Reconsidering cell-assisted lipotransfer for breast augmentation: effect of stromal vascular fraction enrichment on graft survival assessed with 3-dimensional laser scanning

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Background: Cell-assisted lipotransfer (CAL) has been proposed to be beneficial for improving graft retention. Clinically, CAL involves the isolation of the stromal vascular fraction (SVF) from a portion of the lipoaspirate at the time of surgery. However, most studies related to SVF breast augmentation lacked a rigorous methodology and well-designed control.

Objective: We aimed to determine the potential improvement of SVF enrichment in fat grafting for breast augmentation with objective volume assessment.

Methods: From April 2015 to January 2016, 169 patients were enrolled after applying the exclusion criteria. Among them, 97 patients who underwent conventional fat grafting for breast augmentation were assigned to group A. The other 72 patients underwent SVF-enriched fat grafting for breast augmentation and were assigned to group B. A retrospective comparative study was conducted to evaluate the graft survival using 3-dimensional laser scanning.

Results: There was no significant difference between the 2 groups in terms of mean age, original breast volume, grafted fat volume, and postoperative weight change. Breast volume assessments revealed that the percentage of graft survival at 12 months was 69.2% in group A and 71.1% in group B, with no significant difference ($p=0.641$). The preoperative body mass index was significantly lower in group A than in group B. The volume of suctioned fat was significantly less in group A. The operation time was significantly shorter in group A. The postoperative complication rates were significantly lower in group A than in group B.

Conclusion: SVF-enriched fat grafting for breast augmentation was associated with a larger amount of harvested fat, a longer operation time, and a higher incidence of complications. The graft retention rate was not significantly increased. The findings of our study do not support the use of SVF in fat grafting for breast augmentation.

Level of Evidence: IV

Keywords: adipose-derived stem cells; autologous fat grafting; breast augmentation; cell-assisted lipotransfer; 3-dimensional laser scanning; stromal vascular fraction

Received February 7, 2019; Revised May 21, 2019; Accepted May 24, 2019

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Introduction

Cell-assisted lipotransfer (CAL) has been proposed to be beneficial for improving graft retention in reconstructive and cosmetic surgeries [1-3]. Isolated mesenchymal stem cells from adipose tissue demonstrate the potential to differentiate into mesenchymal, including adipogenic, lineages [4]. Considering the ease of isolation and abundant supply, isolation and supplementation of adipose-derived regenerative cells during fat grafting surgery has become attractive [5,6].

Studies have revealed that harvesting fat grafts through liposuction reduces the amount of adipose-derived stem cells (ADSCs) [7,8]. This opens room for supplementation of the lipoaspirate with stromal cells and stem cells isolated from another portion of fat tissue during conventional liposuction. Supplementation aims to restore the amount of ADSCs in the graft to approach the amount seen in native adipose tissue. The isolation procedure of adipose tissue creates a stromal vascular fraction (SVF) layer that is composed of a host of cells, including stem cells and others [9,10]. However, most of the published studies with respect to SVF supplementation for breast augmentation are short of a rigorous methodology. Further, the absence of a control group in most of the previous studies makes it difficult to confirm the efficacy of CAL. The current level of evidence surrounding CAL does not allow drawing a solid conclusion about its use in the clinical setting [11-13].

Magnetic resonance imaging (MRI) and 3-dimensional (3D) imaging both provide accuracy and reliability in breast volume measurement [14,15]. MRI is known for its precision in estimating breast volume and detecting internal consistency. However, for frequent postoperative follow-up, repeated MRI examinations would not be practical or cost-effective. Three-dimensional surface imaging, including 3D laser scanning, is a better option in these cases [16,17].

We aimed to determine the potential benefit of SVF enrichment in fat grafting for breast augmentation with objective assessment using 3D laser scanning. A retrospective comparative study was conducted to evaluate the change of breast volume in consecutive patients who underwent conventional fat grafting and those who underwent SVF-enriched fat grafting for breast augmentation.

Materials and methods

This retrospective study was approved by the Institutional Review Board of the Genesis Group-Practice Clinic and was

conducted in compliance with the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [18]. Informed consent was obtained from all patients, who had been informed of the potential adverse events after surgery. In addition, each patient's written consent was obtained for publication. At the time of the study, the surgeon (CH Chiu) had performed more than 500 cases of autologous fat grafting for breast augmentation with predictable results.

We adopted 3D laser scanning in breast volume assessment in early 2015. From February 2015 to January 2016, autologous fat grafting to the breast was performed by the surgeon in 226 patients. After excluding those with previous implant augmentation, surgery for breast tumor, inadequate follow-up (<12 months), and more than 1 session of fat grafting to the breasts, 169 patients were enrolled in this study. Among them, 97 patients who underwent conventional fat grafting for breast augmentation were assigned to group A. The other 72 patients underwent SVF-enriched fat grafting for breast augmentation and were assigned to group B. Third-generation ultrasound-assisted liposuction (UAL) was performed to harvest the lipoaspirate in both groups. The patients' charts were retrospectively reviewed. Their demographics, complications, operation time, and clinical results were recorded and compared using SPSS software ver. 17.0 (SPSS Inc., Chicago, IL, USA), with statistical significance defined as $p < 0.05$.

Fat grafting technique

All procedures were performed by a single surgeon. Potential donor sites included the abdomen, flanks, hips, thighs, and calves. All patients received intravenous sedation and local tumescent anesthesia before graft harvesting. Each donor site was infiltrated with 150 to 300 ml tumescent anesthesia (1,000 ml lactated Ringer's solution, 40 ml lidocaine [2%], and 1 ml epinephrine [1:1,000]) 10 to 15 minutes before liposuction. Third-generation UAL (Ultra-Z system; Zerone Co., Ltd., Seoul, Korea) was applied with a 3.7-mm, 3-ring probe at 100% amplitude in normal mode (10 Hz) to the donor sites. After emulsifying the subcutaneous fat, adipose tissue was harvested with a 3- or 4-mm aspiration cannula attached to a low-pressure suction machine set to -600 mmHg.

Stromal vascular fraction-enriched fat Graft

A portion of harvested fat (100 ml) was mixed with 0.1% collagenase (Sigma-Aldrich, St. Louis, MO, USA) and transferred to a shaking incubator (Beauty Cell multifunctional bio-workstation; N-BIOTEK, Seoul, Korea) at 37°C (200 rpm), where the

mixture remained for 30 minutes to dissolve the adipose tissue. The collagenase-dissolved fat was then centrifuged at 800 *g* for 5 minutes to isolate the SVF. Thereafter, the resulting cone tube showed 4 distinct layers. From top to bottom, the first layer consisted of lysed fat and oil. The second layer was the collagenase solution. The third layer contained the SVF. The bottom layer contained red blood cells (RBCs). After discarding the upper supernatant, autologous serum was used to mix with the stem cell-containing layer for neutralization at 300 *g* for 3 minutes. This process was repeated 3 times until a turbid layer between the clear supernatant of serum and the bottom RBC layer was formed. This dense, grayish layer, which appeared between the RBCs and serum, was the collection of SVF (Fig. 1). The entire procedure took 1 hour. During the isolation, the remaining aspirated fat was prepared for grafting by centrifugation at 800 *g* for 4 minutes to remove free oil and blood components. The freshly isolated SVF was then combined with the aspirated fat, which was then transferred to 10-ml BD syringes (Becton Dickinson, Franklin Lakes, NJ, USA) and connected to a 14 gauge, 15-cm, single-hole cannula for injection.

Culture of adipose-derived stem cells

To scientifically verify that stem cells were transplanted, SVF samples were further processed in an independent laboratory (Scientific Biotech Corp., Taipei, Taiwan) to isolate ADSCs following previously described methods. To assess the stem cell immunophenotype of the isolated ADSCs, the cells were harvested and characterized using flow cytometry, as described previously [4,19].

Volumetric analysis

Non-contact 3D laser surface scanning (Minolta Vivid 910

3D Digitizer; Konica Minolta Inc., Tokyo, Japan) was performed with a portable device to objectively calculate the breast volume. The scanning process lasted less than 60 seconds, taking multiple views for merging. Data from these scans were merged for volumetric analysis, using Rapidform XOV2 software (INUS Technology Inc., Seoul, Korea), for each breast in all patients, by a blinded expert. To increase the intra-observer reliability, volumetric analysis for each merged 3D photograph was performed twice. The average was adopted for statistical analysis (Fig. 2).

Follow-up evaluation

Physical examination and breast ultrasonography were performed at 3, 6, and 12 months after treatment. Clinical data on all post-treatment complications were collected throughout the follow-up for all patients. Breast ultrasonography was performed routinely at follow-up visits to determine the rates of complications (fat necrosis, indurations, and calcifications). If a mass was palpable during physical examination or observed with ultrasonography, MRI for further evaluation was recommended. Patient satisfaction was assessed with an abbreviated version of the BREAST-Q, in the form of a written questionnaire administered by a nurse preoperatively and 12 months after the procedure [20,21].

Results

The mean±standard deviation patient age was 35±8.8 years (range, 20–56 years) in group A and 37.7±14.9 years (range, 23–53 years) in group B. The mean±standard deviation original breast volume was 97±39 ml (range, 32–253 ml) in group A and 102±40 ml (range, 32–193 ml) in group B. The mean±standard deviation grafted fat volume was 316±47 ml (range, 220–400 ml)

Fig. 1. Isolation of stromal vascular fraction (SVF) and culture of adipose-derived stem cell (ADSC). (A) A grayish layer containing SVF was formed between the upper layer of serum and the bottom layer of red blood cell after repeated neutralization with autologous serum. On average, 4.07×10^8 viable cells were yielded in SVF isolated from 100 ml of lipoaspirate. (B) Samples of the SVF were sent to an independent laboratory outside the clinic. After 3 passages of culturing in the lab, about 6×10^6 stem cells were identified. The adipose-derived stem cells showed the typical elongated shape under a phase contrast light microscope ($\times 10$).

Fig. 2. Computing of breast volume using 3-dimensional (3D) laser scanning. (A) Non-contact 3D laser surface scanning (Minolta Vivid 910 3D Digitizer; Konica Minolta Inc., Tokyo, Japan) was performed with a portable device to objectively calculate the volume of the breasts. (B) A 3D photo was merged with the volume of each breast calculated using software Rapidform XOV2 (INUS Technology Inc., Seoul, Korea) on computer. (C) Close-up view of left breast with its volume calculated. (D) Breast volume was computed with the curvature of the thorax taken into consideration.

Table 1. Patients' demographic and clinical characteristics

Characteristic	Group A ^{a)}	Group B ^{b)}	p-value*
Age (yr)	35±8.8	37.7±14.9	0.218
Preoperative BMI (kg/m ²)	18.9±1.7	20.8±2.7	0.006
Postoperative weight change (kg)	0.47±1.30	1.06±1.94	0.301
Total volume of suctioned fat (ml)	1,473±182	1,760±600	0.040
Original breast volume (ml)	97±39	102±40	0.487
Volume of grafted fat (ml)	316±47	323±32	0.350
Operation time (min)	198±36	223±51	0.038
Complication	5 (5.2)	11 (15.3)	<0.001

Values are presented as mean±SD or n (%).

BMI, body mass index.

^{a)}Patients who underwent conventional fat grafting (n=97).

^{b)}Patients who underwent stromal vascular fraction-enriched fat grafting (n=72).

*Statistical significance is considered at p<0.05 (calculated by independent sample t-test).

in group A and 323±32 ml (range, 240–390 ml) in group B. The postoperative weight change was recorded. The mean±standard deviation weight change was 0.47±1.30 kg (range, -0.2 to 2.3 kg) in group A and 1.06±1.94 kg (range, -3.3 to 4.5 kg) in group B. There were no significant differences between the 2 groups in mean age, original breast volume, grafted fat volume, and postoperative weight change. A significant lower body mass index (BMI) was observed in group A (18.9 vs. 20.8 kg/m², p=0.006). The volume of suctioned fat in group A was significantly less than that in group B (1,473 vs. 1,760 ml, p=0.040). The operation time was significantly shorter in group A than in group B (198

vs. 223 minutes, p=0.038). Postoperative complications included nodules, cysts, and fat necrosis. The complication rate was significantly lower in group A than in group B (5.2% vs. 15.3%, p<0.001; Table 1).

Sequential breast volume assessments with 3D laser scanning revealed that the original breast volumes were 97 ml in group A and 102 ml in group B, with no statistically significant difference. Fourteen days after the surgery, the breast volumes increased to 502 ml in group A and 501 ml in group B. The breast volumes after 14 days were larger than the sum of the original breast volume and grafted volume, indicating a certain degree

of breast swelling. At 12 months postoperatively, the breast volume was 316 ml in group A and 332 ml in group B, with no significant difference ($p=0.258$). The survival percentage was defined as the final breast volume minus the original breast volume, divided by the grafted fat volume. The graft survival percentage at 12 months was 69.2% in group A and 71.1% in group B, with no significant difference ($p=0.641$). These values were close to those calculated at 3 and 6 months, suggesting that the grafted fat became stable at 3 months postoperatively (Fig. 3).

On average, 4.07×10^8 viable cells were yielded in SVF isolated from 100 ml lipoaspirate. SVF samples were sent to an independent laboratory. After 3 culture passages, about 6×10^6 stem cells were identified. The laboratory results scientifically verified that stem cells were transplanted along with the fat graft during our surgical procedures.

Discussion

To apply SVF clinically, surgeons have to isolate a portion of the aspirated fat at the time of surgery. The SVF containing ADSCs is mixed with the fat graft in hopes of doubling the amount of stem cells, which is low in aspirated fat. In several human studies, almost half of the lipoaspirate was used for the isolation of SVF, which increased the ADSC concentration by 2 to 5 times as compared with the non-SVF fat graft [7,11]. As the ADSC

concentration in lipoaspirates is only half the concentration in native adipose tissue, a 2 to 5-fold increase would only equalize the original concentration. Thereby, the improvement in fat graft survival that half of the lipoaspirate can induce remains questionable.

Our study demonstrated that isolation of SVF necessitated harvesting a larger amount of fat and a longer operation time. Unfortunately, SVF enrichment did not induce a significant increase in graft retention after 12 months of follow-up with rigorous volumetric assessment with 3D laser scanning. There is yet no consensus on how many cells are needed and how much fat should be used for optimum graft survival [22]. Some authors used half of the aspirated fat to isolate the SVF [2]. According to our experience and previous studies, the amount of purified fat that can be used for injection is usually one-half or two-third of the lipoaspirate amount. To prepare 540 to 600 ml of the graft, as in our study, more than 1,000 ml of fat should be harvested. If half of the aspirated fat were to be used for SVF isolation, surgeons would need to harvest an additional 1,000 ml of fat for SVF preparation according to their method, which could be problematic in thin and slim patients. In a 2015 study, 250 ml lipoaspirate and 500 ml liposuction fluid were used to isolate SVF, and the authors concluded that SVF from this amount of fat and fluid was not enough for optimal result [23]. In a 2013 comparative study, the authors found that the fat survival rate did not significantly differ between the CAL-enriched group (50%) and the non-CAL group (54%), by using 240 to 360 ml of aspirated fat for the isolation of SVF, which is in line with our result [24].

In another study, 100 ml of adipose tissue was reported to contain 100 million stem cells [25]. We followed a previously described protocol to isolate SVF from 100 ml lipoaspirate. Meanwhile, fat graft was prepared from the remainder of the lipoaspirate [26]. In our study, an average of 4.07×10^8 viable cells were yielded in SVF isolated from 100 ml lipoaspirate. The SVF samples were then sent to an independent laboratory. After 3 culture passages, about 6×10^6 stem cells were identified, which scientifically verified that stem cells were transplanted along with the fat graft during our surgical procedure. In a previous study, 1×10^4 SVF cells per 200 μ L of fat graft improved retention by approximately 20% in a xenograft model of CAL [27]. To enrich 600 ml of fat, 3×10^7 SVF cells (1×10^7 for 200 ml, 3×10^7 for 600 ml) would be needed, according to this calculation. In our study, 4.07×10^8 viable cells were yielded, on average, in SVF isolated from 100 ml lipoaspirate, which was 10 times more than that needed for the enrichment of 600 ml fat. However, we did

Fig. 3. Sequential changes of breast volume and percentage of graft survival. Percentage of graft survival was defined as the final breast volume minus original breast volume, divided by the volume of the grafted fat. The percentage of graft survival at 12 months did not differ in the two groups (group A, 69.2% vs. group B, 71.1%; $p=0.641$). Lines indicate percentage of graft survival. Bars indicate breast volume of each group at different time points.

not observe any significant improvement in graft retention in our study.

The mean postoperative body weight change did not differ between the 2 groups, suggesting that graft retention was not influenced by weight change in our study. However, the preoperative BMI of group A was significantly lower than that of group B (18.9 vs. 20.8 kg/m², $p=0.006$), meaning that patients in group A were thinner and more slender. These patients had a narrower chest diameter and less recipient space for fat injection. The pressure inside the breasts would be greater in group A than in group B after injecting an equivalent amount of fat, which could compromise the retention of graft [12]. Theoretically, graft retention in group A should be even lower under such circumstances, but that was not the case in the present study. We speculated that the refinement of our injection technique may have contributed to the success. The surgeon used the “solid injection method” to increase the amount of safe injection and reduce postoperative complications, in addition to the previously recommended principle of structural fat grafting [28-30]. This method was described in detail in previously published articles and was verified again in this study [31,32]. Over time, this method has been proven to reduce fat-grafting-related complications and enhance graft retention in the long term.

Few studies have addressed the operation time for breast augmentation with fat grafting. It was previously reported that an additional operation time of around 2 hours was needed for CAL breast augmentation [33]. In our study, an additional 30 minutes was necessary for SVF isolation if the procedure of graft preparation was performed by an expert other than the surgeon.

Postoperative complications included fat necrosis, nodules, and cyst formation. The complication rate was significantly lower in group A than in group B in our study. The mechanism behind this phenomenon was unclear. In a systematic review, the authors reported that the CAL method was associated with a higher incidence of complications [12], which was compatible with our result. They explained that a larger amount of injected fat positively correlated with a higher incidence of postoperative complications owing to the resultant local hypoxic environment. However, this presumption failed to explain the low incidence of complications in non-CAL with the injection of a similar amount of fat. In our study, the volume of transplanted fat did not significantly differ between the study groups; however, the CAL group showed higher complication rates. Accordingly, we believe that there must be causes other than hypoxia with respect to this issue; further studies are required to unravel

them.

It has been clarified that fat harvested using UAL has comparable SVF counts and graft retention to those of traditional liposuction, both in human and xenograft studies [34], although the harvesting technique has an impact on cell yield and the SVF function [35,36]. Adipose tissue harvested using third-generation UAL is viable on harvest and is a potentially suitable source for autologous fat grafts. A previous study suggested that UAL could provide an efficient method of harvesting adipose tissue without sacrificing its viability, and the authors concluded that their results were in line with those of other reports that demonstrated clinical success with third-generation UAL [37]. In the current study, UAL was performed in all patients of both groups. This was especially important when the patients seeking autologous fat grafting for breast augmentation are thin and slim. UAL has shown particular benefits in superficial liposuction and lysis of fibrous and adhesive tissues [38]. To successfully perform fat grafting to augment the breasts in underweight women, including those who had undergone liposuction, UAL is a useful technique to harvest enough amount of fat without the risk of causing skin irregularities [28,29].

According to a systematic review comparing different volumetric tools to estimate fat survival after fat grafting, MRI provides highly accurate and reproducible results. Three-dimensional breast imaging systems using laser scanning or multiple stereo cameras are also accurate and reproducible for breast volume measurements [39]. MRI and 3D imaging are reliable tools for the comparative assessments of breast volume. In a review comparing various 3D techniques in breast volume analysis, the Konica Minolta Vivid 910 3D scanner was shown to be reliable and highly correlated with MRI. VECTRA 3D scanner (Canfield Scientific Inc., Fairfield, NJ, USA), on the other hand, is accurate, has low mean measurement error, and is also reliable. Although both are expensive, the former has the advantage of being portable [39,40]. For serial assessments in a short-term period, 3D laser scanning and VECTRA 3D seem to be more practical and cost-effective [17].

This study has several limitations. First, we did not perform routine mammography for all patients besides routine ultrasonography and potential MRI during postoperative follow-up. The postoperative complications may have been underestimated. Second, previous studies revealed that when using ADSCs through ex vivo expansion, the ADSC concentration is increased by 1,250 to 6,250 times, which considerably improves graft retention. In our research, this was not analyzed in the clinical setting.

In conclusion, SVF-enriched fat grafting for breast augmentation was associated with a larger amount of harvested fat, a longer operation time, and a higher incidence of complications. Graft retention was not significantly increase in contrast to our expectation. Our findings do not support the use of SVF in autologous fat grafting for breast augmentation.

Conflicts of interest

The authors have nothing to disclose.

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