Engineering Adipose Tissue for Regenerative and Reparative Therapies

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ABSTRACT

The correction or augmentation of soft tissue defects caused by trauma, tumor resection, congenital abnormalities, and aging presents a multitude of challenges in reconstructive surgery. Soft tissue defects run the gamut in terms of volume, from restoring the fullness of the face by removing wrinkles to restoring the breast mound after mastectomy. The limitations of current restorative and reparative techniques have served as drivers for the development of adipose tissue as an application area for tissue engineering. Tissue engineering is a multidisciplinary and maturing field that combines bioengineering, the clinical sciences, and the life sciences to repair or regrow tissues. This article discusses the inadequacies of current methods of correcting soft tissue defects and the innovative adipose tissue engineering strategies under pursuit to abrogate these limitations and improve patients' outcomes and quality of life, and speculates rationally on the future. It does not discuss the applications and technologies involved with adipose-derived stem cells unless directly applied toward adipogenesis.

KEYWORDS: Adipose tissue, biomedical engineering, breast reconstruction, soft tissue augmentation, tissue engineering, tissue scaffolds

Resections of various tumors of the head and neck, breast, soft tissue, and bone as well as trauma, congenital abnormalities, changes associated with aging, and a myriad of other medical conditions commonly result in contour deformities caused by defects in the dermis and underlying subcutaneous adipose tissue.[1–4] These can impair the aesthetic appearance, function, and psychological well-being of patients. Soft tissue restoration as a means of correcting these defects poses particular challenges for both the tissue engineer and the reconstructive surgeon.

The clinical strategy used to date to repair soft tissue defects is largely based on restoring the

Tissue Repair, Regeneration, and Engineering in Plastic Surgery; Guest Editors, C. Randall Harrell, M.D. and Eser Yuksel, M.D. *Seminars in Plastic Surgery*, Volume 19, Number 3, 2005. Address for correspondence and reprint requests: Charles W. Patrick Jr., Ph.D., Director of Research, Laboratory of Reparative Biology and Bioengineering, Department of Plastic Surgery, The University of Texas M.D. Anderson Cancer Center, P.O. Box 301402, Houston, TX 77231. [1]Laboratory of Reparative Biology and Bioengineering, Department of Plastic Surgery, Unit 602, The University of Texas M.D. Anderson Cancer Center, Houston, Texas. 1535-2188,p;2005,19,03,207,215,ftx,en; sps00171x.

missing volume with a natural, synthetic, or hybrid material. The first substances used as filler materials proved to be not only ineffective for soft tissue repair but also dangerous to the patient because of allergic reactions, local chronic edema, lymphadenopathy, scarring, ulcerations, and migration with associated reactive processes (i.e., oil granulomas).[5] Although many second- and third-generation materials are currently used clinically and new materials are being consistently developed, none has proved to be the appropriate material for soft tissue repair. Contemporary materials possess severe limitations including, but not limited to, unpredictable outcome, fibrous capsular contraction, allergic reaction, suboptimal mechanical properties, distortion, migration, and long-term resorption.[2] The ideal material for soft tissue restoration should possess the following attributes: nonallergenic, nonpyrogenic, produce no disease states, permit ambient temperature storage, look and feel natural, economical relative to standard of care, stable after implantation or injection, fully integrated or replaced by host tissue over time, and easy to use in the operating room and outpatient setting. For relatively small defects, the material should be versatile enough to be injected through small-gauge needles yet capable of being molded into a solid implant after injection.[6] These attributes should be employed as design constraints when developing the next generation of materials or reparative strategies, or both.

STANDARD OF CARE FOR SMALL-VOLUME SOFT TISSUE REPAIR

Numerous substances have been used in the last two decades to repair small soft tissue defects (Table 1).[2,3] Nonautologous materials are recognized as foreign and are degraded by collagenases and inflammatory cells. Repeated injections are required to "plump up" even the smallest of defects. Although allergic reactions occur rarely (3–5% incidence), hypersensitivity reactions are observed.[7,8]

Table 1 Small-Volume Soft Tissue Fillers

Autologous collagen (Autologen®)
Autologous fat
Autologous fibroblasts (Isolagen®)
Bovine collagen (Zyderm®, Zyplast®)
Calcium hydroxyapatite (Radiesse™)
Cross-linked polydimethylsiloxane
Fibril
Gelatin powder
Human tissue matrix (Dermalogen™)
Hyaluronic acid (Hylaform®, Hylaform Plus®, Restylane®)
Polylactic acid (Sculptura™)
Polymethylmethacrylate microspheres
Silicone fluid
Teflon paste

In addition, erythema and induration in the presence of circulating antibodies, granulomatous responses, and serum sickness–like illnesses have all been documented. Other synthetic materials are also associated with technical and regulatory difficulties. Allografts or homologous tissue grafts are not ideal because of the potential for viral transmission and potential immunogenic or allergic reactions. Autologous materials have been used as filler materials such as dermal cellular and extracellular matrix constituents and injectable fat. This requires, however, harvesting and processing the patient's tissues. In addition, logistical factors involving transport, preservation, and processing of tissues may limit the usefulness of autologous tissue.

Early on, autologous fat (i.e., mature adipose tissue) was investigated as a virtually limitless source of material for soft tissue repair. It is easily harvested, readily available, and most patients possess excessive amounts that can be harvested without producing significant contour defects. Despite the theoretical advantages, however, autologous fat transplantation has demonstrated poor results, with a 40 to 60% reduction in graft volume because of resorption.[9,10] Adipose tissue resorption is postulated to be related to insufficient revascularization and mechanical damage. Recent work has

demonstrated that microvascular endothelial cells play a critical role in protecting adipose cells from hypoxia.[11] Moreover, adipose tissue mass is governed by the formation of functional vasculature.[12] However, the interplay between adipogenesis and angiogenesis continues to be a poorly understood system. Some limited success has been obtained by using small-diameter "pearls" of fat tissue where mass transport occurs readily by diffusion.[13,14] However, this strategy is not viable for the majority of clinically sized defects. The advent of liposuction led investigators to attempt using single-cell suspensions of mature adipocytes. However, because adipocytes have a cytoplasm composed of 80 to 90% lipid, they tend to be traumatized by the mechanical forces of aspiration, resulting in approximately 90% damaged cells. The remaining 10% tend to form cysts or localized necrosis after injection. Moreover, mature adipocytes cannot be expanded ex vivo because they are terminally differentiated.

STANDARD OF CARE FOR LARGE-VOLUME SOFT TISSUE REPAIR

Breast reconstruction subsequent to tumor resection is perhaps the largest anatomical volume involving adipose tissue as a reparative strategy. Breast cancer is the second leading cause of cancer in women (after skin cancer), affecting one in every nine women in America and one in every three women with cancer. This underscores the important role played by breast reconstruction not only for cosmetic purposes but also for the healthy maintenance of self-esteem and body image.[15] Breast reconstruction is the sixth most common reconstructive procedure performed in the United States.[16] The usual indication is to restore the breast mound following complete removal of the breast performed for cancer treatment by either total or radical mastectomy. Postmastectomy reconstruction involves replacing missing skin and soft tissue volume to recreate the appearance of the breast. Because of the volume often involved and relative paucity of adequate fill, moderate to large breast tissue deficits can be particularly challenging to repair. Current methods use breast implants, soft tissue flaps, or a combination of these.[15]

Synthetic materials involved in breast reconstruction use a process known as tissue expansion to create additional skin first. An inflatable silicone tissue expander is placed submuscularly and is gradually inflated by the injection of physiological saline over weeks to months. When expansion is complete, the tissue expander is removed and is replaced with a permanent breast saline- or silicone-filled implant to provide the volume necessary for breast mound restoration. The disadvantages of permanent breast implants are that they may erode through the skin, become infected, form deforming scars, and cause physical or cosmetic complications related to fibrous capsule formation and internal contracture. They are also difficult to manipulate during shaping and may lead to unnatural cosmesis.[15]

Autologous tissue reconstruction commonly uses skin and fat from the lower abdomen in the form of a flap referred to as a transverse rectus abdominis musculocutaneous (TRAM) flap, although there are other autologous tissue sites such as the gluteal area. The TRAM flap procedure utilizes the rectus abdominis muscle along with the overlying skin and subcutaneous fat.[15] The tissue may be transferred with its blood supply intact by maintaining the superior attachment of the rectus abdominis muscle or by sacrificing the blood supply and performing microvascular anastomoses in the chest to the internal mammary blood vessels or in the axilla to the thoracodorsal blood vessels. The skin and fat can then be reshaped to take on the appearance of a breast. The disadvantages of the TRAM flap procedure are that it is quite extensive and requires surgery at areas other than the breast. This may lead to significant secondary comorbidity, such as scarring or alterations of otherwise normal areas and abdominal hernia. The desire to perform natural tissue reconstructions without the trauma of surgery or complications at the donor site provides the impetus for finding a

tissue-engineered soft tissue alternative that may be used in postmastectomy breast reconstructions.

TISSUE ENGINEERING STRATEGIES FOR SOFT TISSUE REPAIR

Tissue engineering strategies in general involve seeding cells and introducing appropriate tissue induction, differentiation, and maintenance factors in a three-dimensional natural, synthetic, or hybrid scaffold to develop "biological substitutes that restore, maintain, or improve tissue function."[2] Numerous scaffold materials, ranging from nonwoven fiber and hydrogel extracellular matrix structures to biodegradable polymers in the form of foams, nonwoven fibers, and hydrogels, are being investigated.[17] Scaffold materials must mechanically support and guide tissue formation, such as adipogenesis. Materials must also be biocompatible, biodegradable, and easily processed. Ideally, the scaffold should recapitulate the endogenous extracellular matrix as much as possible to facilitate tissue induction. Acellular tissue engineering constructs are implanted in patients and rely on recruitment of surrounding cells or are tailored to remain acellular, whereas cellular tissue engineering constructs either are grown ex vivo in sophisticated bioreactors and then implanted in the patient or are placed directly in vivo with the patient serving as a bioreactor. Each of these tissue engineering modalities is discussed subsequently.

Preadipocyte-Seeded Scaffolds

Preadipocytes, precursor cells that differentiate into mature adipocytes, can be seeded onto a scaffold and allowed to proliferate and differentiate to promote the formation of adipose tissue. Preadipocytes are a preferred cell source over mature adipocytes because they can be readily expanded in culture, are mechanically stable, and can be obtained from fat biopsies or liposuction aspirates.[18] Various materials are being investigated as potential scaffolds for adipose tissue growth. It should be noted that many experimental studies employ 3T3-L1 cells as a preadipocyte surrogate. The reader should be aware that 3T3-L1 cells are a mouse tumor-derived cell line and are not preadipocytes. Thus, limitations exist in interpreting data obtained from 3T3-L1 cells and translating the information to in vivo reparative adipogenesis.

Recently, poly(glycolic acid) (PGA) scaffolds reinforced with poly(L-lactic acid) (PLLA) and infused with human preadipocyte-seeded fibrin demonstrated in vivo adipogenesis for up to 6 weeks in mice.[19] In addition, PGA polymers seeded with 3T3-L1 cells demonstrated fat-like tissues up to 1 month in mice.[20] In earlier studies, rigid poly(L-lactic-co-glycolic-acid) (PLGA) polymer foams seeded with rat preadipocytes were implanted subcutaneously into male Lewis rats in an effort to generate de novo adipose tissue. Results from short-term (2–5 weeks) and long-term (1–12 months) studies demonstrated the successful formation of adipose tissue for up to 2 months.[21,22] The volume of generated adipose tissue then began to decrease and was completely resorbed by 5 months. The resorption of adipose tissue after 2 months may be due to factors such as a lack of adequate vascularization, lack of support structure after the PLGA degraded, the anatomical site of implantation not being conducive to long-term maintenance of adipose tissue, or a limitation related to the small animal model used. The long-term maintenance of generated tissue is a challenge for all tissue engineering applications.

Fluortex monofilament-expanded polytetrafluoroethylene (52 μm pore size) has also been studied in vitro as a potential scaffold for adipose tissue engineering.[23] Preadipocytes do not attach to uncoated polytetrafluoroethylene and, hence, human collagen, albumin, and fibronectin coatings were studied to optimize seeding efficiency. Fibronectin coating resulted in a significantly higher number of attached human preadipocytes than the collagen or albumin coatings. Human preadipocytes were able to proliferate and differentiate into

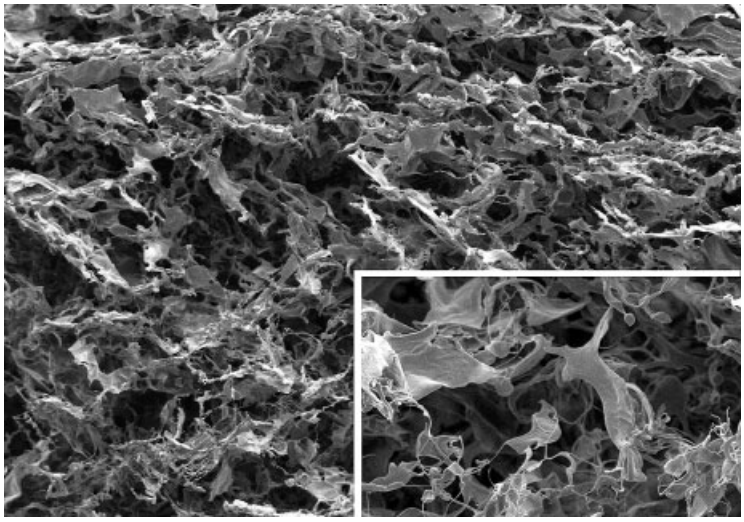

Figure 1 Scanning electron micrograph of a collagen-chitosan blend cross-linked with 0.2% glutaraldehyde. Image is 100× and inset is 500× magnification.

adipocytes on the fibronectin-coated expanded polytetrafluoroethylene in vitro over a period of 120 hours. In vivo studies have not been conducted. Recently, 3T3-L1 cells were shown to adhere to and lipid load on nondegradable fibrous polyethylene terephthalate scaffolds.[24]

In vitro adipogenesis has been demonstrated in porous gelatin sponges (Gelfoam).[25] In addition, Von Heimburg and colleagues have studied porous collagen scaffolds and two types of hyaluronan-based devices for adipose tissue engineering.[26,27] The collagen scaffolds are constructed on the basis of a directional solidification method followed by freeze drying to obtain a uniformly porous structure. The hyaluronic acid–based devices were manufactured into sponges or scaffolds composed of nonwoven fibers. The sponges demonstrated open, interconnecting pores ranging from 50 to 340 μm, and the nonwoven mesh had an interfiber distance of 100 to 300 μm. In vivo studies in mice showed that the hyaluronan sponge proved to be a better scaffold than the freeze-dried collagen construct or the hyaluronan nonwoven carrier because of the larger, interconnected pores. After 8 months, more adipocytes were found in the sponge than in the nonwoven mesh. The large pore size is important for the preadipocytes to incorporate lipids and enlarge during differentiation. Pore size was also found to be a factor in adipogenesis in several proprietary Johnson & Johnson biodegradable polymer sponges and nonwoven fibers.[28] In addition to single-component extracellular matrix scaffolds such as gelatin and hyaluronan, investigators are beginning to investigate the utility of multicomponent blends in an effort to tailor further material properties and cellular interactions (Fig. 1).

Synthetic polymer hydrogels are also actively being studied as potential tissue engineering scaffolds because of the ability to derivatize the polymers with bioactive functional groups and, thereby, control molecular and cellular function.[29–31] Hydrogels are viscoelastic polymeric structures that contain a significant volume fraction of water (usually $>90\%$). The three-dimensional polymeric structures of the hydrogel are held together primarily by cross-linking. Currently, hydrogels are being employed in biomedicine for controlled drug release, soft tissue augmentation, cell separations, and biosensors. In the tissue engineering arena, hydrogels can be used as nascent materials or they can be modified with bioactive peptides that aid them in mimicking cell adhesion properties of the extracellular matrix. Proteolytically degradable peptides can be incorporated into the backbone of the polymer to form hydrogels that are degraded by cell-secreted enzymes.[31] For instance, polyethylene glycol (PEG)

can be modified with the peptide sequence leucine, glycine, proline, and alanine (LGPA) to form a polymer degradable by collagenase.[31] Preadipocyte adhesion sites can also be coupled to PEG using the peptide tyrosine, isoleucine, glycine, serine, and arginine (YIGSR).[31] YIGSR is a cell binding peptide found on laminin. Patrick and Wu have shown that preadipocytes bind preferentially to laminin-1 and that cell adhesion to and migration on laminin-1 is mediated by the $\alpha_1\beta_1$ integrin.[32] The preadipocytes can be mixed into a PEG solution and then photopolymerized into a hydrogel. Moreover, the PEG hydrogels were shown to have material and rheological properties similar or superior to those of human subcutaneous adipose tissue.[30] Combining the degradable PEG with the polymer coupled with cell adhesion sites produces a synthetic hydrogel that has been shown to be adequate scaffold material in vitro. Alhadlaq and colleagues implanted PEG diacrylate hydrogels seeded with mesenchymal stem cells and demonstrated in vivo adipogenesis for up to 4 weeks in mice.[29] The clinical benefit of having a polymerizable hydrogel such as PEG is that the cell-polymer solution can easily be injected into the defect to be corrected and then photopolymerized in situ into a hydrogel. The need for complex surgical intervention would thus be eliminated.

Porous alginate material (a naturally derived hydrogel) has also been investigated as a construct for soft tissue engineering. Halberstadt et al modified the alginate material with the peptide sequence arginine, glycine, and aspartic acid (RGD) to allow cells to adhere to the construct.[33] In vitro studies demonstrated that the porous alginate-RGD material supported cell attachment, adhesion, and proliferation. Small animal studies performed over 6 months showed that the implanted material was also conducive to tissue ingrowth and did not elicit major inflammatory responses.[34] In a large animal model (sheep), the material was seeded with preadipocytes and injected into the nape of the neck. Well-defined adipose tissue was identified within the hydrogel at 1 and 3 months. Unfortunately, it cannot be determined whether the adipose tissue growth resulted from the previously seeded preadipocytes in the material or from resident preadipocytes.[33]

Collagen hydrogels have also been investigated as a three-dimensional biological matrix upon which preadipocytes are cocultured with human mammary epithelial cells to form tissue that closely resembles the normal human breast. Histologic analysis of the collagen gels from in vitro studies indicates a pattern of ductal structures of human mammary epithelial cells within clusters of adipocytes similar to the architecture of breast tissue. These findings indicate that there is a potential for breast tissue to be regenerated in vitro on a three-dimensional scaffold and later implanted for breast reconstruction purposes.

The majority of in vitro investigations have been conducted in static, two-dimensional culture conditions. Three-dimensional bioreactor systems are being developed to mimic the in vivo environment more realistically and to conduct in vitro studies over longer periods of time.[35] Seeding preadipocytes within batch and perfused bioreactors results in viable adipose-like tissue (Fig. 2). The

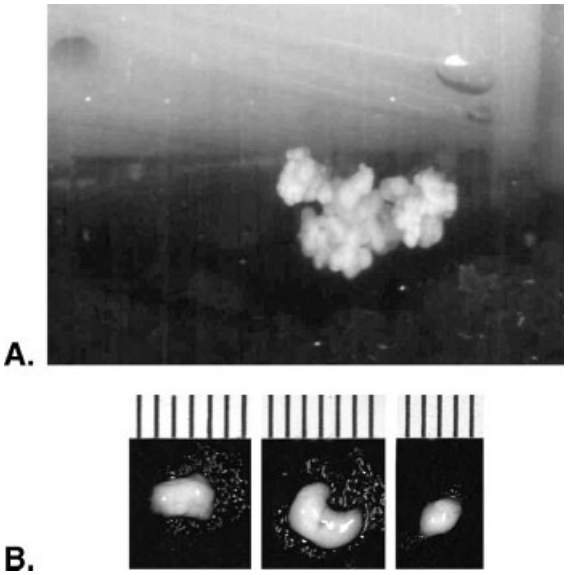

Figure 2 (A) Adipose-like tissue generated in a perfused, low-shear bioreactor. Image is captured through the bioreactor housing during operation. (B) Representative volumes of tissue generated in the bioreactor. The scale tick marks are millimeters.

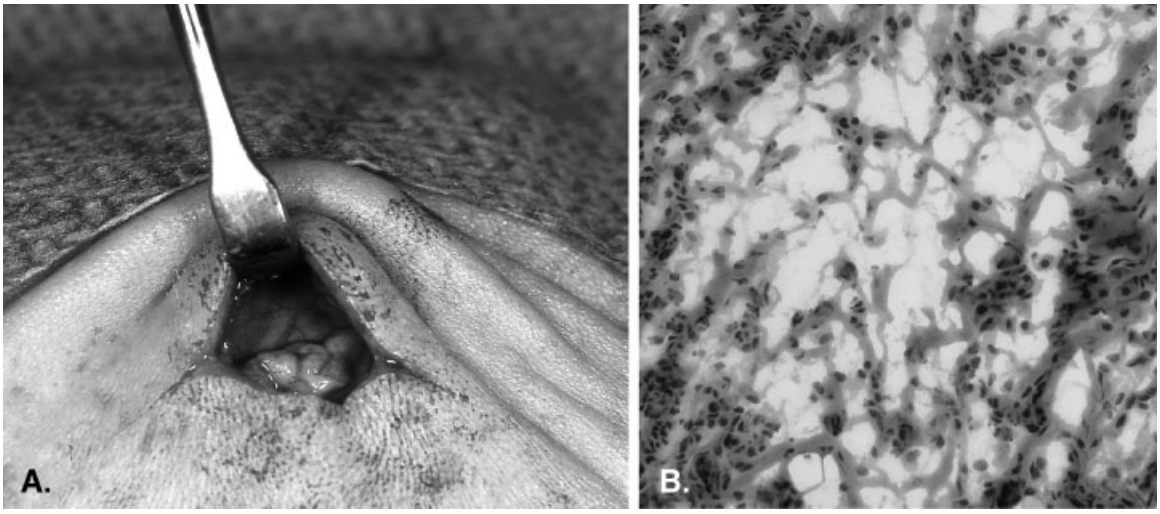

Figure 3 (A) Bioreactor-generated adipose tissue placed in a subcutaneous pocket of a rat. (B) H&E-stained histological section tissue sample from (A) 7 days after implantation.

bioreactor-generated adipose tissue is incorporated in vivo when placed subcutaneously in Lewis rats, as shown in Figure 3.

Preadipocyte Recruitment into Permissive Microenvironments

Acellular tissue engineering constructs that elicit recruitment of surrounding resident preadipocytes are also being investigated for de novo adipose tissue growth. For example, the reconstituted basement membrane of a mouse tumor, or Matrigel, has been shown by several investigators to induce migration, proliferation, and differentiation of preadipocytes when supplemented with basic fibroblast growth factor (bFGF).[36] It is speculated that when Matrigel plus bFGF is injected into a subcutaneous space, preadipocytes residing in the adjacent connective tissue migrate into the extracellular matrix gel. Results demonstrate that endothelial cells are also recruited. It remains unclear whether the observed angiogenesis drives the adipogenesis or vice versa. When gelatin microspheres loaded with bFGF were coimplanted with Matrigel, similar results were obtained.[37] Beahm and colleagues have demonstrated recruitment of resident preadipocytes and formation of de novo adipose tissue using a vascular pedicle model in athymic nude rats.[38,39] Briefly, the superficial inferior epigastric blood vessels were isolated, and silicone molds of various shapes (sheet, hemisphere, sphere) were sutured around the vessels. The molds were packed with PGA fibers. Subsequently, Matrigel and bFGF were injected into the packing fibers. Qualitative assessment over 4 to 20 weeks demonstrated de novo adipogenesis. This approach is currently being translated to a large animal model (Yucatan micropig) to assess adipose tissue engineering strategies.[38]

PLGA/PEG microspheres have been investigated as growth factor delivery vehicles in the absence of Matrigel. Insulin, insulin-like growth factor-1 (IGF-1), and bFGF have been administered to the muscular fascia of the rat abdominal wall via these PLGA/PEG microspheres.[40] At the harvest at 4 weeks, adipose tissue was grossly observed at the site of implantation. Histologic and image analysis showed that the microspheres treated with both insulin and IGF-1 demonstrated statistically greater increases in adipose tissue (composed of fibroblasts and adipocytes) than did empty microspheres or microspheres treated with insulin, IGF-1, or bFGF alone or with all three growth factors. This de novo generation of adipose tissue may result from one of three mechanisms. Stem cells may be present in the fascia and stimulated to

differentiate into preadipocytes and subsequently adipocytes. Another possible explanation is that preadipocytes residing in the fascia are stimulated to differentiate into adipose tissue. The third explanation, and the most speculative, is that the fibroblasts in the fascia dedifferentiate and then differentiate into preadipocytes and subsequently adipocytes. Regardless of the means, results indicate that adipose tissue can be generated in fascia within a short period (4 weeks) by delivery of microspheres treated with insulin and IGF-1.[40]

CONCLUSION

The field of adipose tissue engineering offers great potential in abrogating limitations realized with soft tissue augmentation after tumor resection and trauma. This review has discussed the state-of-the-art techniques in adipose tissue engineering. Numerous synthetic and naturally derived scaffolds are currently being investigated in vitro and in vivo for adipose tissue growth. The implantation of cultured preadipocytes or recruitment of endogenous preadipocytes into a scaffold has resulted in the development of adipose tissue. The generation of adipose tissue has the potential to change the way reconstructive surgery is practiced as well as increase patients' quality of life. Future studies must address the long-term maintenance of newly generated adipose tissue, the interplay between adipogenesis and angiogenesis, as well as the development of appropriate large animal models to assess adipose tissue engineering strategies.

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