Adipose and mammary epithelial tissue engineering

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Keywords: patterning, mammary epithelium, morphogenesis, 3D, organotypic

Abbreviations: ASC, adipose-derived stem cell; BAT, brown adipose tissue; ECM, extracellular matrix; iPSC, induced pluripotent stem cell; TDLU, terminal ductal lobular unit; WAT, white adipose tissue; 3D, three-dimensional

Breast reconstruction is a type of surgery for women who have had a mastectomy, and involves using autologous tissue or prosthetic material to construct a natural-looking breast. Adipose tissue is the major contributor to the volume of the breast, whereas epithelial cells comprise the functional unit of the mammary gland. Adipose-derived stem cells (ASCs) can differentiate into both adipocytes and epithelial cells and can be acquired from autologous sources. ASCs are therefore an attractive candidate for clinical applications to repair or regenerate the breast. Here we review the current state of adipose tissue engineering methods, including the biomaterials used for adipose tissue engineering and the application of these techniques for mammary epithelial tissue engineering. Adipose tissue engineering combined with microfabrication approaches to engineer the epithelium represents a promising avenue to replicate the native structure of the breast.

Anatomy of the Breast

The human breast is comprised of glandular, ductal, connective, and adipose tissues (Fig. 1). The functional unit of the breast is the mammary gland, a tree-like structure of epithelial ducts surrounded by adipose tissue.¹ The glandular and adipose tissues are held together by connective tissue, including Cooper’s ligaments which attach the breast to the dermis of the overlying skin.² Each breast has 15–20 sections (lobes) that branch out from the nipple. Each lobe is further divided into many smaller lobules, at the end of which are tiny bulb-like glands, known as terminal ductal lobular units (TDLUs), wherein milk is produced in response to hormonal signals. The lobes, lobules, and glands are connected by ducts, which deliver milk to openings in the nipple.³,⁴

The breasts of both women and men develop from the same embryonic tissues and are morphologically indistinguishable until the onset of puberty,⁵ at which time ovarian estrogens promote the sprouting, growth, and development of the mammary gland. In men, high levels of testosterone inhibit this development. As a result, the breasts of human males are much less prominent than those of females.⁶

Approximately 1 in 8 women will develop invasive breast cancer in the United States, and up to 40% will require a mastectomy.⁶ Breast reconstruction is a type of plastic surgery that aims to restore the shape, appearance, and size of a breast following its removal by mastectomy. Breast augmentation surgery, also known as augmentation mammoplasty, uses implants to increase the size of the breast or to restore its volume after weight loss or pregnancy. Saline-filled and silicone gel-filled implants are the most common. However, complications derived from the foreign body, such as capsular contracture, malposition, implant rupture, and infection, occur at a relatively high rate and frequently result in the need for implant removal.⁷

Breast reconstruction using the patient’s own tissues, rather than implantable devices, tends to produce better results with fewer complications and better approximates the shape, contour, softness, and fullness of the natural breast.⁸ The softness and suppleness which give the breasts their shape are mainly due to the presence of adipose tissue. Recent studies suggest the use of autologous fat tissue as an alternative implant material for breast augmentation.⁹–¹¹ Stem cells are collected from the patient’s own adipose tissue and then placed, along with appropriate angiogenic and adipogenic growth factors, within a biodegradable scaffold. The transplanted stem cells are able to differentiate into new adipose tissue or vascular endothelial cells.¹² Adipose tissue engineering is an emerging field that combines expertise in areas such as cell culture, cell differentiation, angiogenesis, tissue transfer, and polymer chemistry to regenerate adipose tissue de novo for breast reconstruction.

Adipose Tissue and Adipose Tissue Engineering

Adipose tissue, also known as fat, is the anatomical term for loose connective tissue composed of adipocytes.¹² Adipose tissue is primarily located beneath the skin and is also found around internal organs, in bone marrow, and as described above, is a major component of the human breast.¹³ There are two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT), which have essentially antagonistic functions. WAT stores excess energy as triglycerides and BAT is specialized...
Adipose-Derived Stem Cells for Tissue Engineering

Adipose-derived stem cells (ASCs) represent a readily available source for isolation of potentially useful stem cells. Stem cells are distinguished from other cell types by two properties. First, they have the ability to renew themselves through cell division while maintaining the undifferentiated state. Second, they have the capacity to differentiate into specialized cell types under certain physiologic or experimental conditions. There are primarily two kinds of stem cells that can be isolated from animals and humans: embryonic stem cells and adult stem cells. In 2006, researchers identified a new type of stem cell, called induced pluripotent stem cells (iPSCs), which are generated from somatic cells by the transgenic expression of three transcription factors referred to as OSK: Oct3/4, Sox2 and Klf4. The use of ASCs circumvents ethical issues associated with embryonic stem cells and the potential for oncogenic issues associated with iPSCs.

Ideally, a stem cell used for applications in regenerative medicine should meet the following criteria: (1) available in abundant quantities (millions to billions of cells); (2) harvested using minimally invasive procedures; (3) able to differentiate into multiple cell lineages in a regulatable and reproducible manner; (4) safely and effectively transplanted to either an autologous or allogeneic host; (5) manufactured in accordance with current Good Manufacturing Practice guidelines.

Adipose stem cells can fulfill all of these criteria. ASCs are localized near the vasculature in adipose tissue, and can be retrieved in high number from either liposuction aspirates or fragments of subcutaneous tissue. Furthermore, ASCs are easily expanded in culture, with one gram of adipose tissue yielding approximately 5,000 stem cells, 500-fold greater than the yield from the same volume of bone marrow. ASCs have similar properties to bone marrow stem cells and are capable of osteogenic, chondrogenic, adipogenic, and neurogenic differentiation in culture. ASCs have been shown to be immunoprivileged, to prevent severe graft- vs.-host disease in culture and in vivo, and to be genetically stable in long-term culture. The potential of ASCs to differentiate into cells derived from all three germ layers has been shown in a variety of studies.

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Rodbell and colleagues pioneered the original methods in the 1960s to isolate ASCs from adipose tissue using fat from rats. Several other groups further adapted these methods for human fat. Briefly, raw liposuction aspirate or finely minced adipose tissue is washed, digested with collagenase, and centrifuged to remove blood cells, saline, and local anesthetics. Undifferentiated ASCs can be characterized by several cell-surface markers including CD29, CD44, CD71, CD90 and CD105. One of the most important uses of ASCs is to replace fat tissue itself. ASCs are able to undergo adipogenic differentiation in response to inductive stimuli including dexamethasone, insulin, forskolin, and peroxisome proliferator-activated receptor-γ (PPARγ). During this process, ASCs decrease their proliferation and change in morphology from an elongated fibroblast-like appearance to a rounded shape. In addition, these cells start accumulating intracellular lipid droplets, secrete increased amounts of the adipocyte protein leptin, and express...
adipogenic proteins including fatty acid-binding protein and lipoprotein lipase.\textsuperscript{41,43-45} Large soft tissue defects are common following trauma, burns, and oncological resections including mastectomy, as described above. The ability of ASCs to produce fat tissue definitely represents a promising avenue to reconstruct these various tissue defects.

**Biomaterials for Adipose Tissue Engineering**

Tissue-specific scaffolds are essential to differentiate ASCs and effectively construct three-dimensional (3D) tissues. Ultimately, the scaffold must degrade as it is replaced by healthy host tissue. A number of scaffold biomaterials have been investigated for the purpose of engineering adipose tissue, including synthetic scaffolds and naturally derived materials.\textsuperscript{46} Several factors must be considered when designing a scaffold, including its mechanical properties, degradation characteristics, immunogenicity, cellular response to the material, ease of handling in the clinic, and cost.\textsuperscript{37}

Synthetic scaffolds have been used widely for adipose tissue engineering. The advantages of synthetic polymers include the ability to specifically tailor their mechanical, chemical, and degradation properties.\textsuperscript{48} Considerable work has been performed using synthetic polymers such as poly(lactic acid) (PLA), polyglycolic acid (PGA), polyethylene terephthalate (PET), and poly(lactic-co-glycolic acid) (PLGA).\textsuperscript{48}

PLA and PGA have been used for studies both in culture and in vivo and have the potential to support regenerated adipose tissue.\textsuperscript{49-52} When ASCs were cultured on PLA-based scaffolds in the presence of adipogenic stimulants, they showed significant upregulation of adipogenic transcript levels and substantial lipid accumulation. However, the scaffolds rapidly degraded within 4 weeks after implantation in a rat muscle pouch defect model.\textsuperscript{52} Likewise, PGA, while showing promise to support adipogenesis in culture, also degrades rapidly in vivo.\textsuperscript{50-53,54} When mouse 3T3-L1 cells, a preadipocyte cell line derived from disaggregated Swiss 3T3 mouse embryos,\textsuperscript{55} were seeded on fibrous PET matrices, they acquired morphological and biological features of adipocytes in culture,\textsuperscript{69} but their properties in vivo remain to be investigated. Adipose-derived ECM promotes a favorable microenvironment for adipogenesis, but has yet to be formulated as a 3D porous scaffold.\textsuperscript{70-72} The placenta is also a rich source of ECM and basement membrane components, and contains similar types of collagen as does adipose tissue,\textsuperscript{73} and therefore has great potential for use as a scaffold for adipose tissue engineering. Mature adipocytes were observed 8 weeks after seeding within a decellularized human placenta scaffold.\textsuperscript{74} However, the isolation and decellularization procedure for the placenta is both expensive and time-consuming.\textsuperscript{74}

In summary, several studies have demonstrated adipose tissue formation using both synthetic and natural polymers. On the one hand, synthetic materials offer consistent control of material properties. On the other hand, natural materials offer considerable advantages with respect to biocompatibility and degradation properties. Additional studies are needed to further demonstrate and compare long-term in vivo functionality of each material for clinical applications in soft tissue replacement.

**Epithelial Tissue Engineering**

Epithelial tissues line the cavities and surfaces of structures throughout the body and also form many glands, including the mammary gland. Epithelial cells can arise from each of the three germ layers of the embryo. The epidermis and its appendages, including the mammary gland, originate from the ectoderm. In contrast, the lining of the gastrointestinal tract derives from the endoderm, and the inner linings of body cavities derive from the mesoderm.\textsuperscript{75}

In 2004, two groups demonstrated the capacity of ASCs to differentiate into endothelial cells, a specialized epithelium.\textsuperscript{76,77} Subsequent studies have demonstrated the differentiation...
approach is to incorporate epithelial cells, fibroblasts, endothelial cells, adipocytes, and macrophages into a single platform. This, together with incorporation of adequate matrix scaffolds, will enable the generation of more complex, realistic mammary tissues.90

Conclusions

The field of tissue engineering offers great potential for abrogating the current limitations of breast reconstruction following tumor resection. The primary basis for any tissue-engineered construct is the cellular source that is used to initiate new tissue growth. ASCs provide an abundant and readily accessible source of multipotent stem cells. The use of stem cells expanded in culture and combined with novel biomaterials for organ reconstruction offers a potential solution for tissue replacement. ASCs have several advantages over other sources of stem cells, the most important being their ease of availability. The ability of ASCs to differentiate into epithelial cells makes them a promising tool for breast reconstruction. With the evolution of biological microfabrication, it is plausible to construct tissue models in which the biology, chemistry, geometry, and mechanics can be controlled at every length scale. Future studies are needed to demonstrate the safety and efficacy of adipocytes and epithelial cells derived from ASCs in animal models or clinically, either alone or in combination with novel biomaterial scaffolds.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Work from the authors’ lab was supported in part by the NIH (GM083997 and HL110335), the David and Lucile Packard Foundation, the Alfred P. Sloan Foundation, the Camille and Henry Dreyfus Foundation and Susan G. Komen for the Cure. CMN holds a Career Award at the Scientific Interface from the Burroughs Wellcome Fund.

Figure 2. Reconstructing the breast. Schematic of 3D microfabrication procedure used to build the mammary epithelial tissues. Preadipocytes are seeded in unpolymerized collagen. Cavities of collagen gel are generated by molding the cell-gel mixture around a patterned elastomeric stamp. In the presence of differentiation medium (DM), preadipocytes are induced to differentiate into adipocytes. Epithelial cells are then embedded into the cavities, which form hollow tubules conforming to the size and shape of the collagen cavities.
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