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Review Article

Fat grafts enriched with adipose-derived stem cells

Ki Yong Hong

Department of Plastic and Reconstructive Surgery, Dongguk University Ilsan Hospital, Goyang, Korea

Autologous fat grafts are widely used in soft-tissue augmentation and reconstruction. To reduce the unpredictability of fat grafts and to improve their long-term survival, cell-assisted lipotransfer (CAL) was introduced. In this alternative method, autologous fat is mixed and grafted with stromal vascular fraction cells or adipose-derived stem/stromal cells (ASCs). In regenerative medicine, ASCs exhibit excellent therapeutic potential and are also simple to harvest. Although the efficacy of CAL has been demonstrated in experimental and clinical research, studies on its safety in terms of oncologic risk have reported inconclusive results. In order to establish CAL as a viable stem cell therapeutic approach, it will be necessary to demonstrate its oncologic safety in basic and clinical studies. Doing so could transform the paradigm of clinical strategy and practice for the treatment of a wide variety of diseases.

Keywords: Adipose tissue / Adipose-derived mesenchymal stem cells / Transplants

FAT GRAFTS

Adipose tissue

Autologous fat grafts have been widely used in soft-tissue augmentation and reconstruction surgery. Despite their numerous advantages, including a simple harvest technique, low cost, and easy accessibility, the applicability of autologous fat grafts is limited by their unpredictable long-term outcomes associated with poor graft retention [1,2]. In recent decades, several advances have been made to overcome the low rate of graft survival, including various refinements of existing techniques, the development of nanofat to reduce the size of fat particles used for injection, and the standardization of protocols for fat harvesting and processing [3-5].

Adipose tissue is composed of at least two functionally distinct types of fat: white and brown [6]. The primary roles of white adipose tissue are energy storage and the release of hormones and adipokines that modulate whole-body metabolism [7]. Consequently, the majority of studies related to adipose tissue have focused on the treatment of obesity, which contributes to an increased risk of developing type 2 diabetes, cardiovascular disorders, and cancer [8]. Additionally, white tissue can act as a thermal insulator and protect other organs from mechanical damage [9]. However, for plastic surgeons, the physical properties of white adipose tissue, not its function, make autologous fat grafts a useful component of their surgical strategies.

Fate of fat grafts

Adipose tissue is composed of adipocytes, adipose-derived stem/stromal cells (ASCs) and various other cells, including endothelial, mural, immune, and neuronal cells [10]. In addition, adipose tissue is highly vascularized, as each adipocyte is surrounded by an extensive capillary network [11]. Thus, angiogenesis is closely related to the maintenance and remodeling of adipose tissue. A recent study investigating the fate of adipocytes and ASCs after non-vascularized fat grafts identified three zones (survival, regenerating, and necrotic) in grafts [12]. In the survival zone, which is less than 300 μm thick, both adipocytes

Correspondence: Ki Yong Hong
Department of Plastic and Reconstructive Surgery, Dongguk University Ilsan Hospital, 27 Dongguk-ro, Ilsandong-gu, Goyang 10326, Korea
E-mail: pskyhong@gmail.com

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and ASCs can survive. In the regenerating zone, which varies in thickness depending on aspects of the microenvironment such as vascularity, adipocytes die and only ASCs survive. Grafted adipose tissue in this zone degenerates within the first week, and regenerates into mature adipocytes after 3 months [13]. However, in the necrotic zone, which is at the center of the graft, both adipocytes and ASCs eventually die and are absorbed, after which the zone is filled with fibrous tissue. According to this theory, rapid revascularization of the surrounding recipient tissue and expansion of the regenerating zone resulting from increased vascularity are crucial for ensuring the survival of non-vascularized fat grafts.

ADIPOSE-DERIVED STEM CELLS

ASCs are multipotent mesenchymal stem/stromal cells (MSCs) with potential to differentiate not only into mesodermal lineages [14], such as adipocytes, osteoblasts, chondrocytes, fibroblasts, and myocytes, but also into non-mesodermal cell types, such as neuronal cells [15], hepatocytes [16], endothelial cells [17,18], and cardiomyocytes [19]. In addition to their extensive differentiation potential, ASCs secrete high levels of growth factors, including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and transforming growth factor-beta (TGF-β) [20]. In addition to growth factors, ASCs release cytokines, including fms-related tyrosine kinase 3 (Flt-3) ligand, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage-colony stimulating factor (M-CSF), and various interleukins [21].

Stromal-vascular fraction

Manual isolation of ASCs typically involves enzymatic digestion of adipose tissue using collagenase, followed by centrifugation to separate floating mature adipocytes from other cells in the pellet [3,22]. The isolated cells in the pellet, known as stromal vascular fraction (SVF) cells, are a heterogenous mixture of cells including ASCs, endothelial cells, pericytes, immune cells, and fibroblasts [23]. SVF cells are a primary culture from adipose tissue, and are designated as passage 0 ASCs. While bone marrow stem cells constitute less than 0.002% of stromal cells, it has been estimated that ASCs constitute up to 2% of SVF cells. This underscores the clinical value of adipose tissue as a valuable source of competent adult stem cells [24]. However, the composition ratio of ASCs within SVF cells could vary widely depending on factors including age, sex, clinical history, and the harvest site.

Definition of ASCs

ASCs were first reported by Zuk et al. in 2001 [25]. These cells have been described using a variety of terms, and were finally named as ASCs by the International Federation for Adipose Therapeutics and Science (IFATS) [26]. The IFATS and the International Society for Cellular Therapy published a joint statement to establish minimal definitions of SVF cells and ASCs (Table 1) [27]. According to this declaration, SVF cells and ASCs should have a viability of >70% and >90%, respectively. SVF cells express the following marker profile: CD13, CD29, CD44, CD73, and CD90 positive (>40%), and CD34 positive (>20%), but CD31 (<20%) and CD45 negative (<50%). ASCs should be positive for CD13, CD29, CD44, CD73, CD90, and CD105 (>80%), but negative for CD31, CD45, and CD235a (<2%). ASCs can be distinguished from bone-marrow-derived MSCs as they are CD36-positive and CD106-negative. Moreover, they are expected to be able to differentiate into adipogenic, osteogenic, and chondrogenic lineages (Fig. 1).

Isolation of ASCs

Although it is possible to sort ASCs by flow cytometry using immunophenotypic surface markers, the necessary antibodies and reagents are mostly approved for research use only. Thus, for clinical purposes, ASCs can be obtained from SVF cells by *in vitro* cultivation on cell culture plates. They accumulate as spindle-shaped cells that are grossly indistinguishable from fibroblasts. ASCs from passage 3–7 are usually used for clinical and experimental purposes.

SVF cells can be isolated from adipose tissue by enzymatic digestion or non-enzymatic (mechanical) disruption techniques. The most common isolation protocol consists of enzymatic digestion with collagenase, centrifugation, and red blood cell lysis. In addition to collagenase, trypsin or dispase can be used to digest adipose tissue [27]. The enzymatic method currently

Table 1. Immunophenotypic surface markers of SVF cells and ASCs

	SVF cells	ASCs	MSCs
CD34	+	±	−
CD45	+	−	−
CD13	±	++	++
CD73	±	++	++
CD90	±	++	++
CD105	±	++	++
CD10		++	±
CD36		+	−
CD106		±	+

++, ≥70%; +, ≥30%–70%; ±, ≥2%–30%; −, ≤2%.
SVF, stromal vascular fraction; ASCs, adipose-derived stem/stromal cells; MSCs, mesenchymal stem/stromal cells.

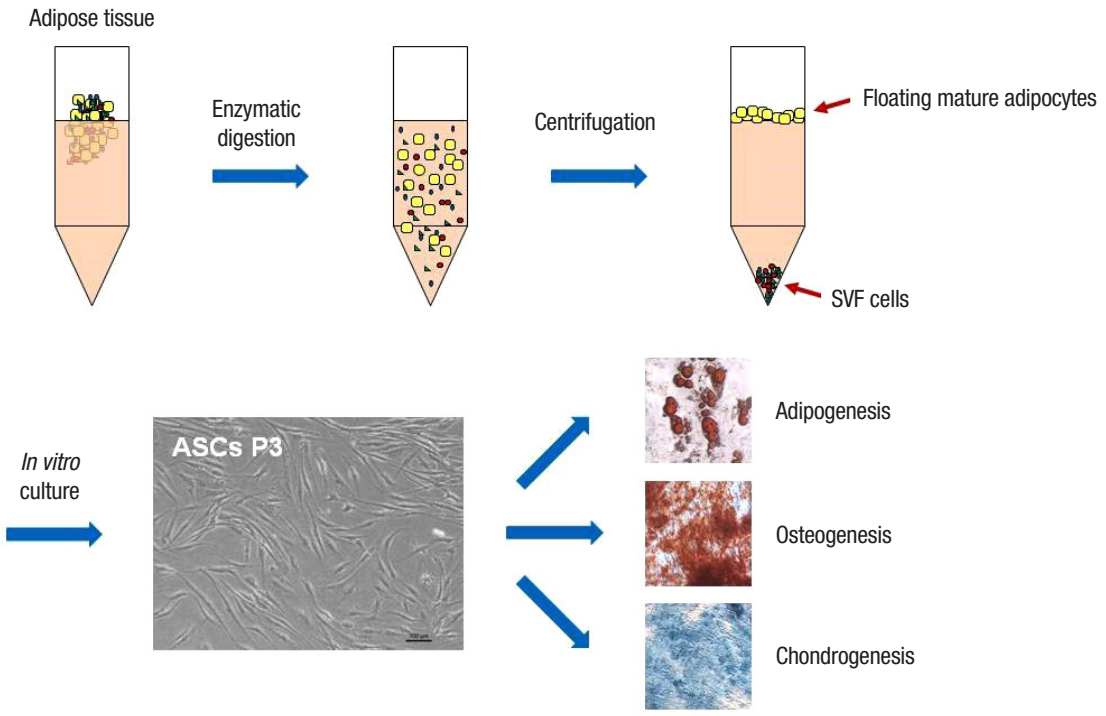

Fig. 1. The isolation procedure, morphology, and differentiation potential of adipose-derived stem/stromal cells (ASCs). Stromal vascular fraction (SVF) cells can be isolated by enzymatic digestion of adipose tissue using collagenase, followed by centrifugation to separate floating mature adipocytes from other cells. The isolated cells in the pellet, known as SVF cells, consist of a mixture of heterogeneous cell types including ASCs. ASCs can be obtained from the SVF cells by *in vitro* cultivation on cell culture plates. They accumulate as cells with a spindle-shaped morphology and the capacity to differentiate into adipogenic, osteogenic, and chondrogenic lineages. Their differentiation potential can be analyzed by histologic staining using Oil Red O for adipogenic differentiation, Alizarin Red for osteogenic differentiation, and Alcian Blue for chondrogenic differentiation.

yields more nucleated cells than the non-enzymatic method, indicating better efficiency in terms of isolation of ASCs. However, this protocol involves the use of xenogeneic components, especially collagenase, and these substances may pose potential risks and safety issues, such as exposure to infectious agents and immune reactions, although residual collagenase itself does not exhibit toxicity [28,29].

As an alternative approach, non-enzymatic methods that use physical force to separate ASCs within the adipose tissue have received increasing attention because they are simple, rapid, and inexpensive. Non-enzymatic disruption involves a combination of the following methods: filtration, centrifugation, red blood cell lysis, mechanical agitation, vortexing, vibration, and ultrasonic cavitation [20]. However, in comparison with enzymatic digestion, the disruptive physical forces employed in the non-enzymatic method are not sufficient to ensure that ASCs are released from the perivascular space, which is assumed to be a niche of ASCs, leading to a low yield of SVF cells. This is a critical disadvantage of this method [30].

CELL-ASSISTED LIPOTRANSFER

Clinical efficacy

Cell-assisted lipotransfer (CAL) has been reported to improve the clinical outcomes of fat grafts [31-33]. In this novel concept, a fat graft is enriched through the intermixture of autologous SVF cells. CAL involves increasing the density of ASCs in the adipose tissue by supplementing the tissue with SVF cells or ASCs (Fig. 2). As a result, ASC-poor aspirated fat can be converted into ASC-rich fat for grafting. The clinical efficiency of CAL was first demonstrated in a randomized controlled study in 2013 [34]. In the study, two fat types (30 mL each), enriched with ASCs (2.0×10^7 cells per mL of fat) or without ASCs, were injected into the subcutaneous plane as a bolus in the posterior part of the right and left upper arm. Magnetic resonance imaging measurements of the volume of the fat grafts at 4 months postoperatively revealed that the ASC-enriched fat grafts had significantly higher residual volumes than the control fat grafts (23.0 cm^3 vs. 4.7 cm^3). Despite several criticisms concerning the exceedingly high cell numbers, bolus injection, and the use of cultured ASCs, CAL has been accepted and studied as a cellular

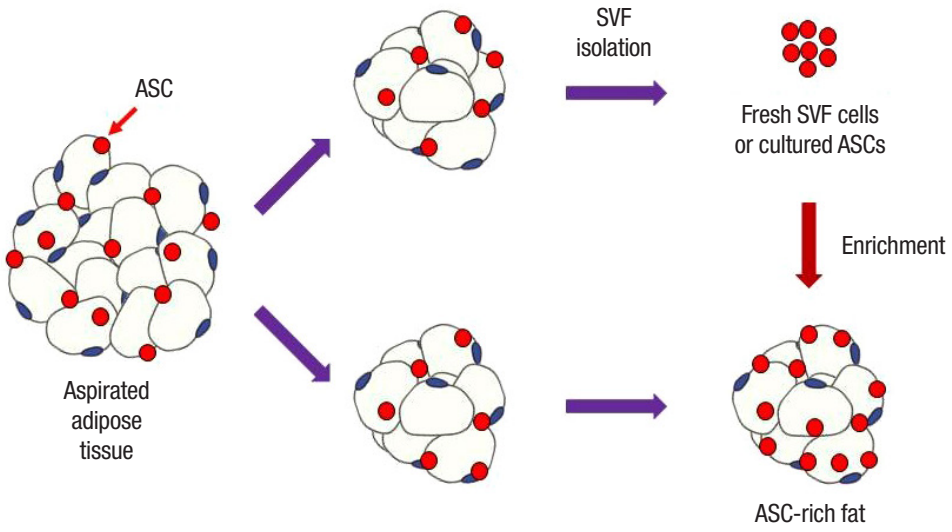

Fig. 2. Schematic illustration of cell-assisted lipotransfer (CAL). Stromal vascular fraction (SVF) cells are isolated using remnant fat other than the amount of fat planned for grafting. In CAL using adipose-derived stem/stromal cells (ASCs), two liposuction procedures are usually required, for ASC culture and fat grafting, respectively. Prior to grafting, the fat is enriched by mixing it with SVF cells or ASCs.

therapy for aesthetic and reconstructive applications. In another study, superior fat graft retention was observed when cultured ASCs were used as a source of cellular enrichment in CAL, instead of SVF cells [35].

Since the introduction of CAL, clinical trials targeting various sites in the body have been reported. The clinical trials of facial fat grafts enriched with SVF cells or ASCs are summarized in Table 2 [33,36-44]. CAL has been applied not only to treat lipodystrophic diseases, such as hemifacial atrophy (Parry-Romberg syndrome) and craniofacial microsomia, but also to perform cosmetic procedures, such as facial contouring and scar revision, and as an adjunct in face lift procedures. A recent meta-analysis suggested that CAL may be more applicable on the face than on the breast [45]. To verify this result, a prospective randomized controlled study investigating the efficacy of CAL in the craniofacial region is required.

Mechanism

Several mechanisms have been proposed to explain the enhancement of fat graft survival mediated by SVF cells or ASCs. According to one hypothesis, ASCs promote angiogenesis and subsequent revascularization by secreting various growth factors [46,47]. The second hypothesis states that ASCs can induce angiogenesis and adipogenesis by differentiating into endothelial cells and adipocytes [48]. To elucidate the fate and microenvironmental changes of fat, ASCs, and recipient tissue associated with CAL, the author generated an experimental animal model using two different transgenic reporter mice, and grafted both fat and ASCs expressing different fluorescent signals into wild-type mice [1]. Tracing analyses revealed that the donor ASCs participated in angiogenesis by differentiating into endothelial cells. Further, newly differentiated fat from donor ASCs and recipient tissue was found to be integrated with the donor fat, leading to improved graft retention. Moreover, ASC supplementation promoted angiogenesis and adipogenesis in a dose-dependent manner. However, in a subsequent experimental study, intravenously injected ASCs concurrent with fat grafts were found to induce angiogenesis and adipogenesis by paracrine action, rather than by direct differentiation, although consistent results were found in terms of enhancing the survival of grafted fat [49].

Safety

The potential of ASCs to create a favorable microenvironment for improved graft retention and regeneration could also induce favorable conditions for tumor cell growth. In particular, the possibility cannot be ruled out that ASCs may stimulate dormant breast cancer cells after CAL in patients with a mild deformity after mastectomy for breast malignancy. To date, several studies, including *in vitro* and *in vivo* experiments, have investigated this issue and reported markedly controversial findings [50-53]. A clinical analysis of a large series of autologous fat grafts without supplementation with ASCs for breast reconstruction demonstrated a low complication rate and no evidence of increased oncologic risk [54-57]. The RESTORE-2 trial, which was the first prospective clinical trial of CAL for reconstruction after breast conservation therapy showed no increase in the local recurrence rate in 67 patients [58]. However,

Table 2. Characteristics of clinical trials of facial fat grafts enriched with SVF cells and ASCs

Study (year)	Enriched cells	No. of patients	Age (yr)	No. of operations	Injected volume (mL)	Volumetric measurement method	Volume gain (mL)	Fat survival rate (%)	Follow-up period (mo)
Sasaki (2015) [44]	SVF	9	65.5 (52–77)	1	9.0 ± 2.0	3D scan	NA	72.9 ± 50.0	12
Gentile et al. (2014) [43]	SVF	10	23–67	2 in 40%	NA	MRI, US	NA	63[a]	12
Chang et al. (2013) [42]	SVF	10	27.5 (19–35)	2 in 30%, 3 in 20%	34.4 ± 13.7	CT	NA	68.3 ± 1.7	6
Li et al. (2013) [41]	SVF	26	29.5 ± 6.8	1	17.5 ± 7.3	CT	11.5 ± 5.3	64.8 ± 10.2	6
Tanikawa et al. (2013) [40]	SVF	7	12.1 ± 2.2	1	27 ± 7	3D CT	NA	88.0 ± 13.0	6
Lee et al. (2012) [39]	SVF	9	43.3 ± 14.7	1	NA	Photography	NA	NA	3
Koh et al. (2012) [38]	ASC	5	28[a]	1	29.9 ± 6.7	3D CT, 3D scan	18.1 ± 5.2	61.1 ± 13.7	15
Sterodimas et al. (2011) [37]	SVF	10	43.9 ± 17.0	1	74.3 ± 47.0	Subjective satisfaction	NA	NA	18
Tiryaki et al. (2011) [36]	SVF	5	NA	1	29.2 ± 16.2	Photography	NA	NA	10
Yoshimura et al. (2008) [33]	SVF	3	38.7 ± 8.1	1	100 ± 10	Photography	NA	NA	10.3

Values are presented as mean (range) or mean ± SD.
SVF, stromal vascular fraction; ASCs, adipose-derived stem/stromal cells; NA, not available; 3D, three-dimensional; MRI, magnetic resonance imaging; US, ultrasound; CT, computed tomography.
[a]Mean.

the findings of 12-month follow-up after post-lumpectomy reconstruction remain inconclusive with regard to the oncologic safety of CAL in breast reconstruction. Thus, a prospective study with a longer follow-up period is needed to prove the oncologic safety of CAL for reconstruction after cancer treatment.

PERSPECTIVE

Autologous fat grafts are a primary choice of treatment for the reconstruction of mild to moderate contour deformities. ASCs have been noted for their simple harvesting procedure and excellent therapeutic potential in regenerative medicine. Nonetheless, several issues need to be elucidated regarding the use of fat grafts and CAL [59].

Clinicians need to know the optimal ratio of adipose tissue to SVF cells or ASCs for CAL. Moreover, it would be invaluable to develop precise surgical plans based on the predicted survival of fat grafts. Prior to answering practical questions, comparative analyses and standardization of fat harvest sites, techniques, and SVF isolation protocols with uniform parameters are necessary [2].

Demonstrating the oncologic safety of stem cell use with CAL is a prerequisite for harnessing its clinical potential. The safety of CAL is controversial due to the lack of experimental models capable of reproducing the complexity of the tumor microenvironment [60]. Therefore, suitable experimental models must be developed to identify potential mechanisms of oncologic risk. Ultimately, a prospective, multi-center case-cohort study based on a registry system will be crucial for providing convincing evidence.

Compared to SVF cells, the use of ASCs in Korea is limited due to legal and regulatory issues. The indications of cell therapy with ASCs are restricted to several diseases. Thus, clinical trials that provide convincing evidence regarding the efficacy and safety of ASCs are needed to obtain scientific, clinical, and legal authorization for their use.

A large number of ongoing studies and clinical trials will broaden the indications of ASC use and highlight the clinical value of ASCs beyond the field of plastic and reconstructive surgery. In the context of advances in fat grafts and CAL, ongoing research should expand to consider both the physical and functional properties of white adipose tissue.

CONCLUSION

Autologous fat grafts have emerged as a primary surgical option for soft-tissue augmentation and reconstruction. The use of fat grafts enriched with SVF cells or ASCs improves the long-term survival of grafts and shows promising results. If oncologic safety is demonstrated by scientific and clinical evidence, stem cell therapeutics such as CAL may shift the paradigm of clinical strategy and practice, with potential to be applied for various diseases.

NOTES

Conflict of interest

No potential conflict of interest relevant to this article was reported.

ORCID

Ki Yong Hong https://orcid.org/0000-0002-8579-0237

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