Improving Low-Density Fat by Condensing Cellular and Collagen Content through a Mechanical Process: Basic Research and Clinical Applications

Huidong Zhu, M.D.
Yuping Quan, M.D.
Jing Wang, M.D.
Shenglu Jiang, M.D.
Feng Lu, M.D., Ph.D.
Junrong Cai, M.D., Ph.D.
Yunjun Liao, M.D., Ph.D.

Guangzhou, Guangdong, People's Republic of China

Background: Large-volume fat grafting results in high absorption and complication rates. Low-density fat includes small numbers of viable cells and considerable oil, resulting in nodules and oil cysts. This study evaluated a strategy for large-volume fat grafting using a mechanical process to condense low-density fat and transplanting it with high-density fat.

Methods: Low-density fat, defined as the upper half of centrifuged lipoaspirates, was emulsified by intersyringe shifting and centrifuged to obtain condensed low-density fat. Fresh condensed low-density fat was analyzed by counting cells in the stromal vascular fraction, and by electron scanning and Western blotting. The retention rate and histologic changes of the product were analyzed using a fat grafting model in nude mice. Transplantation with a combination of condensed low-density fat and high-density fat was tested in patients undergoing breast reconstruction and breast augmentation.

Results: The condensed low-density fat derived from low-density fat contained a large number of stromal vascular fraction cells and collagens, comparable to that of high-density fat and much higher than in low-density fat and Coleman fat. Retention rates 12 weeks after transplantation were higher for condensed low-density fat (55.0 ± 7.5 percent) than for low-density fat (31.1 ± 5.7 percent) and Coleman fat (41.1 ± 6.8 percent), with condensed low-density fat having fewer oil cysts and lower macrophage infiltration. Patients grafted with combined condensed low-density fat and high-density fat showed good long-term volume retention.

Conclusions: Using mechanical methods to condense low-density fat to a level comparable to that of high-density fat is a practical method of improving fat graft retention and avoiding severe complications. This new strategy may improve the quality of lipoaspirates for patients requiring large-volume augmentation. (*Plast. Reconstr. Surg.* 148: 1029, 2021.)

Depression or loss of soft tissue is a common problem in plastic surgery. Following standardization of fat grafting techniques,[1] autologous fat grafting has become a very important and valuable method for volume augmentation and tissue reconstruction, with the advantages of abundant sources, easy acquisition, and no graft rejection reaction. However, some problems with fat transplantation, such as unpredictable retention rates, the need for multiple operations, and high complication rates, remain unsolved.

Centrifugal force was found to have potential impact on the viability of lipoaspirates and long-term graft retention.[2,3] Centrifugation using the Coleman technique was found to yield higher numbers of viable adipocytes than the conventional fat grafting process, making the Coleman technique the preferred method of preparing lipoaspirates.[4] Centrifugation using the standard Coleman technique was shown to create a graded

From the Department of Plastic and Cosmetic Surgery, Nanfang Hospital, Southern Medical University.
Received for publication May 13, 2020; accepted April 13, 2021.
The first two authors contributed equally to this study and should be considered co–first authors.
The last three authors contributed equally to this study and should be considered co–first corresponding authors.

DOI: 10.1097/PRS.0000000000008484

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

Related digital media are available in the full-text version of the article on www.PRSJournal.com.

density of fat. The high-density fat in the lower part of these lipoaspirates following centrifugation was shown to contain more stromal vascular fraction cells and viable adipocytes, and higher concentrations of angiogenic/vasculogenic and anti-inflammatory adipokines, than the low-density fat in the upper part of these centrifuged lipoaspirates.[5] Low-density fat contained large amounts of oil and large-sized adipocytes. Compared with low-density fat, transplantation of high-density fat resulted in a relatively higher fat graft retention rate and showed a greater potential to be a promising alternative for fat grafting.[6]

After transplantation, it is difficult for fragile mature adipocytes to survive under hypoxic and ischemic conditions, with most of these adipocytes dying and forming oil cysts.[7,8] Adipocytes die easily under ischemic conditions,[9] whereas adipose-derived stem/progenitor cells attached to extracellular matrix are activated and contribute to tissue regeneration. Vascularized grafts contain adipose-derived stem cells, which are more likely to survive the hypoxic insult of transplantation and stimulate angiogenesis by releasing angiogenic factors.[10] Furthermore, surviving adipose-derived stem cells were found to contribute to the regeneration of newly formed adipose tissue.[11] Thus, we hypothesized that removing the oil/adipocytes and increasing stromal vascular fraction density in the low-density fat to a level comparable with that in the high-density fat may improve lipoaspirate quality and improve fat graft retention.

Stromal vascular fraction density can be increased and oil within lipoaspirates removed using a pure mechanical process.[12,13] Transplantation of mechanically processed fat tissue achieved better clinical outcomes, including a higher retention rate and better histologic appearance, compared with Coleman fat.[14] During the mechanical process, most mature adipocytes were destroyed and oil was removed by centrifugation, wheras the extracellular matrix and stromal vascular fraction cells were preserved and concentrated. These findings indicate that low-density fat can be concentrated by a mechanical process that removes oil and condenses stromal vascular fraction cells and extracellular matrix proteins.

This study describes a practical strategy for transplantation of large volumes of used high-density fat and condensed low-density fat obtained by mechanical preparation. Results in two patients are presented, as are the results of an experimental study to determine the cellular and collagen contents of the condensed low-density fat and compare these contents with low-density fat, high-density fat, and Coleman fat.

MATERIALS AND METHODS

Fat Harvesting and Condensed Low-Density Fat Preparation

Human abdominal lipoaspirates were obtained from eight healthy women with no systemic diseases. Liposuction at −0.75 atm of suction pressure was performed with a 3-mm multiport cannula, containing several sharp side holes 1 mm in diameter (Tulip Medical Products, San Diego, Calif.). The harvested fat was allowed to stand for 10 minutes in a mixture of ice water, the liquid portion was discarded, and the fat was centrifuged at 1200 *g* for 3 minutes to generate Coleman fat. The liquid portion was discarded, and the remaining Coleman fat was divided into two parts, with the upper half defined as low-density fat and the lower half as high-density fat.

Low-density fat was emulsified by shifting at 20 ml/second between two 20-ml syringes connected by a female-to-female Luer-Lok connector with an internal diameter of 2.4 mm. After intersyringe shifting two, four, and six times, the low-density fat was condensed to 70 percent, 50 percent, and 30 percent, respectively, of its original volume. The emulsified fat was then centrifuged at 1600 *g* for 3 minutes, and the sticky substance under the oil layer was defined as the condensed low-density fat (Fig. 1).

Patients

Between March of 2017 and September of 2019, 54 women underwent large-volume fat grafting with a combination of condensed low-density fat and high-density fat, including 46 who underwent bilateral breast augmentation and 10 who underwent breast reconstruction. The study protocol was approved by the Institutional Review Board of the Nanfang Hospital. All patients provided written, informed consent. Each patient was photographed before and after surgery by the same doctor. To assess the volume change after surgery, a three-dimensional laser light scanner (CASZM MVS-600; Shenzhen Zhongke Zhimei Technology Co., People's Republic of China) and 3D Medical Virtual Simulation System (CASZM MVS-600) was used.[15–17] In brief, patients were asked to stand upright 700 to 900 mm in front of the scanner and raise their hands horizontally. The patients were told to hold their breath at the end of exhalation for 3 seconds when the scanning was performed. After scanning, the three-dimensional model was

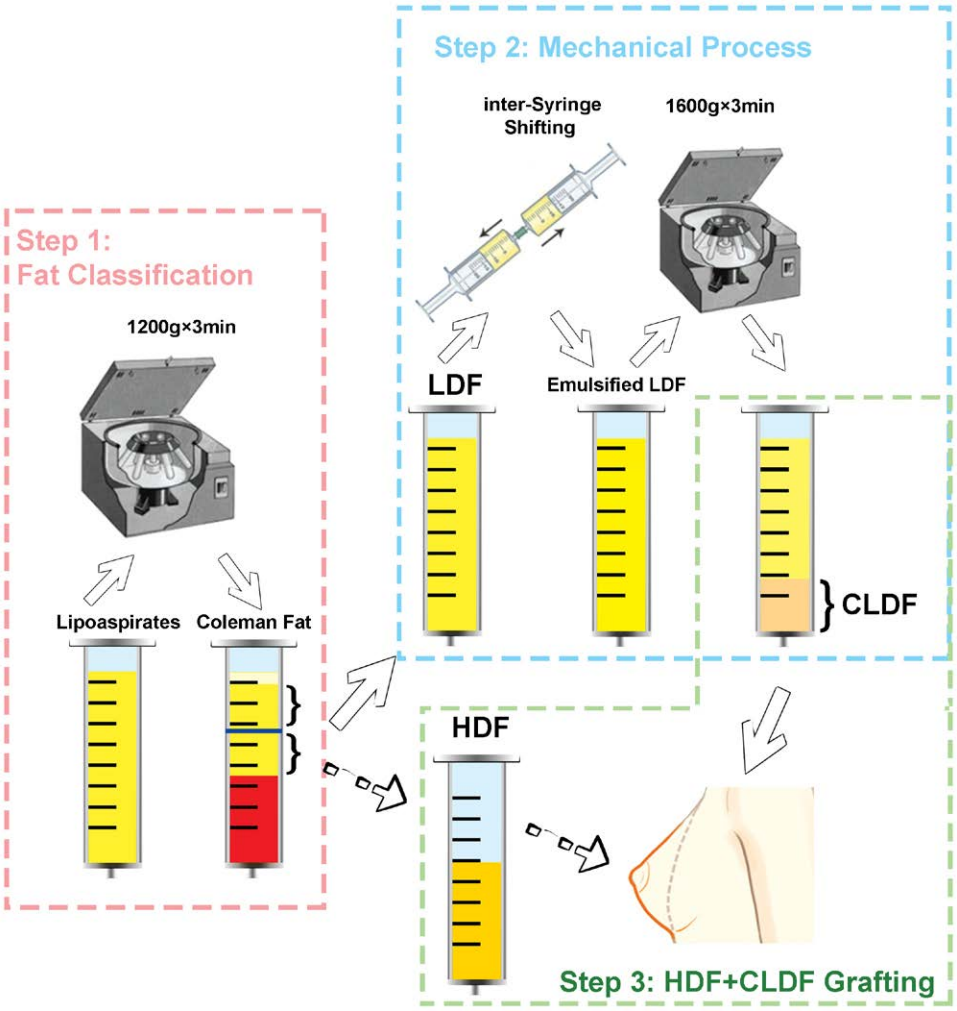

Fig. 1. Schematic depiction showing the processing of condensed low-density fat (*CLDF*). *LDF*, low-density fat; *HDF*, high-density fat.

then generated by the iterative closest point algorithm. Images obtained before and after treatment were recorded and overlapped into a single image to calculate the precise volume change.

Counting of Stromal Vascular Fraction Cells

Stromal vascular fraction cells were isolated from low-density fat, Coleman fat, high-density fat, and condensed low-density fat preparations. Ten milliliters of each sample was digested with 0.075% collagenase in phosphate-buffered saline for 30 minutes at 37°C while shaking. Mature adipocytes and connective tissue were removed by centrifugation at 800 *g* for 5 minutes. The cell pellets were resuspended, filtered through a 100-μm mesh, centrifuged again, and resuspended in 1 ml of phosphate-buffered saline. Each sample (20 μl) was transferred to a plate and stained with trypan blue dye, and the concentration of stromal vascular fraction cells was counted using an automatic cell counting machine (Countstar, Shanghai, People's Republic of China), which consists of a microscope and a digital camera. Each sample was analyzed for 10 random fields to avoid sampling bias.

Flow Cytometric Analysis of Adipose-Derived Stem Cells

Isolated stromal vascular fraction cell samples from low-density fat, Coleman fat, high-density fat, 30 percent condensed low-density fat, 50 percent condensed low-density fat, and 70 percent condensed low-density fat were stained with fluorescein isothiocyanate–conjugated mouse anti-human CD31, CD34, and $CD45^{-}$ antibodies (BD Biosciences, San Jose, Calif.) and analyzed by multicolor flow cytometry (LSR II; BD Biosciences). Changes in cell composition were calculated

according to surface marker expression profiles. $CD34^+/CD31^-/CD45^-$ cells were regarded as adipose-derived stem cells and their populations were determined using Cell Quest Pro software (BD Biosciences).

Scanning Electron Microscopy

Samples derived from low-density fat; high-density fat; and 30 percent, 50 percent, and 70 percent condensed low-density fat were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, and postfixed in 1% osmium tetroxide in the same buffer for 1 hour. The samples were subsequently dehydrated with increasing concentrations of acetone, critical-point dried, fixed to stubs with colloidal silver, sputtered with gold using a MED 010 coater (Oerlikon Balzers, Balzers, Liechtenstein), and examined under an S-3000N scanning electron microscope (Hitachi, Ltd., Tokyo, Japan). The spacial structure and cellular component of different sample were then analyzed.

Evaluation of Collagen Content

Samples of low-density fat; high-density fat; and 30 percent, 50 percent, and 70 percent condensed low-density fat (all 50 ml) were homogenized for 1 minute and centrifuged at 1600 *g* for 3 minutes. The upper layer, mainly composed of broken adipocytes, released oil, and water, was removed; and the lower layer, containing slag-like sediment, was stored at −80°C. After 24 hours, the samples were lyophilized for 24 hours to remove internal water from the sediment and weighed, with the dry weight of the collagen-rich sediment determined by subtracting the weight of the tube.

Western Blot Analysis

Specimens obtained from low-density fat, Coleman fat, high-density fat, and 30 percent condensed low-density fat were lysed in RIPA buffer (0.5% NP-40, 0.1% sodium deoxycholate, 150 mM sodium chloride, 50 mM Tris-Cl, pH 7.5). The lysates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to polyvinylidene fluoride membranes (Millipore, Burlington, Mass.), and incubated with antibodies against collagen I (1:1000; 91144T; Cell Signaling Technology, Danvers, Mass.;), collagen II (1:1000; ab34712, Abcam, Waltham, Mass.), collagen III (1:1000; ab7778; Abcam), collagen IV (1:1000; 86042; Abcam), and glyceraldehyde 3-phosphate dehydrogenase (1:1000 to 1:5000; Cell Signaling Technology), followed by incubation with secondary antibodies. Finally, the proteins were detected using an enhanced chemiluminescence (Pierce, Rockford, Ill.) detection system.

Nude Mouse Fat Grafting Model

All animal experiments were approved by the Nanfang Hospital Animal Ethics Committee Laboratory and were conducted according to the guidelines of the National Health and Medical Research Council of China. Male nude mice, aged 6 to 8 weeks, were housed in individual cages with a 12-hour light/dark cycle, and provided with standard food and water ad libitum. Each mouse was injected subcutaneously in each flank with 0.3 ml of low-density fat, Coleman fat, high-density fat, or 30 percent condensed low-density fat using a 1-ml syringe with a blunt infiltration cannula. Each injected graft formed a spherical shape. Six animals in each group were killed 3 months after the injection. The grafts were harvested and carefully separated from surrounding tissue, and their volume was measured. Each harvested sample was assessed histologically and immunohistochemically.

Histologic Examination

Tissue samples from mice injected subcutaneously with low-density fat, Coleman fat, high-density fat, and 30 percent condensed low-density fat were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin for staining with hematoxylin and eosin. Tissue blocks were sectioned, examined under an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan), and photographed using an Olympus DP71 digital camera. For quantification of oil cysts, each sample was captured for 10 fields per slide.

Immunofluorescent Staining

Tissue samples were fixed in zinc fixative (BD Biosciences), embedded in paraffin, and sectioned at 5 μm. Samples were stained immunofluorescently with the following primary antibodies: rat anti-mouse Mac2 (1:200; Cedarlane Corp., Burlington, Ontario, Canada) and guinea pig anti-mouse perilipin (1:200; Progen, Heidelberg, Germany) antibodies. After washing, the samples were incubated with donkey anti–rat-555 immunoglobulin G (1:200; Abcam) and goat anti–rabbit-430 immunoglobulin G (1:200; Invitrogen, North Ryde, New South Wales, Australia) secondary antibodies, respectively. Nuclei were stained with Hoechst (Sigma, St. Louis, Mo.), and the samples were examined by confocal microscopy (Leica TCSSP2; Leica Microsystems GmbH,

Wetzlar, Germany). Adipocytes were represented as perilipin-positive cells, and macrophages were represented as Mac2-positive cells. Mac2-positive crown-like structures were regarded as oil cysts.

Statistical Analysis

Data were expressed as mean ± SD. Results in multiple groups were compared by repeated measures analysis of variance. Results in two groups at single time points were compared by independent *t* tests, and results in multiple groups at single time points were compared by one-way analysis of variance. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Cellular Density of the Condensed Low-Density Fat

Assessment of the density of stromal vascular fraction cells in fat preparations showed that it was lowest in low-density fat ($1.03 \pm 0.34 \times 10^5$ cells/ml), somewhat higher in Coleman fat ($2.07 \pm 0.51 \times 10^5$ cells/ml), and higher in high-density fat ($4.63 \pm 0.32 \times 10^5$ cells/ml). The stromal vascular fraction cell densities in 30 percent, 50 percent, and 70 percent condensed low-density fat were $5.00 \pm 0.58 \times 10^5$, $3.40 \pm 0.54 \times 10^5$, and $2.58 \pm 0.24 \times 10^5$ cells/ml, respectively (Fig. 2, *left*). The stromal vascular fraction cell densities were significantly higher in high-density fat ($p < 0.01$) and 30 percent ($p < 0.01$) and 50 percent condensed low-density fat ($p < 0.05$) than in low-density fat.

Flow cytometry indicated that the density of adipose-derived stem cells was consistent with the density of stromal vascular fraction cells. The densities of adipose-derived stem cells of low-density fat, Coleman fat, and high-density fat were $1.69 \pm 0.82 \times 10^4$, $5.72 \pm 2.80 \times 10^4$, and $1.56 \pm 0.70 \times 10^5$ cells/ml, respectively. Moreover, the densities of adipose-derived stem cells increased with the increasing condensation, which were $9.27 \pm 5.29 \times 10^4$, $1.15 \pm 0.54 \times 10^5$, and $1.64 \pm 0.83 \times 10^5$ cells/ml in 70 percent, 50 percent, and 30 percent condensed low-density fat, respectively. The density of adipose-derived stem cells was significantly higher in high-density fat and 30 percent condensed low-density fat than in low-density fat ($p < 0.01$ each) (Fig. 2, *right*).

Structural Changes in Condensed Low-Density Fat

Scanning electron microscopy was performed to assess changes in the spatial structure of extracellular matrix after the condensation process. Low-density fat consisted of a large volume of loosely connected unilocular adipocytes, most greater than 100 μm in diameter (Fig. 3, *left*), whereas high-density fat consisted predominately of smaller adipocytes, 50 to 100 μm in diameter, tightly wrapped by fibrous connective tissue (Fig. 3, *center*). Examination of 30 percent condensed low-density fat showed few adipocytes, with the major component being extracellular matrix, indicating that most of the mature adipocytes had ruptured (Fig. 3, *right*).

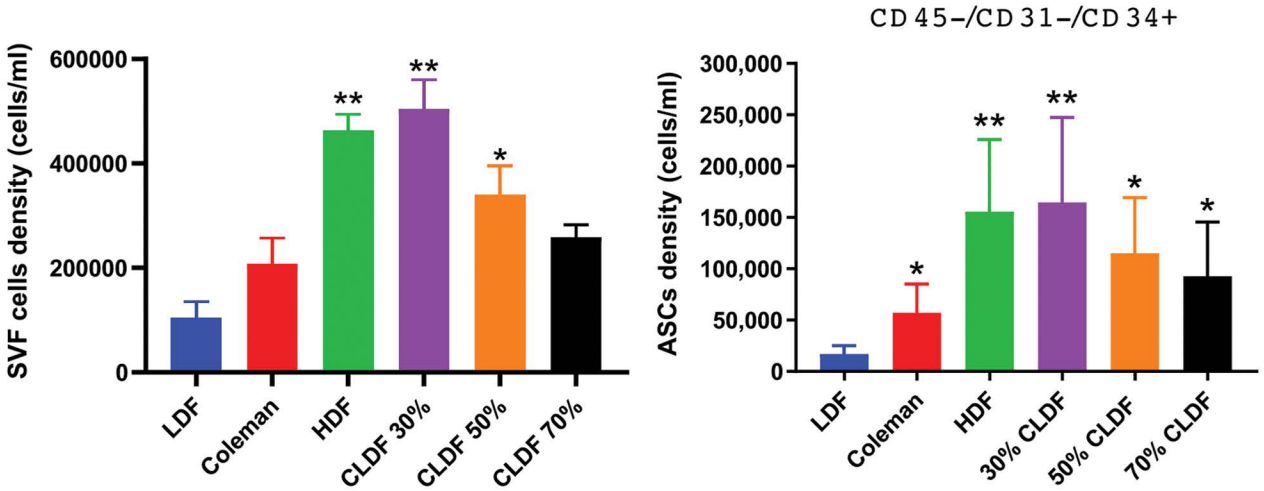

Fig. 2. The cellular component of fat under different processing condition. Stromal vascular fraction (*SVF*) cells were isolated from low-density fat (*LDF*); Coleman fat; high-density fat (*HDF*); and 30 percent, 50 percent, and 70 percent condensed low-density fat (*CLDF*) by collagenase digestion (*left*). The adipose-derived stem cells were identified as $CD45^-/CD31^-/CD34^+$ cells by flow cytometry (*right*) ($n = 6$ for all groups). *$p < 0.05$ and **$p < 0.01$ compared with low-density fat.

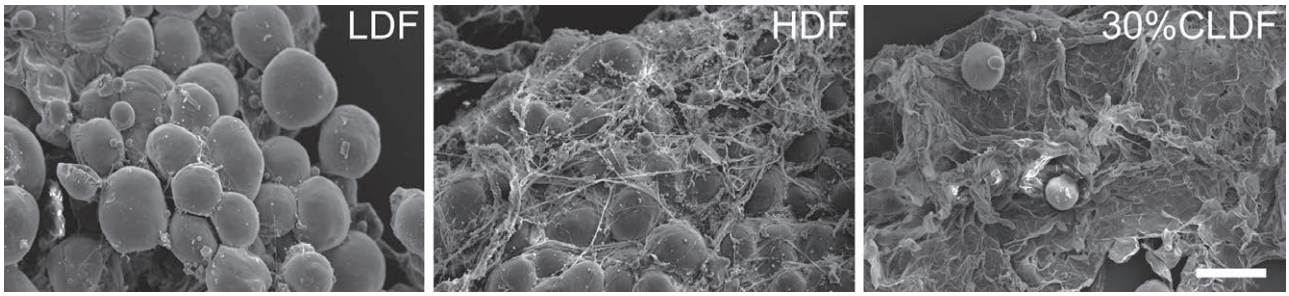

Fig. 3. Structural changes in condensed low-density fat (*CLDF*) as determined by scanning electron microscopy. The low-density fat (*LDF*) samples contained a large volume of loosely connected adipocytes, and little extracellular matrix (*left*), whereas the high-density fat (*HDF*) samples showed high numbers of smaller adipocytes tightly connected by a large amount of extracellular matrix (*center*). Most mature adipocytes were ruptured after the shifting process, with few adipocytes present in the condensed low-density fat, which consisted mainly of extracellular matrix (*right*). *Scale bar* = 100 μm.

Levels of Collagen Proteins

The level of collagen proteins in 50-ml aliquots of condensed low-density fat samples increased with increasing condensation, being 1.340 ± 0.105 g, 1.533 ± 0.119 g, and 2.113 ± 0.120 g in preparations of 70 percent, 50 percent, and 30 percent condensed low-density fat, respectively (Fig. 4, *above, left*), with the latter being closest to that of high-density fat (2.217 ± 0.080 g; $p > 0.05$). The collagen content of high-density fat and 30 percent condensed low-density fat were significantly greater than that of low-density fat (0.893 ± 0.120 g; $p < 0.01$, each).

Immunoblotting results showed that the levels of collagens I, II, and IV were significantly higher in condensed low-density fat and high-density fat compared with low-density fat ($p < 0.01$ each) (Fig. 4, *below*). By contrast, collagen levels did not differ significantly in the high-density fat and condensed low-density fat preparations ($p > 0.05$).

Assessment of Graft Retention

Three months after transplantation of fat preparations into nude mice, the volume of low-density fat grafts had decreased significantly, with a retention rate of approximately 31.1 ± 5.7 percent (Fig. 5, *left*). The retention rate of Coleman fat, condensed low-density fat, and high-density fat were significantly higher than low-density fat, approximately 41.1 ± 6.8 percent ($p < 0.05$), 55.0 ± 7.5 percent ($p < 0.01$), and 53.8 ± 8.9 percent ($p < 0.01$), respectively. The retention rate of condensed low-density fat grafts was comparable to the high-density fat grafts ($p > 0.05$).

Histologic Evaluation of the Grafts

Hematoxylin and eosin staining showed histologic changes in transplanted fat. Both condensed low-density fat and high-density fat grafts showed a normal adipose tissue structure with few oil cysts, whereas low-density fat grafts showed numerous large oil cysts and infiltration of inflammatory cells. [**See Figure, Supplemental Digital Content 1**, which shows histologic evaluation of low-density fat, Coleman fat, high-density fat, and condensed low-density fat grafts 12 weeks after transplantation. The *star* indicates an oil cyst in the graft ($n = 6$ in all groups). *Scale bar* = 100 μm, ***http://links.lww.com/PRS/E642***.] Low-density fat grafts had significantly more oil cysts per view than Coleman fat grafts (14.33 ± 3.01 versus 10.33 ± 3.26; $p < 0.05$), whereas high-density fat grafts (3.17 ± 2.04; $p < 0.01$) and 30 percent condensed low-density fat grafts (3.66 ± 2.40; $p < 0.01$) had significantly fewer oil cysts per view than low-density fat grafts (Fig. 5, *right*). The oil cyst formation between high-density fat and condensed low-density fat did not differ significantly ($p > 0.05$).

Macrophage Infiltration after Grafting

Immunofluorescence staining showed that low-density fat grafts contained few perilipin-positive mature adipocytes, many Mac2-positive cells, and numerous oil cysts at 3 months, indicating the infiltration of macrophages and a severe inflammatory response (Fig. 6, *left*). Staining of high-density fat grafts showed large numbers of perilipin-positive mature adipocytes, few Mac2–positive cells, and a slight inflammatory response (Fig. 6, *center*). Immunofluorescence staining of 30 percent condensed low-density fat grafts showed large numbers of perilipin-positive mature adipocytes, with these adipocytes being uniformly small with a more integrated structure, and few Mac2-positive macrophages (Fig. 6, *right*).

CASE REPORT

Case 1

A 32-year-old woman was unsatisfied with the shape of her breast 2 years after total left

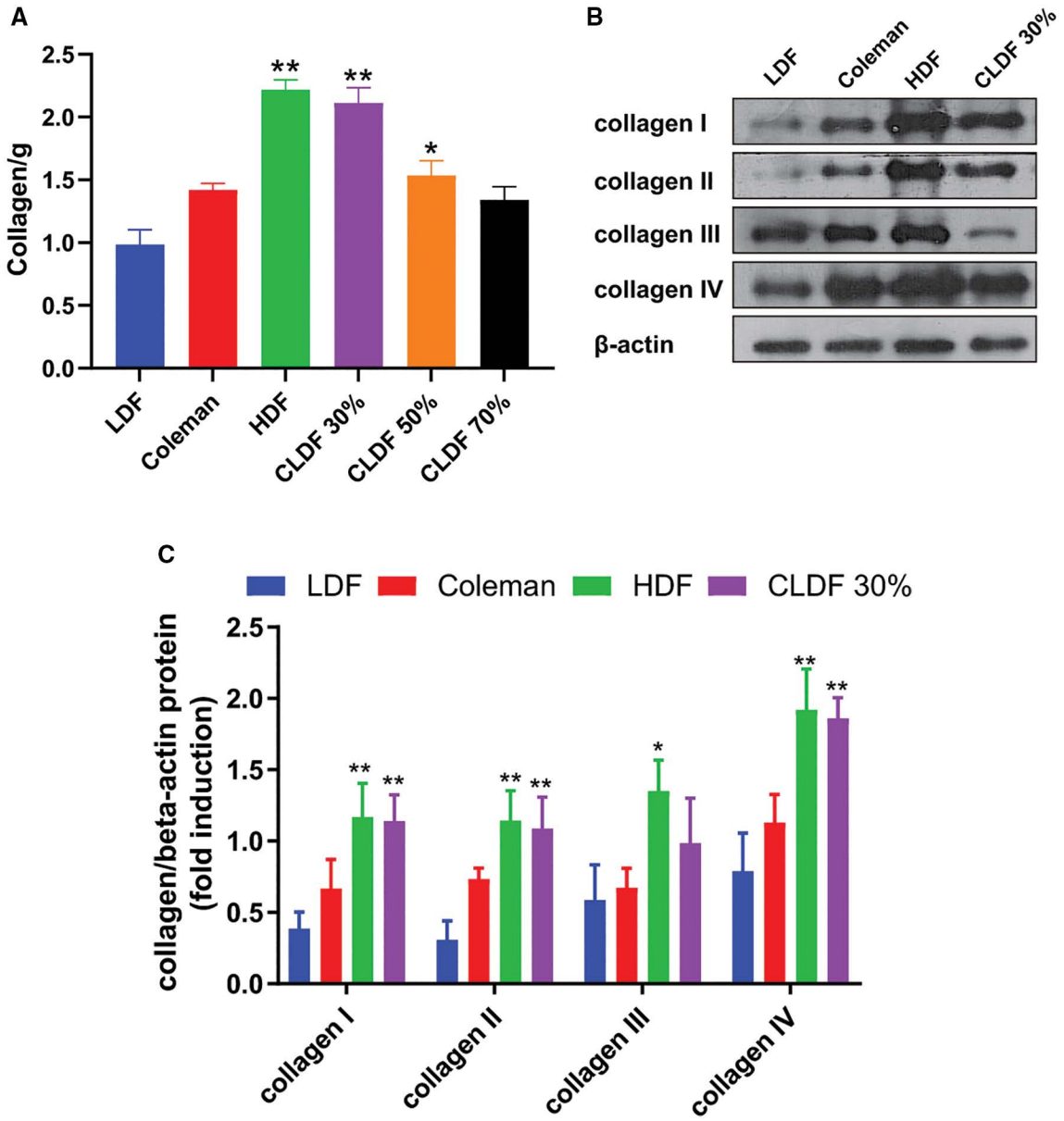

Fig. 4. Relationship between collagen components and the extent of condensation of condensed low-density fat (*CLDF*). (*Above, left*) The amounts of collagen were significantly higher in high-density fat (*HDF*) ($p < 0.05$) and condensed low-density fat ($p < 0.05$) than in low-density fat (*LDF*). (*Above, right*) Protein levels of collagens I through IV in low-density fat, Coleman fat, high-density fat, and condensed low-density fat. (*Below*) Statistical comparisons of protein levels in low-density fat, Coleman fat, high-density fat, and condensed low-density fat. *$p < 0.05$ and **$p < 0.01$ compared with low-density fat ($n = 6$ for all groups).

mastectomy for breast cancer and wished to undergo breast reconstruction (Fig. 7, *left*). She received two sessions of fat grafting for breast reconstruction and was injected with 70 ml condensed low-density fat plus 230 ml of high-density fat in total. Six months after the second fat grafting, she expressed satisfaction with the augmentation of her breast (Fig. 7, *right*). The retention rate of the graft was 43.95 percent evaluated by the three-dimensional laser scanner. [**See Figure, Supplemental Digital Content 2**, which shows three-dimensional images of the breast for a 34-year-old patient before (*left*) and 3 months (*center*) after high-density fat and condensed low-density fat transplantation; images are merged into one image to analyze the volume change (*right*). *Red* represents volume augmentation and *green* represents volume reduction of the breast,

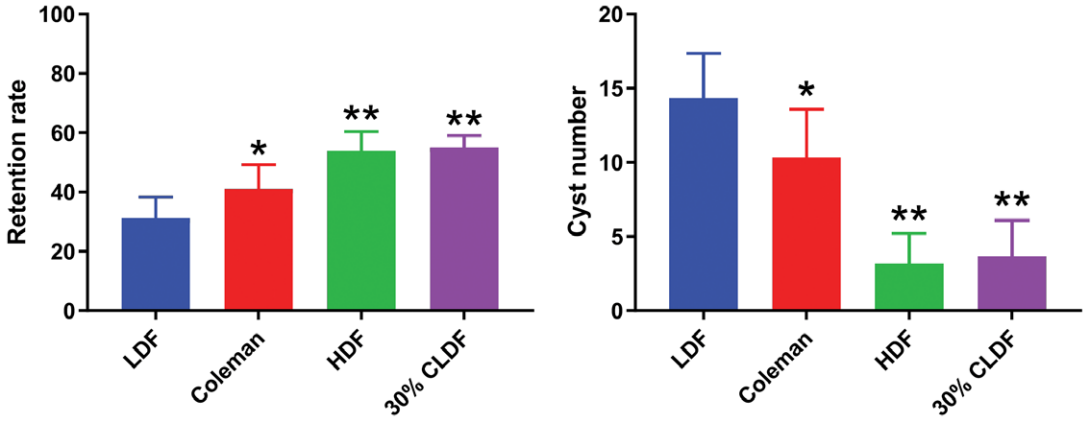

Fig. 5. Graft retention rates and oil cyst formation 12 weeks after graft transplantation. (*Left*) The retention rates of condensed low-density fat (*CLDF*) and high-density fat (*HDF*) grafts were significantly higher than those of low-density fat (*LDF*) grafts ($p < 0.01$), and the retention rates of Coleman fat grafts were higher than those of low-density fat grafts ($p < 0.05$). (*Right*) Oil cyst formation was significantly higher in low-density fat than in Coleman fat ($p < 0.05$), high-density fat ($p < 0.01$), and condensed low-density fat ($p < 0.01$) grafts. *$p < 0.05$ and **$p < 0.01$, compared with low-density fat ($n = 6$ for all groups).

as the deeper color shows greater change, ***http://links.lww.com/PRS/E643***.] There were no palpable nodules, and she reported no pain or any other discomfort complications.

Case 2

A 25-year-old woman was unsatisfied with her breast size and wanted natural augmentation of her breasts (Fig. 8, *left*). In the treatment, each breast was injected with 200 ml of high-density fat and 40 ml of condensed low-density fat. She was satisfied with the augmentation and the natural feel of her breasts 3 months after transplantation (Fig. 8, *right*). The retention rate of the graft was 41.20 percent evaluated by three-dimensional laser scanning. No palpable nodules, pain, or any other discomfort complications were reported.

DISCUSSION

This study describes a new strategy to optimize the components of lipoaspirate for large-volume

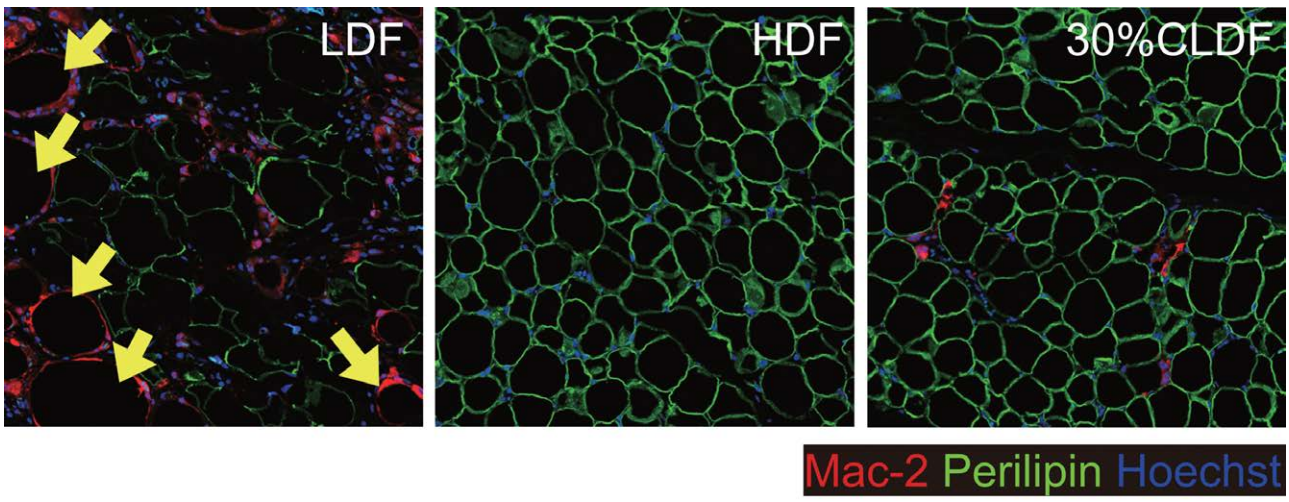

Fig. 6. Changes in macrophage infiltration 12 weeks after graft transplantation. Macrophage infiltration 12 weeks after transplantation was lower following condensed low-density fat (*CLDF*) than low-density fat (*LDF*) grafting. Low-density fat grafts showed large oil droplets (*yellow arrows*) surrounded by Mac2-positive macrophages (*left*). The high-density fat (*HDF*) grafts showed large numbers of perilipin-positive adipocytes, but few macrophages and few oil droplets (*center*). Condensed low-density fat grafts showed large numbers of intact, perilipin-positive adipocytes, but few Mac2-positive macrophages and few oil droplets (*right*). *Scale bar* = 100 μm.

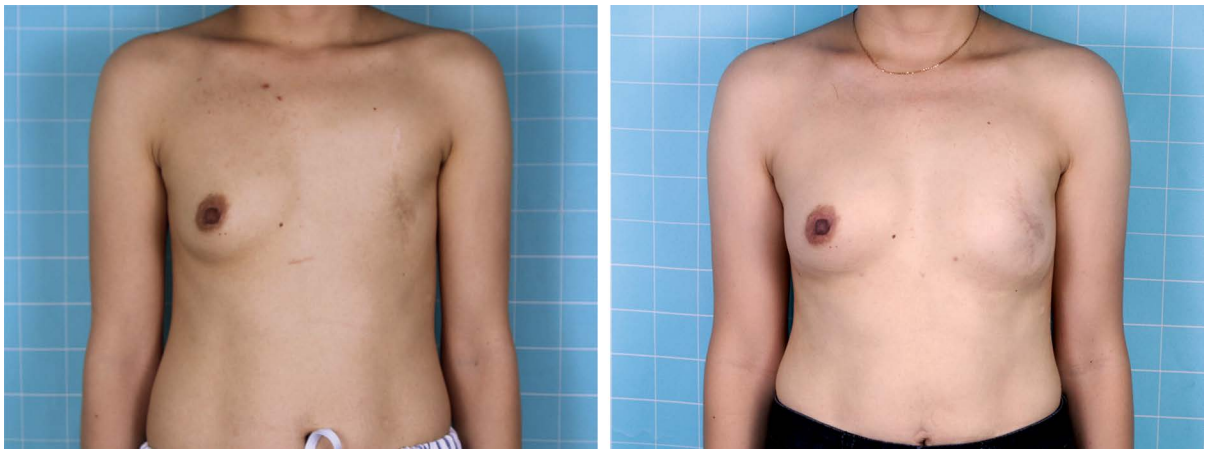

Fig. 7. A 32-year-old woman who was unsatisfied with the shape of her breast 2 years after left total mastectomy underwent two sessions of fat transplantation. Views before the operation (*left*) and 6 months after the second injection of 70 ml of condensed low-density fat and 230 ml of high-density fat (*right*).

transplantation. Coleman fat was centrifuged and divided into two parts, with the upper layer defined as low-density fat and the lower layer as high-density fat. Grafting of high-density fat, which contained high levels of viable cells, into nude mice resulted in a high graft retention rate, whereas grafting of low-density fat, containing considerable oil but few cells, resulted in a poor graft retention rate and large oil cysts. The quality of low-density fat grafts was improved by mechanical condensation, with these condensed low-density fat preparations containing large numbers of viable cells and little oil. High-density fat and condensed low-density fat were combined for large-volume transplantation, yielding better clinical outcomes.

Autologous fat grafting has become an ideal tool for volume augmentation and enhancement of tissue regeneration. However, unlike facial fat grafting, the benefits of large-volume (100 to 300 cc) and megavolume (>300 cc) fat grafting are unclear, as retention rates vary and complications such as fat oil cysts and calcifications are common.[18–20] Classic structural fat grafting emphasized the importance of preserving the native structure of adipose tissue and transferring maximally intact tissue samples into the host area.[21] However, most mature adipocytes are fragile and cannot survive hypoxic and ischemic conditions, such that these cells will become necrotic after transplantation, especially after large-volume fat transplantation.[22] By contrast, adipose-derived stem cells are less fragile and can survive better under ischemic conditions after fat grafting. After transplantation, the surviving adipose-derived stem cells can differentiate into adipocytes, which

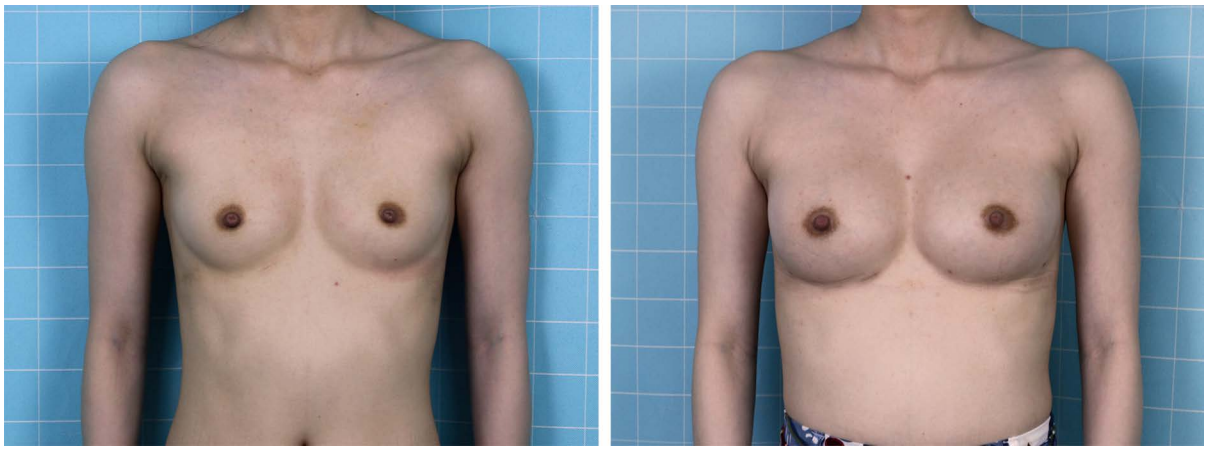

Fig. 8. Fat transplantation in a 30-year-old woman dissatisfied with the size of her breasts who wished to undergo natural breast augmentation. View of her breasts before (*left*) and 3 months after injection of 200 ml of high-density fat and 40 ml of condensed low-density fat into each breast (*right*).

can form new adipose tissue.[22] Thus, good-quality fat should contain high levels of adipose-derived stem cells and fewer mature adipocytes.

Centrifugation of lipoaspirates generates fractions, in which the stromal vascular fraction densities gradually increase from upper to lower layers.[23] Coleman fat has been divided into three layers, with the lowest layer containing more viable adipocytes and stromal vascular fraction cells, leading to the highest volume retention after grafting; and the topmost layer containing mostly fragile adipocytes and few stromal vascular fraction cells, leading to the lowest volume retention. In the present study, Coleman cells were classified into two layers by centrifugation, with the upper and lower layers defined as low-density fat and high-density fat, respectively. The results of these two studies are consistent, as retention rates were higher after transplantation of high-density fat than conventional Coleman fat or low-density fat. In addition, numerous large oil cysts were present in grafts 3 months after low-density fat transplantation, whereas few or no oil cysts were present after high-density fat grafting. Immunofluorescent staining also showed that low-density fat grafts contained few perilipin-positive adipocytes and numerous Mac2-positive crown-like structures.

This study showed that mechanical methods, including intersyringe shifting and centrifugation, could improve low-density fat quality. A similar mechanical method has been used to process Coleman fat, generating a product called stromal vascular fraction gel, which was rich in stromal vascular fraction cells and extracellular matrix.[14,24–26] The transplantation of stromal vascular fraction gel resulted in higher graft retention and lower oil cyst formation rates in a mouse model and in clinical practice. However, the production of stromal vascular fraction gel requires a large volume of adipose tissue, as only 20 percent of the original volume remains, making stromal vascular fraction gel unsuitable for large-volume fat grafting. High-density fat is a good-quality fat and can be used directly, whereas low-density fat requires improvement before use. The processing of condensed low-density fat in this study is similar to that of stromal vascular fraction gel, involving centrifugation after intersyringe shifting of lipoaspirates. One of the major differences was that condensed low-density fat was prepared from low-density fat, whereas stromal vascular fraction gel was prepared from whole Coleman fat. Moreover, the mechanical process of condensed low-density fat requires less destruction of the original product and the condensing rate is lower than that of stromal vascular fraction gel.

Prolongation of intersyringe shifting times resulted in a greater concentration of extracellular matrix and stromal vascular fraction cells, along with other components of low-density fat. Our results suggest that, when low-density fat is concentrated to 30 percent of its original volume, the density of stromal vascular fraction cells and the content of extracellular matrix were close to those of high-density fat. Adipose-derived stem cells and extracellular matrix are two key factors for neovascularization.[27,28] The main function of adipose-derived stem cells in grafts is to participate in the formation of blood vessels with endothelial precursor cells mainly through their secretory function.[10,29] Extracellular matrix provides a spatial background for the signaling of various cell surface growth factor receptors and adhesion molecules, such as integrins and collagen IV, a capillary basal membrane protein. This study found that the mechanical process condensed rather than destroyed extracellular matrix proteins, preserving the spatial structure of the extracellular matrix.

In clinical practice, we have used this strategy of high-density fat combined with condensed low-density fat for large-volume fat grafting since March of 2017. More than 50 patients to date have undergone large-volume fat grafting using this technique. This combination resulted in high graft retention rates and satisfactory results. No patient to date has reported any palpable nodules, pain, or any other discomfort. This strategy improves the components of lipoaspirates, but also requires larger volumes of fat tissue. However, the clinical efficacy of this strategy should be carefully evaluated and more clinical evidence should be provided. We are conducting a clinical trial comparing the efficacy of this strategy with conventional fat grafting.

CONCLUSIONS

Using a mechanical process, low-density fat can be condensed into condensed low-density fat, containing high levels of stromal vascular fraction cells and extracellular matrix proteins, similar to those of high-density fat. This novel strategy of transplantation of condensed low-density fat and high-density fat for large-volume fat grafting can reduce the complication rates associated with low-density fat and improve retention rates. This strategy has shown satisfactory results in patients without any disturbing complications.

Junrong Cai, M.D., Ph.D.
Department of Plastic and Cosmetic Surgery
Nanfang Hospital
Southern Medical University
1838 Guangzhou North Road
Guangzhou, Guangdong 510515,
People's Republic of China
drjunrongcai@outlook.com

Yunjun Liao, M.D., Ph.D.
Department of Plastic and Cosmetic Surgery
Nanfang Hospital
Southern Medical University
1838 Guangzhou North Road
Guangzhou, Guangdong 510515,
People's Republic of China
yunjun1000@sina.com

Feng Lu, M.D., Ph.D.
Department of Plastic and Cosmetic Surgery
Nanfang Hospital
Southern Medical University
1838 Guangzhou North Road
Guangzhou, Guangdong 510515,
People's Republic of China
406604293@qq.com

ACKNOWLEDGMENTS

This work was supported by the National Nature Science Foundation of China (81801932, 81901976, 81971852, 81772101, and 81901975).

REFERENCES

1. Coleman SR. Facial recontouring with lipostructure. *Clin Plast Surg.* 1997;24:347–367.
2. Rohrich RJ, Sorokin ES, Brown SA. In search of improved fat transfer viability: A quantitative analysis of the role of centrifugation and harvest site. *Plast Reconstr Surg.* 2004;113:391–395; discussion 396–397.
3. Smith P, Adams WP Jr, Lipschitz AH, et al. Autologous human fat grafting: Effect of harvesting and preparation techniques on adipocyte graft survival. *Plast Reconstr Surg.* 2006;117:1836–1844.
4. Pu LLQ, Coleman SR, Cui X, Ferguson REH Jr, Vasconez HC. Autologous fat grafts harvested and refined by the Coleman technique: A comparative study. *Plast Reconstr Surg.* 2008;122:932–937.
5. De Francesco F, Guastafierro A, Nicoletti G, Razzano S, Riccio M, Ferraro GA. The selective centrifugation ensures a better in vitro isolation of ASCs and restores a soft tissue regeneration in vivo. *Int J Mol Sci.* 2017;18:E1038.
6. Qiu L, Su Y, Zhang D, et al. Identification of the centrifuged lipoaspirate fractions suitable for postgrafting survival. *Plast Reconstr Surg.* 2016;137:67e–76e.
7. Dong Z, Peng Z, Chang Q, Lu F. The survival condition and immunoregulatory function of adipose stromal vascular fraction (SVF) in the early stage of nonvascularized adipose transplantation. *PLoS One* 2013;8:e80364.
8. Eto H, Kato H, Suga H, et al. The fate of adipocytes after nonvascularized fat grafting: Evidence of early death and replacement of adipocytes. *Plast Reconstr Surg.* 2012;129:1081–1092.
9. Toyserkani NM, Quaade ML, Sørensen JA. Cell-assisted lipotransfer: A systematic review of its efficacy. *Aesthetic Plast Surg.* 2016;40:309–318.
10. Kapur SK, Katz AJ. Review of the adipose derived stem cell secretome. *Biochimie* 2013;95:2222–2228.
11. Paik KJ, Zielins ER, Atashroo DA, et al. Studies in fat grafting: Part V. Cell-assisted lipotransfer to enhance fat graft retention is dose dependent. *Plast Reconstr Surg.* 2015;136:67–75.
12. Yao Y, Dong Z, Liao Y, et al. Adipose extracellular matrix/stromal vascular fraction gel: A novel adipose tissue-derived injectable for stem cell therapy. *Plast Reconstr Surg.* 2017;139:867–879.
13. Zhang Y, Cai J, Zhou T, Yao Y, Dong Z, Lu F. Improved long-term volume retention of stromal vascular fraction gel grafting with enhanced angiogenesis and adipogenesis. *Plast Reconstr Surg.* 2018;141:676e–686e.
14. Yao Y, Cai J, Zhang P, et al. Adipose stromal vascular fraction gel grafting: A new method for tissue volumization and rejuvenation. *Dermatol Surg.* 2018;44:1278–1286.
15. Ye Y, Chang H, Song Z, Zhao J. Accurate infrared structured light sensing system for dynamic 3D acquisition. *Appl Opt.* 2020;59:E80–E88.
16. Ye Y, Song Z, Guo J, Qiao Y. SIAT-3DFE: A high-resolution 3D facial expression dataset. *IEEE Access* 2020;8:48205–48211.
17. Ye Y, Song Z. A practical means for the optimization of structured light system calibration parameters. Paper presented at: 2016 IEEE International Conference on Image Processing; September 25–28, 2016; Phoenix, Ariz.
18. Khouri RK, Rigotti G, Cardoso E, Khouri RK Jr, Biggs TM. Megavolume autologous fat transfer: Part II. Practice and techniques. *Plast Reconstr Surg.* 2014;133:1369–1377.
19. Asserson DB, Kulinich AG, Orbay H, Sahar DE. Differences in complication rates of gluteoplasty procedures that utilize autologous fat grafting, implants, or local flaps. *Ann Plast Surg.* 2019;82(Suppl 4):S342–S344.
20. Rasmussen BS, Lykke Sørensen C, Vester-Glowinski PV, et al. Effect, feasibility, and clinical relevance of cell enrichment in large volume fat grafting: A systematic review. *Aesthet Surg J.* 2017;37(Suppl 3):S46–S58.
21. Coleman SR. Structural fat grafting: More than a permanent filler. *Plast Reconstr Surg.* 2006;118(Suppl):108S–120S.
22. Del VD, Del VS. The graft-to-capacity ratio: Volumetric planning in large-volume fat transplantation. *Plast Reconstr Surg.* 2014;133:561–569.
23. Ferraro GA, De Francesco F, Tirino V, et al. Effects of a new centrifugation method on adipose cell viability for autologous fat grafting. *Aesthetic Plast Surg.* 2011;35:341–348.
24. Zhang P, Feng J, Liao Y, et al. Ischemic flap survival improvement by composition-selective fat grafting with novel adipose tissue derived product: Stromal vascular fraction gel. *Biochem Biophys Res Commun.* 2018;495:2249–2256.
25. Sun M, He Y, Zhou T, Zhang P, Gao J, Lu F. Adipose extracellular matrix/stromal vascular fraction gel secretes angiogenic factors and enhances skin wound healing in a murine model. *Biomed Res Int.* 2017;2017:3105780.
26. Zhu M, Dong Z, Gao J, et al. Adipocyte regeneration after free fat transplantation: Promotion by stromal vascular fraction cells. *Cell Transplant.* 2015;24:49–62.
27. Suga H, Eto H, Aoi N, et al. Adipose tissue remodeling under ischemia: Death of adipocytes and activation of stem/progenitor cells. *Plast Reconstr Surg.* 2010;126:1911–1923.
28. He Y, Lin M, Wang X, et al. Optimized adipose tissue engineering strategy based on a neo-mechanical processing method. *Wound Repair Regen.* 2018;26:163–171.
29. Guo J, Hu H, Gorecka J, et al. Adipose-derived mesenchymal stem cells accelerate diabetic wound healing in a similar fashion as bone marrow-derived cells. *Am J Physiol Cell Physiol.* 2018;315:C885–C896.