Lipoplasty: From Body Contouring to Tissue Engineering

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Learning Objectives:

The reader is presumed to have a broad understanding of plastic surgical procedures and concepts. After studying this article, the participant should be able to:

1. Discuss the differences between various stem cell populations.
2. Describe the technique of stem cell harvest from adipose tissue.
3. Explain the multilineage potential of processed lipoaspirate cells.

Physicians may earn 1 hour of Category 1 CME credit by successfully completing the examination based on material covered in this article. The examination begins on page **.

Background: The rapid development of disciplines such as cell therapy and tissue engineering has focused attention on stem cells as the ideal cellular substrate for new tissues. Human adipose tissue is a potential source of such stem cells.

Objective: We review the role of human adipose tissue in stem cell research and describe the procurement of stem cells from the stromal vascular fraction of human adipose tissue obtained through suction-assisted lipoplasty.

Methods: Raw lipoaspirate obtained through suction-assisted lipoplasty was washed in phosphate-buffered saline and digested with collagenase. The collagenase was then inactivated by fetal bovine serum and the cells were centrifuged for 10 minutes at 1200 x g. The resulting cell pellet was resuspended, plated, and maintained in nondifferentiating control media.

Results: Processing of 250 to 500 mL of suctioned tissue routinely yielded 2 to 6 x 10^6 processed lipoaspirate cells. Cell viability was typically >95%. These cells have been shown to differentiate in vitro into at least the adipogenic, chondrogenic, myogenic, neurogenic, and osteogenic lineages in the presence of specific induction factors.

Conclusions: Adipose tissue may be an ideal source of stem cells, because it is abundant, easy to obtain in large quantities, and safe to procure. Such a development could place the plastic surgeon at the epicenter of medical research. Issues that require further research include elucidation of site-specific differences in fat cells, the use of vacuum-assisted lipoplasty and ultrasound-assisted lipoplasty in procuring stem cells, and the development of more efficient and convenient tissue processing techniques. (Aesthetic Surg J 2002;22:121-127.)
F or decades, surgeons have attempted to patch or rebuild tissues ravaged by injury and disease, often achieving only limited success. For example, methods for soft tissue reconstruction have included the utilization of alloplastic materials (eg, Teflon, silicone) and autologous tissue transplantation (eg, free fat transplantation). Each of these techniques has been plagued with unique drawbacks ranging from complex immunologic interactions to time-dependent graft resorption. An essential principle of reconstructive surgery is the replacement of “like with like.” However, techniques such as allograft transplantation are associated with attendant complications of rejection and immunosuppression. Autologous tissue transplantation (eg, free tissue transfer to replace tissue lost after radical neck dissection) is currently the standard of care in many major reconstructive procedures. These complex and delicate techniques are accompanied by operative risk, technical difficulty, and donor site morbidity. The ideal replacement tissue is immunocompatible, abundant, easily accessible, and maintains its volume and function over time.

The combination of the rapidly expanding disciplines of developmental and cellular biology with the fabrication of biocompatible scaffolds helped give birth to the field of tissue engineering. Tissue engineering can be defined as the application of scientific principles to the design, construction, modification, growth, and maintenance of living tissues.1 Most future tissue engineering strategies will likely include one or more of the following components: (1) stem or progenitor cells, (2) biodegradable or bioincorporated scaffolds, (3) growth factors or cytokines, and (4) gene therapy.2 The clinical potential of the principles of tissue engineering is immense, ranging from reconstruction (eg, congenital craniofacial microsomia) to cosmetic improvement (eg, breast augmentation).

At the heart of this process lies the stem or progenitor cell. These cells are important because they provide an expandable and renewable source of diverse cell types that can be manipulated for tissue regeneration. The identification of a readily available source of stem cells, when coupled with a comprehensive understanding of developmental biology, will enable surgeons to harvest autologous multilineage stem cells from patients, expand and manipulate the cells in vitro to generate a defined population, seed the cells onto a scaffold, induce their differentiation, and ultimately transplant the bioengineered construct or manipulate its growth and differentiation in vivo (Figure 1).

**Stem Cells**

Potten and Loeffler3 defined stem cells as uncommitted cells capable of proliferation, self-maintenance, the formation of differentiated functional progeny, and capable of regeneration of tissue after an injury.3 Conceptually, there are 2 general types of stem cells that are potentially valuable for tissue engineering and transplantation: embryonic stem cells (ESCs) and postembryonic or adult stem cells. Embryonic stem cells are derived from the inner cell mass of the developing preimplantation blastocyst.4,5 These cells have the potential to differentiate into cells of ectoderm, mesoderm, and endoderm. Although theoretically appealing because of their pluripotent nature, the practical use of ESCs is limited because of ethical considerations and concerns over cell regulation and control. In contrast, adult stem cells, when used autologously, are immunocompatible and have no ethical issues related to their use. Multipotent stem cells can be cultured from a number of adult sources. Perhaps the best-known source is bone marrow, which contains both hematopoietic stem cells (HSCs) and marrow-derived mesenchymal stem cells (MSCs).6,7 Several researchers have shown that MSCs may be as pluripotent as ESCs and capable of differentiation into a variety of lineages, including adipocytes, chondrocytes, myocytes, neurons, and osteocytes (Figure 2).8-17 Stem cells capable of mesodermal differentiation are essential components of tissue engineering strategies for soft tissue reconstruction and enhancement.

Despite the vast potential of marrow-derived MSCs, their practical use has several limitations. Traditional bone marrow procurement is painful, frequently requires general or spinal anesthesia, and yields relatively low numbers of MSCs upon processing. The rarity of these stem cells will likely necessitate an ex vivo expansion step before clinical use. Such a step is clinically cumbersome, expensive, and risks cell contamination and loss. In light of these aspects, an abundant, easily obtainable source of mesodermal stem cells would be advantageous.

**Adipose Tissue**

Connective tissue is the term traditionally applied to a basic type of tissue of mesodermal origin that provides struc-
tural and metabolic support for other tissues and organs throughout the body. The role of connective tissue is critical to the function of the organism as a whole, ranging from physical support (dermis of skin) to metabolism (storage of fat in adipose tissue). Human adipose tissue is such a connective tissue and contains a variety of cells, including mature adipocytes, preadipocytes, fibroblasts, smooth muscle cells, and endothelial cells within its stroma. Adipocytes, derived from the primitive mesoderm, are specifically adapted for the storage of lipids. Stored fat within adipocytes comes from 3 main sources: dietary fat circulating in the bloodstream, fat synthesized in the liver, and fat synthesized from glucose within adipocytes. Because of the essential role of adipose tissue in metabolism, it has a rich blood supply. Adipose tissue comprises approximately 20% of total body weight in healthy adult men and up to 25% in women.\textsuperscript{18}

Figure 1. Schematic illustration of the strategy for the use of processed lipoaspirate (PLA) cells for cell-based tissue engineering and transplantation.

Figure 2. Multilineage potential of bone marrow-derived mesenchymal stem cell (MSC).
The concept that many tissues and organs contain a population of quiescent lineage-committed progenitor cells that act to maintain and repair the tissue is becoming generally accepted (eg, blood and immune system, skin epithelium, epithelium of the small intestinal crypts, etc). Furthermore, several studies have suggested the presence of uncommitted stem cells within the connective tissue matrices of several organs in birds, mice, rats, and rabbits. Like their lineage-committed counterparts, these pluripotent stem cells may be directly involved in tissue maintenance and restoration, although their exact function has yet to be determined. Like bone marrow, adipose tissue is thought to originate from multipotent stem cells within the embryonic mesoderm and similarly contains a supporting stroma. These similarities, combined with the fact that stem cells appear to be present in several animal tissue stromal compartments, support the general concept that adipose tissue may be a potential source of stem cells. Proteolytic digestion of intact human adipose tissue yields a “fibroblastic” population of cells known as the stromal vascular fraction that is known to contain lineage-committed preadipocyte cells.

Our data suggest that, in addition to other cell types such as preadipocytes, a population of cells phenotypically similar to MSCs can be isolated from the stromal vascular fraction of human adipose tissue. These cells, which we term processed lipoaspirate (PLA) cells, appear to possess multilineage potential and are capable of differentiation in vitro into at least the adipo-, osteo-, chondro-, neuro-, and myogenic lineages in the presence of lineage-specific induction factors.

Briefly, human PLA cells were harvested from healthy adult patients via lipoplasty. After maintenance in non-differentiating media, PLA cells were selected for differentiation down various mesodermal lineages. Culturing PLA cells in adipogenic-media for 2 weeks induced adipogenesis. After this time, many flat, spindle-shaped PLA cells began to accumulate intracytoplasmic lipid vacuoles, confirmed with Oil Red O stain, an indicator of intracellular lipid accumulation (Figure 3).

We have also shown that human PLA cells can be successfully infected with an adenovirus transfected with the bone morphogenetic protein-2 (BMP-2) and subsequently undergo a more potent osteogenic response than bone-marrow derived stem cells. We have further induced...
these cells toward chondrogenic differentiation capable of cartilaginous nodule formation in culture, making this a clinically practical chondrogenic precursor population for future tissue engineering strategies. Erickson et al. similarly demonstrated the chondrogenic potential of adipose-derived stromal cells within an alginate matrix. Mizuno et al. demonstrated the potential of human PLA cells to differentiate into myoblasts. They showed that after 6 weeks in myogenic culture, PLA cells became multinucleated and expressed MyoD1 and myosin, consistent with early myogenic differentiation. Processed lipoaspirate cells may also have a non-mesodermal differentiation potential. Initial studies have shown that under neural differentiation conditions, PLA cells take on morphologic characteristics of neurons and express early neural markers, neuron-specific enolase and neuron-specific nuclear protein (Figure 4).

Our data indicate that PLA cells can be maintained in vitro for extended periods with stable population doubling and low levels of senescence. Our laboratory has data that suggest that PLA cells obtained from younger patients appear to have advantages compared with cells obtained from older persons. This age phenomenon is well described in other cell types and suggests a potential role for the clinical banking of one’s own stem cells for future use. The autologous nature of these stem cells, together with their multilineage potential and ease of collection, may make these cells an excellent choice for many tissue engineering strategies.

In addition to the multipotent differentiation potential of PLAs, our laboratory has shown that these cells are capable of viral infection and protein secretion, thus serving as a possible gene therapy vehicle. Infected cells have been demonstrated. Given that diffusion and mass transfer limit the size of successful tissue engineered constructs, neovascularization strategies will play a critical role in large-volume scaffold implantations. Basic fibroblast growth factor (FGF-2) and vascular endothelial-cell growth factor (VEGF) are 2 important pro-angiogenic growth factors that have been shown to facilitate endothelial cell proliferation, differentiation. Processed lipoaspirate cells infected with adenoviral-FGF-2 successfully expressed FGF-2 (Figure 5). In the future, biocompatible scaffolds may potentially be seeded with virally infected stem cells that will secrete pro-angiogenic growth factors, yet maintain their ability to differentiate into specific tissues.

**Procurement of Stem Cells**

Lipoplasty has become the single most performed aesthetic surgery procedure in the United States. Currently, there are 2 forms of lipoplasty available: suction-assisted lipoplasty (SAL) and ultrasound-assisted lipoplasty (UAL). Suction-assisted aspiration of subcutaneous fat through small incisions (6 to 8 mm) has been performed for nearly 25 years. The procedure is generally indicated for the removal of fat collections from clearly defined anatomic regions. It is currently a relatively safe and effective means of removing undesirable fat tissue.
Compared with UAL, SAL is less effective in regions of the body that contain more fibrous tissue (e.g., the flank and upper abdomen).39

In addition to cosmetic applications, we believe traditional SAL is the ideal technique for the harvest of autologous multipotent stem cells within the stromal vascular fraction of adipose tissue. Our data thus far have focused on the harvest of PLA cells via SAL. Briefly, after intravenous sedation or general anesthesia, the region to be aspirated was infiltrated with fluid containing lactated Ringer’s mixture, lidocaine, and epinephrine for pain control and hemostasis. Adequate hemostasis using epinephrine was essential to minimize contamination of the lipoaspirate by peripheral blood cells. Then, hollow blunt-tipped metal cannulas were introduced into the subcutaneous space through small skin incisions. The cannulas, attached to gentle suction, were moved through the adipose compartment, mechanically disrupting the fat tissue. The neurovascular bundles were resistant to the suction device secondary to a strong fibrous capsule; thus, nerves and large blood vessels within the subcutaneous tissue remained intact. The raw lipoaspirates were washed extensively with sterile phosphate-buffered saline and digested with collagenase. After 30 minutes, the collagenase was inactivated by 10% fetal bovine serum and the cells were centrifuged for 10 minutes at 1200×g. The resulting cell pellet was resuspended, plated, and maintained in nondifferentiating control media at 37°C/5% CO2. This cell population was termed the processed lipoaspirate (PLA) and contained our adult multipotent stem cells. Cell viability is typically higher than 95%. Processing of 250 mL to 500 mL of suctioned tissue routinely yields 2 to 6 × 10^8 PLA cells. Significant differences in growth characteristics and differentiation potential of fat cells from different adipose tissue sites have been shown.40,41 Site-specific differences need to be further elucidated so that ideal locations of fat procurement can be tailored to specific tissue engineering requirements. Also, further studies are required to evaluate the relative differences in the harvest of PLA cells between SAL and UAL.

A practical consideration for harvesting PLA cells from fat includes the development of “bedside” (i.e., no ex vivo expansion step) tissue-processing techniques. A device that facilitates enzymatic digestion of the raw lipoaspirate as the fat is suctioned from the patient would allow the harvest of large numbers of stem cells within a few hours. While the patient remains in the operating room facility, the stem cell fraction could be automatically processed and enriched. Then, the stem cells could be combined with appropriate inductive factors or scaffolds and replaced directly back to the patient.

Conclusion

The rapid pace of discovery in the area of developmental biology has resulted in the simultaneous emergence of new cell-based tissue engineering strategies, designed to help regenerate, repair, or enhance tissues in the adult. Autologous adult pluripotent stem cells may be the cornerstone of this new paradigm. Although several types of stem cells appear to exist, most have significant, if not limiting, issues to their broad clinical use. We have been able to isolate an alternate source of adult stem cells from human adipose tissue by SAL. Adipose tissue may be an ideal source of stem cells because it is abundant (more than 60% of the US population is considered obese and/or overweight),42 simple to obtain in large quantities, and safe to procure. This simple and curious finding may place the plastic surgeon at the epicenter of a new era in medicine and surgery.

References


