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Adipose and mammary epithelial tissue engineering

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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.[5]

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.[4] 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.[7-11] 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

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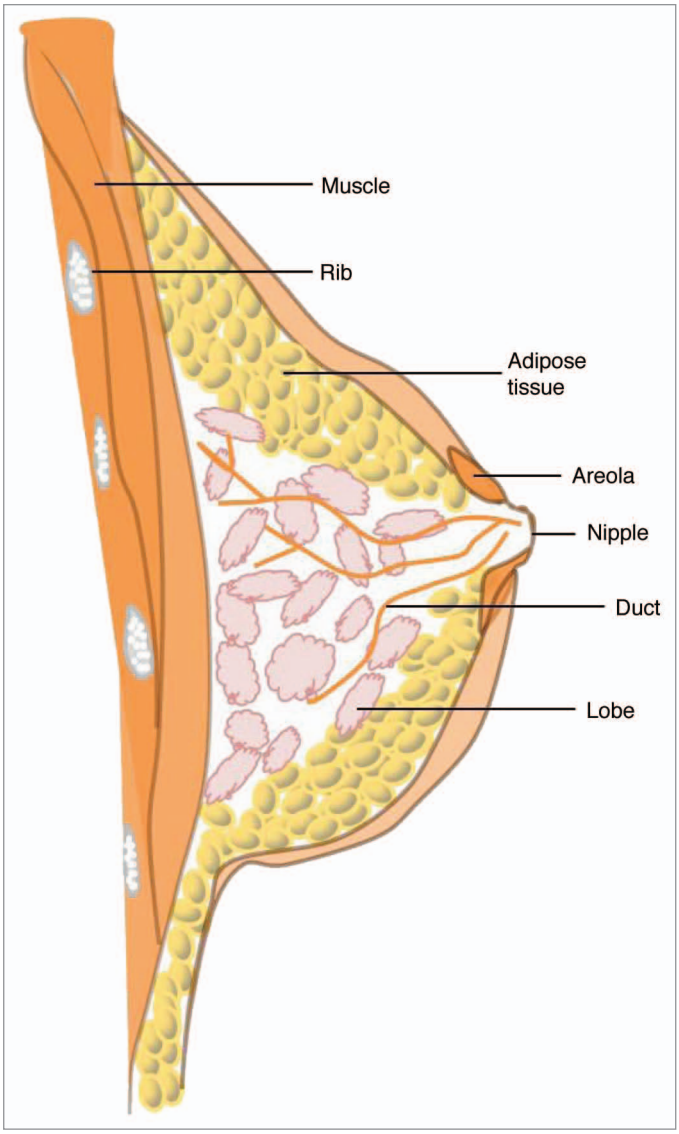

Figure 1. Anatomy of the breast. The female breast consists of glandular, ductal, connective, and adipose tissues.

to dissipate energy via the production of heat.[14] BAT is especially abundant in newborns and in hibernating mammals to enable survival in cold temperatures.[15] As an animal ages, BAT is gradually replaced by WAT.[15] Adipose tissue contains several cell types in addition to adipocytes, including fibroblasts, macrophages, and endothelial cells.[16,17]

Tissue engineering involves the use of living cells, biomaterials, and suitable biochemical and physico-chemical factors to improve or replace biological function.[18] Most patients possess excess adipocytes that can be harvested without creating deformities.[19] The therapeutic use of adipocytes in preclinical studies and clinical trials has been well documented.[20] Adipose tissue has also been identified as a source of multipotent cells that have the capacity to differentiate into myogenic, osteogenic, and neurogenic lineages when cultured with the appropriate lineage-specific stimuli.[21]

Adipose-Derived Stem Cells for Tissue Engineering

Adipose-derived stem cells (ASCs) represent a readily available source for isolation of potentially useful stem cells.[20] 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),[22] which are generated from somatic cells by the transgenic expression of three transcription factors referred to as OSK: Oct3/4, Sox2 and Klf4.[22,23] 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:[24] (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,[25] and can be retrieved in high number from either liposuction aspirates or fragments of subcutaneous tissue. Furthermore, ASCs are easily expanded in culture,[26] with one gram of adipose tissue yielding approximately 5,000 stem cells,[27] 500-fold greater than the yield from the same volume of bone marrow.[28] 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.[29] The potential of ASCs to differentiate into cells derived from all three germ layers has been shown in a variety of studies.[30]

Rodbell and colleagues pioneered the original methods in the 1960s to isolate ASCs from adipose tissue using fat from rats.[31-33] Several other groups further adapted these methods for human fat.[34-36] Briefly, raw liposuction aspirate or finely minced adipose tissue is washed, digested with collagenase, and centrifuged to remove blood cells, saline, and local anesthetics.[24] Undifferentiated ASCs can be characterized by several cell-surface markers including CD29, CD44, CD71, CD90 and CD105.[37-39] 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).[39-42] During this process, ASCs decrease their proliferation and change in morphology from an elongated fibroblast-like appearance to a rounded shape.[43] 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.[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.[46] Several factors must be considered when designing a scaffold, including its mechanical properties, degradation characteristics, immunogenicity, cellular response to the material, ease of handing in the clinic, and cost.[47]

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.[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).[48]

PLA and PGA have been used for studies both in culture and in vivo and have the potential to support regenerated adipose tissue.[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.[52] Likewise, PGA, while showing promise to support adipogenesis in culture, also degrades rapidly in vivo.[49-51,53,54] When mouse 3T3-L1 cells, a preadipocyte cell line derived from disaggregated Swiss 3T3 mouse embryos,[55] were seeded on fibrous PET matrices, they acquired morphological and biological features of mature adipocytes.[56] However, long-term studies have yet to be performed.

Newly formed adipose tissue was obtained when a combination of ASCs and PLGA spheres was injected into subcutaneous tissue of immune-deficient mice.[57] Unfortunately, the new adipose tissue could not retain a specific shape because the implanted PLGA support rapidly disappeared after degradation. A separate study used PLGA scaffolds seeded with rat preadipocytes prior to implantation and found maximum formation of adipose tissue at 2 months, followed by a decrease at 3 months, and complete absence of adipose tissue and PLGA at 5–12 mo.[58] Other synthetic materials have also been explored for adipose tissue engineering applications, and some show promise for potential soft tissue replacement. These include polytetrafluoroethylene (PTFE),[59] polyethylene glycol diacrylate (PEGDA),[60] and polyethylene glycol (PEG).[60,61] Whereas adipose tissue can be formed in vivo using synthetic scaffold-based tissue engineering strategies, the long-term maintenance of adipose tissue remains elusive.

Natural polymers are found as part of the native extracellular matrix (ECM).[62] Compared with synthetic materials, natural polymers tend to be more biocompatible, and their mechanical and biological properties tend to match those found in vivo. The most common natural polymers used recently for adipose tissue engineering include collagen, derivatives of hyaluronic acid (HA), adipose-derived ECM, matrigel, gelatin, and decellularized human placenta.[46,48]

Collagen is the most prevalent natural polymer used in current adipose tissue engineering research due to its biodegradability, biocompatibility, weak antigenicity, and the ability to be shaped to fill a specific defect.[63] Several studies have demonstrated that 3D collagen sponges can support adipogenesis from a variety of cell sources,[64-67] as well as promote the development of new adipose tissue in vivo after 12 weeks.[68] Mauney et al. studied the ability of collagen and PLA matrices to support adipogenesis both in culture and in vivo, and found that although collagen scaffolds supported greater cell attachment upon seeding and greater lipid accumulation in culture, both collagen and PLA scaffolds were undetectable after 4 weeks in vivo due to rapid degradation.[48,52] Human preadipocytes could be successfully and reproducibly inoculated and cultured on 3D HA-based scaffolds, and were able to differentiate into adipocytes in culture,[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.[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,[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.[74] However, the isolation and decellularization procedure for the placenta is both expensive and time-consuming.[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.[75]

In 2004, two groups demonstrated the capacity of ASCs to differentiate into endothelial cells, a specialized epithelium.[76,77] Subsequent studies have demonstrated the differentiation

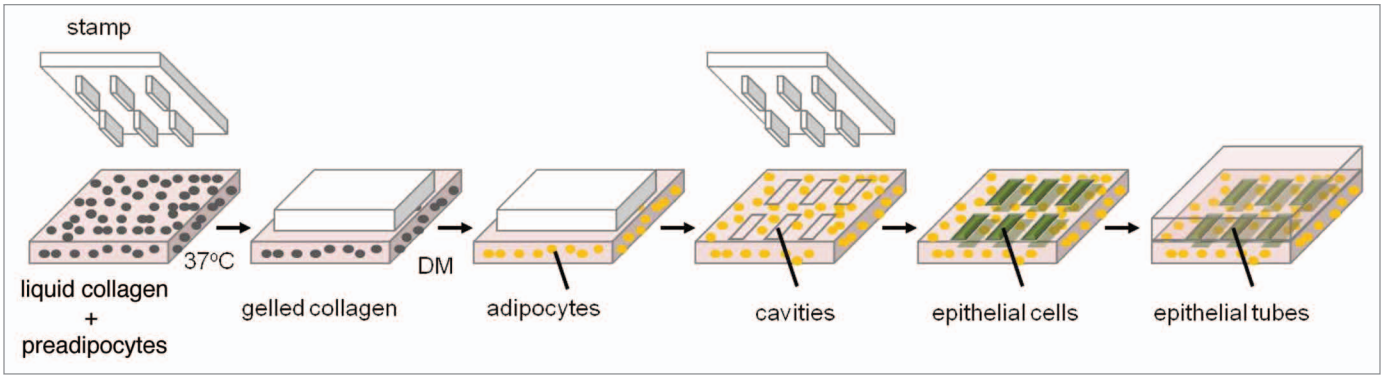

Figure 2. Reconstructing the breast. Schematic of 3D microfabrication procedure used to build the mammary epithelial tissues. Preadipocytes are seeded in unpolymerized collagen. Cavities of collagen gel are generated by molding the cell-gel mixture around a patterned elastomeric stamp. In the presence 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.

potential of ASCs into an epithelial lineage. Human ASCs were able to undergo epithelial differentiation in culture in the presence of all-trans retinoic acid. The differentiated cells displayed several epithelial characteristics including monolayer formation, the expression of the epithelial-specific marker cytokeratin 18, and the formation of keratin fibers. The percentage of ASCs able to undergo epithelial differentiation as quantified by flow cytometry analysis was greater than 80%.[78] Studies by several other groups suggest that the epithelial differentiation of ASCs can also be initiated by direct cell-cell or cell-matrix contacts,[79,80] or by secreted factors such as cytokines, interleukins, or growth factors present in conditioned media.[75]

Cells typically reside in a 3D microenvironment in vivo. Several recently developed techniques for microfabricating 3D epithelial tissues hold promise for engineering these structures with a higher level of physiological and histological realism.[81-83] Our lab has developed an engineered tissue model of the mammary epithelial duct comprised of murine mammary epithelial tubules of arbitrary geometry embedded within a 3D type I collagen gel.[81] A concentrated suspension of mammary epithelial cells is placed within micro-scale collagen cavities prepared by replica micro-molding. Over a 24-h period, the cells form contacts with their neighbors, synthesize and assemble a basement membrane, and rearrange into a polarized epithelial tubule. When induced with growth factors, such as those that act downstream of ovarian hormones in vivo, these 3D epithelial tissues undergo branching morphogenesis, thus expanding the epithelial tree.[81] Importantly, these epithelial tissues can also be engineered within gels containing adipocytes,[84] thus more closely approximating the native structure of the mammary gland (**Fig. 2**). This model has been used to analyze quantitatively the spatial and temporal dynamics of gene expression[85,86] and the mechanical stress profile during branching morphogenesis,[87,88] as well as how the biophysical characteristics of the host microenvironment affect the invasive phenotype of breast tumor cells.[89] Given that the mammary gland is composed of multiple cell types, one of the major challenges of the microfabrication 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.

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