Title of the article:
Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation

Authors:
Kotaro Yoshimura, M.D., Hirotaka Suga, M.D., Hitomi Eto, M.D.

Affiliations:
Department of Plastic Surgery, University of Tokyo School of Medicine;
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Corresponding author:
Kotaro Yoshimura, M.D.
Department of Plastic Surgery, University of Tokyo School of Medicine;
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Telephone: +81-3-5800-8948; Fax: +81-3-5800-8947
E-mail: yoshimura-pla@h.u-tokyo.ac.jp
Summary

Many features of adipose tissue-specific stem/progenitor cells, such as physiological function and localization, have recently been examined. Adipose tissue turns over very slowly and its perivascular progenitor cells differentiate into adipocytes in the next generation. The progenitor cells play important roles in physiological turnover, hyperplasia, and atrophy of adipose tissue; as well as in incidental remodeling, such as post-injury repair. Adipose tissue has been used as an autologous filler for soft tissue defects, despite unpredictable clinical results and a low rate of graft survival, which may be due to the relative deficiency of progenitor cells in graft materials. A novel transplantation strategy, called cell-assisted lipotransfer, involves the enrichment of adipose progenitor cells in grafts; preliminary results suggest this approach to be safe and effective.

Keywords:
adipose-derived stromal cells, stromal vascular fraction, endothelial progenitor cells, pericytes, breast augmentation, adipocyte, angiogenesis, fibrosis, hepatocyte growth factor
Introduction
Adipose tissue has been considered an organ of energy storage, an endocrine organ, a soft tissue filler (augmentation by micro-fat grafting), and a cosmetically unnecessary tissue discarded by liposuction. It is now regarded also as a promising source of adult stem cells, as adipose tissue has plenty of progenitor cells, some of which can differentiate into diverse lineages [1]. A component of fibroblast-like stromal cells obtained from liposuction aspirates can differentiate into various cell lineages [1], including adipogenic, osteogenic [2], chondrogenic [3], myogenic [4], cardiomyogenic [5], and neurogenic [6]. Thus, adipose tissue-derived stromal cells are now called “adipose-derived stem/stromal/progenitor cells (ASCs)” and are expected to become a valuable tool for a wide range of cell-based therapies [7]. ASCs are believed to act as progenitors of adipocytes and vascular cells [8], they reside between adipocytes, around vessels or in the extracellular matrix, and contribute to the turnover of adipose tissue [9]. Adipose tissue turnover is known to be very slow in humans (2 to 10 years) [9,10]. Human ASCs are distinct from other mesenchymal progenitors in their surface marker expression profile; notably, ASCs express stem-cell–associated marker CD34 in higher percentages than bone-marrow–derived mesenchymal stem cells and dermal fibroblasts [11].

A huge body of basic and translational research using adipose-derived stem/progenitor cells has been conducted and ASCs are currently being used in some clinical trials, including treatments for bone defects (autologous fresh ASCs) [12], rectovaginal fistula (autologous cultured ASCs) [13], graft-versus-host disease (non-autologous ASCs) [14], and soft tissue augmentation by progenitor-enriched fat tissue grafting (autologous fresh ASCs) [15,16]. ASCs have been found to have potential similar to bone marrow-derived mesenchymal stem cells and are now of great interest as a tool for cell therapies.

Adipose tissue and its cellular components
Adipose tissue consists predominantly of adipocytes, ASCs, vascular endothelial cells, pericytes, fibroblasts, macrophages, and extracellular matrix [11]. Adipocytes constitute more than 90% of tissue volume due to their large size (50-130 μm diameter), yet the number of adipocytes is estimated to be less than 50% [17,18]. Our recent survey, from whole mount staining of living adipose tissue, suggested the adipocyte percentage is less than 30%. Adipose tissue is known to be rich in microvasculature [19] and every adipocyte has contact with capillaries [20], though the capillaries are a substantial distance from each other due to the large cell size of adipocytes.

Since the discovery of leptin, adipose tissue has been recognized as the largest endocrine organ. In addition to fatty acids, multiple factors such as leptin and adiponectin are released from adipose tissue [18]. It has been shown most inflammatory adipokines are released predominantly from cells other than adipocytes, such as ASCs and resident macrophages [18].
**Stromal vascular fraction**

Through collagenase digestion, a heterogeneous cell mixture containing all cell types, except adipocytes, can be extracted from adipose tissue (or liposuction aspirates) as a cell pellet. Adipocytes are disrupted into oil during the process and discarded as floating tissue and oil after centrifugation. The sedimented cell fraction is called the “stromal vascular fraction (SVF)” and is basically stromal cells along with vascular endothelial and mural cells. The SVF also contains a substantial amount of circulating blood-derived cells such as leucocytes and erythrocytes, although most of the contaminated erythrocytes can be disrupted with hypotonic solution processing. Our study identified freshly isolated ASCs as CD31–CD34+CD45–CD90+CD105–CD146– cells, but they become CD105+ when plated [11]. Nucleate cells contained in the SVF obtained from lipoaspirates are composed of 37% leucocytes (CD45+), 35% ASCs (CD31–CD34+CD45–), 15% endothelial cells (CD31+CD34+CD45–), and other cells (CD31–CD34–CD45–); although, the percentage of blood-derived cells strongly depends on individual hemorrhage volume [17]. ASCs can be extracted from the floating fatty portion and fluid portion of liposuction aspirates; although the fluid portion contains much fewer adipose-derived cells and many more blood-derived cells. In mice, the adipocyte progenitor subpopulation was recently identified as Lin–Sca1+CD24+CD29+CD34+ cells [21].

ASCs can be used clinically without cell expansion if harvested from a large volume of lipoaspirates because a sufficient number of cells can be obtained; 0.1–1 billion nucleate cells can be obtained from 200 ml of aspirated fat tissue and at least 10% of these cells are adipose-derived stromal cells. The use of minimally manipulated fresh cells likely leads to higher safety and efficacy in treatments compared to cells expanded by culture. Some ongoing clinical trials employ freshly isolated SVF, rather than purified or cultured ASCs. As the SVF contains other cells such as vascular endothelial cells or macrophages, synergistic effects may be expected.

**Adipose-derived stem/progenitor cells in adipose tissue remodeling**

ASCs have been shown to have angiogenic characteristics, to release angiogenic factors under ischemia [22,23] or stimulation of growth factors [24], and to experimentally differentiate into vascular endothelial cells [2,25-28]. Thus, ASCs are now considered to be bipotent progenitor cells for both adipocytes and vascular cells, although the differentiation into vascular endothelial cells was not frequently detected in *in vivo* studies [29,30] and a standard *in vitro* protocol for endothelial differentiation is not yet established. ASCs are thought to localize between adipocytes (co-localize with capillaries), in the vessel walls or in the connective tissue; most of them show perivascular localization. Some studies suggest the existence of a cell population localized in the vascular wall which can differentiate into vessels [31,32]; while other studies indicate a possible identity between ASCs and vascular pericytes [33-36]. In addition, ASCs have been shown to be
incorporated into microvascular remodeling and exhibit a perivascular phenotype [34]. Very recently, it was discovered in mice that adipocyte progenitor cells are present in adipose vasculature as mural cells [36].

ASCs contribute to adipose tissue turnover and provide cells in the next generation [9] (Figure 1). Adipocytes have a life span of several to ten years and are replaced with next generation cells derived from ASCs after apoptosis [9,10]. ASCs are thought to be the main proliferating cell population in adipose tissue remodeling, such as the repair process after ischemia-reperfusion injury [24], or in adipose tissue expansion induced by external forces (unpublished data). Adipose tissue grows in adolescence or obesity and atrophies with age or after tissue injury. These remodeling processes are thought to be in balance with adipocyte (either physiological or incidental) apoptosis/necrosis and adipogenesis managed by ASCs; these degenerative and regenerative changes are always accompanied by capillary remodeling (Figure 2). Adipose tissue atrophy with age is likely due to a decrease in ASCs and consequent impaired replacement in the next generation, as is commonly seen in other tissues and organs [37].

Adipose tissue grafting for soft tissue augmentation

Soft tissue augmentation is performed by grafting autologous tissues or artificial materials to correct inborn or acquired tissue defects, such as breast reconstruction, or for purely cosmetic purposes, such as breast augmentation. Although it is not a life saving procedure, the demand is huge; the incidence of breast cancer is increasing and one of eight women suffers in western countries [101]. Breast augmentation is the most frequently performed cosmetic surgery [102], carried out more than 300,000 times annually in the USA. Autologous fat transplantation is a promising treatment for soft tissue augmentation because there is no associated incisional scarring or complications because of foreign materials. Although many innovative efforts to refine autologous lipoinjection have been reported, problems such as unpredictability and a low rate of graft survival due to partial necrosis remain. Implantation of artificial prostheses has been the standard method of breast augmentation, with lipoinjection used by a limited number of plastic surgeons [38]. The use of fat tissue in breasts has been controversial due to the lack of consensus on its safety and appropriateness because of microcalcifications that may cause confusion in the evaluation of mammograms. However, autologous fat injection was recently re-evaluated as a potential alternative to artificial implants for breast augmentation [15,38,39], reflecting recent advances in autologous fat transfer techniques and the radiological detection of breast cancer.

It has not been well documented how adipose grafts survive after lipoinjection. With lipoinjection, the recipient tissue is injured and bleeding occurs. The grafted non-vascularized adipose tissue is placed under ischemia (hypoxia) and is temporarily nourished by diffusion from the surrounding host tissue for a few days until direct capillary attachment is formed (Figure 3). In response to injury, basic fibroblast growth
factor (bFGF) is released from the injured host tissue, especially from extra-cellular matrix and dying cells. Other factors such as platelet derived growth factor (PDGF), epidermal growth factor (EGF), and transforming growth factor (TGF)-β are released from activated platelets in response to local bleeding [40]. Inflammatory cells such as macrophages and lymphocytes are infiltrated and inflammatory cytokines such as interleukins are secreted. During the repairing process, adipocytes, known to be very sensitive to hypoxia, are subject to die within 24 hours if the oxygen pressure is lower than its threshold; while ASCs are likely as resistant to ischemia as bone marrow-derived mesenchymal stem cells, which can be functional for 72 hours under ischemia [41]. In animal models for ischemia-reperfusion injury of adipose tissue [24], ASCs were involved in the repairing process after fat grafting and played key roles in adipogenesis and angiogenesis. A preliminary study suggested even surviving adipocytes die within a few months after transplantation and are replaced with next generation cells, possibly due to the stress of temporary ischemia. Therefore, the number of functional ASCs is likely to be important for tissue repairing and remodeling.

**Aspirated and intact fat tissue**

Aspirated fat tissue, but not excised (intact) fat tissue, can be used as lipoinjection material. Skin incision is necessary to harvest excised fat tissue, which is not acceptable for cosmetic purposes. When aspirated, the fragile parts of adipose tissue are removed with negative pressure through a small suction cannula while honeycomb-like fibrous structures (connective tissues, vasculatures, and nerves) remain in aspirated fatty layers. We have found many adipocytes and capillaries are ruptured and a larger number of dead cells are contained in aspirated fat tissue. In addition, aspirated fat tissue contains only half of the ASCs compared to intact fat tissue [28] (Figure 4). This relative deficiency of ASCs in aspirated fat tissue may be due to: 1) a substantial portion of ASCs are located around large vessels (within tunica adventitia) and left in the donor tissue and 2) some ASCs are released into the fluid portion of liposuction aspirates [11]. Large-sized vessels are located in the fibrous part of the tissue, present in intact but not aspirated fat tissue. Thus, aspirated fat tissue is regarded as relatively progenitor-poor as compared to intact fat tissue [28].

**Supplementation of adipose progenitor cells in micro fat grafting (cell-assisted lipotransfer)**

As discussed above, aspirated fat tissue has a significantly lower progenitor/mature cell ratio. This low ASC/adipocyte ratio may be the main reason for long-term atrophy of transplanted adipose tissue, because ASCs are supposed to contribute to adipose tissue turnover by replacing adipocytes lost in the atrophying process with next generation adipocytes. There are at least three experimental studies, including ours [28,42,43], demonstrating supplementation of adipose progenitor cells enhances the volume or weight of surviving adipose tissue. Enrichment of adipose progenitor cells by supplementation
with the SVF improves the progenitor/adipocyte ratio; progenitor-poor aspirated fat tissue is converted to progenitor-rich tissue. In cell-assisted lipotransfer (CAL) [15,16,28], a novel approach to autologous fat transplantation, freshly isolated SVF cells containing ASCs are attached to the aspirated fat with the fat tissue acting as a living bioscaffold before transplantation (Figure 5).

There are four possible roles for ASCs in CAL treatment, which were partly confirmed in pre-clinical studies [28,42,43]. First, ASCs can differentiate into adipocytes and contribute to regeneration of adipose tissue. Second, ASCs can differentiate into vascular endothelial cells and also probably into vascular mural cells [2,25-28,30], resulting in the promotion of angiogenesis and graft survival. Third, ASCs are known to release angiogenic growth factors such as hepatocyte growth factor (HGF) and stromal cell-derived factor 1 (SDF-1) in response to injury, hypoxia, and other conditions [22-24,29,30] and these factors influence surrounding host tissue. The final, possibly most influential role is ASCs survive as original ASCs [28]. As mentioned above, ASCs reside between adipocytes or in the extracellular matrix, especially around vessels, and contribute to the turnover of adipose tissue, which is known to be very slow (2–10 years) [9,10]. However, surviving adipose grafts probably turn over within the first 2 to 3 months after transplantation. The grafts experience temporary ischemia followed by reperfusion injury; therefore, the number of ASCs may affect the replacement process and post-operative atrophy of grafted fat, which is known to commonly occur during the first 6 months after lipoinjection.

Clinical trials using cell-assisted lipotransfer for soft tissue augmentation, including breast enhancement
As a potential alternative to artificial implants for soft tissue augmentation, including breast enhancement, we started a clinical trial of CAL in 2003. Preliminary results are reported elsewhere [15,16]. Currently, 262 patients (258 females and 4 males) have undergone CAL; on the breast in 230 patients (including 201 cosmetic breast augmentations, 44 breast implant replacements, and 29 post-mastectomy breast reconstructions), on the face in 42 patients (including 3 lupus erythematosus profundus, 2 Romberg’s disease, and 1 scleroderma), on the hip in 4 patients, and on the hand in 3 patients (CAL was performed at multiple sites in fifteen patients).

Preliminary clinical results are generally satisfactory with the natural texture, softness, and contour resulting and an absence of foreign materials. Three-dimensional measurements of breast volume showed graft take ranged from approximately 40% to 80%. Possible complications included cyst formation and calcification derived from fat necrosis, mainly due to inappropriate tissue preparation and injection technique. CT scans and MRI showed transplanted fat tissue survives well and forms a considerably thick fatty layer subcutaneously, on and around the mammary glands, and also between the mammary
glands and the pectoralis muscles. ASC supplementation dramatically improves the ASC/adipocyte ratio and is suggested to minimize adipose atrophy after transplantation, although further studies are needed to elucidate the effects of ASC supplementation.

In the clinical trial, ectopic fibrogenesis was observed in two patients injected with SVF cells as a cell suspension separately from fat grafts [44]. In cell-based therapies using adherent mesenchymal stem cells, unfavorable behaviors such as differentiation into myofibroblasts have been reported [45,46]. This possibility of unexpected behaviors should be taken into account even if cells are derived from adult tissue and have not been substantially manipulated. A resolution may be to adhere adipose-derived adherent stem/progenitor cells to cells, tissue, extracellular matrix, or biological scaffold before administration.

**Future perspective**

Literature reports of ASCs have increased rapidly in the last three years and are continuing to rise. More than ten kinds of clinical trials using freshly isolated or cultured ASCs are ongoing in more than ten countries. Clinical applications are designed based on either (or both) potential ASC differentiation into adipose, vasculature, heart muscle, etc. or the secretory capacity of ASCs for growth factors or hormones. New strategies will be developed to maximize the therapeutic potential of adult stem cells; likely, a microenvironment will be produced to activate and control the administrated cells. Such microenvironments can be prepared by developing novel methods of cell administration or preconditioning of the recipient tissue, e.g. combined administration with bioscaffolds (such as tissues, cells, extracellular matrix, or their combined constructs) or factor-releasing nanometer-sized natural or synthesized products. In the next five years, the safety and efficacy of ongoing clinical trials will be verified and new or modified trials will be initiated. Given the physiological functions of ASCs, promoting angiogenesis would be the most promising outcome. Careful design of cell delivery protocol and selection of target diseases will be critical to the success of each clinical application.

**Executive summary**

- It was recently revealed adipose tissue turns over very slowly (several to 10 years) and adipocyte progenitor cells are present in the vasculature of adipose tissue. Recent works suggested the identity of the progenitor cells and vascular pericytes.

- Adipose tissue contains a substantial amount (greater than adipocytes in number) of stromal cells, called adipose-derived stromal cells (ASCs). Some ASCs are multipotent and can differentiate into various lineages.
· The stromal vascular fraction (SVF) is the heterogeneous cell population obtained from collagenase digestion of adipose tissue; it contains ASCs and vascular endothelial cells, as well as blood-derived cell populations.

· ASCs divide, migrate, and differentiate into adipocytes or vascular cells during the turnover or remodeling process that adipose tissue undergoes to compensate for apoptotic or degenerative changes. Every adipocyte needs direct contact with a capillary, thus adipogenesis is always accompanied by capillary angiogenesis.

· Micro-fat grafting is a promising method of soft tissue augmentation due to the absence of both foreign materials and conspicuous scarring. There are still some issues to be resolved, including unpredictable effectiveness and long-term atrophy of grafted fat tissue.

· Aspirated fat tissue, the material used in micro-fat grafting, has fewer adipose progenitor cells than intact fat tissue. This may impair remodeling of the grafted tissue and lead to long-term atrophy of the grafts.

· Preliminary results from our clinical trials using progenitor-enriched fat tissue grafting suggested supplementation with vascular stromal fractions, containing adipose progenitor cells, may boost the efficacy and safety of conventional autologous micro-fat grafting.

· ASCs are a promising adult stem/progenitor cell population for use as a therapeutic tool, especially in the initial stages of regenerative medicine. A substantial (therapeutic) amount of cells can be obtained without cell culture and the anticipated therapeutic potentials are similar to marrow-derived mesenchymal stem cells.
References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


**Adipose tissue turns over and approximately 10% of adipocytes are renewed annually in all adult ages.


*Adipocyte progenitor cells resident in murine adipose tissue were identified as Lin–:CD29+:CD34+:Sca-1+:CD24+ cell population.


*Endothelial stem/progenitor cells are resident at the transitional area from smooth muscle layer to adventitial layer of vascular walls.


*Human mesenchymal stem cells found throughout fetal and adult tissues are suggested to be perivascular cells (pericytes).

**Adipocyte progenitor cells reside in the mural cell compartment of the adipose vasculature.


**Web site**


**Financial disclosure**

We have nothing to declare.
Legends

Figure 1. Adipose tissue turnover.
It was recently revealed adipose tissue turns over [9] and adipocyte progenitor cells are resident in the vasculature [34]. The extracellular matrix (ECM) also turns over. The average adipocyte life span is ~10 years [9]. Adipocytes and capillaries may be derived from common progenitor cells [8].
Figure 2. **Balance of cell division and death in adipose tissue.**

Adipose tissue turnover is the physiological balance between cell growth and apoptosis. In hyperplasia (not hypertrophy), adipocyte progenitor cells divide more frequently than adipocytes undergo apoptosis. In response to a decrease in progenitor cells with age, tissue turnover results in atrophy. Adipose tissue remodeling responds to various types of stimuli; degenerative and regenerative events are seen depending on the type and extent of stimuli. In all of the processes above, adipose tissue progenitor cells are involved in growth, replacement, or repair processes.
Figure 3. Cellular and molecular events after adipose tissue grafting.
Adipose grafting induces injury in the recipient tissue; bleeding from the host tissue activates platelets and various soluble factors derived from platelets are released. At the same time, basic fibroblast growth factor (bFGF) is released from injured tissue or cells and stimulates adipose-derived progenitor cells to release hepatocyte growth factor (HGF), which promotes angiogenesis and inhibits fibrogenesis [24]. Tissue injury further induces inflammatory cell infiltration and release of inflammatory cytokines. Transplanted adipose tissue is temporarily placed under ischemia and part of the tissue survives after establishing vascularization. In the ischemia-reperfusion process, necrotizing and repairing processes occur and adipose progenitor cells play key roles in the adipose tissue remodeling. ECM, extracellular matrix; PDGF: platelet derived growth factor; EGF: epidermal growth factor; TGFβ: transforming growth factor-β; IL: interleukin.
Figure 4. Comparison of human aspirated and excised (intact) fat tissue obtained from a single site of a single patient
Macroscopic views (left, top), schematic views (left, middle), electromicroscopic (right, top and middle; red scale bars = 200 μm, white scale bars = 40 μm), and whole mount staining images (bottom; scale bars = 100 μm). The basic structure of adipose tissue was preserved in the aspirated fat, while some adipocytes and capillaries were disrupted. Vascular vessels, especially those of large size, were notably less in aspirated fat tissue compared to the excised fat tissue. ASC yield from aspirated fat tissue was considerably less (56 ± 12 %) than that from excised fat tissue [28].
Figure 5. Concept of cell-assisted lipotransfer (CAL)
Relatively progenitor-poor aspirated fat tissue is converted to progenitor-enriched fat tissue by supplementation with the stromal vascular fraction (SVF), which can be obtained from adipose and fluid portions of liposuction aspirates through collagenase digestion. SVF cells are attached to the aspirated fat tissue, which acts as a scaffold in this strategy. The SVF contains 10-40% of adipose-derived stromal cells (ASCs) (CD34+CD31-CD45-), some of which have multipotency and can differentiate into several lineages in vitro. The SVF also contains blood-derived cells (CD45+ cells) such as leukocytes.