



Review

Stem cells and adipose tissue engineering

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Abstract

A large proportion of the plastic and reconstructive surgical procedures performed each year are to repair soft tissue defects that result from traumatic injury, tumor resection, and congenital defects. These defects typically result from the loss of a large volume of adipose tissue. To date, no ideal filler material which is successful in all cases has been developed. Additionally, the success of using autologous fat tissue grafts to repair soft tissue defects has been limited. Researchers are thus investigating strategies to engineer volumes of adipose tissue that may be used in these cases. A necessary component for engineering a viable tissue construct is an appropriate cell source. Attempts to engineer adipose tissue have involved the use of preadipocytes and adipocytes as the base cell source. Increased interest surrounding the research and development of stem cells as a source of cells for tissue engineering has, however, led to a new path of investigation for developing adipose tissue-engineering strategies. This manuscript serves as a review of the current state of adipose tissue-engineering methods and describes the shift toward tissue-engineering strategies using stem cells.

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Keywords: Adipose tissue; Soft tissue reconstruction; Stem cells; Tissue engineering

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1. Introduction

A soft tissue defect is generally defined as a large tissue void within the subcutaneous fat layer of the skin that often results in a change in the “normal” tissue contour [1].

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Restoration of natural tissue function is not often the primary goal in reconstruction; rather, restoration of the soft tissue aesthetic function is targeted in order to minimize the anxiety and negative psychological feelings associated with disfigurement. Millions of plastic and reconstructive surgical procedures are performed each year to repair soft tissue defects that result from traumatic injury (i.e., significant burns), tumor resections (i.e., mastectomy and carcinoma removal), and congenital defects [2]. The American Society of Plastic Surgeons reported that over 5 million reconstructive procedures were performed in 2004, approximately 4 million of which were due to tumor removal [3]. Strategies to repair soft tissue defects, e.g. breast reconstruction procedures, collagen injections, and the use of autologous tissue transfers (i.e. free fat tissue grafts and tissue flaps) [4,5], include the use of implants and fillers [6,7]; however, there is likely no single filler material that will satisfy all clinical needs. Excess amounts of adipose tissue are found all over the human body, and may be readily obtained through liposuction and transplanted to a target location. The use of autologous fat tissue to repair soft tissue defects is logical in its approach, but the use of this method has not been consistently successful in patients [1,8–10]. When autologous fat tissue is transplanted from one location to the defect site, the common occurrence is significant resorption of the transplanted tissue over time, resulting in 40–60% of the graft volume loss. One proposed reason for tissue resorption is lack of sufficient revascularization of the tissue following transplantation to a new location [1,8,11]. The fat grafts never acquire sufficient vascularity, so centralized graft blood flow is not adequate for long-term survival of the tissue, and often leads to tissue resorption [10]. This insufficient tissue vascularization limits the supply of oxygen and nutrients to the tissue, limiting the chances for long-term tissue viability [12]. Tissue-engineering strategies are thus being investigated to develop methods for generating adipose tissue.

The primary goal of tissue engineering is to regenerate healthy tissues or organs for patients in need, thus eliminating the need for tissue or organ transplants and mechanical devices. With organ and tissue transplants, immunological rejection is often a primary concern for patients receiving donated tissues. Tissue-engineering strategies are suggested to eliminate these concerns [13]. Specifically, healthy cells taken from a patient may be cultured in a laboratory to attain a larger number of healthy cells. These cells may then be seeded onto a scaffold that will support cell growth and proliferation. The cell-covered scaffold may then be implanted into the patient at the needed site. As the cells grow, the scaffold material degrades or absorbs, and ultimately, a new tissue mass remains [14,15]. In this method of scaffold-guided tissue regeneration, scaffolds are used as support structures that provide a surface for cells to adhere to, and that provide a shape for the tissue that the construct is mimicking [8,16]. Tissue-engineering methods are being

used to develop a wide range of tissues, including bone, skin, cartilage, vascular, and adipose tissues [17]. The development of adipose tissue-engineering strategies will be essential in the restoration of tissue at soft tissue defect sites.

2. Adipose tissue engineering

The development of a clinically translatable method of engineering adipose tissue for soft tissue reconstruction requires investigation of several components. There must be coordination between all key aspects of the tissue engineering process, including the selection of cell source, scaffold material, cellular environment, and means of device delivery in order for the engineering of any tissue to be successful. This review focuses specifically on the evaluation of the cellular aspect of engineering adipose tissue, which in turn influences the selection of appropriate scaffolding materials.

2.1. Cellular components of adipose tissue

Adipose tissue is a highly specialized connective tissue found in two forms: white and brown [18]. Both of these forms serve to insulate and cushion the body, but they each have specialized function as well. Brown adipose tissue is termed such because of its color, attributed to its high vascularity. Brown adipose tissue functions primarily as a heat source in the body. As the aging process occurs, the brown adipose tissue is gradually replaced by white adipose tissue, whose primary function is to provide an energy source for the body [19]. The primary cellular component for adipose tissue is a collection of lipid filled cells known as adipocytes that are held in place by collagen fibers. Cytoplasm in the mature adipocyte contains approximately 90% lipid [11]. Other cellular components contained in adipose tissue are stromal-vascular cells including smooth muscle cells, endothelial cells, fibroblasts, blood cells, and preadipocytes [18,20]. While white adipose tissue is not as highly vascularized as its brown counterpart, each fat cell in white adipose tissue is in contact with at least one capillary, providing a vascular network that allows continued growth of the tissue [8,10,18].

Adipose tissue-engineering strategies have involved the use of transplantation of preadipocytes and adipocytes in order to restore the volume of tissue lost at defect sites. Indeed, the use of autologous fat tissue obtained through liposuction and aspiration procedures has been shown to be largely unsuccessful in restoring tissue volume due to insufficient angiogenesis of the transplanted tissue. The lipid filled cytoplasm of the adipocyte is susceptible to damage during aspiration procedures [11,21,22]. This procedure induced damage results in a large cell population that will not retain the desired cell volume *in vivo* [10]. Mature adipocytes in culture have also been shown to be limited in their proliferative capabilities, and are not readily expandable. Their limited growth capacity is attributed to their

terminally differentiated state, which further discourages their use in tissue-engineering methods [8,11,22].

Preadipocytes, precursor cells committed to the adipocyte lineage, are contained within the stromal-vascular fraction of enzymatically digested tissue. These cells are fusiform or fibroblast-like in appearance before they are differentiated [10,11]. Differentiation of these cells results in morphological, and biochemical changes where the cells become rounded in shape, and begin to accumulate triacylglycerol and lipid vacuoles [8,22]. Preadipocytes are deemed more advantageous as a cell source than mature adipocytes because they are easily cultured, easily expanded, and easily obtained. They are capable of both proliferating to obtain larger cell numbers, and of differentiating to obtain the tissue of interest [21,23–25].

2.2. Current adipose tissue-engineering strategies

Numerous strategies to engineer adipose tissue have been investigated. Several of these strategies, further discussed here in this text, are summarized in Table 1. These strategies have commonly involved seeding preadipocyte cells on polymer scaffolds made of materials such as polyester based absorbables [26–31], hyaluronic acid [23,32,33], collagen [5,34,35], polyethylene glycol (PEG) [36], and chemically modified alginate [37,38]. The shape of the scaffolds used in these studies varied from polymer disks, sponges, foams, and injectable microspheres.

Traditional research strategies for adipose tissue engineering may be observed through the work of Patrick and coworkers. Preadipocytes and capillary endothelial cells are isolated through enzymatic digestion of fat obtained from patients during liposuction or fat biopsy [8,10]. The preadipocytes are cultured with the appropriate growth factors on a polymeric scaffold. The concept is that the scaffold would then be implanted into the breast envelope of the patient to fill the defect site. Ideally, as the scaffold

remodels or absorbs, the preadipocytes would grow, proliferate, and eventually mature into adipose tissue. Differentiation of preadipocytes into mature adipocytes was demonstrated using this method, specifically isolating preadipocytes from the epididymal fat pads of Sprague-Dawley and Lewis rats. Adipocyte production was identified through the use of histological staining and fluorescence labeling, and the cells, seeded on PLG scaffolds, demonstrated viability post implantation for extended periods of time [9,26,27,39]. This general process is commonly used for preadipocyte-based methods of adipose tissue engineering, regardless of the material being used for formation of the scaffold.

Variations of this process could include surface modifications to the materials of interest in an effort to increase cellular adhesion and implant biocompatibility. Specifically, for adipose tissue-engineering strategies, the surfaces of polymeric scaffolds may be chemically altered by incorporating cell adhesion molecules, such as arginine–glycine–aspartic acid (RGD) peptides [1,37,40,41] or tyrosine–isoleucine–glycine–serine–arginine (YIGSR) peptides, which bind fibronectin and laminin, respectively [8,22,36]. The addition of binding sites on a scaffold surface will enhance the attachment of preadipocyte cells, thereby enhancing growth and proliferation of the cells as well. Additionally, specific mechanical properties of the designed scaffolds may be altered to enhance the performance of the tissue-engineered construct. Scaffolds for adipose tissue engineering have typically been designed for restoration of tissue volume, as opposed to the restoration of tissue function. As a result, the scaffolds would ideally restore the aesthetic function of the tissue by imparting a soft, smooth feel closely resembling that of natural tissue. The rigidity and stiffness of scaffolds used for adipose tissue engineering are therefore properties that must be considered. An ideal scaffold would meet the aesthetic requirements while also providing a surface that will still allow and promote

Table 1
Current adipose tissue engineering strategies

Proposed strategy	Description	References
Scaffold guided tissue regeneration	Preadipocyte cells cultured on absorbable polymeric scaffolds and implanted <i>in vivo</i> such that simultaneous cellular proliferation and scaffold resorption results in mature adipose tissue	[5,8–10,23,26–39]
Injectable composite system	Injectable microcarrier beads combined with a hydrogel delivery medium to form a minimally invasive implant that will stimulate regeneration of host adipose cells and fill a soft-tissue void upon injection <i>in vivo</i>	[31,34,35,50–52]
Fragmented omentum based-tissue regeneration	The highly vascularized tissue of the omentum fragmented and combined with preadipocyte cells such that implantation <i>in vivo</i> results in a tissue mass consisting of high triacylglycerol content	[53]
<i>De novo</i> adipogenesis	A stimulus, such as appropriate growth factors, applied <i>in vivo</i> induces the migration of preadipocytes to the implant site. The cells subsequently proliferate and differentiate to form adipose tissue depots	[54–58]

Several strategies have been proposed and investigated in an attempt to develop successful adipose tissue engineering methods. These methods have consisted of using cellular and polymeric-based scaffolds to support adipose cell growth, and the formation of tissue to fill a void or defect site.

cellular attachment. Synthetic polymers that can be produced under controlled conditions may be advantageous for tissue engineering applications because uniform batches of materials that can be altered chemically or structurally will provide a means of forming application-specific scaffolds tailored to a particular application [14,22,42,43]. Additionally, the surface topography of the pores within a scaffold may be altered to influence cellular behavior. Pore size and shape have been shown to effect cellular attachment by providing formed binding sites that cells may grow into. In the case of adipose tissue engineering, when seeding preadipocyte cells on porous scaffolds, the pores would ideally be large enough so as not to inhibit the proliferation and differentiation of preadipocytes. As preadipocytes mature, they significantly increase in size due to an increase in cell number and an increase in volume associated with lipid formation [5,23,32,44–49]. Adequate sizing of pores is essential in scaffold design for adipose tissue engineering.

Both preadipocytes and adipocytes are anchorage-dependent cells that must be seeded on an appropriate scaffold surface that provides traction for cell differentiation and proliferation to occur. Because preadipocytes and adipocytes are generally function limited when embedded directly in a gel and because a minimally invasive reconstructive option is advantageous to a patient, Burg and co-workers proposed and tested an injectable composite system [50,51]. The system is comprised of cells seeded on biodegradable beads of an injectable size; these cellular constructs are then mixed with a hydrogel delivery medium, resulting in a composite that may be injected into a patient through a syringe at the defect site. *In vitro* studies using 3T3-L1 preadipocyte cells cultured on various surfaces showed that the selection of injectable bead chemistry and topography, with and without therapeutic components, may be used to influence cellular differentiation. Preadipocyte cells were cultured *in vitro* on highly porous gelatin beads and relatively smooth polylactide beads. Assays to measure lipid production and to evaluate the genes expressed by the cells revealed that the scaffold material and/or surface topography did influence cell attachment, lipid production, and gene expression [34]. *In vivo* studies in several large animal models have demonstrated efficacy of the composite system. Implantation of cellular and acellular injectable composite constructs within a bovine animal model revealed that the selected bead materials were biocompatible and capable of supporting adipose cell growth [31,35]. Burg and coworkers have suggested the concurrent use of absorbable tissue expanders as temporary “space fillers” to allow the injection of composite cellular systems and thus facilitate the serial development of breast tissue in large defects [52]. Potentially, an initial injection of composite cellular systems would be made at the defect site within the patient. With the use of biodegradable or absorbable beads, it is assumed that some volume will be lost once the beads resorb. Following this slight volume loss, subsequent injections of additional

cellular composites may be made at the defect site, restoring the volume once again. This process would continue until the original volume was completely restored.

Another novel strategy that has been investigated in an attempt to engineer adipose tissue involves the use of fragmented omentum [53]. The omentum extends from above the stomach (lesser omentum) to below the transverse colon and small intestine (greater omentum) and serves to cover and support various abdominal organs. The omentum is highly vascularized and filled with adipose tissue, both ideal characteristics for engineering adipose tissue [53]. In studies conducted by Masuda and coworkers, fragmented omentum tissue was combined with preadipocytes *in vivo*. Results following implantation indicated that the omentum implanted with preadipocytes had the ability to form a tissue mass with high triacylglycerol content, indicative of fat [53].

Other methods of adipose tissue engineering are based on the use of acellular tissue-engineering devices. Here, an appropriate stimulus, applied *in vivo*, is able to induce the migration of preadipocytes, which subsequently proliferate and differentiate into mature adipocytes. This *de novo* adipogenesis has been demonstrated using subcutaneous injections consisting of Matrigel (a collagen-based gel derived from the basement membrane of a murine tumor) with basic fibroblast growth factor (bFGF) [54–57]. Within the implantation period, a visible fat pad was formed at the injection site, likely attributable to preadipocyte and endothelial cell migration to the injection site. Other attempts using *de novo* tissue-engineering principles have included coinjections of photocured styrenated gelatin microspheres (SGMs) with bFGF or insulin and insulin-like growth factor I (IGF-1), factors that stimulate both angiogenesis and adipogenesis [54,56,58]. Implantation studies using this method resulted in the formation of fat pads *in vivo*, however the details must be further investigated to improve methods for maximizing the quantity of tissue formed [58]. Several other growth factors including glucocorticoids such as dexamethasone, thyroid hormone, epidermal growth factor (EGF), transforming growth factor- β (TGF- β), and platelet-derived growth factor (PDGF) have been shown to positively influence adipogenesis. The addition of such factors to culture mediums may accelerate the rate at which proliferation of preadipocytes occurs *in vitro*, thereby accelerating the rate of differentiation [9,55,59–61]. Secretion of these factors *in vivo* may influence the rate at which mature adipose tissue forms within the body as well, aiding in further development of a successful tissue-engineering construct.

For the previously described methods for engineering adipose tissue, one common factor in each method was a cellular base for the specific method. One of the basic requirements for developing a suitable tissue replacement is an adequate source of viable cells that will stimulate the growth and formation of new, healthy tissue. Specifically, the ability to engineer an adipose tissue graft that will remain viable and that will retain its volume once

implanted is essential. Though several strategies using polymeric materials seeded with preadipocyte cells have been shown to induce adipose tissue formation *in vivo*, one noted shortcoming with these strategies has been delayed growth in volume of the tissue constructs, which has been attributed to slow vascularization of the tissue [53].

Current methods for neovascularization, or the formation of a new vascular network, within a tissue-engineered construct are limited. Methods for adipose tissue angiogenesis include inducing vessel formation with co-cultures of adipose cells and endothelial cells in tubular scaffolds. Growth factors aid in the induction of capillary formation, however, the vessels are not long lasting [8,11]. The creation of vasculature prior to implantation allows the tissue to receive nutrients faster because it does not have to form its vasculature, however, the tissue may still only receive minimal nutrition [62]. Additional methods for vascularization include revascularization of the implanted material, whereby vessels from the host tissue grow into the tissue site. This is often limited because the diffusion of nutrients is minimal to the center of an implanted graft [8]. Further research investigating mechanisms to induce neovascularization is ongoing. The relationships between certain vascular growth factors and stromal cells must be understood to progress towards creating a viable graft. The potential to create a vascularized construct is great considering the advances in cellular research [8,63].

Stem cells, which have the ability to differentiate into various cell types, may provide an ideal source of cells for engineering adipose tissue. The ability of stem cells to differentiate to several tissue types will mean fewer culture medium requirements, since the base medium for the cells will essentially be the same. Additionally, the capacity of stem cells to differentiate into endothelial cells and adipocytes upon receipt of the proper stimuli may be advantageous for developing a vascularized fat graft with sustained and increased volume for reconstructive purposes.

3. Stem cells as a cell source for tissue engineering

Different cell types that could be used for repair and regeneration include mature cells obtained from the patient or stem cells (either adult or embryonic) [64,65]. The use of

mature cells obtained from the patient minimizes the need for immunosuppressive therapy after implantation, but these cells may not be the best source of cells for tissue regeneration, primarily because these adult cells have already differentiated and committed to a specific cell type. This option provides little potential for further growth and limits the source of harvested tissue for repair to the site of the initial damage [64]. Stem cells, on the other hand, are by definition a population of cells able to provide replacement cells for a specific differentiated cell type [66]. These unique cells are different from other cell types in three defined respects. First, stem cells are able to divide and renew themselves over long periods of time [65,67–69], and are able to replicate or proliferate several times. By virtue of their ability to self-replicate, stem cells are said to be self-renewing [65,70]. Secondly, stem cells are not specialized and they are immature, meaning that they do not have any tissue specificity and are not required to perform specialized, tissue-specific functions. Third, stem cells differentiate into specialized cells [65,67]. Stem cells are capable of differentiating into at least one type of specific cell. How potent a stem cell is, or how many different cell phenotypes it can differentiate into (also known as stem cell plasticity) [67,71] may be described using several terms to classify stem cells. Stem cells may be defined as either totipotent, pluripotent, or multipotent whereby the stem cell is able to form all, most, or a small number of cells and/or tissues of an organism, respectively. Additionally, stem cells capable of forming the blood cells of the body are defined as hematopoietic stem cells [65].

3.1. Stem cell sources and applications

There are several potential sources for obtaining stem cells for tissue regeneration or repair purposes. These sources are summarized in Table 2, and the most commonly used cell types, adult and embryonic, are outlined in the following sections.

3.2. Embryonic stem cells

Embryonic stem cells are pluripotent stem cells that are harvested from the inner cell mass of the pre-implantation

Table 2
Types of human stem cells

Stem cell type	Source	Cell lineages produced
Embryonic	Embryonic tissue	All types
Mesenchymal	Bone marrow, adipose tissue	Osteogenic, adipogenic, chondrogenic, myogenic, neurogenic, and marrow stromal
Hematopoietic	Bone marrow	Blood cells (red, white, platelets), endothelial, muscle, immune system lineages
Neural	Brain	Neurons, astrocytes, oligodendrocytes, blood cells

Various sources for stem cells include embryonic tissue, bone marrow, adipose tissue, and the brain. Each stem cell type has been shown to have the capacity for differentiating to cell types of multiple lineages [66,75].

blastocyst (3–5-days-old embryo), and have been obtained from mice, non-human primates, and humans [64,72]. Those embryonic stem cells obtained from mice are able to remain unspecialized when they are cultured along with leukemia inhibitory factor (LIF) [72]. The *in vitro* isolation of human embryonic stem cells involves transferring the inner cell mass of the blastocyst to a culture medium that is supplemented with a feeder layer [73] of mice embryonic fibroblast cells that prevents cellular differentiation in human embryonic stem cells. After a period of about 6 months, the embryonic stem cells, which have not differentiated even after all of this time, may be referred to as a complete embryonic stem cell line [64,65,72,74]. When the cells begin to aggregate, they form embryoid bodies and are no longer undifferentiated or unspecialized cells. Cells may differentiate spontaneously, but it is preferred to manipulate them so that specific cell types are formed. The potency of these cells indicates that they are capable of producing a large range of specific phenotypes including blood cells, neural cells, adipocytes, muscle cells, and chondrocytes, among others [64,65,72]. The ability of researchers to efficiently manipulate embryonic stem cells to differentiate into specifically directed cells will provide means of an unlimited supply of cells that may be used, not only for the growth of implantable tissues, but also for testing new drugs to cure diseases, and in the identification of potentially problematic genes [65,70,72,75,76].

The use of embryonic stem cells in all areas of biomedical research has been met with opposition due to large controversy surrounding the sources from which these cells are obtained. The debate surrounding the use of stem cells involves government officials, religious affiliates, members of the scientific community, and members of the general public, all of whom have very decisive views on the use of these cells [77,78]. Opponents of embryonic stem cell use most often question the morality of using cells obtained from destroyed embryos, an act they consider equivalent to destroying human life [79,80]. Current research has been directed towards developing methods that will permit the use of embryonic stem cells while eliminating the ethical concerns surrounding their use. Methods to produce human embryonic stem cells without destroying embryos were proposed in the President's Council on Bioethics report in 2004 [81]. Two of these methods were further investigated in individual proof of concept studies [82–84] which gleaned promising results. Questions, however, still point to the ethical use of these methods for many involved in these debates [83]. The use of stem cells derived from adult tissues, and not those derived from embryonic tissues, avoids many of the ethical concerns that may arise from the use of stem cells in research applications.

3.3. Adult stem cells

Adult stem cells, also referred to as somatic stem cells or mesenchymal stem cells, are those mature, adult cells that

are undifferentiated and found in a specific tissue or organ. These cells are self-renewing, and are able to differentiate into major specialized cell types that serve to maintain the integrity of and repair the tissues in which they are found [64,65,85]. Mesenchymal stem cells may undergo self-renewal for several generations while continuing to maintain their specific cell characteristics. Mesenchymal stem cells are multipotent cells that are easily isolated, easily cultured, and readily expanded in the laboratory setting. All of these attributes make mesenchymal stem cells an attractive cell source for use in several clinical applications [75], including cell-based therapies for treatment of diseases such as Parkinson's and Alzheimer's diseases, spinal cord injuries, burns, heart disease, and osteoarthritis, among other conditions [65]. These adult stem cells typically include hematopoietic stem cells, neural stem cells, bone marrow stromal cells, dermal stem cells, and fetal cord blood stem cells among others.

Bone marrow contains hematopoietic stem cells and it is also the most recognized source of mesenchymal stem cells. Stem cells obtained from bone marrow are found in the stroma of the marrow. These cells are multipotent, and are therefore able to differentiate into lineages of cells such as adipocytes, osteocytes, myocytes, tenocytes, and neural cells [66,70,75,86–90]. These cells are typically obtained from bone marrow aspirates from marrow transplant donors. When cultured *in vitro*, bone marrow stem cells exhibit a fibroblast-like morphology. Marrow stromal cells have been studied and, to date, certain cell surface markers have been identified that are useful in cell selection and determination of preparation of marrow stem cell populations [86]. In addition to their ability to differentiate into multiple cell lineages, the use of marrow stem cells is advantageous because they offer a source of cells that is isolated and expanded *in vitro* with relative ease. The number of cells may be significantly increased by sub-culturing a small sample of donor tissue [75,86].

In addition to bone marrow, adipose tissue has been identified as a source of multipotent cells that have the capacity to differentiate to cells of adipogenic [91–96], chondrogenic [88,89,91–94,97–104], myogenic [91–94], and osteogenic [28,91–94,96,105,106] lineages when cultured with the appropriate lineage specific stimuli, as shown in Table 3 [24,107–110]. Adipose-derived stem cells (ADSCs) may be obtained from tissue harvested through liposuction (termed processed lipoaspirate cells (PLAs)), or through abdominoplasty procedures. These cells have also been identified as mesenchymal cells because they are derived from adipose tissue which is, in turn, derived from mesenchyme, much like bone marrow [92]. ADSCs have been shown to be very similar to marrow-derived stem cells in morphology and phenotype [111]. In addition to their common multipotency, several CD marker antigens found on the surface of marrow stem cells have been found on the surface of ADSCs [92,96,107,108]. Several common stem cell surface markers are summarized in Table 4. ADSCs are advantageous for tissue-engineering applications because

Table 3
Multilineage potential of adipose-derived stem cells

Cell lineage	Culture medium	Differentiation stimulants	Lineage determinants
Adipogenic	DMEM + 10% FBS	Insulin, IBMX (3-isobutyl-1-methylxanthine), dexamethasone, Indomethacin	Lipid accumulation apparent by positive Oil Red O stain
Chondrogenic	DMEM + 1% FBS	Insulin, TGF- β 1, Ascorbate-2-phosphate	Sulfated proteoglycan rich matrix detected with Alcian Blue stain; synthesis of collagen II detected by immunostain with collagen II-specific antibody
Myogenic	DMEM + 10% FBS + 5% HS	Dexamethasone, hydrocortisone	Identification of multinucleation with light microscopy; expression of skeletal muscle myosin heavy-chain and MyoD1 expression identified with myosin- and MyoD1-specific antibodies
Osteogenic	DMEM + 10% FBS	Dexamethasone, ascorbate-2-phosphate, β -glycerophosphate	Alkaline phosphatase activity apparent by specific stain; production of calcified matrix apparent with von Kossa stain

Stem cells derived from adipose tissue have demonstrated the capacity to differentiate to cells of multiple lineages, upon receipt of the appropriate chemical stimulus. Normal cell culture mediums for each pathway included Dulbecco's modified eagle medium (DMEM) supplemented with fetal bovine serum (FBS) or horse serum (HS), and the identified stimulants to induce differentiation. Differentiation of the cells to adipo-, chondro-, myo-, or osteogenic lineages may be characterized using lineage specific histological or immunological assays [91–93,108,110].

they are largely available. Adipose tissue is often available in an abundant, expendable quantity. It is also easy to harvest, in contrast to marrow stromal cell extraction which results in significant pain [24,108]. ADSCs are limited, however, by several factors. First, ADSCs have not been classified as immortal. ADSCs display obvious signs of “old age”, thus limiting their capacity for subculturing. Additionally, adipose tissue is known to vary in metabolic activity and in its capacity for proliferation and differentiation, depending on the location of the tissue depot and the age and gender of the patient [108,112].

3.4. Stem cells for engineering adipose tissue

Stem cells derived from adipose tissue and bone marrow show great promise as an alternate source of cells for adipose tissue engineering. These stem cells can be easily obtained, easily purified, and are readily expanded in culture. Stem cells offer a potentially unlimited source of cells for tissue engineering; thus, research in using stem cells to produce adipose tissue has become increasingly popular [113–115]. The use of stem cells not only potentially provides an unlimited supply of cells, but also increases the ability of researchers to define and control cellular constituents. And, if adult stem cells are used, the patient's own cells may be used, thus eliminating other biocompatibility complications [116].

Attempts to engineer adipose tissue are most commonly based on the use of adipose cells obtained from samples of healthy, mature adipose tissue. Preadipocytes, found in the stromal-vascular fraction of adipose tissue, are able to differentiate to mature adipocytes. These cells, however, following elevated numbers of passages, lose their capacity to differentiate [117]. The discovery of a population of stem cells contained within adipose tissue has provided an

alternative source of cells from which adipose cells may be obtained. Rodriguez and coworkers have investigated methods to culture human multipotent adipose-derived stem cells (hMADS) in serum-free conditions that will maintain the adipogenic potential of these multipotent cells. These hMADS were shown to maintain their ability to undergo adipogenesis over 160 population doublings, which could prove significant when culturing cells for formation of an adipose tissue-engineered construct [117]. Toward developing adipose tissue-engineering strategies, the use of ADSCs, differentiated to adipocytes, may provide an alternate cell source with high proliferation capacity that would be essential in the clinical setting.

One of the first steps towards developing successful adipose tissue-engineering strategies is garnering an understanding of adipogenesis, and identifying a cell source for use in these methods. Evaluating stem cell behavior and identifying factors that affect their differentiation in strategies for adipose tissue engineering are areas where questions must be answered. In a study conducted by Cui and coworkers, adipogenesis was induced in D1 cells, a murine bone marrow stromal cell line [118]. The D1 cells were treated with the steroid dexamethasone, which caused the cells to produce triglyceride-containing vesicles, and to express the 422 (aP2) gene, which is indicative of adipocyte differentiation [118]. The results of the study indicated that the dexamethasone stimulated the cells to differentiate to adipocytes [118]. Further investigation into the properties of this steroid as well as other biochemical and physicochemical factors affecting stem cell differentiation could potentially lead to more efficient methods of generating adipose tissue.

Additionally, a study conducted by Xiong and coworkers demonstrated the ability of human embryonic stem

Table 4
Common stem cell surface markers [124]

Surface marker	BMS cell expression	ADS cell expression	References
CD9 (tetraspan)	+	+	[92,94,96,97,107,110,125]
CD10 (CALLA)	+	+	[92,96,97,107,110,125]
CD11 (α -integrin)	+	–	[86,110]
CD13 (aminopeptidase)	+	+	[92,96,97,107,110,125]
CD14	–	–	[75,92,104,110,111]
CD18 (β -2 integrin)	–	–	[110]
CD29 (β -1 integrin)	+	+	[92,94,96,97,107,110,111,125,126]
CD31 (PECAM-1)	–	–	[92,107,109,110,125]
CD34	–	±	[75,92,96,97,104,107,109–111,125–127]
CD44 (hyaluronate receptor or phagocytic glycoprotein-1)	+	+	[75,92,96,97,109,110,126]
CD45 (LCA)	–	–	[75,92,104,107,109,110,125,126]
CD49d (α -4 integrin)	–	+	[92,96,97,107,109,110,125]
CD49e (α -5 integrin)	+	+	[92,96,97,107,125]
CD50 (ICAM-3)	–	–	[75,110]
CD51 (α -V integrin)	±	+	[124]
CD54 (ICAM-1)	+	+	[92,96,97,107,110,125]
CD55 (DAF)	+	+	[92,96,97,107,110,125]
CD56 (NCAM)	–	–	[92,110]
CD59 (complement protectin)	+	+	[92,96,97,107,110,125]
CD61 (β -3 integrin)	±	±	[124]
CD62 (endothelial-selectin)	–	–	[92,110]
CD71 (transferrin receptor)	+	+	[92,110,126]
CD73 (5' nucleotidase)	–	+	[110]
CD90 (Thy-1)	+	+	[92,104,107,110,111,125,126]
CD104	–	–	[92,110]
CD105 (Endoglin)	+	+	[92,94,96,97,104,105,107,109–111,125,126,128]
CD106 (VCAM)	+	±	[92,96,97,104,107,109,110,125,126,128]
CD117 (c-Kit)	+	+	[92,107,125]
CD133 (MDR-1)	+	–	[81]
CD146 (Muc18)	+	+	[92,96,97,107,110,125]
CD166 (ALCAM)	+	+	[92,94,96,97,105,107,110,125]
α -smooth muscle actin	+	+	[75,110]
Collagen type I	+	+	[75,110]
Collagen type II	+	+	[75,110]
HLA-ABC	+	+	[86,110,126]
Osteopontin	+	+	[75,110]
Osteonectin	+	+	[110]
Vimentin	+	+	[75,110]
Factor VIII-related Ag	–	–	[110]
HLA-DR	–	–	[86,110]
Stro-1	+	±	[90,92,96,107,109,110,125,126,128]
CFU-F (colony forming unit-fibroblast)	+	–	[90]

The expression of several cell surface markers of bone marrow mesenchymal cells and adipose-derived stem cells has been investigated. The expression of some surface markers has been reported with differing outcomes in the literature. Those markers that have been reported positively or negatively expressed are denoted with (+) in the preceding table.

Abbreviations—BMS: bone marrow stem; ADS: adipose-derived stem; CALLA: common acute lymphocytic leukemia antigen; PECAM: platelet endothelial cell adhesion molecule; LCA: leukocyte common antigen; ICAM: intercellular adhesion molecule; DAF: decay accelerating factor; NCAM: neural cell adhesion molecule; VCAM: vascular cell adhesion molecule; ALCAM: activated lymphocyte cell adhesion molecule; HLA: histocompatibility locus antigens.

cells to differentiate into adipocytes during *in vitro* [119] culture. The use of human embryonic stem cells, particularly for formation of adipocytes has been limited. The researchers in this case used a WiCellH1 cell line, cultured in medium containing a peroxisome proliferator-activated receptor γ (PPAR γ) agonist. Assays specific for adipogenic markers were performed and indicated that the human embryonic stem cells indeed differentiated to adipocytes. The ability to induce these cells to differentiate into

adipocytes could provide great insight on questions that still exist regarding adipogenesis [119].

Only recently have more attempts been made to engineer adipose tissue using mesenchymal stem cells. Neubauer and coworkers obtained rat marrow stromal cells and exposed them to medium containing bFGF. The cells were cultured on porous polylactide-co-glycolide (PLG) scaffolds *in vitro*, and were evaluated to assess their level of adipogenic differentiation. Following culture, the cells exhibited a high

density of differentiated adipocytes within the scaffolds and high levels of glycerol-3-phosphate dehydrogenase (GDPH), proving this method promising [113]. Additionally, Hong and coworkers evaluated the use of human bone marrow stromal cells cultured on gelatin sponges for engineering adipose tissue [120]. The isolated cells were evaluated *in vitro* using a monolayer culture which demonstrated the adipogenic capabilities of the stromal cells after receiving a specific differentiation medium. *Ex vivo* culture of the cells on the gelatin sponges further demonstrated the ability of the cells to differentiate to form mature tissue. These studies also offer promising results; however, long-term evaluation of these constructs *in vivo* is essential to determining the overall effectiveness of this method [120].

Studies evaluating the potential of using bone marrow-derived mesenchymal stem cells for tissue engineering *in vivo* have been conducted as well. Alhadlaq and coworkers performed *in vitro* and *in vivo* studies using human mesenchymal stem cells encapsulated in photopolymerized poly(ethylene glycol) diacrylate (PEGDA) [121]. The constructs cultured *in vitro* successfully demonstrated a capacity for adipogenic differentiation. The *in vivo* samples were implanted within a subcutaneous pocket on SCID mice. Following the implantation period, RT-PCR experiments demonstrated the expression of adipocyte specific genes. Oil red O staining was also positive for the *in vivo* experimental samples, indicating the presence of lipid containing cells within the tissue samples [121]. Additionally, bone marrow mesenchymal stem cells derived from rabbits were used in an *in vivo* study conducted by Choi and coworkers [115]. Their study, however, used PLG microspheres as the cellular carrier. Cellular microspheres, cultured both with and without an adipogenic medium, were injected into nude mice for 2 weeks. Subsequent evaluation of the implanted samples revealed that new adipose tissue was evident in those samples cultured with the adipogenic medium, indicating that this method may be useful in developing a successful method for engineering soft tissue [115].

The work reviewed to this point has focused on using embryonic or adult stem cells to specifically form adipose tissue for soft tissue reconstruction purposes. Stem cells, particularly human ADSCs have demonstrated the capacity to differentiate to endothelial cells [122]. These cells have also been shown to secrete angiogenic factors such as vascular endothelial growth factor (VEGF) [123]. In considering strategies for adipose tissue engineering, it is necessary to consider methods for supplying the newly formed tissue with nutrients to maintain its viability, e.g., for engineering vasculature within the tissue. Several factors have to be considered before this may be accomplished, but it is logical that a co-culture of stem cells that could be differentiated to vascular cells after receipt of the appropriate stimulus, with cells of an adipogenic lineage, would be a possible first-step toward developing a vascularized adipose tissue graft that would

maintain its volume and viability for the duration of implantation *in vivo*.

4. Concerns and implications

As with all areas of research, there are specific areas of scientific concern to consider for furthering the development of methodologies. With the use of stem cells for tissue-engineering applications, there will be numerous concerns to address, including standardization of methods for tissue procurement, cell isolation, and cell culture. Currently, adipose tissue derived stem cells are obtained from liposuction aspirates or abdominoplasty procedures. The methods for harvesting the tissue may have an effect on the ability of the cells to proliferate and differentiate during *in vitro* culture, thereby introducing variability into the process of cell retrieval and culture for each tissue sample. Additionally, variability exists with the use of stem cells for tissue-engineering applications because, to date, no definitive adult stem cell markers have been discovered to ensure the purity of all stem cell populations [67]. The absence of such markers could potentially minimize the ability to reproduce populations of viable cells that are in fact multipotent stem cells.

Successfully engineering any tissue construct requires careful consideration of all aspects of the device. The material chosen for a particular scaffold is often selected based on the mechanical properties that are best suited to a specific application. Therefore, characteristics of stem cell biomaterial carriers will vary according to the particular application. Because the use of stem cells in tissue engineering is a research area in infancy, there is no clear method of determining how the cells, once cultured on biomaterials will behave. Cell–surface interactions will have to be further investigated to more fully understand the behavior of the stem cells in tissue-engineering applications.

5. Conclusion

The field of tissue engineering has significant potential for developing viable, natural tissue constructs. The primary basis for any tissue-engineered construct is the cellular source that is used to initiate new tissue growth. Preadipocytes and adipocytes have been the logical cell source for soft tissue-engineering reconstruction. The investigations of strategies that incorporate stem cells, however, have shown promising results for engineering soft tissue. The use of stem cells for tissue-engineering applications is still met with ethical concerns and scientific obstacles that must be addressed, but the potential progress that could be made towards developing a successful strategy for adipose tissue engineering should not be ignored.

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