Condensation of Tissue and Stem Cells for Fat Grafting

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KEYWORDS

• Fat grafting • Cell assisted lipotransfer • Adipose-derived stem/stromal cell • Tissue regeneration • Macrophages • Vascular endothelial cells

KEY POINTS

- Adequate centrifugation purifies and condenses aspirated adipose tissue and improves graft retention.
- We can condense tissue by removing unnecessary components of grafted tissue through decantation, filtrations or centrifugation.
- Condensation of adipose-derived stem/stromal cells (ASCs) is important to get better adipocyte regeneration after fat grafting and achieve tissue revitalizing effects.
- ASCs can be condensed by reducing adipocytes from the graft through mechanical processing or strong centrifugation.
- Supplementation of stromal vascular fraction or ASCs can also improve ASC/adipocyte ratio in the graft and is expected to obtain better outcomes for tissue volumization and revitalization.

INTRODUCTION

Adipose tissue has many types of cells other than adipocytes, which can be extracted as a cell pellet called stromal vascular fraction (SVF) through collagenase digestion of aspirated adipose tissue. SVF contains adipose-derived stem/stromal cells (ASCs), vascular endothelial cells, pericytes, adipose-resident macrophages, lymphocytes, and so on.[1] ASCs are regarded as a potent tool for cell base therapies because they have biological functions such as multidirection differentiation, growth factor secretion, and immunomodulation, and can be obtained readily in a large amount through liposuction.

Condensation of grafting adipose tissue is a key to achieve better volumizing effects (better volume retention) by fat grafting. It is particularly important when these is a limitation of injection volume (eg, breast) owing to the limited skin envelop, because an injection of excessive volume leads to severe ischemia and fat necrosis. Condensation of grafting fat can be achieved by means of removal of unnecessary components, such as water, oil, dead cells, and blood cells. Because aspirated fat tissue is relatively poor in stem cells (ASCs),[2] condensation of ASCs in the graft is another issue for seeking better volumizing effects.

Recently, regenerative effects of fat grafting are appreciated by many clinicians. Stem cell–depleted tissues such as irradiated tissue, chronically inflammatory tissue, and ischemic fibrous tissue are improved by fat grafting in quality, vascularity, and healing and expanding capacity.[3,4] It has been reported frequently that hypertrophic scarring and

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scar contracture are softer and that skin hyperpigmentation disappears after fat grafting.[5] Such regenerative/revitalizing effects of fat grafting are considered to be derived from ASCs in the tissue. Therefore, condensation of ASCs in the graft may be also crucial for such regenerating/revitalizing applications.

BASIC SCIENCE

Difference Between Aspirated Fat Tissue and Intact Adipose Tissue

Adipose tissue contains various types of cells including adipocytes and ASCs, as well as connective tissue (see the article by Mashiko and Yoshimura elsewhere in this issue for details). When surgeons aspirate fat, only fragile parts of adipose tissue are harvested through a suction cannula, whereas the honeycomb-like fibrous structures remain intact in the donor site.[6] The fibrous structure is predominantly composed of connective tissues and large vasculatures, which are considered to contain many ASCs. We have found that aspirated fat tissue contains only one-half the number of ASCs compared with intact fat tissue.[2] Stage-specific embryonic antigen-3–positive cells, which may be highly multipotent stem cells (muse cells),[7] locate around large vasculatures. These cells are also deficient in aspirated adipose tissue (unpublished data, Doi K et al, 2012). The relative deficiency of ASCs in aspirated fat tissue may be owing to (1) a substantial portion of ASCs being left in the donor tissue and (2) some ASCs being released into the fluid portion of liposuction aspirates, possibly owing to the act of an endogenous enzyme.[1,6] Thus, aspirated fat tissue is regarded as relatively ASC poor compared with intact fat tissue. This low ASC/adipocyte ratio may be a reason for long-term atrophy after fat grafting.[2]

Importance of Adipose-Derived Stem/Stromal Cells in the Grafted Tissue for Adipose Regeneration after Fat Grafting

ASCs have the potential to modulate or suppress immunoreaction,[8] differentiate into adipocytes,[9,10] vascular endothelial cells, or others and release angiogenic growth factors, such as hepatocyte growth factor and vascular endothelial growth factor, especially under hypoxic conditions.[11] ASCs were reported to contribute to angiogenesis during the adipose remodeling process after ischemia or fat grafting.[9–11] Our recent study using green fluorescent protein mice revealed that regenerated adipocytes after fat grafting are mostly originated from ASCs in the graft tissue, but not from other host-derived stem/progenitor cells, although new ASCs can be provided partly by bone marrow or other tissues.[12] It was suggested that only ASCs originally located adjacent to dying adipocytes can become adipocytes, although other ASCs can contribute in other ways, such as angiogenesis or release of growth factors.

RELEVANCE TO CLINICIANS

Graft Tissue Condensation

Liposuction aspirates contain some components unnecessary for adipose tissue engraftment/regeneration; water, oil (broken adipocytes), and blood cells (red blood cells and white blood cells). It is recommended to remove such components and reduce the graft volume without reducing the number of viable adipocytes and ASCs; this is called condensation of graft tissue. Tissue condensation is important, especially when there is a maximum limit in graft volume, such as with breast augmentation. There are 3 major methods for graft tissue condensation: decantation (gravity sedimentation), filtration with or without a vacuum, and centrifugation. Among the 3, centrifugation is most effective to remove the water content without losing ASCs, although some adipocytes can be broken by the mechanical force and the resulting condensed fat may become more viscous and need higher pressure to inject through a small cannula (**Fig. 1**).[13] Oil released from damaged adipocytes causes inflammation-like foreign materials, suggesting that removal of oil should be important for better healing after fat grafting.

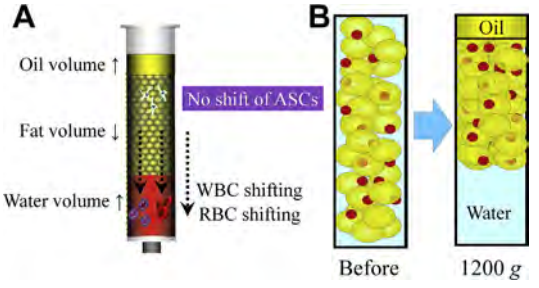

Fig. 1. Tissue and adipose-derived stem/stromal cell (ASC) condensation by centrifugation. (*A*) By centrifugation, fat volume becomes compact, water volume increases and oil will be clearly separated as a top layer. Many of red blood cells (RBCs) and white blood cells (WBCs) in the aspirated fat shift into the water portion after centrifugation, but most of ASCs remain in the fat portion. (*B*) By centrifugation at 1200×g for 3 minutes, fat volume decreases by 30%. Some adipocytes are broken and become oil as a top layer, but all ASCs remain intact and are concentrated in the condensed fat tissue.

Ratio of Adipocytes and Adipose-Derived Stem/Stromal Cells in the Graft

Recent studies indicate that ASCs in the graft are key components to contribute adipogenesis and angiogenesis after fat grafting. If the graft tissue is ASC deficient in number, it may be reasonable to normalize stem cell density in graft tissue.[6] There are theoretically 2 ways to improve the adipocyte/ASC ratio in the graft (ASC condensation): one is to reduce the number of adipocytes and tissue volume, and the other is to increase the number of ASCs (**Fig. 2**).

Reducing the number of adipocytes and tissue volume can be done by mechanical removal of adipocytes, such as mechanical crushing/mincing, aggressive centrifugation, and ultrasonic cavitation. Such destructive processes have to be done with great care, because too much damage, heat, or pressure to the tissue could kill ASCs as well. Increasing the number of ASCs can be done by supplementing freshly isolated SVF or cultured/purified ASCs to the graft (cell-assisted lipotransfer).[2] SVF can be achieved through collagenase digestion or other ways if extra liposuction aspirates are available. ASCs can be purified readily and expanded by adherent culture of SVF, and can also be banked in a liquid nitrogen for a long period if needed.

Adipose-Derived Stem/Stromal Cells Condensation by Reduction of Adipocytes and Tissue Volume

Reduction of adipocyte number in the tissue without losing ASC viability, which leads to tissue volume reduction and ASC condensation at the same time, can be done by various methods, such as aggressive centrifugation, mechanical crushing or mincing, and ultrasound cavitation.

Centrifugation not only separates water and oil from fat tissue, but also mechanically breaks some adipocytes, depending on the magnitude of centrifugal force, although ASCs in the tissue

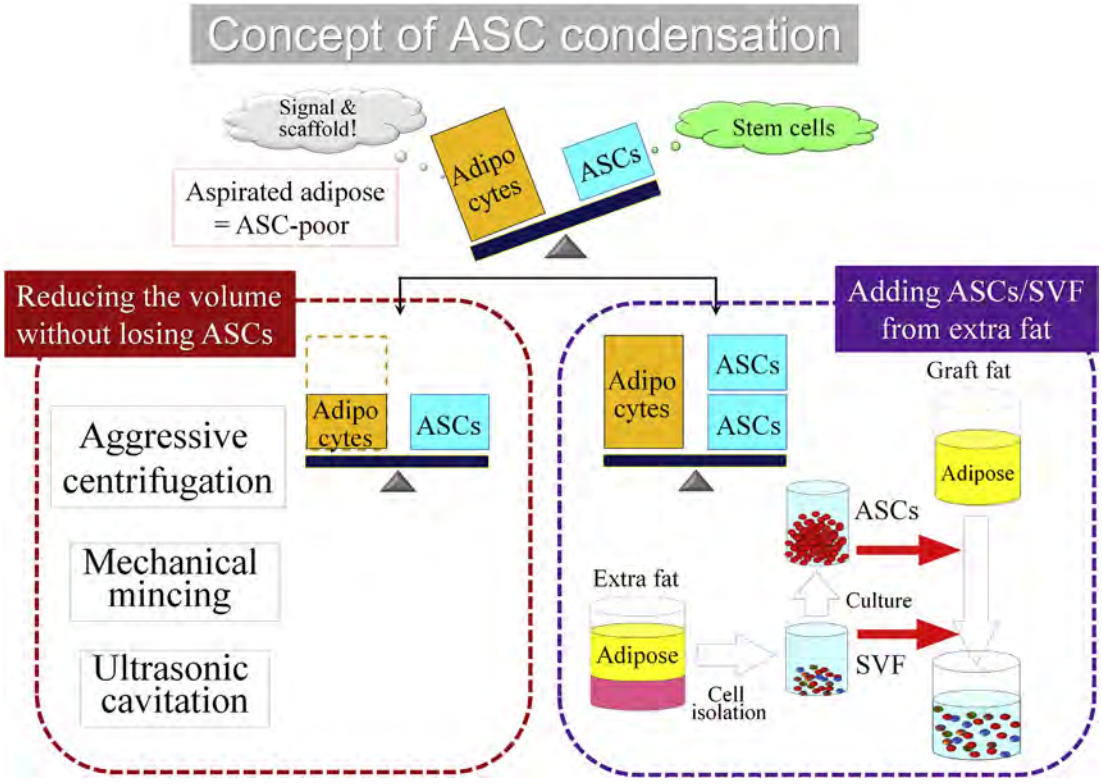

Fig. 2. Two concepts for adipose-derived stem/stromal cell (ASC) condensation in the graft tissue. Aspirated fat tissue is relatively ASC-poor compared with intact adipose tissue, and there are 2 concepts to normalize the ASC number in the tissue. One is to reduce the number of adipocytes without losing ASCs, which results in substantial volume reduction. After this process, adipose tissue and ASCs are condensed with a greater ratio of ASCs/adipocytes. Another is to supplement freshly isolated stromal vascular fraction (SVF) or culture expanded ASCs. Isolated ASCs cannot function unless they are properly incorporated into the tissue.

are well preserved when centrifuged at below 3000×*g*.[13] Thus, the adipocyte/ASC ratio can be increased after strong centrifugation (by 20% when centrifuged at 1200×*g*; see **Fig. 1**B). Adequate centrifugation condenses tissue and ASCs, and also improves fat graft survival, although too strong centrifugation may worsen graft survival.[13,14]

There are some other attempts to further condense adipose graft tissue. Mechanical chopping, shredding, pureeing, or mincing, manually or with specific devices (like homogenizers or food processors), can further fragment aspirated fat tissue and rupture adipocytes. Appropriately, such mechanical processing can reduce substantially adipocytes, which become oil and can be removed by subsequent centrifugation. As a result, condensed fat tissue with a reduced volume can be obtained, although excessive processing can kill ASCs as well as endothelial cells, and has to be avoided. Ultrasonic cavitation may be also useful to damage selectively adipocytes in the future.

Adipose-Derived Stem/Stromal Cells Condensation by Supplementing Stromal Vascular Fraction or Adipose-Derived Stem/Stromal Cells

Another strategy is supplementing freshly isolated SVF or culture-expanded ASCs to aspirated fat tissue and called cell-assisted (enhanced) lipotransfer (**Fig. 3**). We can achieve SVF cells from lipoaspirates through collagenase digestion (processed lipoaspirate cells), although a much smaller number of SVF is also obtained from the fluid infranatant portion of liposuction aspirates (liposuction aspirate fluid cells).[1] Other nonenzymatic methods, such as mechanical processing and ultrasonic cavitation, have been attempted, but there are no established, efficient methods so far.

Stromal Vascular Fraction Isolation Procedures

For processed lipoaspirate cells, suctioned fat tissue is digested with 0.075% collagenase in phosphate-buffered saline for 30 minutes on a

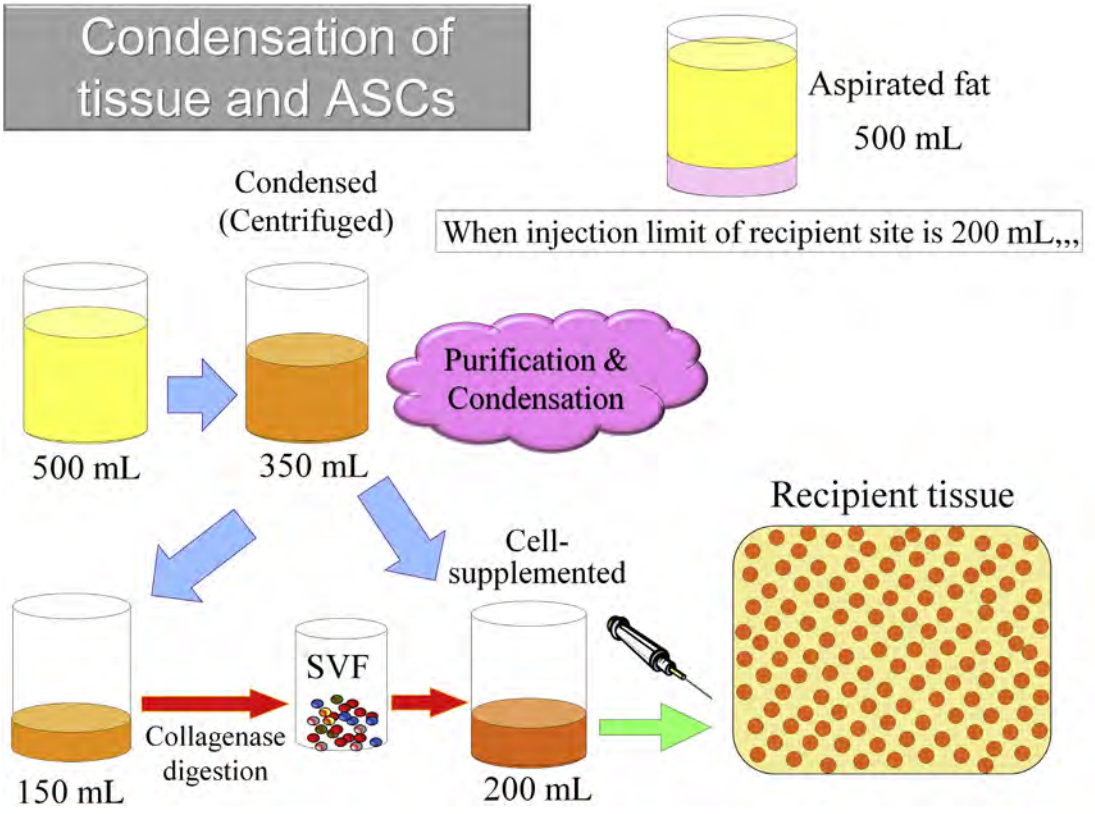

Fig. 3. An example of how to use aspirated fat. In this case, when we have 500 mL aspirated fat but the recipient tissue accepts a 200-mL injection at maximum (eg, owing to limited skin envelop), we can process and condense the graft tissue and adipose-derived stem/stromal cells (ASCs) before transplantation. Even after strong centrifugation, 350 mL of condensed fat tissue remains. Then the excessive 150 mL of centrifuged fat can be used for stromal vascular fraction (SVF) isolation for further condensation of ASCs in the graft material.

shaker at 37°C.[15] After centrifugation (800×*g* for 10 minutes), floating tissue (adipocytes and connective tissues) are discarded. The cell pellets are resuspended in Dulbecco's modified Eagle's medium and passed through a 100-mm mesh filter. To eliminate the remaining collagenase, the cells pellets are washed by resuspension in Dulbecco's modified Eagle's medium and after centrifugation at least 3 times. The process takes about 80 minutes (**Fig. 4**). For liposuction aspirate fluid cells, the suctioned fluid is centrifuged (400×*g* for 10 minutes) and the pellets are resuspended in distilled water (for 30 seconds) for erythrocyte lysis, followed by osmic normalization by adding 10% volume of 10× phosphate-buffered saline (or 9% NaCl solution). After centrifugation (and filtration), cell pellets are obtained as liposuction aspirate fluid cells; the process takes about 20 minutes. For these SVF cells, cell counting for erythrocytes and nucleated cells is performed using a hematocytometer used for blood testing. The SVF cell number is affected largely by hemorrhage contamination. Normal viable nucleate processed lipoaspirate cells cell number is 300,000 to 800,000 per 1 mL of aspirated adipose tissue.[1] Before injection, freshly isolated SVF cells or cultured ASCs are added to graft materials, followed by gentle mixing and a 5- to 10-minute incubation period to achieve appropriate cell adhesion to the graft tissue.

Clinical Reports of Cell-Assisted Lipotransfer

There are some studies reporting the clinical outcomes of cell-assisted lipotransfer. Although lipotransfer supplemented with ASCs or SVF cells have shown therapeutic potential in uncontrolled trials and comparative case series,[16–22] the clinical results remain controversial. Recently, some comparative studies of cell-assisted lipotransfer were reported. Chang and colleagues[23] reported a volumetric analysis of SVF-supplemented fat grafting and regular fat grafting to progressive hemifacial atrophy patients, concluding that fat survival and clinical improvement was greater with SVF-supplemented grafting than fat grafting alone after 6 months. Tanikawa and colleagues[24] reported that SVF-supplemented fat grafting for patients with craniofacial microsomia was effective. Survival fat volume was 88% at 6 months, which was significantly greater that nonsupplemented fat grafting (54%). Gentile and colleagues[25] applied SVF-supplemented fat grafting to the face and reported significantly better contouring maintenance compared with fat grafting alone. In contrast, Peltoniemi and colleagues[26] and Wang

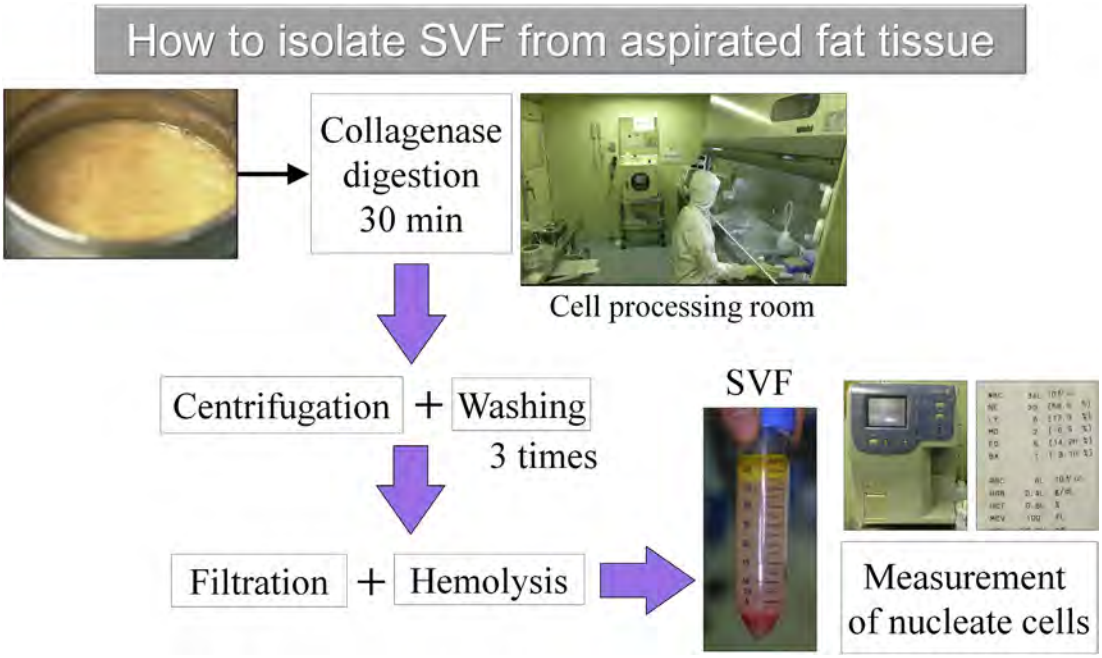

Fig. 4. Collagenase digestion process of aspirated fat for stromal vascular fraction (SVF) isolation. Aspirated fat is digested with collagenase for 30 minutes and the isolated cell fraction can be collected after spinning. Then, we wash the SVF cells, remove red blood cells by incubation with hemolysis buffer, and count the obtained nucleate cells with a hematocytometer or cell counter. The whole process should be performed in a clean room and takes about 80 minutes.

and colleagues[27] reported that cell-assisted lipotransfer using SVF cells did not contribute to improve the outcomes.

Kølle and colleagues[28] reported a randomized, placebo-controlled trial to compare the volumizing effects of ASC-enhanced fat graft and regular fat graft in the same patients. In this study, ASCs were expanded by adherent culture and 20 million cultured ASCs were supplemented to 1 g fat graft, showing a significantly greater volume retention (80.9%) in ASC-supplemented group compared with 16.3% in the nonadditive control.

Taken together, the results of fat grafting with SVF/ASC supplementation seem to be affected by many factors. There is no standard or optimized method of SVF isolation and cell supplementation to the graft tissue. SVF contains many other cells, such as leukocytes, and some may have unfavorable effects on fat grafting. The number of ASCs isolated in SVF through collagenase is only 10% to 20% of those contained in the original tissue. ASCs have to be attached to the tissue or cells to function properly and avoid unwanted migration or differentiation.[29] Volume retention is not a reliable index to evaluate fat grafting results because oil cyst formation from large fat necrosis also increases the clinical score of volume retention. Further studies are necessary to achieve clinical benefits of ASCs with greater magnitude and consistency.

Further Condensation of Adipose-Derived Stem/Stromal Cells for Other Therapeutic Use

Fresh SVF and cultured ASCs have been used in numerous clinical trials, including autoimmune diseases, osteoarthritis, and myocardial infarction. These trials are expecting ASCs to reduce immunoreaction, release growth factors, and/or accelerate tissue repair and angiogenesis. However, there are other attempts to prepare ASC-containing tissues by removing adipocytes from adipose tissue and further condensing ASCs. Both adipocytes and ASCs are needed for tissue enlargement (adipose regeneration after fat grafting), but therapies for improving the quality (vascularity, inflammation, elasticity, and healing capacity) of tissue may not need any adipocytes. Fat grafting is showing clinical success for rejuvenating and revitalizing tissue. Such new types of processed adipose tissue (without adipocytes) are expected to be used in the future as an alternative to fat grafting for treating stem cell-depleted tissues.

SUMMARY

Adipose tissue has many types of cells other than adipocytes, which can be extracted as a cell pellet called SVF, which contains ASCs, vascular endothelial cells, pericytes, adipose-resident macrophages, and lymphocytes, among others. Condensation of grafting adipose tissue is a key to achieve better volumizing effects (better volume retention) by fat grafting. Because aspirated fat tissue is relatively poor in stem cells (ASCs), condensation of ASCs in the graft is another issue for seeking better volumizing and regenerating effects. One way to improve the adipocyte/ASC ratio in the graft (ASC condensation) is to reduce the number of adipocytes and tissue volume, and the other way is to increase the number of ASCs. ASC condensation can be done by mechanical removal of adipocytes, and increasing the number of ASCs can be achieved by supplementing freshly isolated SVF or cultured/purified ASCs to the graft (cell-assisted lipotransfer). Clinical trials of fat grafting with supplementation of SVF/ASCs suggested beneficial effects of supplementation, although further studies are needed to confirm and achieve benefits of ASCs with greater magnitude and consistency. For nonvolumizing purposes, such as revitalization of stem cell-depleted tissue and treatment of inflammatory conditions, a new style of processing of adipose tissue may be utilized in the future.

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