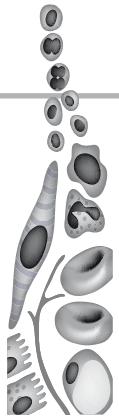

Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation

Many features of adipose tissue-specific stem/progenitor cells, such as physiological function and localization, have recently been examined. Adipose-tissue turnover is very slow 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 postinjury 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, termed cell-assisted lipotransfer, involves the enrichment of adipose progenitor cells in grafts; preliminary results suggest this approach to be safe and effective.

KEYWORDS: adipocyte ▪ adipose-derived stromal cells ▪ angiogenesis ▪ breast augmentation ▪ endothelial progenitor cells ▪ fibrosis ▪ HGF ▪ pericytes ▪ stromal vascular fraction

Kotaro Yoshimura†, Hirotaka Suga & Hitomi Eto

†Author for correspondence: Department of Plastic Surgery, University of Tokyo School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Tel.: +81 3 5800 8948; Fax: +81 3 5800 8947; E-mail: yoshimura-pla@h.u-tokyo.ac.jp

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 also regarded 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], 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 (2–10 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 ASCs 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 (nonautologous ASCs) [14] and soft tissue augmentation by progenitor-enriched fat tissue grafting (autologous fresh ASCs) [15–18]. ASCs have been found to have potential similarities to bone marrow-derived mesenchymal stem cells and are now of great interest as a tool for cell therapies.

Adipose tissue & 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 owing to their large size (50–130 µm diameter), yet the number of adipocytes is estimated to be less than 50% [19,20]. 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 [21] and every adipocyte has contact with capillaries [22], although the capillaries are a substantial distance from each other owing 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 [20]. It has been shown that most inflammatory adipokines

future medicine part of fsg

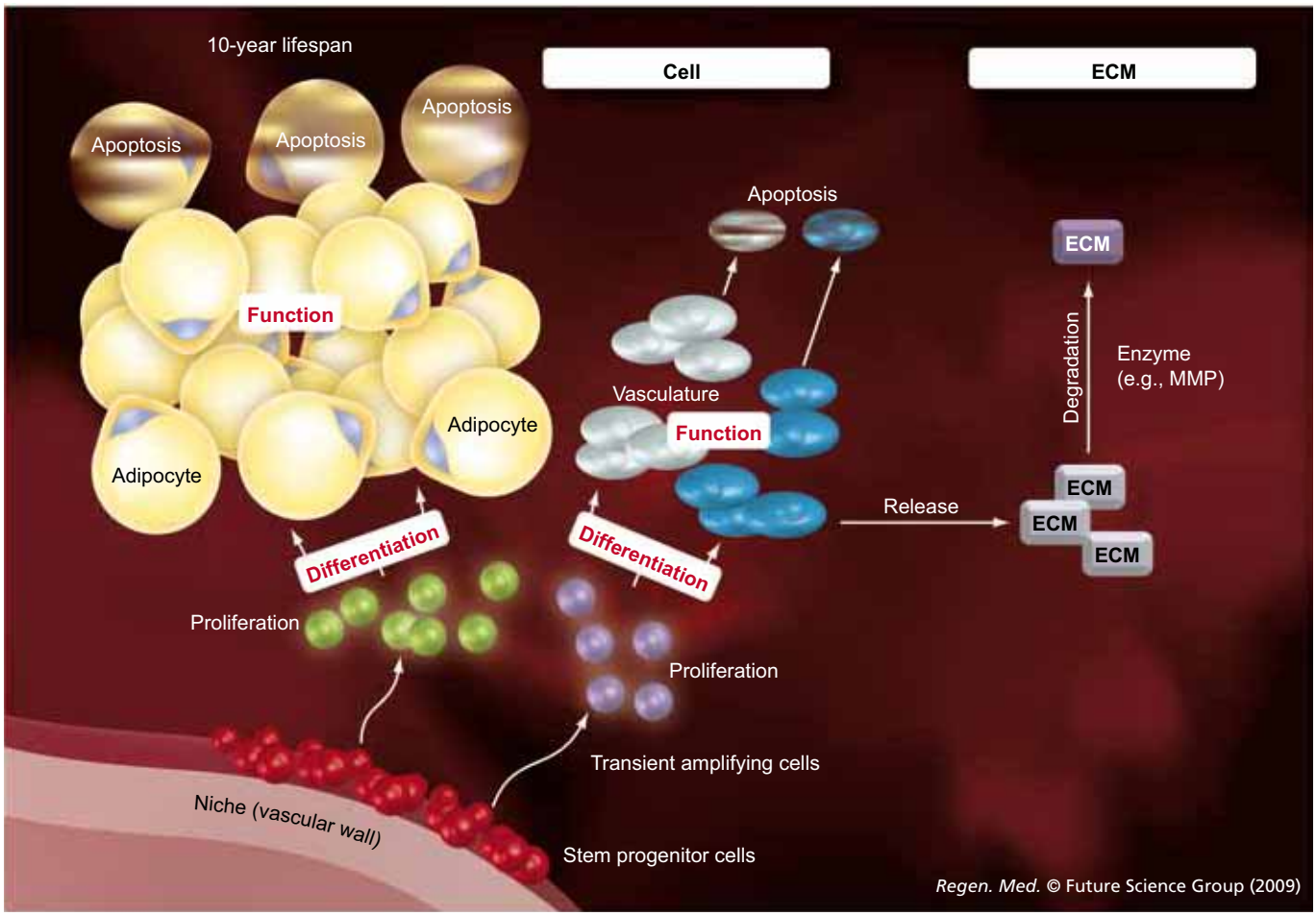

Figure 1. Adipose-tissue turnover. It was recently revealed that adipose-tissue turnover is slow [9] and adipocyte progenitor cells are resident in the vasculature [42]. The ECM also renews itself. The average adipocyte lifespan is approximately 10 years [9]. Adipocytes and capillaries may be derived from common progenitor cells [8].
ECM: Extracellular matrix; MMP: Matrix metalloproteinase.

are released predominantly from cells other than adipocytes, such as ASCs and resident macrophages [20].

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 number 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 [19]. ASCs can be extracted from both the floating fatty portion and fluid portion of liposuction aspirates; however, 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 [23].

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 ASCs. The use of freshly isolated cells likely leads to higher safety and efficacy in treatments compared with

cells expanded by culture; the US FDA (21 CFR Parts 16, 1270 and 1271) regards cells cultured even overnight as more-than-minimally manipulated cells, and cultured ASCs are known to show a distinct phenotype to fresh ASCs [11,24]. 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, as suggested by many studies [25–28].

ASCs in adipose tissue remodeling

ASCs have been shown to have angiogenic characteristics, to release angiogenic factors under ischemia [29,30] or stimulation of growth factors [31], and to experimentally differentiate into vascular endothelial cells [2,32–35]. 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 [28,36] 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 that can differentiate into vessels [37,38], while other studies indicate a possible identity between ASCs and vascular pericytes [39–42]. In addition, ASCs have been shown to be incorporated into microvascular remodeling and exhibit a perivascular phenotype [40]. Recently, it was discovered in mice that adipocyte progenitor cells are present in adipose vasculature as mural cells [42].

ASCs contribute to adipose-tissue turnover and provide cells in the next generation (Figure 1) [9]. Adipocytes have a lifespan of 2–10 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 [31], or in adipose tissue expansion induced by external forces [Kato H *et al.*, 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 subsequent impaired replacement in the next generation, as is commonly seen in other tissues and organs [43].

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 with one in eight women now suffering from this disease 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

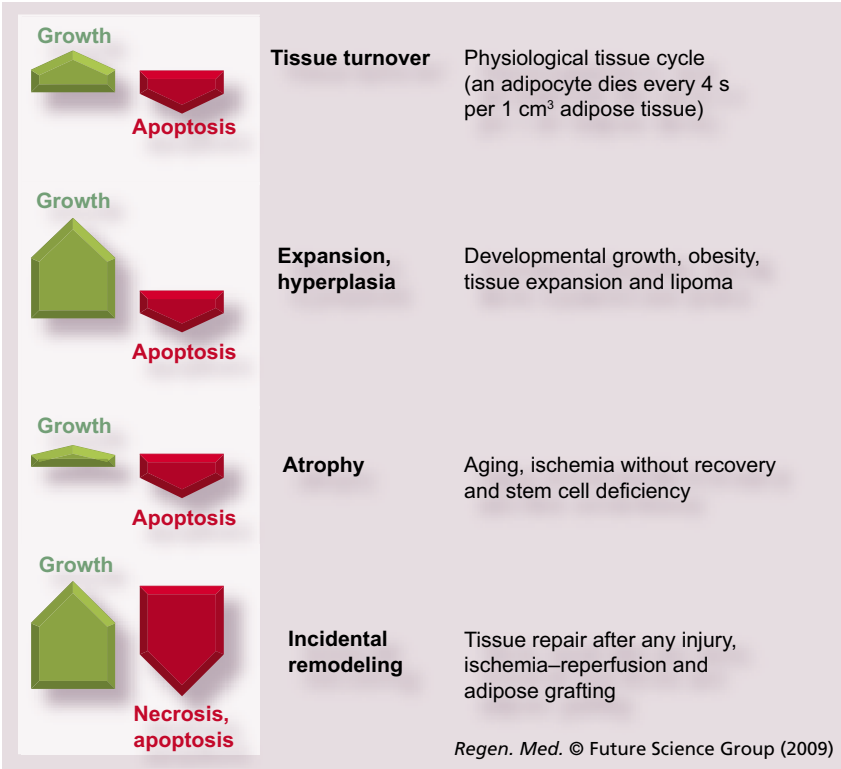

Figure 2. Balance between cell division and death in adipose tissue. Adipose-tissue turnover is the physiological balance between cell growth and apoptosis. In hyperplasia (not hypertrophy), adipocyte progenitor cells divide more frequently than adipocytes undergo apoptosis. In response to a decrease in progenitor cells with age, tissue turnover results in atrophy. Adipose tissue remodeling responds to various types of stimuli; degenerative and regenerative events depend on the type and extent of stimuli. In all of the processes in the figure, adipose tissue progenitor cells are involved in growth, replacement and repair processes.

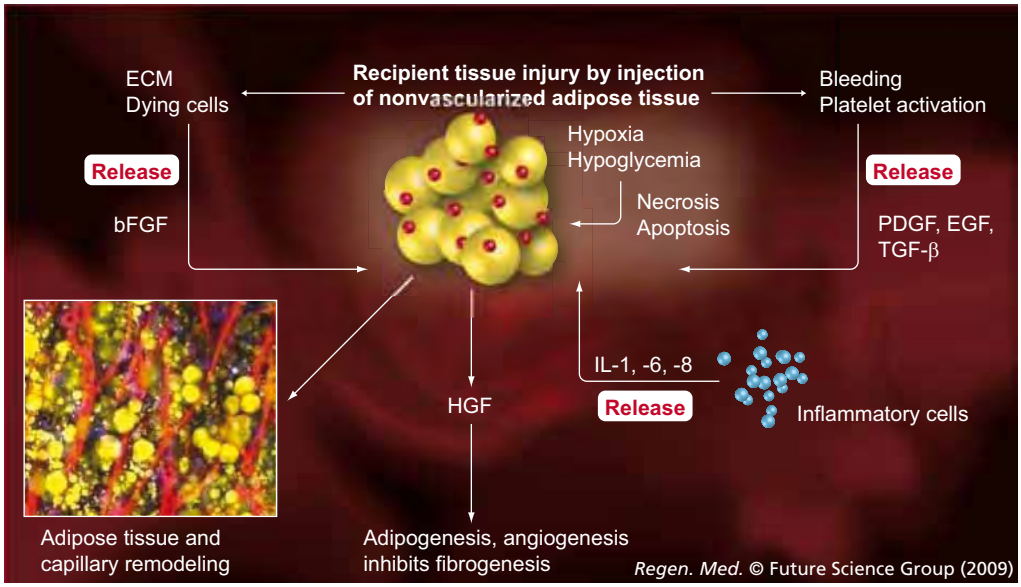

Figure 3. Cellular and molecular events after adipose tissue grafting. Adipose grafting induces injury in the recipient tissue; bleeding from the host tissue activates platelets and various soluble factors derived from platelets are released. At the same time, bFGF is released from injured tissue or cells and stimulates adipose-derived progenitor cells to release HGF, which promotes angiogenesis and inhibits fibrogenesis [31]. Tissue injury further induces inflammatory cell infiltration and the 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 adipose-tissue remodeling. bFGF: Basic FGF; ECM: Extracellular matrix.

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 [44]. The use of fat tissue in breasts has been controversial owing 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 [15,17,18,44,45], 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 nonvasculralized 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, tissue-bound basic FGF is released from the injured host tissue, especially from the extracellular matrix and dying cells [46]. Other factors such as PDGF, EGF and TGF-β are released from activated platelets in response to local bleeding [47]. 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 likely to die within 24 h if the oxygen pressure is lower than its threshold. ASCs, however, are thought to be more resistant to ischemia as is the case for bone marrow-derived mesenchymal stem cells, which can be functional for 72 h under ischemia [48]. In animal models for ischemia–reperfusion injury of adipose tissue [31], ASCs were involved in the repair 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 owing to the stress of temporary ischemia. Therefore, the number of functional ASCs is likely to be important for tissue repair and remodeling.

Aspirated & 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 with intact fat tissue (FIGURE 4) [35]. This relative deficiency of ASCs in aspirated fat tissue may be because a substantial portion of ASCs are located around large vessels (within tunica adventitia) and left in the donor tissue; and 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 with intact fat tissue [35].

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

As discussed earlier, 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

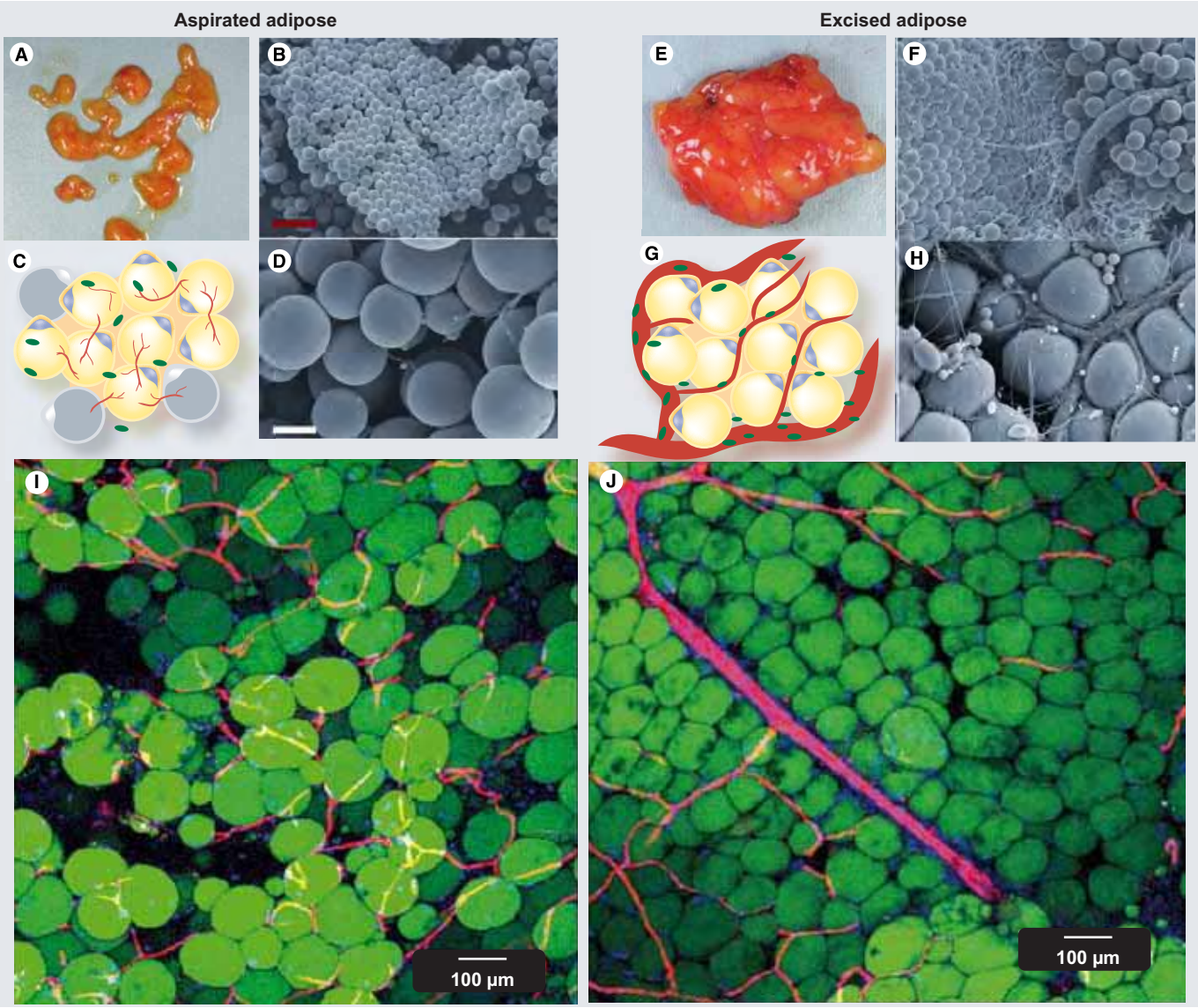

Figure 4. Comparison of human aspirated and excised (intact) fat tissue obtained from a single site from one patient. Macroscopic views **(A & E)**, schematic **(C & G)** and electromicroscopic views (**B, D, F & H**; red scale bars = 200 µm, white scale bars = 40 µm), and whole mount staining images (**I & J**; 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 with the excised fat tissue. Adipose-derived stromal cell yield from aspirated fat tissue was considerably less (56 ± 12%) than that from excised fat tissue. B, D, F and H taken from [35].

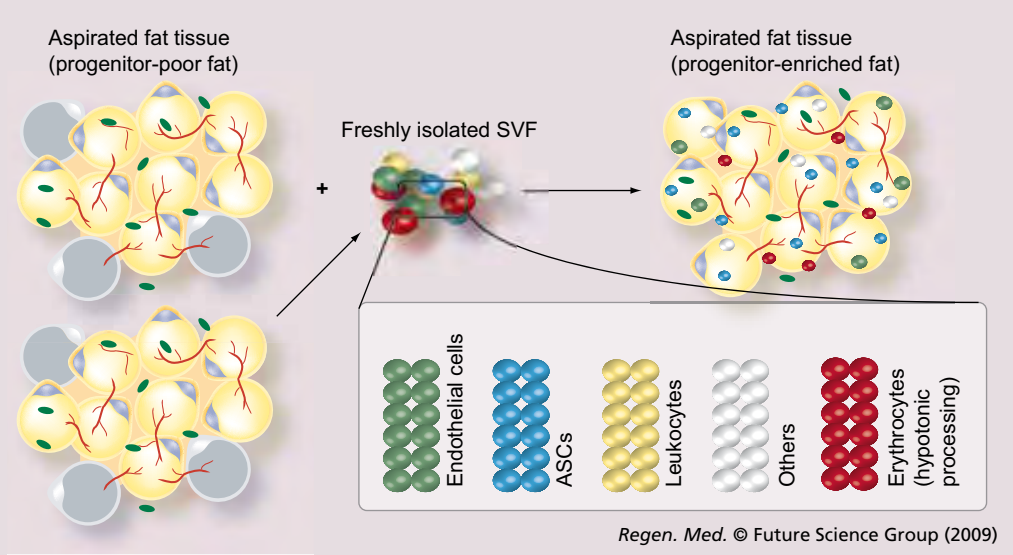

Figure 5. Concept of cell-assisted lipotransfer. Relatively progenitor-poor aspirated fat tissue is converted to progenitor-enriched fat tissue by supplementation with the SVF, which can be obtained from adipose and fluid portions of liposuction aspirates through collagenase digestion. SVF cells are attached to the aspirated fat tissue, which acts a scaffold in this strategy. The SVF contains 10–40% of 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.
ASC: Adipose-derived stromal cell; SVF: Stromal vascular fraction.

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 [35,49,50], demonstrating that 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) [15–18,35], 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 preclinical studies [35,49,50]. 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 [2,32–35,37], resulting in the promotion of angiogenesis and graft survival. Third, ASCs are known to release angiogenic growth factors such as HGF and SDF-1 in response to injury, hypoxia and other conditions [28–31,36], and these factors influence surrounding host tissue. The final, possibly most influential role, is that ASCs survive as original ASCs [35]. As mentioned earlier, 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 turnover within the first 2–3 months after transplantation, as suggested by adipose-tissue remodeling after ischemia–reperfusion injury [31] and other unpublished data. The grafts experience temporary ischemia followed by reperfusion injury; therefore, the number of ASCs may affect the replacement process and postoperative 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 [15–18]. Currently, 307 patients (303 females and four males) have undergone CAL; on the breast in 269 patients (including 177 cosmetic breast augmentations, 52 breast implant replacements and 40 postmastectomy breast reconstructions), on the face in 48 patients (including three lupus erythematosus profundus, two Romberg's disease and one scleroderma), on the hip in four patients, and on the hand in three patients (CAL was performed at multiple sites in 15 patients).

Preliminary clinical results are generally satisfactory in terms of the natural texture, softness, and contour resulting from the soft tissue augmentation and an absence of foreign materials. 3D measurements (Sppd-3E, Kiisya Co. Ltd., Tokyo, Japan) that enabled volumetric evaluation of the breast mound with the patient in a standing position showed that graft take ranged from approximately 40 to 80% [17,18]. Surviving fat volume varies substantially among patients, and multiple factors are likely to affect the clinical results; patient factors include skin redundancy of the breast and technical factors include devices, graft fat preparation and injection techniques. 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 that 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 such as a blinded and controlled comparison of CAL and traditional fat grafting are needed to elucidate the effects of ASC supplementation (i.e., conversion to 'progenitor-rich tissue').

In the clinical trial, ectopic fibrogenesis was observed in two patients injected with SVF cells as a cell suspension separately from fat grafts [51]. In cell-based therapies using adherent mesenchymal stem cells, unfavorable behaviors such as differentiation into myofibroblasts have been reported [52,53]. 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 solution may be to adhere adipose-derived adherent stem/progenitor cells to cells, tissue, extracellular matrix or biological scaffold before administration.

Executive summary

- It was recently revealed that adipose-tissue turnover is very slow (2–10 years) and adipocyte progenitor cells are present in the vasculature of adipose tissue. Recent studies have suggested the identity of the progenitor cells and vascular pericytes.
- Adipose tissue contains a substantial amount (greater than adipocytes in number) of stromal cells, termed adipose-derived stromal cells (ASCs). Some ASCs are multipotent and can differentiate into various lineages.

Adipose tissue & its cellular components

- The stromal vascular fraction 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.

Adipose-derived stem/progenitor cells in adipose tissue remodeling

- 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.

Adipose tissue grafting for soft tissue augmentation

- Micro-fat grafting is a promising method of soft tissue augmentation owing 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 & intact 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.

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

- Preliminary results from our clinical trials using progenitor-enriched fat tissue grafting suggested supplementation with stromal vascular 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 potential is similar to that of bone marrow-derived mesenchymal stem cells.

Future perspective

Literature reports of ASCs have increased rapidly in the last 3 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 and heart muscle, 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, for example, combined administration with bioscaffolds (e.g., tissues, cells, extracellular matrix or their combined constructs) or factor-releasing nanometer-sized natural or synthesized products. In the next 5 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.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Zuk PA, Zhu M, Ashjian P *et al.*: Human adipose tissue is a source of multipotent stem cells. *Mol. Biol. Cell* 13(12), 4279–4295 (2002).

2 Cowan CM, Shi YY, Aalami OO *et al.*: Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. *Nat. Biotechnol.* 22(5), 560–567 (2004).

3 Erickson GR, Gimble JM, Franklin DM, Rice HE, Awad H, Guilak F: Chondrogenic potential of adipose tissue-derived stromal cells *in vitro* and *in vivo*. *Biochem. Biophys. Res. Commun.* 290(2), 763–769 (2002).

4 Rodriguez AM, Pisani D, Dechesne CA *et al.*: Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. *J. Exp. Med.* 201(9), 1397–1405 (2005).

5 Miyahara Y, Nagaya N, Kataoka M *et al.*: Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat. Med.* 12(4), 459–465 (2006).

6 Safford KM, Hicok KC, Safford SD *et al.*: Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem. Biophys. Res. Commun.* 294(2), 371–379 (2002).

7 Gimble JM, Katz AJ, Bunnell BA: Adipose-derived stem cells for regenerative medicine. *Circ. Res.* 100(9), 1249–1260 (2007).

8 Planat-Benard V, Silvestre JS, Cousin B *et al.*: Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. *Circulation* 109(5), 656–663 (2004).

9 Spalding KL, Arner E, Westermark PO *et al.*: Dynamics of fat cell turnover in humans. *Nature* 453(7196), 783–787 (2008).

▪▪ **Adipose-tissue turnover is very slow and approximately 10% of adipocytes are renewed annually in adults of all ages.**

10 Strawford A, Antelo F, Christiansen M, Hellerstein MK: Adipose tissue triglyceride turnover, *de novo* lipogenesis, and cell proliferation in humans measured with 2H2O. *Am. J. Physiol. Endocrinol. Metab.* 286(4), E577–E588 (2004).

11 Yoshimura K, Shigeura T, Matsumoto D *et al.*: Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J. Cell. Physiol.* 208(1), 64–76 (2006).

12 Lendeckel S, Jodicke A, Christophis P *et al.*: Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. *J. Craniomaxillofac. Surg.* 32(6), 370–373 (2004).

13 Garcia-Olmo D, Garcia-Arranz M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA: A Phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis. Colon Rectum.* 48(7), 1416–1423 (2005).

14 Fang B, Song Y, Lin Q *et al.*: Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs-host disease in two children. *Pediatr. Transplant.* 11(7), 814–817 (2007).

15 Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K: Cell-assisted lipotransfer (CAL) for cosmetic breast augmentation – supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast. Surg.* 32(1), 48–55 (2008).

16 Yoshimura K, Sato K, Aoi N *et al.*: Cell-assisted lipotransfer for facial lipoatrophy: efficacy of clinical use of adipose-derived stem cells. *Dermatol. Surg.* 34(9), 1178–1185 (2008).

17 Yoshimura K, Asano Y, Aoi N *et al.*: Progenitor-enriched adipose tissue transplantation as rescue for breast implant complications. *Breast J.* (2008) (In Press).

18 Yoshimura K, Asano Y: Fat injection to the breasts: cosmetic augmentation, implant replacement, inborn deformity, and reconstruction after mastectomy. In: *Aesthetic and Reconstructive Surgery of the Breast.* Hall-Findlay EJ, Evans GRD (Eds). Elsevier Ltd, London, UK (2008).

19 Suga H, Matsumoto D, Inoue K *et al.*: Numerical measurement of viable and nonviable adipocytes and other cellular components in aspirated fat tissue. *Plast. Reconstr. Surg.* 122(1), 103–114 (2008).

20 Trayhurn P, Wang B, Wood IS: Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? *Br. J. Nutr.* 100(2), 227–235 (2008).

21 van Harmelen V, Skurk T, Hauner H: Primary culture and differentiation of human adipocyte precursor cells. *Methods Mol. Med.* 107, 125–135 (2005).

22 Kubik S, Kretz O: Initial lymph vascular system of various tissues and organs. In: *Foldi's Textbook of Lymphology (2nd Edition).* Foldi M *et al.* (Eds). Elsivier GmbH, Munich, Germany 24–41 (2006).

23 Rodeheffer MS, Birsoy K, Friedman JM: Identification of white adipocyte progenitor cells *in vivo*. *Cell* 135(2), 240–249 (2008).

▪ **Adipocyte progenitor cells resident in murine adipose tissue were identified as the Lin$^-$CD29$^+$CD34$^+$Sca-1$^+$CD24$^+$ cell population.**

24 Varma MJ, Breuls RG, Schouten TE *et al.*: Phenotypical and functional characterization of freshly isolated adipose tissue-derived stem cells. *Stem Cells Dev.* 16(1), 91–104 (2007).

25 Borges J, Müller MC, Momeni A *et al.*: *In vitro* analysis of the interactions between preadipocytes and endothelial cells in a 3D fibrin matrix. *Minim. Invasive Ther. Allied Technol.* 16(3), 141–148 (2007).

26 Rajashekhar G, Traktuev DO, Roell WC *et al.*: IFATS collection: Adipose stromal cell differentiation is reduced by endothelial cell contact and paracrine communication: role of canonical Wnt signaling. *Stem Cells* 26(10), 2674–2681 (2008).

27 Lai N, Jayaraman A, Lee K: Enhanced proliferation of human umbilical vein endothelial cells and differentiation of 3T3-L1 adipocytes in coculture. *Tissue Eng. Part A* DOI:10.1089/ten.tea.2008.0101. (2008) (Epub ahead of print).

28 Nakagami H, Maeda K, Morishita R *et al.*: Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. *Arterioscler. Thromb. Vasc. Biol.* 25(12), 2542–2547 (2005).

29 Rehman J, Traktuev D, Li J *et al.*: Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109(10), 1292–1298 (2004).

30 Thangarajah H, Vial IN, Chang E *et al.*: IFATS series: Adipose stromal cells adopt a proangiogenic phenotype under the influence of hypoxia. *Stem Cells* DOI: 10.1634/stemcells.2008–0277 (2008) (Epub ahead of print).

31 Suga H, Eto H, Shigeura T *et al.*: IFATS collection: FGF-2-induced HGF secretion by adipose-derived stromal cells inhibits post-injury fibrogenesis through a JNK-dependent mechanism. *Stem Cells* 27(1), 238–249 (2009).

32 Miranville A, Heeschen C, Sengenes C *et al.*: Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation* 110(3), 349–355 (2004).

33 Cao Y, Sun Z, Liao L *et al.*: Human adipose tissue-derived stem cells differentiate into endothelial cells *in vitro* and improve postnatal neovascularization *in vivo*. *Biochem. Biophys. Res. Commun.* 332(2), 370–379 (2005).

34 Moon MH, Kim SY, Kim YJ *et al.*: Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. *Cell Physiol. Biochem.* 17(5–6), 279–290 (2006).

35 Matsumoto D, Sato K, Gonda K *et al.*: Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. *Tissue Eng.* 12(12), 3375–3382 (2006).

36 Kondo K, Shintani S, Shibata R *et al.*: Implantation of adipose-derived regenerative cells enhances ischemia-induced angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* 29(1), 61–66 (2008).

37 Alessandri G, Girelli M, Taccagni G *et al.*: Human vasculogenesis *ex vivo*: embryonal aorta as a tool for isolation of endothelial cell progenitors. *Lab.Invest.* 81(6), 875–885 (2001).

38 Zengin E, Chalajour F, Gehling UM *et al.*: Vascular wall resident progenitor cells: a source for postnatal vasculogenesis. *Development* 133(8), 1543–1551 (2006).

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

39 Traktuev DO, Merfeld-Clauss S, Li J *et al.*: A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ. Res.* 102(1), 77–85 (2008).

40 Amos PJ, Shang H, Bailey AM, Taylor A, Katz AJ, Peirce SM: IFATS series: The role of human adipose-derived stromal cells in inflammatory microvascular remodeling and evidence of a perivascular phenotype. *Stem Cells* 26(10), 2682–2690 (2008)

41 Crisan M, Yap S, Casteilla L *et al.*: A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3(3), 301–313 (2008).

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

42 Tang W, Zeve D, Suh JM *et al.*: White fat progenitor cells reside in the adipose vasculature. *Science* 322(5901), 583–586 (2008)

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

43 Caplan AI: Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J. Cell. Physiol.* 213(2), 341–347 (2007).

44 Coleman SR, Saboeiro AP: Fat grafting to the breast revisited: safety and efficacy. *Plast. Reconstr. Surg.* 119(3), 775–785 (2007).

45 Spear SL, Wilson HB, Lockwood MD: Fat injection to correct contour deformities in the reconstructed breast. *Plast. Reconstr. Surg.* 116(5), 1300–1305 (2005).

46 Muthukrishnan L, Warder E, McNeil PL: Basic fibroblast growth factor is efficiently released from a cytolsolic storage site through plasma membrane disruptions of endothelial cells. *J. Cell Physiol.* 148(1), 1–16 (1991).

47 Aiba-Kojima E, Tsuno NH, Inoue K *et al.*: Characterization of wound drainage fluids as a source of soluble factors associated with wound healing: comparison with platelet-rich plasma and potential use in cell culture. *Wound Repair Regen.* 15(4), 511–520 (2007).

48 Myolotte LA, Duffy AM, Murphy M *et al.*: Metabolic flexibility permits mesenchymal stem cell survival in an ischemic environment. *Stem Cells* 26(5), 1325–1336 (2008)

49 Masuda T, Furue M, Matsuda T: Novel strategy for soft tissue augmentation based on transplantation of fragmented omentum and preadipocytes. *Tissue Eng.* 10(11–12), 1672–1683 (2004).

50 Moseley TA, Zhu M, Hedrick MH: Adipose-derived stem and progenitor cells as fillers in plastic and reconstructive surgery. *Plast. Reconstr. Surg.* 118(Suppl. 3), S121–S128 (2006).

51 Yoshimura K, Aoi N, Suga H *et al.*: Ectopic fibrogenesis induced by transplantation of adipose-derived progenitor cell suspension immediately after lipoinjection. *Transplantation* 85(12), 1868–1869 (2008).

52 Russo FP, Alison MR, Bigger BW *et al.*: The bone marrow functionally contributes to liver fibrosis. *Gastroenterology* 130(6), 1807–1821 (2006).

53 di Bonzo LV, Ferrero I, Cravanzola C *et al.*: Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. *Gut* 57(2), 223–231 (2008).

Websites

101 National Cancer Institute. Probability of Breast Cancer in American Women. www.cancer.gov/cancertopics/factsheet/Detection/probability-breast-cancer

102 American Society for Plastic Surgery. National plastic surgery statistics 2007. www.plasticsurgery.org/media/statistics/loader.cfm?url=/commonspot/security/getfile.cfm&PageID=29287