+

Eppley, 1992	1280367 Bioactivation of free-fat transfers: a potential new approach to improving graft survival [39]	Rat	Inguinal region	Back	5	Bioactive fat grafts were created by the addition of basic fibroblasts via dextran beads. There were 4 grafts for each animal: FGF and beads, free fat alone, free fat plus beads, and beads plus a control solution (n=40).	1 and 12 months	The growth factor grafts maintained nearly their entire weight 1 year after the transplant. The free fat group lost more than half of the original graft weight after 1 year. There were more small-sized adipocytes among the larger adipocytes in the growth factor treated group.	+
Eppley, 1992	1374121 Autologous facial fat transplantation: improved graft maintenance by microbead bioactivation [40]	Rat	Inguinal region	Face	5	Fat grafts are either mixed with either bFGF alone or dextran beads pretreated with bFGF. Grafts were compared by weight and histology at one and 6 months after the transplant (n=20)	1 and 6 months	The grafts were very similar after 1 month, but after 6 months the bead- containing grafts maintained nearly complete weight and had better overall graft form	+
Eppley, 1991	1897414 A physiochemical approach to improving free fat graft survival: preliminary observations [41]	Rats	Inguinal fat pad	Lateral face	5	Bioactive free fat grafts were made using bFGF carried on positively charged, hydrophilic, dextran beads. The bioactive fat grafts were compared with contralateral free fat grafts (n=15).	90 days	Complete retention of the bioactive fat grafts was observed after 90 days, while the free fat grafts lost nearly half their original weight	+

Table 9-3. IGF Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Yuksel, 2000	10809102 Increased free fat-graft survival with the long-term, local delivery of insulin, insulin-like growth factor-I, and basic fibroblast growth factor by PLGA/PEG microspheres [31]	Rats	Inguinal fat	Sub-dermal pockets in the dorso-lumbar region	5	Group 1: fat + insulin microspheres (n=6) Group 2: fat + IGF-1 microspheres (n=6) Group 3: fat + basic FGF microspheres (n=6) Group 4: fat + insulin and IGF-1 microspheres (n=6) Group 5: fat + insulin, IGF-1, and basic FGF microspheres (n=6) Control: fat + blank microsphere (n=6) Control: untreated fat (n=6)	12 weeks	All growth factor treatments significantly increased fat graft weight and volume in comparison with the untreated and blank microsphere-treated controls. Treatment with insulin and IGF-1, alone or in combination, was found to increase the adipocyte area percentage in comparison with fat grafts treated with b-FGF alone or in combination with other growth factors	+

Table 9-4. EGF Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Concl.
Park, 2011	21461630 The effect of epidermal growth factor on autogenous fat graft [51]	New Zealand rabbits	Inguinal region	Ear	5	Group 1: fat + EGF (n=24) Control: fat + normal saline (n=24)	3 months	The EGF fat graft had a significantly higher survival rate compared to the control group. There was also an increase in neovascularization and maintenance of fat cell morphology observed in the EGF group	+

Table 9-5. PDGF Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Concl.
Craft, 2009	18178534 Effect of local, long-term delivery of platelet-derived growth factor (PDGF) on injected fat graft survival in severe combined immunodeficient (SCID) mice [55]	Mice with SCID	Human subcutaneous adipose tissue from the abdomen	Pericranial region	5	Group 1: fat + PDGF bound to microspheres (n=8) Group 2: fat + free PDGF (n=8) Control: fat + blank microspheres (n=8) Control: fat graft (n=8)	12 weeks	The group with PDGF bound to microspheres had an increased weight maintenance and preservation of adipose tissue architecture, as compared to the control groups	+

Table 9-6. PDGF Human Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Concl .
Fontdevila, 2014	25068344 Double-blind clinical trial to compare autologous fat grafts versus autologous fat grafts with PDGF: no effect of PDGF [54]	Human	Various depending on patient	Face	2b	Group 1: fat + PDGF Control: fat	2 and 12 months	Both groups exhibited significant increase of volume in the facial area and improvement in clinical facial atrophy grade after treatment. There was no statistically significant difference in the volume gain between the groups.	-

Table 9-7. EPO Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Hamed, 2010	21085572 Erythropoietin improves the survival of fat tissue after its transplantation in nude mice [60]	Nude mice	Human thigh	Scalp	5	Experimental: low-dose EPO + fat graft (n=10) Experimental: high-dose EPO + fat graft (n=10) Control: VEGF + fat graft (n=10) Control: PBS + fat graft (n=10)	15 weeks	The weight and volume of EPO-treated grafts were higher than PBS-treated and VEGF-treated grafts. EPO treatment increased the expression of microvascular density and angiogenic factors, while reducing inflammation and fat cell apoptosis in a dose-dependent manner	+

Table 9-8. EPO Human Studies

Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Sabbatini, 2015	26034645 Effects of erythropoietin on adipose tissue: a possible strategy in refilling [59]	In vitro	Anterior mid-face	N/A	5	Fat graft was seeded on culture dishes for 24 hours and then treated for 3 weeks with either 0.15 µg/ml of EPO, 0.30 µg/ml of EPO, or 0.60 µg/ml of EPO. After staining with CD31 and CD68, quantification of cell infiltration in fat grafts was estimated.	3 weeks	EPO increased the number of CD31-positive microvessels and CD31-positive small lymphocytes in the fat grafts. EPO decreased the number of CD68-positive cells and macrophages in the fat grafts.	+

Table 9-9. PRP Animal Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Li, 2015	25805284 Improved fat graft survival by different volume fractions of platelet-rich plasma and adipose-derived stem cells. [67]	Nude mice	Human fat tissue and blood	Subcutaneous (mouse)	5	Lipoinjection of granular fat, PRP (0%, 10%, 20%, and 30%; volume/volume [v/v]) and ASCs	10, 30, 60 and 90 days	20% (v/v) PRP and ASCs had higher residual volumes and improves graft survival. No significant difference between 20% and 30% PRP in terms of retaining fat grafts and histology.	+
Por, 2009	18550460 Platelet-rich plasma has no effect on increasing free fat graft survival in the nude mouse [68]	Nude mice	Human lipoaspirate from subcutaneous abdominal adipose tissue	Scalp	5	Group 1: fat + PRP (n=12) Control: fat + normal saline (n=12)	16 weeks	The mean weight, volume and histological parameters between the experimental and control groups were not statistically significant	no sig diff
Nakamura, 2010	20548232 Platelet-rich plasma (PRP) promotes survival of fat-grafts in rats [69]	Rats	Inguinal region	Subcutaneous dorsal pockets	5	Fat transplants prepared with PRP and without PRP (control) were transplanted into subcutaneous dorsal pockets on the right and left sides of 64 rats Group 1: fat + PRP + fat graft Control: fat	10, 20, 3, 120 days	The control group was significantly resorbed by day 30. The PRP group showed little resorption from day 30-120. The number of normal adipocytes in the control group decreased after day 20 but the PRP group sustained normal adipocytes. PRP had more granulation tissue and capillary formation. PRP increased angiogenesis and viable adipocytes	+

Pires Fraga, 2010	20708988 Increased survival of free fat grafts with platelet-rich plasma in rabbits [70]	New Zealand rabbits	Scapula r area	Ear	5	Group 1: fat + PRP (n=15) Control: fat + normal saline (n=15)	6 mont hs	PRP group had a significantly higher fat survival weight, number of adipocytes and blood vessels. There was more necrosis and fibrosis in the control group	+
Rodriguez- Flores, 2011	21184066 Influence of platelet-rich plasma on the histologic characteristics of the autologous fat graft to the upper lip of rabbits [71]	New Zealand rabbits	Groin fat pad	Upper lip	5	One side of the rabbit's upper lip was injected with fat + PRP while the other side was injected with fat alone (n=8)	8 and 12 week s	PRP group had less inflammatory reactions and fewer oil cysts compared to control	+
Oh, 2011	21492303 Activated platelet- rich plasma improves fat graft survival in nude mice: a pilot study [72]	Nude mice	Abdome n	Scalp	5	Group 1: fat + PRP Control: fat + normal saline	10 week s	Increase in weight, volume, and vascularity; reduced cysts, vacuoles, and fibrosis; no difference in the cellular integrity and inflammation between the two groups	+

Table 9-10. PRP Human Studies

Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Sasaki, 2015	26335660 The Safety and Efficacy of Cell-Assisted Fat Grafting to Traditional Fat Grafting in the Anterior Mid-Face: An Indirect Assessment by 3D Imaging. [73]	Human	Anterior mid face	Site specific depending on patient	3	On the voluntary principle, candidates selected one of four techniques for volumization of their mid-face: conventional fat grafting; PRP-assisted fat grafting; SVF-assisted fat grafting; and PRP/SVF-assisted fat grafting. For comparison data, comparable fat volumes, SVF volumes and nucleated cells, and PRP volumes and platelet concentrations were injected into each designated group.	Up to one year	PRP, SVF, and PRP/SVF cell supplementation of processed fat resulted in statistically significant percent mean graft retention over their baseline control at 12 months ($p < 0.01$). The use of either PRP or SVF alone resulted in almost equal outcomes. Combining cell populations provided no additional advantage over single cellular therapy.	+
Tajima, 2014	25287591 Direct and indirect effects of a combination of adipose-derived stem cells and platelet-rich plasma on bone regeneration[74]	Rat	Inguinal fat pads	Calvarial defect	5	ASCs were isolated from inguinal fat pads of F344 inbred rats, while PRP was prepared from these rats. The ASC/PRP admixture was transplanted into the rat calvarial defect.	4 and 8 weeks	Transplantation of the ASC/PRP admixture had dramatic effects on bone regeneration overtime in comparison with rats that received other transplants. Furthermore, some ASCs directly differentiated into osteogenic cells	+
Gentile, 2013	22964262 Breast reconstruction with autologous fat graft mixed with platelet-rich plasma [65]	PRP	Abdomen	Breast	2b	Group 1: fat + PRP (n=50) Control: fat (n=50)	1 year	69% maintenance of the contour restoring and volume after 1 year in the PRP group compared with 39% in the fat only group	+
Keyhan, 2013	22883321 Use of platelet-rich fibrin and platelet-rich plasma in combination with fat graft: which is more effective during facial liposuction? [75]	PRP and platelet rich fibrin (PRF)	Inner side of the knee	Face	1b	Patients underwent bilateral facial liposuction: one side was treated with PRP and the other side was treated with PRF. Each patient was evaluated for fat resorption (n=25)	1 month and 1 year	After 1 year, the hemiface treated with PRP showed greater fat resorption compared to the side treated with PRF	+

Niță, 2013	24398999 The synergy between lasers and adipose tissues surgery in cervicofacial rejuvenation: histopathological aspects [76]	PRP, adipolaser rejuvenation	Flanks or abdomen	Face	2b	Adipolaser rejuvenation was compared in two zones of the inferior abdomen in patients (n=50) preparing for abdominoplasty. The histology of fat alone (area A) or stimulation of fat graft with fractional CO2 laser and activated PRP (area B) were compared	10 days and 4 months	Significant histological difference between stimulated and non-stimulated fat grafts with regard to number of young adipocytes present, pre-adipocytes, local cell growth, neovascularization, and dermal matrix remodeling. CO2 with PRP prolonged the life and take of the facial fat graft, increased collagen formation, and led to better remodeling of the dermal matrix	+
Cervelli, 2012	22182435 Treatment of traumatic scars using fat grafts mixed with platelet-rich plasma, and resurfacing of skin with the 1540 nm nonablative laser [77]	PRP	Abdomen	Traumatic scars of different body parts	1b	Group 1: fat + PRP (n=20) Group 2: nonablative laser (n=20) Group 3: treated with both procedures 1 and 2 (n=20)	3 and 6 months interval for up to 24 months	Group 3 was the most effective treatment (11% increase of wound healing vs. Group 2 and 22% increase of wound healing vs. Group 1)	+
Gentile, 2012	23197813 A comparative translational study: the combined use of enhanced stromal vascular fraction and platelet-rich plasma improves fat grafting maintenance in breast reconstruction [78]	PRP, e-SVF (enhanced stromal vascular fraction)	Abdomen	Breast	3b	Group 1: fat + e-SVF (n=10) Group 2: fat + PRP (n=13) Control: fat (n=10)	1 year	After 1 year, the e-SVF fat grafts had a 63% maintenance of contour restoring and volume; PRP-enhanced fat grafts had a 69% maintenance; non-treated fat grafts had a 39% maintenance	+

Salgarello, 2011	21617451 Breast fat grafting with platelet-rich plasma: a comparative clinical study and current state of the art [66]	PRP	Abdomen, flanks, trochanter regions, inner thigh, and medial aspect of the knees	Breast	3b	Group 1: fat + PRP (10%) (n=17) Control: fat (n=25)	3 and 6 months	Fat graft enriched with PRP (10%) was not better than Coleman fat graft using parameters of clinical outcomes (from surgeons and patients), rate of liponecrosis, and necessity for further fat graft	no sig diff
Cervelli, 2010	20489388 Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid [79]	PRP	Abdomen	Inferior limbs ulcers or loss of substance	3b	Fat + PRP (n=30)	0, 1, 2, and 3 weeks; 1, 3, and 6 months; and 1 year	Wound healing improvement after 3 weeks in 100% of patients; healing in less than 6 weeks in 47% of patients; complete wound healing within 3 months of 57% patients	+
Cervelli, 2009	19156458 Regenerative surgery: use of fat grafting combined with platelet-rich plasma for chronic lower-extremity ulcers [80]	PRP	Abdomen	Lower limb chronic ulcers	2b	Group 1: fat + PRP (n=20) Control: fat + hyaluronic acid and collagen (n=10)	2 and 5 weeks; 3, 6, and 12 months; then annual	16/20 patients had ulcers re-epithelialize during an average of 9.7 weeks compared with 2/10 treated with hyaluronic acid and collagen based treatment	+

Table 9-11. ASC Animal Studies

Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Jung, 2015	26207545 Improvement of Fat Transplantation: Fat Graft With Adipose-Derived Stem Cells and Oxygen-Generating Microspheres [86]	Rat	Inguinal fat pads	Back	5	Group 1: Fat transplantation only Group 2: Fat transplantation with oxygen-generating microspheres Group 3: Fat transplantation with ASC's Group 4: Fat transplantation with oxygen-generating microspheres and ASC's	2 weeks	Compared to control group, weight and volume increased significantly in group 3 and 4; group 3 and 4 had the largest survival distance of fat cells from transplanted tissue. Group 3 had the most FGF-2 at 14 days and Group 4 had the most FGF-2, IGF-1, EGF at 14 days.	+
Kashimura, 2015	26375247 The Effect of Mature Adipocyte-Derived Dedifferentiated Fat (DFAT) Cells on a Dorsal Skin Flap Model [87]	Sprague-Dawley rat	Intraperitoneal adipose tissue from male SD rat	Back	5	Group 1: Flap base injection where DFAT cells injected 2 cm from the flap base Group 2: Flap center injection where DFAT cells injected in flap center	14 days	Connective tissue thickened in the flap base injection group of rats. The survival rates for flap base injection group were the highest.	+
Loder, 2015	25185931 Wound Healing after thermal injury is improved by fat and adipose-derived stem cell isografts [88]	C57BL/6 mice	Inguinal fat pads	Burn sites	5	Group 1: processed adipose Group 2: ASCs Group 3: mixed adipose (adipose and ASCs) Group 4: saline	5 and 14 days	No significant changes in proliferation or vascularization were seen at 5 days between groups. All groups showed improved healing over controls.	no sig diff
Zhu, 2015	24172865 Adipocyte regeneration after free fat transplantation: Promotion by stromal vascular fraction cells [89]	BALB/c-nu/nu mice	Human abdominal lipoaspirate	Back	5	Group 1: fat with SVF cells Control: fat with DMEM	1, 4, 7, 14, 30, 60, 90 days	Adipose tissue mixed with SVF cells showed a higher retention rate, new vessels on graft at day 3, neonatal adipocytes surrounding new vessels, and higher levels of VEGF and bFGF compared to the control group (adipose tissue with	+

								culture medium and no SVF cells).	
Derby, 2014	24334307 Adipose-derived stem cell to epithelial stem cell transdifferentiation: a mechanism to potentially improve understanding of fat grafting's impact on skin rejuvenation [90]	Mice	Abdomino-inguinal fat pads from GFP+ mice	Parascapular region	5	Group 1: whole fat graft (n=6) Group 2: ASC + peptide hydrogel carrier (n=6) Control: Contralateral side	8 weeks	Both whole fat and ASC groups have increased dermal vessels. GFP+ cells express p63. Significantly higher levels of p63 were expressed in ADSC + hydrogel group versus gel alone and control	+
Koellensperger, 2014	24703751 Intracutaneously injected human adipose tissue-derived stem cells in a mouse model stay at the site of injection [91]	Immuno deficient BALBc/nude mice	Human abdominal lipoaspirate	Back	5	Group 1: MSC injected (n=21) Control: Culture medium injected	4 weeks, 6 months, 12 months	MSCs move deeper in subcutaneous tissue and exhibit partial differentiation to adipocytes, successfully stay at the injection site and survive up to one year. Some differentiate into adipocytes with no inflammation, ulceration, or tumor induction	+
Kono, 2014	24300011 Phenotypic and functional properties of feline dedifferentiated fat cells and adipose-derived stem cells [92]	Animal	Feline omental fat tissue	N/A	5	Mature adipocyte-derived dedifferentiated fat (DFAT) cells were compared to ASCs for growth kinetics, colony-forming unit fibroblast (CFU-F) frequency, immunophenotypic properties, and multilineage differentiation potential	N/A	DFAT cells and ASCs had similar immunophenotypes. CFU-F's were significantly higher in DFAT and both cells exhibited multipotent activity <i>in vitro</i>	No sig diff
Tian, 2014	25608789 Effects of rat allogeneic adipose-derived stem cells	Sprague-Dawley rats	Inguinal region	Back	5	A = allogeneic adipose granule (AG) group; B=autologous adipose granule group;	7 days	Groups C and D had higher wet weights and more surrounding cells	+

	on the early neovascularization of autologous fat transplantation [93]					C= autologous ADSCs+autologous AG group; D= allogeneic ADSCs+autologous AG group		with positive CD31 than groups A and B.	
Trivisonno, 2014	24687265 Harvest of superficial layers of fat with a microcannula and isolation of adipose tissue-derived stromal and vascular cells [94]	Mice	Abdomen and hip of human females (n=6)	N/A	5	Group 1: rounded-tip cannula with a length of 170 mm, a diameter of 3 mm, and a single elliptic suction port on the side near its distal end (port diameter: 3 × 9 mm) Group 2: rounded-tip infiltration cannula with a length of 170 mm, a diameter of 2 mm, and 5 round ports placed spirally along the sides of the distal cannula shaft (each port diameter: 1 mm) ASCs expressing firefly luciferase were injected into immunosuppressed mice	Several weeks	Number of cells collected by microcannula were significantly higher and cells implanted into the mice survived multiple weeks in areas experiencing neovascularization	+
Dong, 2013	24260375 The survival condition and immunoregulatory function of adipose stromal vascular fraction (SVF) in the early stage of nonvascularized adipose transplantation [95]	Nude mice	Human abdominal lipoaspirate	Flank	5	Mice (n=28) were injected on contralateral sides with: Group 1: human lipoaspirate mixed with marked SVF cells Control: human lipoaspirate mixed with PBS	1, 4, 7 and 14 day	SVF cells contained blood-derived cells, adipose-derived stromal cells (ADSCs), and endothelial cells. ADSCs moved to the periphery of the implant	+
He, 2013	23210496 Effect of ASCs on the graft survival rates of fat particles in rabbits [96]	Rabbit	Inguinal regions	Dorsal midline	5	Experimental: SVF containing ASC transplanted with fat graft into one side of rabbit's dorsal midline; Control: untreated fat graft transplanted into the other side of the dorsal midline	3 months	Survival rate of ASC treated grafts after 3 months was 23.56 +/- 2.49%, while the survival rate of the untreated control grafts was 11.06 +/- 2.10%.	+

Lee, 2013	23471894 Orbital volume augmentation after injection of human orbital adipose-derived stem cells in rabbits [97]	Rabbits	Human orbital adipose tissue	Rabbit orbit	5	Group 1: injection with hyaluronic acid gel (HAG); Group 2: injection with HAG + human orbital stromal vascular fraction (hoSVF); Group 3: injection with HAG + hoADSCs	4 weeks, 8 weeks, and 12 weeks	Group with HAG+hoADSCs had the largest exophthalmometric value difference from injection to 4 weeks at 2.43 mm and 2.56 at 12 weeks. Specific inflammation at week 4 but went away 8 weeks after injection (all groups).	+
Liu, 2013	22681647 The adjuvant use of stromal vascular fraction and platelet-rich fibrin for autologous adipose tissue transplantation [98]	Rabbit	Scapular region (rabbit)	Rabbit Ear	5	Group 1: adipose granules (AG)+saline; Group 2: (AG)+SVF; Group 3: (AG+PRF); Group 4: (AG+SVF+PRF).	Weekly up to 24 weeks	Group 4 had a higher microvessel density at 4 weeks post-implant and the resorption rates were the lowest of all the groups	+
Philips, 2013	23783061 Prevalence of endogenous CD34+ adipose stem cells predicts human fat graft retention in a xenograft model [99]	Nude mice	Human lipoaspirate	Flank	5	Lipoaspirate from human subjects (n=8) were injected into mice. The fat was evaluated for SVF percentage and relevant markers.	8 weeks	High correlation between SVF percentage of CD34+ progenitors and high graft retention	+
Wang, 2013	23816649 Combining decellularized human adipose tissue extracellular matrix and adipose-derived stem cells for adipose tissue engineering [100]	Fischer rats	Human abdominal lipoaspirate	Back	5	Group 1: engineered fat grafts (ASCs with hDAM microparticles and fresh fat) Control: fresh fat grafts harvested via the Coleman technique	1, 2, 4, and 8 weeks	Engineered fat grafts maintained their volume for 8 weeks and the ASCs contributed to adipose tissue formation	+
Zamperone, 2013	23777308 Isolation and characterization of a spontaneously immortalized multipotent mesenchymal cell line derived from mouse subcutaneous adipose tissue [101]	Mice	epididymal/parametrial fat pads of mice	Intrasplenic injection	5	NOD/SCID-gnull male mice were treated with monocrotalin and the next day, 2×10^6 GFP-m17 ASCs were infused	Daily, varying 1 week up to 6 weeks	Despite exposure to pro-tumor agents, this cell line was non-tumorigenic and immortalized as it was found stable after 180 passages.	+

Zhu, 2013	23392800 Effects of xenogeneic adipose-derived stem cell transplantation on acute-on-chronic liver failure [102]	Rabbit	Porcine fat tissue	Rabbits	5	Rabbits with acute-on chronic liver failure (ACLF) received either ASC transplantation or the same volume of saline	2, 7, 14, 21, and 28 days	ASC transplantation were found in the periportal region of the liver, improved survival rate, biochemical parameters, and there was less histomorphological scoring	+
Zografou, 2013	23636118 Autologous transplantation of adipose-derived stem cells enhances skin graft survival and wound healing in diabetic rats [103]	Rats	Panniculus carnosus tissue from the midline of the back	Muscle-fascial layer of midline of the back	5	A full thickness skin graft model was used with ASCs (n=10) or without ASCs (n=10) in diabetic mice. The stem cells were derived from the same rat's inguinal fat pad. The grafts were assessed after 1 week	1 week	Increased survival, angiogenesis and epithelialization in the ASC group. In addition, the ASC group had less necrosis and increased capillary density, collagen intensity, VEGF and TGF-beta expression	+
Butler, 2012	22655687 Cotransplantation of adipose-derived mesenchymal stromal cells and endothelial cells in a modular construct drives vascularization in SCID/bg mice [104]	Mice	Abdominal fat samples (human)	Dorsum	5	Group1: modules coated with EC, without embedded adMSC; Group 2: modules embedded with adMSC and coated with EC; Group 3: modules with embedded adMSC, without EC	3, 7, 14, 21, 30, 60, and 90 days	Vessel number increased up to day 14, then decreased afterwards (pointing to maturation of vessels). Vessel perfusion was confirmed (day 21). Implant volumes decreased over time but HMEC+adMSC implants were larger than control (adMSC without HMEC coating).	+
Venugopal, 2012	22374846 Adipogenesis on biphasic calcium phosphate using rat adipose-derived mesenchymal stem cells: in vitro and in vivo [105]	Sprague Dawley rat	Subcutaneous fat pad	Dorsal muscle	5	Cell-ceramic-engineered construct with ASCs and BCP implanted into rat dorsal muscle	3 weeks	Histology of implanted tissue construct showed chicken wire net-like adipose cells within the construct	+

Li, 2011	21774351 The effect of adipose-derived stem cells on viability of random pattern skin flap in rabbits [106]	Rabbits	Human fat	Back	5	Random pattern skin flaps were designed on the backs of rabbits and then injected with either ADSCs or medium (without ADSCs) as a control on the contralateral side.	1 week	side with ADSCs had a significant increase of flap survival and significantly higher capillary density	+
Ko, 2011	21448310 Effects of expanded human adipose tissue-derived mesenchymal stem cells on the viability of cryopreserved fat grafts in the nude mouse [107]	Nude mice	Human adipose	Subcutaneous injection over the skull	5	Single donor ADSC were mixed with fat that had been cryopreserved at -70c for 8 weeks. This mixture was injected subcutaneously into nude mice.	4 and 15 weeks	At 4 weeks post-transplantation, the adipose-derived stem cell group showed significantly improved fat cell integrity as well as greater volume and weight. At 15 weeks the two groups showed no significant difference in size, weight or histological parameters.	+
Fu, 2010	21046778 Experimental study of the effect of adipose stromal vascular fraction cells on the survival rate of fat transplantation. [108]	Rabbit	Autologous fat tissue	Back	5	Fat graft mixed with SVFs (Group A), ASCs (Group B), or untreated (Group C)	6 months	Groups treated with SVFs (Groups A) and ASCs (Group B) consisted of adipose tissue with less fat necrosis and fibrosis and higher capillary density as compared to untreated fat grafts (Group C).	+
Moyer, 2010	20842001 Alginate microencapsulation technology for the percutaneous delivery of adipose-derived stem cells [109]	Nude mice	Human	Dorsal subcutaneous pocket	5	Experimental: Nude mice were implanted with ADSC-treated alginate microspheres; Control: untreated microspheres	1 and 3 months	Microspheres showed durability of up to 3 months, and ADSCs remained viable and demonstrated signs of mitosis.	+
Jiang, 2009	19873723 Proliferation of the mesenchymal stem cells in a delayed fat flap: an experimental study in rabbits [110]	Rabbit	Delayed fat flap in inguinal region of rabbit	N/A	5	Expression rates of ASCs taken from a delayed fat flap	21 days	In the delayed fat flap, CD29 and CD44 were higher but CD14 and CD45 were lower.	+

Li, 2009	19558169 Experimental study of the effect of adipose tissue derived stem cells on the survival rate of free fat transplantation [111]	Nude mice	Human liposuction	Back	5	ASCs were harvested from liposuction and differentiated into adipogenic, chondrogenic and osteogenic lines. These stem cells were mixed with fat and injected to group A. Group B was insulin + fat and group C was medium +fat. Each preparation was injected subcutaneously on the backs of the 18 mice.	6 months	Wet weight of the transplant was highest in group A (165.97mg), followed by B(93.42mg) followed by C(67.64mg). The rate of fibrosis and steatonecrosis was significantly lower in the ASC group than either of the other two. It was also shown that some of the remaining adipose-derived stem cells expressed CD31 and FITC suggesting the differentiated into Vascular endothelial cells	+
Li, 2009	19275095 Comparison between kinds of myofascial flap encapsulating adipose-derived stromal cells carrier complex in terms of adipogenic efficacy in vivo [112]	Rabbits	Neck	(Autogenous ASCs transplant)	5	Group A: muscle flap and collagen protein scaffold to encapsulate ADSCs; Group B: dextral gluteus maximus fascial flap with no specific vessel pedicle	8 weeks	Neovascularization was seen in group A, but very little in group B. Group A had a higher weight of cambium. There were regenerated adipocytes and partial capillary endothelium in both groups.	+
Lu, 2009	20009828 Improvement of the survival of human autologous fat transplantation by using VEGF-transfected adipose-derived stem cells [17]	Nude mice	Liposuction aspirate from human thigh	Four random locations on the mouse (1 spot per group, four injections per mouse)	5	Group 1; VEGF transfected ASCs; Group 2: stem cells; Group 3: 10 micromol/liter insulin; Group 4: Dulbecco's modified Eagle medium.	6 months	Group 1 showed the highest survival percent (74.1 +/- 12.6) and significantly higher capillary density	+

Yoo, 2009	19270821 Tissue engineering of injectable soft tissue filler: using adipose stem cells and micronized acellular dermal matrix [113]	Nude mice	Human abdomen	Dorsal cranial region	5	Implants included ASCs and micronized acellular dermal matrix (Alloderm).	2 months	ASCs differentiated into adipocytes.	+
Lu, 2008	18176205 Improved viability of random pattern skin flaps through the use of adipose-derived stem cells [114]	ICR mice	Inguinal fat pads of ICR mice	Pedicle base or 1.5 cm distal to the pedicle	5	Skin flap was elevated, then injected with either ASCs from inguinal pads of ICR mice or a combination of mature adipocytes and bFGF (control group).	1 week	Addition of ADSCs resulted in a significant increase in flap viability and capillary density compared to untreated control groups.	+
Mizuno, 2008	17975300 In vivo adipose tissue regeneration by adipose-derived stromal cells isolated from GFP transgenic mice [115]	Nude mice	Inguinal fat pads of GFP mice	Dorsum	5	ASCs isolated from inguinal fat pads of GFP mice were incubated in an induction medium to induce adipogenesis. These induced ASCs were combined with fibrin glue and injected subcutaneously into the dorsum of athymic mice.	4 and 8 weeks	Regenerated tissue was soft with slight angiogenesis. Fluorescence microscopy revealed strong emitted green fluorescence, suggesting the ASCs contributed to adipogenesis.	+
Matsumoto, 2006	17518674 Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection [116]	Mice with SCID - severe combined immunodeficiency	Abdominal or thigh liposuction (human)	Back	5	Experimental: Human aspirated fat transplanted with vascular stromal fractions containing isolated ASCs; Control: Human aspirated fat transplanted without vascular stromal fractions	4 weeks	Injected fat containing ASCs survived better by approximately 35% and contained more microvasculature than untreated fat.	+
Clavijo-Alvarez, 2006	17016179 A novel perfluoroelastomer seeded with adipose-derived stem cells for soft-tissue repair [117]	Nude mice	Human abdomen	Back	5	One of two fluoropolymers that differ in pore size (U48 and P54) were mixed with human adipose-derived stem cells, and then implanted subcutaneously in a nude mouse for 30 days.	30 days	Fluoropolymer U48 increased the adherence and provided a superior surface for proliferation of adipose-derived stem cells.	+

Hong, 2006	17108684 Adipose tissue engineering by human adipose- derived stromal cells [118]	Mice with SCID	Human ASCs	Back	5	ASCs seeded on gelatin sponges were exposed to adipogenic differentiation medium and then implanted into the backs of mice with SCID.	4 weeks	Adipogenic constructs turned into fat tissue within 4 weeks after transplantation. No fat formation was observed when ADSCS were exposed to a control medium without differentiation factors.	+
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Table 9-12. ASC Human Studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Charles-de-Sá, 2015	25811565 Antiaging treatment of the facial skin by fat graft and adipose-derived stem cells. [119]	Human	Liposuction from abdomen	Face	4	Preauricular areas on the patient were injected with fat and its stromal vascular fraction or with fat and expanded mesenchymal stem cells.	3 months	Modified reticular dermis architecture and evidence of a richer microvascular bed	+
Hanson, 2013	23908304 Comparative analysis of adipose-derived mesenchymal stem cells isolated from abdominal and breast tissue [120]	Human cells	Abdomen and Breast	N/A	5	Comparison of surface marker expression, differentiation capabilities, fibroblast growth factor (FGF) and receptor expression, and immunophenotype of macrophages between ASCs taken from breast and abdominal tissue.	N/A	Between the two sets of ASC they showed similar cell surface phenotype and both showed multilineage differentiation. The ASCs taken from breast tissue had a higher FGF expression level versus the ASCs taken from abdominal tissue.	+
Kølle, 2013	24075051 Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial [83]	Human	Abdomen	Posterior part of right and left upper arms	2b	Fat grafts with or without ASCs injected into upper arms	121 days	ASC-enriched fat grafts had significantly higher residual volume than control grafts	+
Marino, 2013	23773718 Therapy with autologous adipose-derived regenerative cells for the care of chronic ulcer of lower limbs in patients with peripheral arterial disease [121]	Human	Abdomen	Lower limb ulcer	4	ASCs were injected into edges of lower limb ulcer	4, 10, 20, 60 and 90 days	With ASC injection there was a decrease in diameter, depth and pain. Also, 6/10 patients experienced total healing of the ulcer	+

Tanikawa, 2013	23806916 Fat grafts supplemented with adipose-derived stromal cells in the rehabilitation of patients with craniofacial microsomia [85]	Human	Abdomen	Face	2b	Patients with craniofacial microsomia received a graft with adipose-derived stromal cells or without (control group). They were assessed preoperatively and 6 months post-operatively.	6 months	88% of the original fat volume survived for the patients with ASC and only 54% fat volume survived for the control group.	+
Doornaert, 2012	22777036 Intrinsic dynamics of the fat graft: in vitro interactions between the main cell actors [122]	Human	Abdomen	N/A	5	CD34+ ASCs were co-cultured with mature adipocytes.	N/A	CD34+ ASCs proliferate and differentiate into adipose tissue upon exposure to mature adipose cells.	+
Koh, 2012	22907186 Clinical application of human adipose tissue-derived mesenchymal stem cells in progressive hemifacial atrophy (Parry-Romberg disease) with microfat grafting techniques using 3-dimensional computed tomography and 3-dimensional camera [123]	Human	Abdomen	Face (Parry-Romberg disease)	2b	Group 1: Received microfat grafts with ASC enrichment Control: Received microfat grafts without ASCs	15 months	Fat graft survival was better in the patients with the adipose-derived stem cell enrichment. Overall resorption was 20.59% in ASC group and 46.81 in control group.	+
Sterodimas, 2011	21813882 Autologous fat transplantation versus adipose-derived stem cell-enriched lipografts: a study [84]	Human	Autologous fat or ASCs	Face (congenital or acquired facial tissue defects)	2b	Ten patients were treated with autologous fat transplantation, and the other ten received adipose-derived stem cell-enriched lipografts.	6, 12, and 18 months	The patients with the adipose-derived stem cell treatment only needed one treatment, while only 3/10 control patients achieved the desired result in one session. There was significantly higher patient satisfaction in the experimental group after 6 months, but by the 18 month evaluation the groups were not significantly different.	+

Tiryaki, 2011	21487913 Staged stem cell- enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: a preliminary report [124]	Human	Various sites	"Hostile recipient areas"	4	Autologous ASCs were injected into recently grafted sites.	Monitore d over 3 years	Postoperative atrophy was minimal and did not change after 8 weeks. This was compared to historical atrophy rates of 20-80%.	+
Yoshimura , 2008	17763894 Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells [125]	Human	Thighs, abdomen, and lower legs	Breast	4	Stromal vascular fraction containing ASCs combined with aspirated fat and injected into breasts for cosmetic breast augmentation (n=40)	2 months	Postoperative atrophy of injected fat was minimal and did not change drastically after 2 months. Cyst formation was detected in 4 patients. Almost all patients were satisfied with the augmentation.	+

Table 9-13. Other studies									
Author, Year	PMID, Title	Model	Donor site	Recipient site	LOE	Technique	Follow-up	Result	Conc
Bae, 2015	25899889 Effects of human adipose-derived stem cells and stromal vascular fraction on cryopreserved fat transfer. [131]	Human to nude mice	Lower abdomen of human	Back of nude mice	5	The cryopreserved fat grafts were treated with ADSC, SVF, or normal saline in 30 six-week-old male nude mice to test whether ADSC and SVF could improve the survival of the transplanted fat tissue.	8 weeks	There was no difference between the control and SVF groups with respect to weight, volume, and histological findings. However, the ADSC group showed a significant increase in weight and volume compared with the control and SVF groups. Histological examination showed that the ADSC supplementation improved the quality of the transplanted fat grafts.	+
Garza, 2015	25502860 Studies in fat grafting: Part IV. Adipose-derived stromal cell gene expression in cell-assisted lipotransfer [132]	Human to mouse	Flank, thigh, and abdomen (Human)	Scalp (Mice)	5	Adipose-derived stromal cells isolated from human lipoaspirate were labeled with green fluorescent protein and luciferase. Fat grafts enhanced with adipose-derived stromal cells were injected into the scalp and bioluminescent imaging was performed to follow retention of adipose-derived stromal cells within the fat graft.	1,5, and 10 days	Although adipose-derived stromal cell survival in the hypoxic graft environment decreases significantly over time, these cells provide multiple angiogenic growth factors. Therefore, improved fat graft volume retention with adipose-derived stromal cell enrichment may be attributable to improved graft vascularization.	+

Gillis, 2015	26218392 Effect of N-Acetylcysteine on Adipose-Derived Stem Cell and Autologous Fat Graft Survival in a Mouse Model [133]	Mouse	Inguinal	Scalp	5	Inguinal fat pads were harvested using tumescent solution with or without N-acetylcysteine. N-Acetylcysteine-treated or control grafts were injected under recipient mouse scalps.	3 months	In vivo, N-acetylcysteine treatment resulted in improved graft retention compared with control N-Acetylcysteine-treated grafts demonstrated less fibrosis and inflammation, and a 33 percent increase in adipocyte density compared with controls ($p < 0.001$) that was not associated with increased vascularity.	+
Li, 2015	23509085 Construction of engineering adipose-like tissue in vivo utilizing human insulin gene-modified umbilical cord mesenchymal stromal cells with silk fibroin 3D scaffolds [134]	Rat	Human insulin gene-modified umbilical cord mesenchymal stromal cells (hUMSCs)	Under dorsal skin	5	hUMSCs infected with Ade-insuline-EGFP were seeded in silk fibroin 3D scaffolds, cultured for 4 days in adipogenic medium, and transplanted. A control group received the silk fibroin 3D scaffolds with untransfected hUMSCs. Both groups were analyzed at 8 and 12 weeks.	8 and 12 weeks	More tissue regeneration, more fat-like cells with larger volume, and more degradation of the scaffolds in the group with hUMSCs infected with Ade-insulin-EGFP in the scaffolds.	+

Luo, 2015	25675023 Coimplanted endothelial cells improve adipose tissue grafts' survival by increasing vascularization [135]	Human to Mice	Human	Mice (Subcutaneous)	5	The isolated ECs were labeled, then added to 0.5-mL fat grafts at different numbers ($0.5 \times 10(6)$, $1 \times 10(6)$, $2 \times 10(6)$, and $4 \times 10(6)$ cells) before subcutaneous implantation in nude mice. Grafts were harvested at 1 week, 1 month, and 2 months after transplantation.	1 week, 1 month, and 2 months	Stromal vascular fraction-derived vascular cells exhibited typical EC characteristics. The observed differences in explanted graft weight, vessel density, vascular gene expression, and cell tracking result indicated that coimplantation with ECs accelerated vascularization that increased graft survival in a concentration-dependent manner.	+
Osinga, 2015	26017597 Effects of intersyringe processing on adipose tissue and its cellular components: implications in autologous fat grafting [136]	Human in vitro	Abdominal	NA	5	Lipoaspiration was performed, followed by shuffling the fat either zero, five, or 30 times between two 10-cc syringes. Thereafter, fat was applied through a 1.5-mm cannula for autologous fat grafting.	N/A	The process of shuffling changed the macroscopic but not the microscopic structure. No difference in cell number, viability, number of lipid droplets, vascular architecture, or ratio of cell composition was found. Analysis of the stromal vascular fraction did not show significant changes.	+

Phipps, 2015	25539302 Alternatively activated M2 macrophages improve autologous Fat Graft survival in a mouse model through induction of angiogenesis [137]	Mouse	Inguinal	Scalp	5	Grafts from C57BL/6 mouse inguinal fat pads were supplemented with M2 macrophages generated by intraperitoneal Brewer's thioglycollate injection and in vitro culture. Grafts with saline or M2 macrophages were injected under recipient mouse scalps and assessed by serial micro- computed tomographic analysis.	1 month and 3 months	One month after graft injection, no significant difference was noted between M2 macrophage- supplemented (105 ± 7.0 mm) and control graft volumes (72 ± 22 mm). By 3 months after injection, M2 macrophage- supplemented grafts remained stable, whereas controls experienced further volume loss (103 ± 8 mm versus 39.4 ± 15 mm; $p = 0.015$).	+
Soares, 2015	25626795 Targeted protection of donor graft vasculature using a phosphodiesterase inhibitor increases survival and predictability of autologous fat grafts [138]	Mice	Inguinal	Dorsa	5	Inguinal fat of donor Tie2/LacZ mice was infiltrated with sildenafil or saline, harvested, and transplanted onto the dorsa of recipient FVB mice. Additional donor mice were perfused with intraarterial trypsin.	12 and 24 weeks	Compared with controls, targeted sildenafil treatment improved early graft perfusion, doubled graft retention at 12 weeks (83 percent versus 39 percent; $p < 0.05$), ultimately retaining 64 percent of the original graft volume by 24 weeks (compared to 4 percent; $p < 0.05$) with superior histologic features.	+

Yu, 2015	26446419 Traditional Chinese Medicine: Salvia miltiorrhiza Enhances Survival Rate of Autologous Adipose Tissue Transplantation in Rabbit Model [139]	Rabbit	Scapular region	Dorsum of the ear	5	Minced adipose tissue harvested from the scapular region was transplanted into the dorsum of the ears of New Zealand rabbits. The experimental groups were intra-peritoneally injected with S. miltiorrhiza for a total 4 weeks. Levels of VEGF, CD31, perilipin and cell survival were monitored.	2,4,and 12 weeks	At 12 weeks, the survival rates in the experimental group were statistically greater than that in the control group, respectively ($p < 0.05$). Plasma levels of VEGF in the experimental group at different time points were significantly higher than that in the control group ($p < 0.05$). Histologically, grafts in the experimental group showed better survival of adipocytes and neo- vascularization. By perilipin immuno- histochemical staining, the experimental group demonstrated better adipocyte survival.	+
Beitzel, 2014	24581253 Properties of biologic scaffolds and their response to mesenchymal stem cells [140]	Human rotator cuff	Bone marrow, proximal humerus	Various various biologic scaffolds	5	MSCs were cultured on human rotator cuff tendon, human highly cross-linked collagen membrane, porcine non-cross-linked collagen membrane, a human platelet-rich fibrin matrix, and a fibrin matrix based on platelet-rich plasma and then counted for adhesion, proliferation and live/dead stain.	N/A	More cells adhered to both the non- cross-linked porcine collagen scaffold and PRF-M. Cell proliferation was higher in the non- cross-linked porcine collagen scaffold compared with PRF- M and fibrin matrix. based on platelet- rich plasma	+
Chung, 2014	24622574 Studies in fat grafting: Part I. Effects of injection technique on in vitro fat viability and in vivo volume	Mice	Not specified (Human)	Scalp	5	Lipoaspiration samples were obtained from five donors, and cellular viability, proliferation, and lipolysis were evaluated following injection using either a modified coleman	4,6,8, and 12 weeks	In vivo fat volume retention was significantly greater than with the modified Coleman technique	+

	retention [141]					technique or an automated, low shear device			
Li, 2014	24740717 Improvement in autologous human fat transplant survival with SVF plus VEGF-PLA nano-sustained release-microspheres [21]	Human cells into mice	Human	Mouse	5	SVF cells were harvested and constructed VEGF-PLA nano-sustained release-microspheres in vitro. Human fat tissues were mixed with SVF cells plus VEGF-PLA, SVF cells alone or Dulbecco's modified Eagle's medium as the control. These three mixtures were injected into random sites in 18 nude mice.	2 months	The mean wet weight of fat in the SVF plus VEGF-PLA, SVF alone, and control transplants were 0.18 ± 0.013 g, 0.16 ± 0.015 g, and 0.071 ± 0.12 g, respectively; the differences between groups were statistically significant. More vessels were present in the SVF plus VEGF-PLA transplants than in the other two types. Transplants mixed with SVF cells also had an acceptable density of capillaries.	+
Saliba, 2014	24307457 Growth factors expression in hyaluronic acid fat graft myringoplasty [142]	Guinea pigs	Nuchal area of guinea pig	Tympanic membrane	5	Hyaluronic acid fat graft myringoplasty (HAFGM) technique and levels of EGF, IGF, TNF α , VEGF, and KGF were investigated.	0,3,8, 21 days	HAFGM had higher levels of all growth factors tested except KGF and the tympanic membrane closure was neovascularized and scarless, versus spontaneous closure in the control group.	+

Sezgin, 2014	24529693 Improving fat graft survival through preconditioning of the recipient site with microneedling [143]	Albino rats	Inguinal fat pad	Dorsal area	5	The dorsal area was preconditioned with standard technique micro-needling 1-week prior to fat graft transfer in the study group while the control group did not undergo micro-needling	15 weeks	Fat grafts in the study group had better integrity and a higher level of vascularity compared to the control group. Volume analysis demonstrated higher graft survival in the study group in comparison to the control group	+
Willem sen, 2014	24984784 The effects of platelet-rich plasma on recovery time and aesthetic outcome in facial rejuvenation: preliminary retrospective observations [144]	Human	Upper legs	Face	3a	For the first part of this study, i.e., evaluation of recovery time after surgery, the following selection criteria were used: nonsmoking females, aged 35-65 years, with a complete documented follow-up. For the second part of the study, evaluation of potential differences in aesthetic outcome, the records of these 82 patients were screened for the presence of pre- and postoperative standardized photographs in three views.	N/A	Adding PRP to facial lipofilling reduces recovery time and improves the overall aesthetic outcome of a MACS-lift.	+
Xu, 2014	25562157 Human breast adipose-derived stem cells transfected with the stromal cell-derived factor-1 receptor CXCR4 exhibit enhanced viability in human autologous free fat grafts [145]	Human to mouse	Breast adipose-derived stem cells (Human)	Random (Mice)	5	Human breast adipose-derived stem cells (HBASCs) were expanded ex vivo for 3 passages, labeled with green fluorescent protein (GFP) and transfected with CXCR4 or left untransfected. Autologous fat tissues were mixed with the GFP-labeled, CXCR4-transfected HBASCs (group A), GFP-labeled HBASCs (group B), the known vascularization-promoting agent VEGF (group C), or medium (group D) and then injected subcutaneously into 32 nude mice at 4 spots in a random fashion.	6 months	Our data demonstrated that HBASCs can enhance the survival and quality of transplanted free fat tissues. Moreover, CXCR4 transfection of these HBASCs could augment this effect.	+

Zhou, 2014	25405913 In vivo bioimaging analysis of stromal vascular fraction-assisted fat grafting: the interaction and mutualism of cells and grafted fat [146]	Mouse	Inguinal	Back	5	Fat tissue and SVF separated from luciferase (Luc)-transgenic rats were applied for bioimaging analysis. The Luc-fat (0.2 mL) was subcutaneously injected into the back of nude mice with or without SVFs from 0.2 mL wild type rat fat	Up to 63 days	The bioimaging results showed that fat tissues transplanted with SVFs had higher survival ratio than those transplanted without SVFs. Stromal vascular fraction-assisted fat grafts had more integral structure and less necrosis cysts. The results showed that, with the existence of grafted fat, transplanted SVF survived for a significantly longer time and could contribute to fat graft survival and regeneration by differentiating into structural cells.	+
Aronowitz, 2013	24281640 Adipose stromal vascular fraction isolation: a head-to-head comparison of four commercial cell separation systems [147]	Human	Abdomen	N/A	5	Comparison of commercially available systems for isolating stromal vascular fraction cells	N/A	Each system (1) PNC's Multi Station, (2) CHA Biotech Cha-Station, (3) Cytori Celution 800/CRS System, and (4) Medi-Khan's Lipokit with MaxStem yielded stromal vascular fraction cells but there are differences in the viability, number, and safety profiles of the collections.	N/A

Bulgin, 2013	24024064 Autologous bone-marrow-derived-mononuclear-cells-enriched fat transplantation in breast augmentation: evaluation of clinical outcomes and aesthetic results in a 30-year-old female [148]	Human	BMMNCs from bone marrow in posterior iliac crest; fat from abdomen, thighs, flanks	Breasts	3b	BMMNCs and purified fat mixture injected directly into patient's breasts	2 weeks, 4 weeks, 3 months, 6 months, and 12 months	Postoperative atrophy of injected fat was minimal and did not change significantly after 12 months; patient experienced improvement in circumferential breast measurement	+
Hamed, 2013	23897352 The chemokine stromal cell-derived factor-1 α promotes endothelial progenitor cell-mediated neovascularization of human transplanted fat tissue in diabetic immunocompromised mice [60]	Mice	Human fat tissue	Scalps of mice	5	Human fat tissue with phosphate-buffered saline or stromal cell-derived factor-1 α was injected into the scalp of each mouse.	18 days and 15 weeks	After 18 days:group with stromal cell-derived factor-1 α treatment of the grafts in the diabetic mice expressed higher levels of VEGF, increased VEGF receptor 2, CXCR4, endothelial nitric oxide synthase, and protein kinase B expression levels, and lower caspase 3 and cytochrome c levels. After 15 weeks:that same group had increased vascularization and prevented resorption of the fat graft.	+
Luo, 2013	23509085 Construction of engineering adipose-like tissue in vivo utilizing human insulin gene-modified umbilical cord mesenchymal stromal cells with silk fibroin 3D scaffolds[149]	Nude mice	Human thigh liposuction	Back of nude mice	5	Mice received human ADSC fat grafts with or without estradiol enrichment, or culture medium alone. Survival rate of the fat grafts was calculated.	12 weeks	Estradiol enriched fat grafts had a higher tissue survival rate than the ADSC alone group (76.9% vs 55.5%). there was also increased capillary formation	+

Yanaga , 2013	24281577 Two-stage transplantation of cell-engineered autologous auricular chondrocytes to regenerate chondrofat composite tissue: clinical application in regenerative surgery [150]	Human	Auricular concha	Nasal/chin area	2b	Two-stage transplantation: 1st = abdomen for growth (6 months), 2=nasal/chin area for reconstruction	1 to 5 years	Chondrofat tissue was stable after 1-5 years, maintained good shape, no infections, 5.6% absorption	+
Zhao, 2013	24165597 Enhancement of fat graft survival by bone marrow-derived mesenchymal stem cell therapy[151]	Nude mice	Inguinal area	Paraverteb ral	5	Three groups of nude mice recieved transplants of cells containing .3ml of adipose granula and .2ml of cell components. Group A received mesenchymal stem cells, group B received expanded mesenchymal stem cells, group C received Dulbecco's medium. Four months later the grafts were harvested measured.	4 months	Fat graft survival rates were .2052 in group A (mesenchymal), .1761 in group B (expanded mesenchymal) and .1350 in group C (Dulbecco's)	+
Alghou l, 2012	22745452 The effect of hyaluronan hydrogel on fat graft survival [152]	Rat	Groin	Dorsum	5	Two groups. Group 1: fat alone; Group 2: fat and hyaluronan hydrogel in a 1:1 mix. In vivo scans at 4, 12, and 20 weeks to quantify fat-HA graft volume and volume of fat alone	4,12 and 20 weeks	Fat-HA yielded reduced fat necrosis (statistical diff. at 12 and 20 weeks), higher blood vessel density (sig. at 12 weeks only) and less volume loss (at 20 weeks).	+
Butala, 2012	22495210 Endogenous stem cell therapy enhances fat graft survival [153]	Mice	Abdomen and thigh (Human)	Tail	5	Male 8-week-old FVB mice were grafted with either high density or low-density human lipoaspirate. Half of the mice receiving low- density fat were treated with a stem cell mobilizer for 14 days	2 and 10 weeks	Endogenous progenitor cell mobilization enhances low- density fat neovascularization, increases vasculogenic cytokine expression, and improves graft survival to a level equal to that of high-density fat grafts	+

Frerich , 2012	22023101 Comparison of different fabrication techniques for human adipose tissue engineering in severe combined immunodeficient mice [154]	SCID mice	Grown on microcarriers	Under the skin	5	Mice received implantations of human adipose tissue derived stromal cells with or without endothelial cells. Another group of mice received the particles in a fibrin matrix with and without endothelial cells. The mice were harvested 12 days, 4 weeks and 4 months and grafts analyzed.	12 days, 4 weeks and 4 months	There was a limited improvement in the group with the endothelial cells after 4 weeks. There were significantly fewer necrotic regions after 4 weeks and 4 months.	+
Lee, 2012	23094251 Facial Soft Tissue Augmentation using Autologous Fat Mixed with Stromal Vascular Fraction [155]	Human	Lower abdomen, hip and thigh	Face	3a	SVF cells were freshly isolated from half of the aspirated fat and were used in combination with the other half of the aspirated fat during the procedure. Between March 2007 and February 2008, a total of 9 SVF-assisted fat grafts were performed in 9 patients.	1 time a week up to 12 weeks, 1 time a month up to 11 months	Scores of the left facial area grafted with adipose tissue mixed with SVF cells were significantly higher compared with those of the right facial area grafted with adipose tissue without SVF cells.	+
Ma, 2012	21607534 Utilizing muscle- derived stem cells to enhance long- term retention and aesthetic outcome of autologous fat grafting: pilot study in mice [156]	Mice	Autologous	Intramuscular	5	MDSCs and fat were transplanted intramuscularly in mice. The mice were assessed after 3 months.	3 months	Group containing MDSCs showed higher fat signal, intact fat cells, less fibrosis, less fat graft loss, and higher capillary density.	+

Sarkan en, 2012	22738319 Bioactive acellular implant induces angiogenesis and adipogenesis and sustained soft tissue restoration in vivo [157]	Rat	Subcutan eous fat (Human and mice)	Dorsal subcutis of rodents	5	Groups: rat (rATE, allograft) or human (hATE, xenograft) derived ATE included in implant to be injected into rat through a hyaluronan hydrogel (HA)	12 weeks to 9 months	ATE-HA implant showed bioactivity, compatibility, sustainability, microvessel induction, adipose tissue deposition (starting at week 12), included capillaries, nerve bundles and healthy connective tissue, and had large fat pads at the end of the study.	+
Yang, 2012	21629060 Role of anti-TNF- α therapy in fat graft preservation [158]	Rat	Autologo us	Back	5	Two groups: Group 1: antirat TNF-alpha monoclonal antibody was added to fat graft; Group 2: fat graft on its own. 8 rats were killed and assessed on days 7, 14, 30 and 60	7,14,30,6 0 days	Group 1 had a higher preservation ratio of tissue compared to Group 2 at 60 days (no significant difference in days before this). Group 1 had lower numbers of apoptotic cells at each time point and expression of TNF alpha was lower in Group 1 versus Group 2 for days 7 and 14.	+
Keck, 2012	21964747 Coenzyme Q10 does not enhance preadipocyte viability in an in vitro lipotransfer model [159]	In vitro	Subcutan eous adipose	N/A	5	Preadipocytes were treated with coenzyme Q10 or a control and incubated with lidocaine. FACS and western blot were used to assess viability and apoptosis.	NA	Coenzyme Q10 did not improve viability or have any impact on the observed apoptosis parameters	-
Koh, 2011	21393582 Stromal vascular fraction from adipose tissue forms profound vascular network through the dynamic reassembly of blood endothelial	Mice	Epididym al adipose tissue	Flank region	5	Cells were mixed with Matrigel or PBS, BSA, VEGF or cartilage oligomeric matrix protein-angiopoietin and implanted into the flank region	1 week	Freshly isolated SVF can effectively induce new vessel formation through the dynamic reassembly of blood endothelial cells	+

	cells [160]								
Mojalla l, 2011	21590499 Stem cells, mature adipocytes, and extracellular scaffold: what does each contribute to fat graft survival? [161]	Nude mice	Human fat	Nude mice	5	Preparations of purified adipose tissue, isolated mature adipocytes, cultured adipose derived stem cells without scaffold, collagen scaffold only, cultured adipose-derived stem cells in a collagen scaffold with and without bioactive factors and freshly isolated adipose derived stem cells in a collagen scaffold were used. Each preparation was grafted onto the nude mice	2 months	Free-cell grafts were resorbed in 50% of the mature adipose group and 60% of the ADSC group. Purified adipose tissue had an 81.8% survival rate. The remaining weight was greater in the Scaffold groups, but the difference was not statistically significant.	no sig diff
Kamakura, 2011	21533662 Autologous cell-enriched fat grafting for breast augmentation [162]	Human	Thighs, hips, buttocks, or abdominal	Breast	3b	After adipose harvesting using syringe liposuction, the tissue is processed in the Celution 800 System. The mean cell viability measured using an automated cell counting system before graft delivery was 85.3%.	3 and 9 months	All patients demonstrated improvement in circumferential breast measurement (BRM) from their baseline state, and breast measurements were stable by 3 months after surgery. T	+
Conde-Green, 2010	20442104 Effects of centrifugation on cell composition and viability of aspirated adipose tissue processed for transplantation [163]	Human	Abdomen	N/A	5	Samples of adipose tissue were centrifuged and samples of adipose tissue that were not centrifuged were compared.	N/A	The pellet in the centrifuged sample had the highest concentration of MSCs and endothelial cells	

Keck, 2010	21042106 Local anesthetics have a major impact on viability of preadipocytes and their differentiation into adipocytes [164]	Human	Subcutaneous	N/A	5	Human preadipocytes were isolated from subcutaneous adipose tissue of 15 patients and treated with bupivacaine, mepivacaine, ropivacaine, articaïne/epinephrine, and lidocaine for 30 minutes.	N/A	There is a marked influence of local anesthetics not only on the quantity but also on the quality of viable preadipocytes as determined by their ability to differentiate into mature adipocytes.	+
Zhu, 2010	20098110 Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention [18]	Mice	Inguinal fat pads	Skull	5	Adipose-derived regenerative cells and fat was transplanted into mice and compared to mice that were injected with non-treated fat graft. Fat grafts were analyzed at both 6 and 9 months after the transplantation.	6 months, 9 months	At both 6 and 9 months, ADRCs increased graft retention by 2-fold, enhanced the quality of the fat grafts, and had a higher capillary density.	+
Umeno, 2009	19460200 Efficacy of autologous fat injection laryngoplasty with an adenoviral vector expressing hepatocyte growth factor in a canine model [165]	Dogs	Dog	True vocal cords	5	Autologous fat plus an adenoviral vector expressing hepatocyte growth factor was injected into the right vocal cord. Harvested fat plus an adenoviral vector with no gene was injected into the left vocal cord. One year later a total laryngectomy was performed one year after the intracordal fat injection.	1 year	The fat area was significantly larger and the number of vasculoendothelial cells surrounding adipocytes was significantly greater in the intracordal injection with hepatocyte growth factor.	+
Zhong, 2009	18162449 Improved fat graft viability by delayed fat flap with ischaemic pretreatment [166]	New Zealand rabbits	Inguinal region	Dorsal midline	5	U-shaped fat flap was raised in the inguinal region to induce ischaemia. Three weeks later, fat flap was transferred to a pocket next to the dorsal midline of the rabbit.	Up to 12 months	VEGF protein in the ischaemia treated fat flaps was significantly higher than the controls at 12 hours after the treatment. There was no difference in number of vessels, fat graft size, and weight at 1 and 3 months after transplantation. The fat grafts in control group disappeared after 6 months,	+

								while the treated group maintained the fat graft up to 12 months.	
Mojallal, 2008	19065020 Does adipose tissue cultured with collagen matrix and preadipocytes give comparable results to the standard technique in plastic surgery? [167]	Nude mice	Various – with scaffoldin g	interscapular	5	Preadipocytes were seeded on collagen scaffolds and then implanted. The other group, adipose tissue taken by the Coleman technique was also implanted but in a different site for comparison. Grafts were assessed after 8 weeks.	8 weeks	The group with the preadipocytes showed adipose appearance and peripheral neovascularization.	+
Piasecki, 2008	19083503 Purified viable fat suspended in matrigel improves volume longevity [168]	Mice	Mice (unspecified)	Mice (Unspecified)	5	Purified fat cells were suspended in GFR Matrigel and then implanted. The other experimental groups were unpurified fat (group 2), and then a control of GFR-Matrigel (group 3). The grafts were then assessed at 1 week, 1 month and 3 months	1 week, 1 month, and 3 months	Fat in GFR Matrigel grafts had a higher fat volume at 3 months, whereas, the unpurified fats developed fibrosis.	N/A

Piasecki, 2008	19083542 Beyond the cells: scaffold matrix character affects the in vivo performance of purified adipocyte fat grafts [169]	Mice	Mice (unspecified)	Mice (unspecified)	5	PuraMatrix (peptide hydrogel) with adipocytes were compared against just PuraMatrix and then against syringe-harvested fat alone	1 week, 1 month and 3 months	PuraMatrix with purified fat had higher volume and longer fat graft survival	N/A
Torio-Padron, 2007	17380359 Engineering of adipose tissue by injection of human preadipocytes in fibrin [170]	Nude mice	Subcutaneous (human)	Back (mouse)	5	Different concentrations of undifferentiated human preadipocytes in fibrin were injected into athymic mice, while the control group was injected with just fibrin.	1,3,6 and 9 months	Within 4 weeks after initial volume reduction of the implants, the volume and shape of the implants with preadipocytes remained stable. The control group implants with just fibrin were completely resorbed within 3 weeks. The best results were observed after implantation of 30 million preadipocytes.	+
Shoshani, 2005	15731687 The effect of interleukin-8 on the viability of injected adipose tissue in nude mice [171]	Mice	Suction-assisted lipectomy	Scalp	5	Group 1: interleukin-8 (0.25ng) injected 24 hours before fat graft with 25 ng of interleukin-8 per 1 cc of injected fat. Group 2: fat without interleukin-8	15	Group 1 had significantly less cyst formation. No significant difference between the groups concerning weight or volume of graft, adipose cell size.	+
Yamaguchi, 2005	16246901 Revascularization determines volume retention and gene expression by fat grafts in mice [172]	Mice	Epididymal fat	Subcutaneous	5	Fat grafts were transplanted into mice either with TNP-470 (an inhibitor of angiogenesis) or without.		Mice with TNP-470 had fat grafts with lower weight and smaller adipocytes than mice without inhibitor. VEGF and leptin were also	+

								lower in TNP mice.	
Moore, 1995	7484471 Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine (173)	Human- in vitro	Abdomen	N/A	5	Fat obtained intraoperatively and by lipectomy was digested with collagenase to isolate adipocytes. These cells were exposed to lidocaine	10 days	Lidocaine potently inhibited glucose transport and lipolysis in adipocytes and their growth in culture.	+

Chapter 10: Conclusions

Autologous fat grafting is a common surgical procedure that involves the transfer of fat from one area of the body to another in order to improve contour deformities. There is tremendous clinical interest in the utilization of fat grafting for soft tissue reconstruction, with thousands of cases performed each year in the treatment of volume loss due to trauma, scars, wounds, fistulas, disease, congenital defects, or the natural process of aging. In the setting of breast reconstruction, fat grafting provides significant contour improvement and improves patient satisfaction. Although fat could become the ideal soft tissue filler, it is plagued by its tremendous variability in long-term graft retention, with volume survival rates of 20-80%, resulting in suboptimal outcomes and repetitive procedures. The goals of this dissertation were to generate and critically appraise evidence for the safety and efficacy of fat grafting in the setting of breast reconstruction and to provide evidence-based recommendations to guide future therapies.

While there is tremendous interest in the use of fat grafting for aesthetic and reconstructive breast surgery, concerns have been raised regarding efficacy and safety of adipose-derived stem cells and fat grafting in the setting of breast reconstruction. In light of the recent FDA regulatory changes regarding the processing of fat, a systematic review was undertaken to determine the safety, efficacy, satisfaction, and oncological outcomes of fat grafting to the breast. Based on our review of the literature and the data collected from our institution, fat grafting appears to be oncologically safe in breast reconstruction. Several adipose graft enrichment strategies encompassing growth factors, platelet-rich

plasma, adipose-derived and bone marrow stem cells, gene therapy and tissue engineering have been attempted to augment and improve the viability of fat grafts. While these strategies are promising, most of the evidence is level five and only in animal models, however, well-designed clinical trials are indicated to establish safety and efficacy of interventions.

Due to the significant variability in fat grafting outcomes and tremendous interest of ASCs graft retention, the final study attempts to describe the impact of a specific donor physiological condition, obesity, on ASC functionality. Understanding how obesity affects ASC function may help to elucidate why lean patients anecdotally have better fat graft retention compared to obese patients. Our research suggests that obese patients may have lower levels of satisfaction with fat grafting procedures and lower retention rates, possibly due to reduced ASC proliferation and differentiation potential. Collectively, the studies that comprise this dissertation generate and critically appraise evidence for the safety, efficacy and outcomes of fat grafting in the setting of breast reconstruction.

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Vita

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Education

Research Fellowship in Surgery, Department of Surgery 05/2014-05/2015
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Master of Health Science (M.H.S.), Molecular Microbiology and Immunology 08/2009-05/2010
Johns Hopkins Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD

Certificate in Vaccine Science and Policy, Department of International Health 08/2009-05/2010
Johns Hopkins Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD

Bachelor of Science (B.S.), Department of Biology 08/2006-05/2009
University of Kentucky, University of Kentucky College of Arts & Sciences, Lexington, KY

Commonwealth Diploma 08/2002-05/2006
Pikeville Independent School District, Pikeville High School, Pikeville, KY

Honors and Awards

Medical School: *Magna Cum Laude / Graduation with Distinction / Graduating Medical Student Research Award / Academic Excellence Scholarship / Dean's Interprofessional Honors Colloquium / Best Paper Award from the 2011 American Academy of Pediatrics Council on Clinical Information Technology / 2011 Medical Student Research Award / 2012-2013 Center for Clinical and Translational Science Professional Student Mentored Research Fellowship (CCTS PSMRF) / Outstanding Leadership and Community Service Awards 2012 and 2013 / College of Medicine Medical Education Award / Glancy Award Co-Investigator - Southeastern Society of Plastic and Reconstructive Surgeons / Best Research Presentation, Kentucky Society of Plastic Surgeons*

College: *Phi Beta Kappa / Summa Cum Laude / Departmental Honors / The Honors Program / Honors in The Honors Program / William C. Parker Diversity Scholarship / Dean's List, all semesters / Gaines Fellowship for the Humanities Awardee / Presidential Scholarship / British Marshall Scholarship 2008 Nominee / Barry Goldwater Scholarship 2007 Nominee / Kentucky Governor's Scholar Scholarship / Kentucky Educational Excellence Scholarship / Chellgren Fellows Program 2006-2009 / Presidential Freedom Volunteer Scholarship 2006 / AMSTEMM/National Science Foundation Research Stipends 2006-2009 / American Medical Student Association Grant 2009*

High School: *Salutatorian / Kentucky Governor's Scholar / Perfect Attendance / Academic Team / All "A" Quick Recall State Winner / Governor's Cup (Quiz Bowl) State Finals / KAAC Achievement Award / Governor's Cup (Quiz Bowl) District and Regional (1st) / Presidential Freedom Award / Booth Scholars Program / Varsity Tennis / Kentucky State Art Fair Winner (2nd, 3rd) / Coca-Cola Scholarship Semi-Finalist / Commonwealth Accountability Testing System Distinguished Scholar / National AAA Travel Challenge State / Prudential Spirit of Community Award / CEDAR Coal Education Scholarship / Best Buy Scholarship / Presidential Volunteer Service Award / MVP Future-Problem Solving Team / Coal Fair Winner (2nd) / FBLA State Economics Winner (5th) / WYMT Television Scholarship / Hugh O'Brian Youth Leadership Ambassador / Senate and House of Representatives Academic Achievement Award / University of Rochester Xerox Innovation and Information Technology Award / Kodak Young Leaders Award / Academic Team / Medical Explorers / Proficiency Advisory Council / National Youth Leadership Forum on Medicine / Eastern KY Science Convention Overall Scholarship Winner 2006*

Editorial Activities

Associate Editor, The *SAGE Encyclopedia of Stem Cell Research*, Second Edition

Editorial Board, *Annals of Plastic Surgery*

Editorial Board, *Aesthetic Plastic Surgery*

Editorial Board, *Wounds*

Editorial Board, *Journal of Translational Medicine*

Editorial Board, *ePlasty* (formerly *Journal of Burns and Wounds*)

Reviewer, *Plastic and Reconstructive Surgery*

Reviewer, *Plastic and Reconstructive Surgery – Global Open*

Reviewer, *Journal of Plastic, Reconstructive & Aesthetic Surgery*

Reviewer, *Aesthetic Surgery Journal*

Reviewer, *European Journal of Plastic Surgery*

Reviewer, *Indian Journal of Plastic Surgery*

Reviewer, *Annals of Surgery*

Reviewer, *American Journal of Surgery*

Reviewer, *Journal of Cutaneous and Aesthetic Surgery*

Reviewer, *Journal of the American Academy of Dermatology*

Reviewer, *Surgical Innovation*

Reviewer, *Cochrane Wounds Group of the Cochrane Collaboration*

Leadership

Treasurer, International Federation of Medical Students' Association
University of Kentucky College of Medicine

2011-2013

Member, Dean's Committee on Admissions
University of Kentucky College of Medicine

2011-2012

Manager, Salvation Army Clinic
University of Kentucky College of Medicine

2011-2012

Membership Chair, American Medical Association
University of Kentucky College of Medicine

2011-2012

Parliamentarian, Student National Medical Association
University of Kentucky College of Medicine

2011-2012

President, American Medical Student Association
University of Kentucky College of Medicine

2010-2014

Member, MedTones Choir
University of Kentucky College of Medicine

2010-2012

Member, Rhythm and Bones Dance Troupe
University of Kentucky College of Medicine

2010-2012

Research Fellow, Department of Neuroscience (Dr. Solomon Snyder)
Johns Hopkins University

2010

Member, Alpha Phi Omega Fraternity
University of Kentucky

2008-2009

Chair, University of Kentucky Global Health Symposium
University of Kentucky

2008-2009

Mentor , AMSTEMM University of Kentucky	2008-2009
Member , Student Affiliates of the American Chemical Society (SAACS) University of Kentucky	2008-2009
Organic Chemistry II Workshop Instructor , Department of Chemistry University of Kentucky	2008
President , The Roosevelt Institution University of Kentucky	2007-2009
Member , Beta Beta Beta Biology Honor Society University of Kentucky	2007-2009
Editor in Chief , Honors Program Newsletter University of Kentucky	2007-2008
Research Assistant , National Association for the Blind Mumbai, India	2007
Research Assistant , UNICEF: Food Fortification Project Gujarat, India	2007
Board Director , Alpha Epsilon Delta Honor Society University of Kentucky	2006-2009
President , Vice President, Pre-Med American Medical Student Association University of Kentucky	2006-2009
Chapter Chair and Volunteer , DanceBlue University of Kentucky	2006-2009
Student Teacher , Sacred Heart Elementary School Williamson, WV	2006-2009
Research Fellow , Gill Heart Institute University of Kentucky	2006-2008
Office Assistant , Appalachian Regional Healthcare Professional Offices South Williamson, KY	2003-2009
Volunteer , Academic/Quiz Bowl Team Pikeville High School	2003-2006
Board Member , Pike County Youth Leadership Council Pikeville High School	2002-2006

Peer-Reviewed Publications

1. Morrison SD, Vyas KS, Motakef S, Gast KM, Chung MT, Rashidi V, Satterwhite T, Kuzon W, Cederna PS. Facial Feminization: Systematic Review of the Literature. *Plast Reconstr Surg*. 2016 Jun;137(6):1759-70.

2. **Vyas KS**, Kim U, North WD, Stewart D. Frontalis Sling for the Treatment of Congenital Ptosis. *Eplasty*. 2016 April 4;16:ic8. eCollection 2016.
3. **Vyas KS**, Whitaker J, Saha SP. Surgical Management of Tumors of the Heart: a rare but important pathology. *J Surg* 2(2). 2016.
4. Wong L, Wilson RM, Snapp WK, Bole R, **Vyas KS**. Nipple Pathology in Total Skin-Sparing Mastectomy: Implications for Immediate Reconstruction. *Ann Plast Surg*. 2016 Feb 24.
5. **Vyas KS**, Mardini S, Phillips LG, Gosman AA, Vasconez HC. Financial Planning for the Plastic Surgery Residency Applicant. *Plast Reconstr Surg*. 2016 Feb;137(2):497e-9e.
6. Vyas KS, Snapp WK, Rinker BD, Vasconez HC. A Critical Analysis of the 2012 Centers for Medicare and Medicaid Services Provider Use and Payment Database. *Plast Reconstr Surg*. 2016 Feb;137(2):462e-73e.
7. Rinker BD, **Vyas KS**. Do Stem Cells Have an Effect When We Fat Graft? Fat Versus Fiction. *Ann Plast Surg*. 2015 Nov 5.
8. **Vyas KS**, Vasconez HC. Regulatory Issues Regarding Fat Grafting. *Plastic Surgery Pulse*. 7(2):1. November 2015.
9. **Vyas KS**, Saha SP. "Recognizing and Preventing Stroke." *Lexington Herald Leader*. 1 November 2015. Print.
10. **Vyas KS**, Vasconez HC. "The Use of Fat in Plastic Surgery." *Lexington Herald Leader*. 1 November 2015. Print.
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5. Morrison SD, Shakir A, **Vyas KS**, Kirby J, Song J, Crane C, Kennedy WA, Lee GK. Total Phalloplasty: A Systematic Review of Operative Techniques and Outcomes. *Plast Reconstr Surg*.
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2. **Vyas KS**, Ryan D, Burns C, Wong L. Prolonged Allograft Survival in a Patient with Chronic Immunosuppression.
3. Cheng I, **Vyas KS**, Velaga Santhosh, Davenport DL, Saha SP. Long Term Outcomes of Carotid Endarterectomy with Primary Closure.
4. **Vyas KS**. A Systematic Review of the Use of Telemedicine in Plastic and Reconstructive Surgery
5. Morrison SD, Shakir A, **Vyas KS**. Penile Replantation: A Systematic Review of Outcomes and Complications

6. **Vyas KS**, Motakef S, Mardini S. Microvascular Free Tissue Transfer Perioperative Issues.
7. **Vyas KS**, Morrison SD, Vasconez HC, Longaker MT. Fat Grafting Enrichment Strategies.
8. **Vyas KS**, Vasconez HC. Fat Grafting in Aesthetic and Reconstructive Surgery: Ideal Donor Sites and Longevity in Different Body Regions.
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10. **Vyas KS**. Dendritic Cells for Composite Tissue Allotransplantation.
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12. **Vyas KS**, Arsenescu V, Arsenescu R. Coordinated oral-intestinal immune response in patients with Inflammatory Bowel Diseases.
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Acknowledgments

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Book Chapters

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Poster Presentations

1. **Vyas KS**, Vasconez HC. Adipose Graft Enrichment Strategies. Southeastern Society of Plastic and Reconstructive Surgeons. Orlando, FL. 12 June 2016. Abstract and Poster.
2. **Vyas KS**, Whitaker J, Saha SP. Surgical Management of Benign Tumors of the Heart: A Single Center Experience. Center for Clinical and Translational Science 10th Annual Spring Conference. University of Kentucky. Lexington, KY. 25 March 2015. Abstract and Poster.
3. **Vyas KS**, Starr ME, Saito H, Lynch MP, Vasconez HC. Elucidating molecular mechanisms for fat graft retention. Center for Clinical and Translational Science 10th Annual Spring Conference. University of Kentucky. Lexington, KY. 25 March 2015. Abstract and Poster.
4. **Vyas KS**, Snapp WK, Vasconez HC. Plastic Surgery Medicare Reimbursement: Analysis of Claims and Payments. Center for Clinical and Translational Science 10th Annual Spring Conference. University of Kentucky. Lexington, KY. 25 March 2015. Abstract and Poster.
5. **Vyas KS**, Yan B, Vasconez HC. How easy, reliable, and understandable is online information about plastic surgery procedures? Center for Clinical and Translational Science 10th Annual Spring Conference. University of Kentucky. Lexington, KY. 25 March 2015. Abstract and Poster.
6. **Vyas KS**, Noonan JA. Pulmonary Hypertension in the RASopathies. Center for Clinical and Translational Science 9th Annual Spring Conference. University of Kentucky. Lexington, KY. 27 March 2014. Abstract and Poster.
7. **Vyas KS**, Wong LK. Intraoperative Management of Free Flaps: Current Practice. Alpha Omega Alpha Groves Memorial and MD/PhD Program Student Research Day. University of Kentucky. Lexington, KY. 21 February 2014. Abstract and Poster.
8. **Vyas KS**, Stratton T, Soares N. Medical Student Stress across the Undergraduate Continuum: A Multi-School Study. Generalists in Medical Education. Philadelphia, PA. 1 November 2013. Abstract and Poster.
9. **Vyas KS**, Wong LK. Intraoperative Management of Free Flaps: Current Practice. Southeastern Society of Plastic and Reconstructive Surgeons. Bonita Springs, FL. 1 June 2013. Abstract and Poster.
10. **Vyas K**, Stratton T, Soares N. Medical Student Health and Wellness. Center for Clinical and Translational Science 8th Annual Spring Conference. University of Kentucky. Lexington, KY. 8 April 2013. Abstract and Poster.
11. **Vyas KS**, Saha SP. Mediastinoscopy: Trends and Practice Patterns in the United States. Alpha Omega Alpha Groves Memorial and MD/PhD Program Student Research Day. University of Kentucky. Lexington, KY. 20 February 2013. Abstract and Poster.
12. **Vyas KS**, Soares NS. Trend Toward Use of WHO Growth Charts and Impact On Overweight Identification. Regional Global Health Conference – Crossing Borders and Disciplines: New Horizons in Global Health. Lexington, KY. 26 October 2012. Abstract and Poster.

13. **Vyas K**, Stratton T, Bonasso P, Nease E, Harris V, Pampati S, Rowland M, Batra S, Gress T, Soares N. Medical Students' Health and Wellness: A Pilot Study of SGEA Institutions. Regional Conference of the Southern Group on Educational Affairs (SGEA). 19 April 2012. Poster.
14. **Vyas KS**, Soares NS. Trend Toward Use of WHO Growth Charts and Impact On Overweight Identification. Alpha Omega Alpha Groves Memorial and MD/PhD Program Student Research Day. University of Kentucky. Lexington, KY. 1 March 2012. Abstract and Poster.
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16. **Vyas KS**, Sumandea MP. PKC-dependent phosphorylation of cTnT. Gill Heart Cardiovascular Research Day, University of Kentucky. Lexington, KY. 13 October 2007. Abstract and Poster.
17. **Vyas KS**, García-Cazarín ML, Sumandea MP. Altered Phosphorylation of cTnT and cTnI in SHR Myocardium. Gill Heart Cardiovascular Research Day, University of Kentucky. Lexington, KY. 13 October 2007. Abstract and Poster.
18. **Vyas KS**, García-Cazarín ML, Sumandea MP. Altered Phosphorylation of cTnT and cTnI in SHR Myocardium. Showcase of Undergraduate Scholars, University of Kentucky. Lexington, KY. April 2008. Abstract and Poster.
19. **Vyas KS**, Sumandea MP. PKC-dependent phosphorylation of cardiac troponin T. Showcase of Undergraduate Scholars, University of Kentucky. Lexington, KY. April 2007. Abstract and Poster.
20. **Vyas KS**, García-Cazarín ML, Sumandea MP. Altered Phosphorylation of cTnT and cTnI in SHR Myocardium. Joint Meeting of the Biophysical Society 52nd Annual Meeting & 16th International Biophysics Congress (IUPAB). Long Beach, CA. February 2008. Abstract and Poster.
21. **Vyas KS**, García-Cazarín ML, Sumandea MP. Altered Phosphorylation of cTnT and cTnI in SHR Myocardium. National Conference on Undergraduate Research, Salisbury University. Salisbury, MD. April 2008. Abstract and Poster.
22. Bozio C, **Vyas KS**, Sumandea MP. Novel Protein Mechanisms in Heart Failure. Posters at the Capitol, Capitol Building. Frankfort, KY. January 31, 2008. Abstract and Poster.

Oral Presentations

1. **Vyas KS**, Vasconez HC. Regulatory Issues Regarding Fat Grafting. Kentucky Medical Association and Kentucky Society of Plastic Surgeons. Louisville, KY. 1 Sept 2015. Lecture.
2. Vasconez HC, **Vyas KS**. The "Mutton Chop" Flap Revisited. Southeastern Society of Plastic and Reconstructive Surgeons. Amelia Island, Florida. 6 June 2015. Lecture.
3. Lynch MP, **Vyas KS**, Vasconez HC. The Effect of Obesity on Adipose-Derived Stem Cells. Southeastern Society of Plastic and Reconstructive Surgeons. Amelia Island, Florida. 6 June 2015. Lecture.
4. **Vyas KS**, Lynch MP, Vasconez HC. The Effect of Obesity on Adipose-Derived Stem Cells. Kentucky Medical Association and Kentucky Society of Plastic Surgeons. Louisville, KY. 16 Sept 2014. Lecture.

5. **Vyas KS**, Saha SP. Mediastinoscopy: Trends and Practice Patterns in the United States. Cardiothoracic Surgery Research Colloquium. Lexington, KY. 18 Feb 2014. Lecture.
6. **Vyas K**, Stratton T, Soares N. Medical Student Health and Wellness. Introduction to Clinical Research Summer Workshop. University of Kentucky. Lexington, KY. 25 July 2013. Lecture.
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