Mechanical Micronization of Lipoaspirates: Squeeze and Emulsification Techniques

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Background: Condensation of grafted fat has been considered a key for achieving better outcomes after fat grafting. The authors investigated the therapeutic potential of two mechanical tissue micronizing procedures: squeeze and emulsification.

Methods: Human aspirated fat was centrifuged (centrifuged fat) and fragmented with an automated slicer (squeezed fat). Alternatively, centrifuged fat was emulsified by repeated transfer between two syringes through a small-hole connecter and then separated by mesh filtration into two portions: residual tissue of emulsified fat and filtrated fluid of emulsified fat. The four products were examined for cellular components.

Results: Histologic and electron microscopic analyses revealed that squeezed fat and residual tissue of emulsified fat contained broken adipocytes and fragmented capillaries. Compared with centrifuged fat, the squeezed fat and residual fat products exhibited increased specific gravity and increased numbers of adipose-derived stem/stromal cells and endothelial cells per volume, suggesting successful cell/tissue condensation in both squeezed fat and residual tissue of emulsified fat. Although cell number and viability in the stromal vascular fraction were well maintained in both squeezed fat and residual fat, stromal vascular fraction culture assay showed that adipose-derived stromal cells were relatively damaged in residual tissue of emulsified fat but not in squeezed fat. By contrast, no adipose-derived stromal cells were cultured from filtrated fluid of emulsified fat.

Conclusions: The authors' results demonstrated that mechanical micronization is easily conducted as a minimal manipulation procedure, which can condense the tissue by selectively removing adipocytes without damaging key components, such as adipose-derived stromal cells and endothelial cells. Depending on the extent of adipocyte removal, the product may be a useful therapeutic tool for efficient tissue volumization or therapeutic revitalization/fertilization. (*Plast. Reconstr. Surg.* 139: 79, 2017.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

Condensation of fat tissue, such as by centrifugation, has been considered one of the keys for achieving better outcomes of fat grafting.[1] The importance of adipose-derived stem/stromal cells in adipose tissue remodeling/revitalization has been documented,[2] and we have recently demonstrated the therapeutic potential of micronized fat tissue fragments containing adipose-derived stromal cells, vascular endothelial cells, and extracellular matrix.[3] As lipoaspirates have more hematopoietic cells and fewer adipose-derived stromal cells than intact fat tissue,[4,5] there is much interest in determining the best method of condensing lipoaspirates for producing superior outcomes

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after fat grafting for tissue volumization and revitalization/fertilization.[6] Fat grafting is a simple filling procedure but has been shown to have regenerating potential such as converting pathologic tissues with ischemia, fibrosis, stem cell deficiency, and poor healing capacity into healthier tissue with better vascularity and healing potential. Stem cells in the fat tissue are considered to be the key component to induce such beneficial clinical effects.

Centrifugation, which is the most popular processing method, not only separates water and oil from lipoaspirates but can also rupture superficial fragile adipocytes (depending on the magnitude of centrifugal force), leading to further condensation of adipose-derived stromal cells in the tissue.[7] There are also many other ways to rupture adipocytes without damaging adipose-derived stromal cells, such as mechanical chopping, shredding, pureeing, or mincing.[3,6] Such mechanical manipulations are expected to condense the tissue and adipose-derived stromal cells by mechanically disrupting adipocytes, which then transform into oil and can be removed by subsequent centrifugation; however, excessive mechanical processing can result in loss of viability of adipose-derived stromal cells and endothelial cells.[7] A number of mechanical processing procedures have been reported in an effort to obtain better clinical outcomes,[7–13] but detailed analyses of the final products are lacking.

In this study, we evaluated two mechanical procedures for condensation and micronization of lipoaspirates: squeeze[8] and emulsification.[9] We examined the cellular components of the final products, with the aim of determining which method generates the potentially most useful product for clinical applications.

PATIENTS AND METHODS

Human Tissue Sampling

We obtained aspirated fat tissue from 10 nonobese female patients aged 41.0 ± 8.2 years (mean ± SD) and a mean body mass index of 21.8 ± 1.3 kg/m^2 who underwent liposuction under general anesthesia. Aspirated fat tissue used in this study was harvested from the thigh after infiltration with a tumescent solution (1 liter of normal saline sodium chloride with 1:1,000,000 adrenaline). Liposuction was performed using a multiport 3-mm cannula with side holes 2 mm in diameter. Intact excised fat was obtained from one patient who simultaneously underwent abdominoplasty. Informed consent was obtained from each patient using a protocol approved by our institutional review board.

Lipoaspirate Mechanical Processing Procedures

Human aspirated fat was centrifuged (1200 *g* for 3 minutes) to discard the oil and water layers. We termed the resulting fat layer centrifuged fat (Fig. 1, *left*). Centrifuged fat was mechanically processed in two ways: by squeeze (Fig. 1, *above*, *right*) and by emulsification (Fig. 1, *below*, *right*). Squeeze (cutting into small fragments) was performed

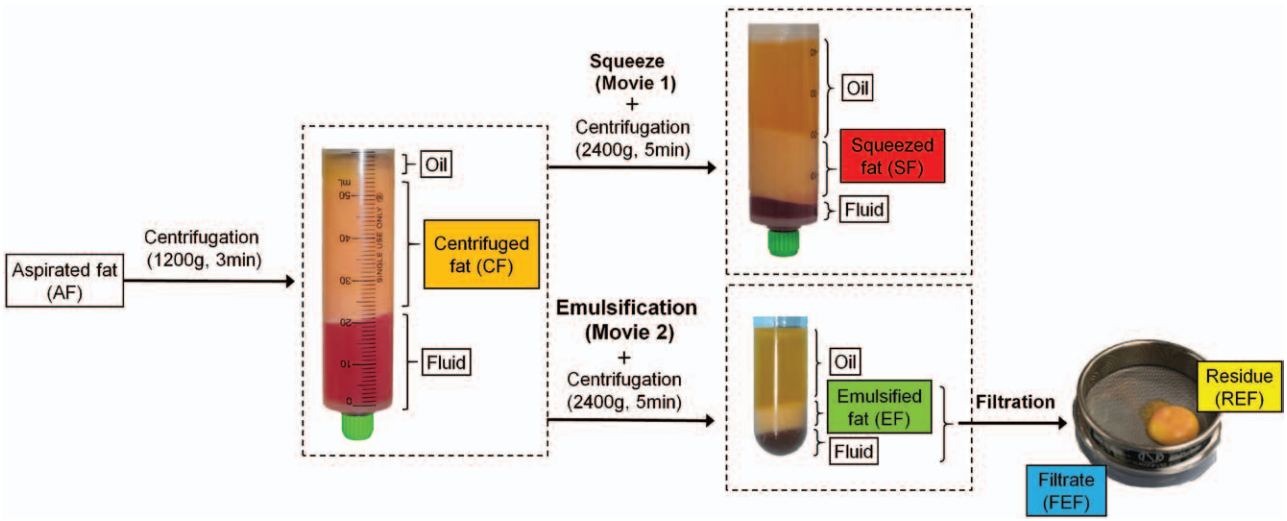
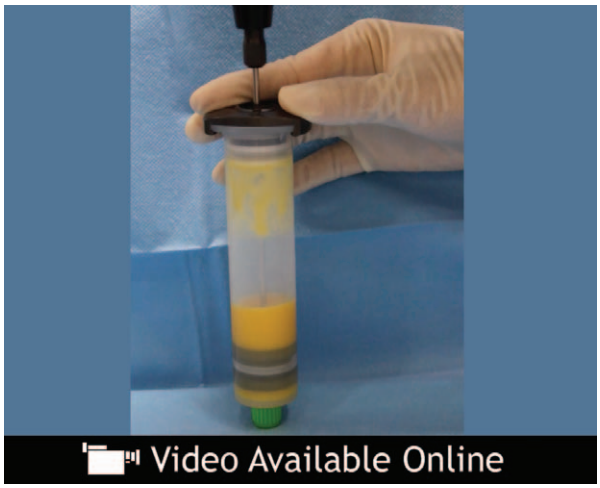


Fig. 1. Illustration of two mechanical processing procedures: squeeze and emulsification. Aspirated fat (*AF*) was first centrifuged to obtain centrifuged fat (*CF*). Centrifuged fat was then either squeezed or emulsified. Squeezed fat (*SF*) was obtained by removing the oil and fluid layers after squeezing and centrifugation. After emulsification, the tissue was centrifuged and filtered. The residual tissue of emulsified fat (*REF*) and filtrated fluid of emulsified fat (*FEF*) were obtained on and under the filter, respectively.



Video 1. Supplemental Digital Content 1 shows the centrifuged fat mechanical micronizing process for the production of squeezed fat. Sharp propeller-like blades in the piston were electronically spinning; they moved up and down twice during the preparation process, ***http://links.lww.com/PRS/B964***.

using an automated slicer with a spinning sharp blade (Medikan, Seoul, Republic of Korea) (**See Video, Supplemental Digital Content 1**, which shows the centrifuged fat mechanical micronizing process for the production of squeezed fat. Sharp propeller-like blades in the piston were electronically spinning; they moved up and down twice during the preparation process, ***http://links.lww.com/PRS/B964***.), followed by centrifugation (1200 *g* for 3 minutes) to discard the oil and water portions; the final product was termed squeezed fat (Fig. 1, *above*, *right*). By contrast, emulsification was performed manually with regular disposable syringes as follows: centrifuged fat was transferred a total of 30 times between two Luer-lock 2.5-ml syringes joined to each other by a connector with three small holes (Transfer Emulsifier; Tulip Medical, San Diego, Calif.). (**See Video, Supplemental Digital Content 2**, which shows the centrifuged fat mechanical micronizing process for the production of emulsified fat. Centrifuged fat was transferred manually 30 times between two syringes through a Luer-lock connecter containing three small holes, ***http://links.lww.com/PRS/B965***.) The emulsified product was centrifuged again (1200 *g* for 3 minutes) to discard the oil, and the remainder of the product was separated by decanting filtration through a stainless steel mesh with a 500-μm pore size (Tokyo Screen, Tokyo, Japan) to generate filtrated fluid of emulsified fat and residual tissue of emulsified fat.

The volume and weight of each product were measured, and the specific gravity and volume ratio to centrifuged fat were calculated. In addition, the specific gravity of the oil and connective tissue obtained through homogenization and strong centrifugation (22,000 *g* for 10 minutes) of aspirated fat were measured.

Whole-Mount Staining of Living Fat Tissue

Centrifuged fat, squeezed fat, and residual tissue of emulsified fat were incubated with the following reagents for 30 minutes: BODIPY 568 (Molecular Probes, Eugene, Ore.) to stain adipocytes, Alexa Fluor 488–conjugated isolectin GS-IB4 (Molecular Probes) to stain endothelial

Video 2. Supplemental Digital Content 2 shows the centrifuged fat mechanical micronizing process for the production of emulsified fat. Centrifuged fat was transferred manually 30 times between two syringes through a Luer-lock connecter containing three small holes, ***http://links.lww.com/PRS/B965***.

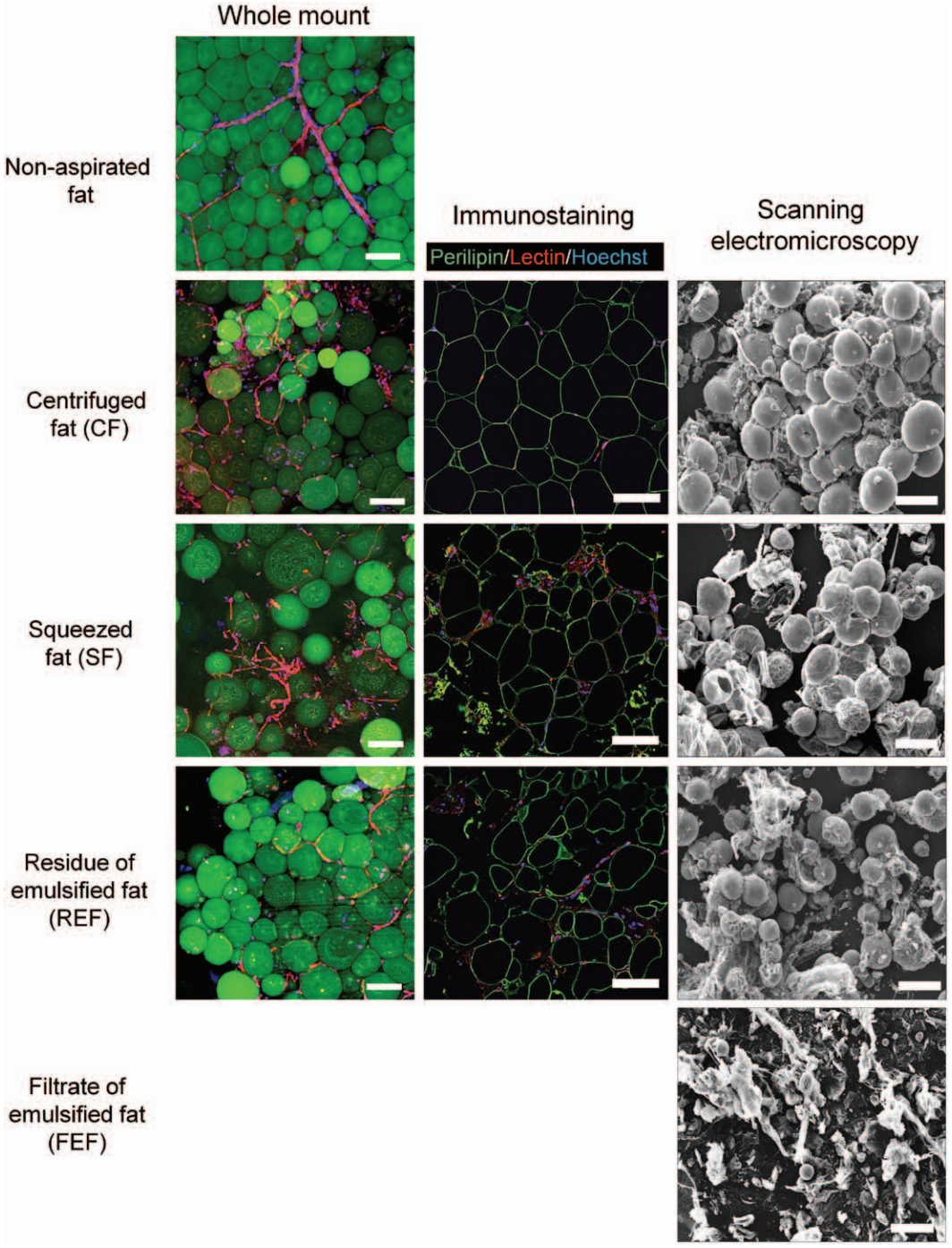


Fig. 2. Microscopic and electron microscopic analyses. (*Left*) Nonfixed living tissue samples were stained with BODIPY (adipocytes and lipid drops; *green*), lectin (endothelial cells; *red*), and Hoechst 33342 (nuclei; *blue*) for whole-mount staining. (*Center*) Paraffin-embedded sections were stained for perilipin (adipocytes; *green*), lectin (endothelial cells; *red*), and Hoechst 33342 (nuclei; *blue*). (*Right*) Scanning electron microscopic images are shown. *Scale bars* = 100 μm.

cells, and Hoechst 33342 (Dojindo, Kumamoto, Japan) to stain all nuclei. The samples were then washed and observed directly with a confocal microscope system (Leica TCS SP2; Leica Microsystems GmbH, Wetzlar, Germany). Fifty images acquired at 0.4-μm intervals were used for reconstructing 20-μm-thick three-dimensional images. Intact (excised) adipose tissue was evaluated in the same manner for use as a control. Filtrated fluid of emulsified fat could

not be processed in this manner because of its liquid state.

Immunohistochemistry

Centrifuged fat, squeezed fat, and residual tissue of emulsified fat were fixed (Zinc Fixative; BD Biosciences, San Jose, Calif.), paraffin-embedded, and sectioned at 5 μm. Immunostaining was performed with the primary antibody of guinea pig anti-perilipin (dilution, 1:200; Progen, Heidelberg, Germany). An isotype immunoglobulin was used as a negative control for immunostaining. Nuclei and endothelial cells were delineated by Hoechst 33342 (Dojindo, Tokyo, Japan) and Alexa Fluor 647-conjugated lectin (Life Technologies, Carlsbad, Calif.), respectively. Stained sections were examined with a fluorescence microscope (Keyence, Osaka, Japan). Filtrated fluid of emulsified fat could not be processed in this manner because of its liquid state.

Scanning Electron Microscopy

The small fragments of centrifuged fat, squeezed fat, residual tissue of emulsified fat, and filtrated fluid of emulsified fat were fixed in 2% paraformaldehyde; 2.5% glutaraldehyde in 0.1 M, pH 7.2 sodium cacodylate buffer; and then postfixed in 2% osmium tetroxide with the same buffer at room temperature for 2 hours. The specimens were dehydrated by passage through ascending alcohol concentrations and isoamyl acetate, and then critical point dried with liquid carbon dioxide and sputter coated with platinum-palladium. The specimens were observed with a scanning electron microscope (S-3500N; Hitachi, Tokyo, Japan) at 25 kV.

Stromal Vascular Fraction Isolation, Culture, and Analysis

The stromal vascular fraction was isolated from centrifuged fat, squeezed fat, and residual tissue of emulsified fat as described previously.[14] In brief, each tissue was washed and digested in phosphate-buffered saline containing 0.075% collagenase (Wako Pure Chemical, Osaka, Japan) for 30 minutes at 37°C in a shaking water bath. After centrifugation (800 *g* for 10 minutes) and resuspension of the cell pellets, stromal vascular fraction was obtained by filtering the cell suspension through 100-, 70-, and 40-μm meshes (Millipore, Billerica, Mass.). Filtrated fluid of emulsified fat, which was originally liquid effluent, was simply filtered through these meshes, without first undergoing enzymatic digestion.

Cell numbers and viability of stromal vascular fraction cells were measured with an automated cell counter (NucleoCounter NC-100; ChemoMetec, Allerød, Denmark). The isolated stromal vascular fraction from centrifuged fat, squeezed fat, and residual tissue of emulsified fat, and the filtrated fluid of emulsified fat, were plated on medium at a density of 5×10^5 nucleated cells per 100-mm plastic dish. Cells were cultured at 37°C in 5% carbon dioxide and humidified air. The culture medium was Dulbecco's Modified Eagle Medium with Ham's F-12 (Wako Pure Chemical) supplemented with 10% fetal bovine serum, 100 IU penicillin, and 100 mg/ml streptomycin. The medium was replaced every 3 days, and after primary culture for 7 days, the attached cells were trypsinized and counted.

Flow Cytometry

The stromal vascular fraction from centrifuged fat, squeezed fat, and residual tissue of emulsified fat, and the filtrated fluid of emulsified fat, were examined for surface marker expression using flow cytometry. The following monoclonal antibodies conjugated to fluorochromes were used: anti–CD31-phycoerythrin, anti–CD34-phycoerythrin-Cy7, and anti–CD45-fluorescein isothiocyanate (BD Biosciences). Multicolor flow cytometry was performed with a MACS-Quant (Miltenyi Biotec, Bergisch Gladbach, Germany), and cell composition was calculated.

Table 1. Volume, Specific Gravity, and Assumed Tissue Composition of Each Product*

	Volume (% Relative to CF)	Specific Gravity (g/ml)	Mass Fraction		
			Adipocytes (%)	ECM (%)	ECM/Adipocytes
CF	100	0.96 ± 0.02	58.3	41.7	0.7
SF	48.3 ± 5.6	1.04 ± 0.06	25.0	75.0	3.0
REF	39.3 ± 4.8	0.99 ± 0.11	45.8	54.2	1.2
FEF	10.1 ± 2.6	0.90 ± 0.03	83.3	16.7	0.2
Oil		0.86 ± 0.01			
ECM		1.10 ± 0.02			

CF, centrifuged fat; ECM, extracellular matrix; SF, squeezed fat; REF, residual tissue of emulsified fat; FEF, filtrated fluid of emulsified fat.
*Data are mean ± SD.

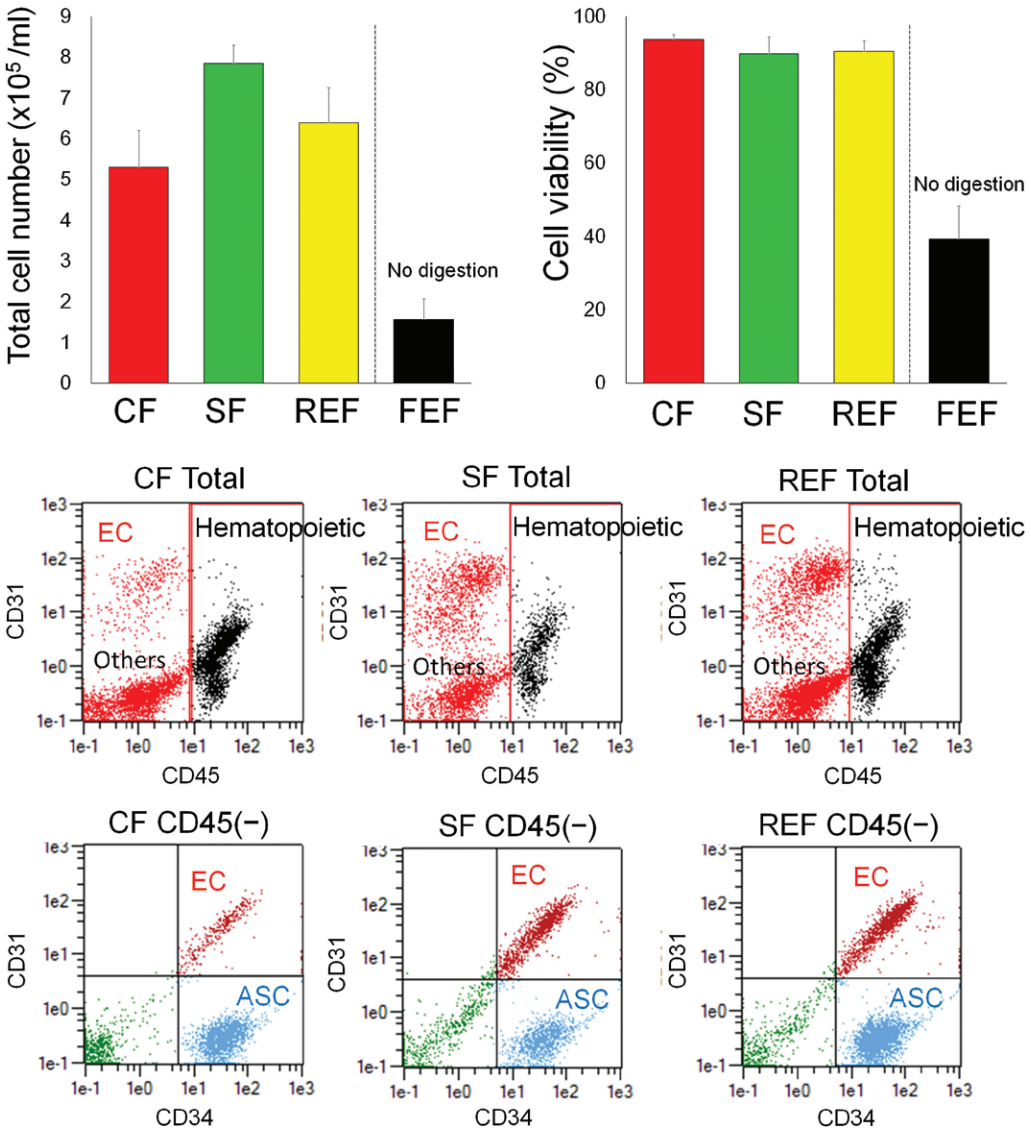


Fig. 3. Cellular analyses of stromal vascular fraction. (*Above*) Total cell number of the stromal vascular fraction obtained through digestion from 1 ml of each product such as squeezed fat (*above*, *left*). Cell viability of stromal vascular fraction from each product (*above*, *right*). Filtrated fluid of emulsified fat was not digested; thus, the filtrated fluid of emulsified fat number and viability data refer to the cells contained in the filtrated fluid. (*Center* and *below*) Representative plots of flow cytometry data. Hematopoietic cells are $CD45^+$, adipose-derived stem/stromal cells (*ASCs*) are $CD45^-/CD31^-/CD34^+$, and vascular endothelial cells (*ECs*) are $CD45^-/CD31^+/CD34^+$. *CF*, centrifuged fat; *SF*, squeezed fat; *REF*, residual tissue of emulsified fat; *FEF*, filtrate fluid of emulsified fat.

RESULTS

Whole-Mount Staining

The three-dimensional structure of living tissue was visualized by whole-mount staining (Fig. 2, *left*). Although nonaspirated adipose tissue showed compactly packed adipocytes and large vessels, aspirated fat after centrifugation (centrifuged fat) exhibited dead space and disrupted capillaries between adipocytes and a lack of large vessels. Squeezed fat and residual tissue of emulsified fat showed further structural damage, and squeezed fat exhibited occasional aggregations of capillary fragments.

Immunohistochemistry

Immunohistochemically stained sections showed adipocytes (perilipin), capillary (lectin), and nuclei (Hoechst) (Fig. 2, *center*). Centrifuged fat contained nondamaged parts of tissue, filled with viable adipocytes. By contrast, every field of the

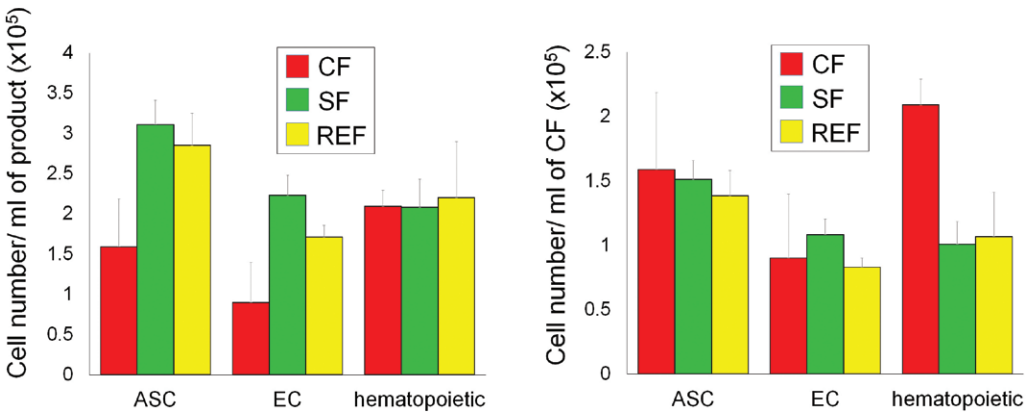


Fig. 4. Cellular analyses of stromal vascular fraction. Cell number of each cellular component in the stromal vascular fraction from each product. The absolute numbers (*left*) and the calculated numbers normalized by the volume of the original material (centrifuged fat) (*right*) are shown. *CF*, centrifuged fat; *SF*, squeezed fat; *REF*, residual tissue of emulsified fat; *FEF*, filtrate fluid of emulsified fat.

squeezed fat and residual tissue of emulsified fat sections showed irregular-sized/shaped adipocytes, and lipid droplets and fragmented capillaries. The irregular-sized/shaped adipocytes seemed to be damaged or dead, although they still expressed perilipin. Perilipin expression of adipocytes is usually lost several days after death (our previous finding).

Scanning Electron Microscopy

Visualization of ultrastructure was performed using a scanning electron microscope (Fig. 2, *right*). Centrifuged fat exhibited occasional damaged adipocytes, which were not present in nonaspirated fat. Compared to centrifuged fat, the squeezed fat and filtrated fluid of emulsified fat products generally had considerably more damaged adipocytes. Filtrated fluid of emulsified fat was filled with extracellular matrix fragments and contained few intact adipocytes.

Physiologic Measurement of Tissue

The volume and weight of each product relative to centrifuged fat were measured (Table 1). The relative volume for squeezed fat and residual tissue of emulsified fat were 48.3 ± 5.6 percent and 39.3 ± 4.8 percent, respectively. As the lost volume was oil and water (Fig. 1), more than half of the adipocytes were theoretically broken during the mechanical processing procedures.

The specific gravity for oil and extracellular matrix were 0.86 and 1.10, respectively (Table 1). Assuming that only oil (content of adipocytes) and extracellular matrix contribute to the weight of fat tissue, the mass fraction of adipocytes and extracellular matrix can be calculated based on the specific gravity of each product. The mass fraction values, including the extracellular matrix/adipocyte ratio, of each product are shown in Table 1.

Number and Viability of Stromal Vascular Fraction Cells

The number and viability of stromal vascular fraction cells were measured after collagenase digestion, whereas the number and viability of isolated cells in filtrated fluid of emulsified fat were measured without digestion (Fig. 3, *above*). The viability of stromal vascular fraction was well-maintained after processing in squeezed fat (89.9 ± 4.6 percent) and residual tissue of emulsified fat (90.6 ± 2.8 percent), compared with centrifuged fat (93.8 ± 1.2 percent). On the contrary, filtrated fluid of emulsified fat contained a smaller number of isolated cells and many dead cells (viability, 39.3 ± 9.1 percent).

Flow Cytometry

Characterization and classification of the stromal vascular fraction cells from centrifuged fat, squeezed fat, and residual tissue of emulsified fat, and the cells in filtrated fluid of emulsified fat, were performed by flow cytometry for multiple cell surface antigens (Figs. 3, *below*, and 4). The flow cytometric results for filtrated fluid of emulsified fat were inconsistent, primarily because of the low cell number and viability; many debris particles (consisting mainly of extracellular matrix fragments) were also detected in filtrated fluid of emulsified fat.

After $CD45^+$ hematopoietic cells were gated out, adipose-derived stromal cells ($CD45^-/CD31^-/CD34^+$) and endothelial cells ($CD45^-/CD31^+/CD34^-$) were measured (Fig. 3, *below*).

Compared with centrifuged fat, the squeezed fat and residual tissue of emulsified fat products exhibited a higher composition of adipose-derived stromal cells (2.1 and 1.8 times, respectively) and endothelial cells (2.6 and 1.8 times, respectively) (Fig. 4, *left*). By contrast, when normalized as "from 1 ml of centrifuged fat," the number of adipose-derived stromal cells and endothelial cells were similar in squeezed fat (1.5×10^5 and 1.1×10^5) and residual tissue of emulsified fat (1.4×10^5 and 0.8×10^5) compared with those of centrifuged fat (1.6×10^5 and 0.9×10^5) (Fig. 4, *right*). The number of hematopoietic cells in squeezed fat (1.0×10^5) and residual tissue of emulsified fat (1.1×10^5) from 1 ml of centrifuged fat was approximately half the number in centrifuged fat (2.1×10^5). These results suggest that both mechanical processing procedures partly removed other cells, such as hematopoietic cells, and maintained adipose-derived stromal cells and endothelial cells in the products.

Stromal Vascular Fraction Cell Culture and Measurement of Viable Adipose-Derived Stromal Cells

Viable adipose-derived stromal cells contained in each product were measured by adherent cell culture of stromal vascular fractions. Cultured stromal vascular fraction cells from centrifuged fat, squeezed fat, and residual tissue of emulsified fat showed adipose-derived stromal cell–like morphology and were expanded, although few cells were cultured in filtrated fluid of emulsified fat (Fig. 5). Cell yield was counted and normalized as being from 1 ml of centrifuged fat. After 1 week of culture, the normalized number of cultured adipose-derived stromal cells was significantly greater in squeezed fat ($1.1 \pm 0.1 \times 10^6$) and centrifuged fat ($9.2 \pm 0.8 \times 10^5$) than in residual tissue of emulsified fat ($5.1 \pm 0.7 \times 10^5$) (Fig. 6). These results suggest that the number and viability of adipose-derived stromal cells in squeezed fat was well maintained, although adipose-derived stromal cells in residual tissue of emulsified fat were partly damaged. No adipose-derived stromal cells were expanded from filtrated fluid of emulsified fat.

DISCUSSION

Biological Features of Adipose-Derived Products Obtained through Mechanical Processing

Our study demonstrated that the squeeze and emulsification methods effectively "condense" fat tissue by selectively reducing the number of adipocytes. In this article, "condensed fat tissue" refers to an increase in the number of adipose-derived stromal cells per tissue volume. Centrifuged fat is a type of condensed fat tissue, containing not only more adipocytes and adipose-derived stromal cells per volume compared to aspirated fat but also a higher adipose-derived stromal cell–to-adipocyte ratio.[7] Squeezed fat and residual tissue of emulsified fat exhibited similar microscopic features: massively broken adipocytes and fragmented capillaries secondary to mechanical processing. After squeeze or emulsification, centrifugation was performed to clearly discard oil drops, which were derived from adipocytes destroyed by the mechanical processing. Filtrated fluid of emulsified fat was a fluid containing water, oil, and many extracellular matrix fragments. The volume of squeezed fat and emulsified fat (i.e., sum of residual tissue of emulsified fat plus filtrated fluid of emulsified fat) was slightly less than half the volume of centrifuged fat; this volume reduction resulted from the partial removal of adipocytes. The extracellular matrix–to-adipocyte ratio (calculated based on specific gravity) was higher in both squeezed fat (3.0) and residual tissue of emulsified fat (1.2), compared with centrifuged fat (0.7). The extracellular matrix of squeezed fat and residual tissue of emulsified fat contained a substantial portion of adipose-derived stromal cells and endothelial cells, supporting the suggestion that these products may be useful therapeutically.[3]

The stromal vascular fraction cells obtained from squeezed fat and residual tissue of emulsified fat demonstrated high viability (approximately 90 percent), whereas the viability of cells in filtrated fluid of emulsified fat was only 39.3 percent. Multicolor flow cytometry showed that squeezed fat and residual tissue of emulsified fat contained approximately twice the number of adipose-derived stromal cells and endothelial cells per volume compared with centrifuged fat, indicating that squeezed fat and residual tissue of emulsified fat were produced through mechanical volume reduction while preserving most of the original adipose-derived stromal cells and endothelial cells. When standardized as being from 1 ml of centrifuged fat, squeezed fat and residual tissue of emulsified fat contained approximately half the number of hematopoietic cells compared with centrifuged fat, suggesting the shift of some blood-derived cells into the fluid portion.

Thus, our results indicate that squeezing and emulsification micronized the adipose tissue and

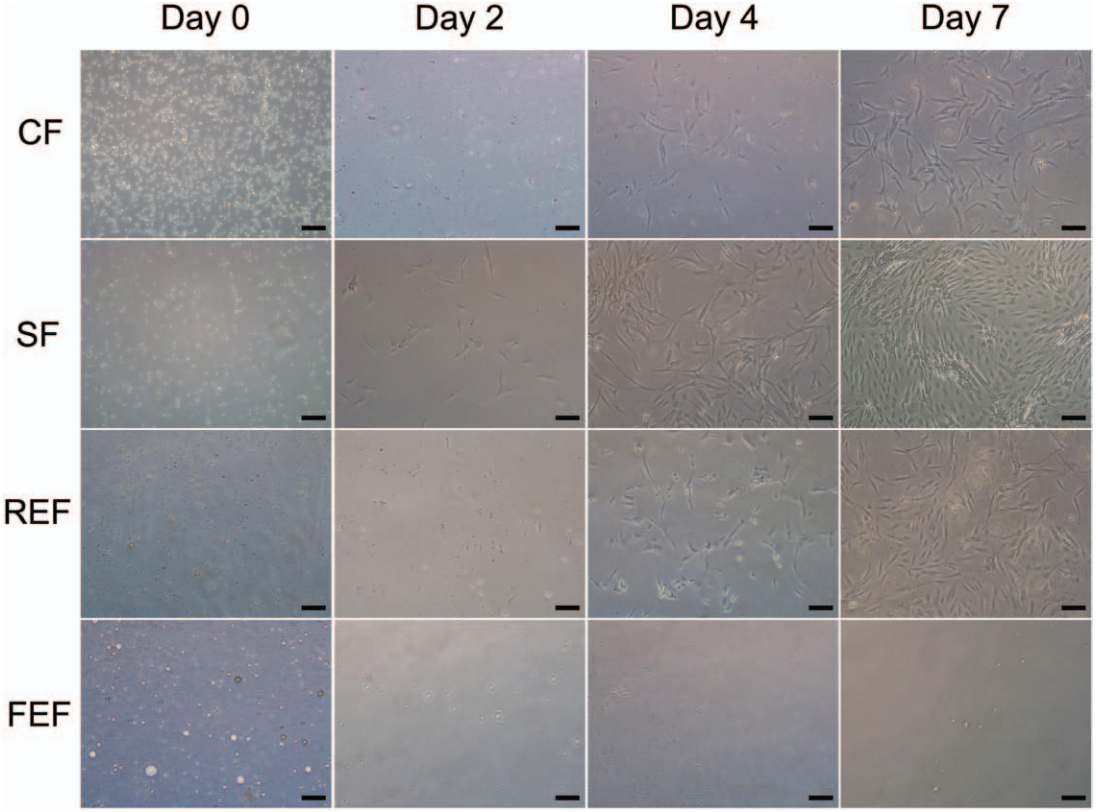


Fig. 5. Primary culture of stromal vascular fraction. Microscopic views of the cell culture of stromal vascular fraction from each product and from the filtrate of emulsified fat (*FEF*) after seeding of the same number of nucleate stromal vascular fraction cells (before standardization). Adipose-derived stromal cells were selectively expanded in the culture dish. *Scale bars* = 100 μm. *CF*, centrifuged fat; *SF*, squeezed fat; *REF*, residual tissue of emulsified fat.

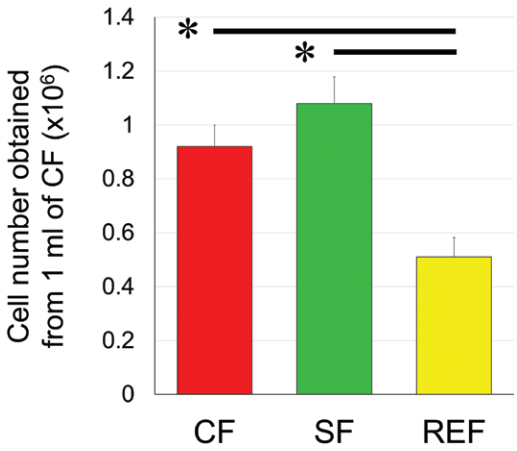


Fig. 6. Primary culture of stromal vascular fraction. Adipose-derived stromal cell yield on day 7, which was standardized as being from 1 ml of centrifuged fat. The viability and number of adipose-derived stromal cells in the tissue were well preserved in squeezed fat, whereas the adipose-derived stromal cells in emulsified fat (*REF*) seemed to be more damaged. *CF*, centrifuged fat; *SF*, squeezed fat; *REF*, residual tissue of emulsified fat.

partially removed adipocytes and hematopoietic cells and retained viable adipose-derived stromal cells and endothelial cells (Fig. 7). Filtrated fluid of emulsified fat contained few living cells and numerous fragmented extracellular matrix particles, and thus we could not assess its cellular composition. The syringe connector that we used had holes that were smaller than the holes in the connector used in a previous study,[9] and this hole size may have affected cell viability and particle size. A fundamental difference between squeeze and emulsification in this study is that the tissue cutting/micronization is conducted using a sharp blade for the squeezed fat but not in the emulsified fat. Because adipose-derived stromal cell culture assay indicated a better yield of viable adipose-derived stromal cells in squeezed fat than in residual tissue of emulsified fat, that sharp cutting (squeeze) of the tissue may be less traumatic to cellular components than blunt cutting (emulsification).

Clinical Perspectives of Mechanically Processed Products

Fat grafting is now considered to be important for not only tissue volumization but also tissue regeneration and revitalization/fertilization. The

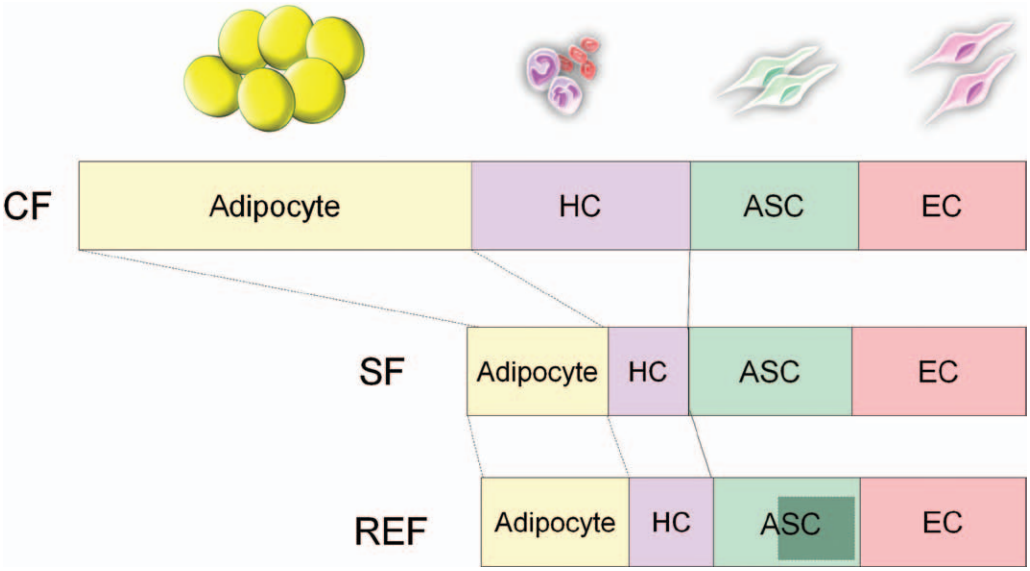


Fig. 7. Simplified schema of changes in cell composition by mechanical processing. Squeeze and emulsification selectively removed a portion of the adipocytes and hematopoietic cells and preserved adipose-derived stem/stromal cells and vascular endothelial cells. The cell culture assay results suggested that at least some adipose-derived stromal cells were damaged in residual tissue of emulsified fat (*shaded area*). *CF*, centrifuged fat; *SF*, squeezed fat; *REF*, residual tissue of emulsified fat; *HC*, hematopoietic cells; *ASC*, adipose-derived stem/stromal cells; *EC*, endothelial cells.

clinical effects of fat grafting on aged tissue, diabetic ulcers,[15] irradiated tissue,[16–18] chronic ischemia,[19] systemic sclerosis,[20] and scar contractures[21–23] have been increasingly reported. Although both adipocytes and adipose-derived stromal cells in the graft are critical components for tissue volumization,[24,25] therapies for improving the quality of tissue (i.e., vascularity, inflammation, fibrosis, and healing capacity) may not require adipocytes. For tissue revitalization/fertilization, adipose-derived stromal cells are considered to play a critical role.[6] Adipose-derived stromal cells are potent sources of trophic factors and enzymes, especially under hypoxic conditions,[26] but they also have the potential to modulate or suppress immunoreactions[27] and to differentiate into adipocytes, endothelial cells, or other cells.[28] Moreover, endothelial cells in the stromal vascular fraction are also important because they serve as paracrine mediators and regulate the regenerative potential of adipose-derived stromal cells by means of platelet-derived growth factor signaling, and improve adipose-derived stromal cell functionality.[29] Stromal vascular fraction includes some other cells that may be important for regenerative use, such as $CD146^+/CD31^-$ pericytes.[10,30] However, we focused on $CD45^+/CD31^-/CD34^+$ adipose-derived stromal cells in this study, as they seem to be the most definitive population in the stromal vascular fraction to have the greatest potential for adipogenic[31] and multilineage differentiation.[32]

Previous studies suggested that reduction of adipocytes without losing viable adipose-derived stromal cells can be accomplished by simple centrifugation at an appropriate centrifugal force (<3000 *g*).[7,33] The squeeze and emulsification techniques used in this study further condensed the aspirated fat tissue by intentionally reducing adipocytes (increasing the adipose-derived stromal cell–to-adipocyte ratio). The reduction efficiency may be evaluated by measuring the specific gravity of the product. If the magnitude of adipocyte reduction is small, the product might be good for tissue volumization, as during adipose-derived stromal cell–enriched fat grafting.[34,35] If adipocyte reduction is greater, the micronized product might be a useful injectable tool for tissue revitalization/fertilization or immunomodulation.[6] Potential for tissue volumization may be related to adipogenic differentiation capacity of adipose-derived stromal cells, which was not evaluated in this study.

Mechanical processing has the obvious advantage that it does not require enzymatic digestion, and manipulation is thereby minimal. As collagenase digestion of adipose tissue for stromal vascular fraction isolation is regarded as a more-than-minimal manipulation, even noncultured stromal vascular fraction is strictly regulated for

clinical use in many countries. Although we previously confirmed the therapeutic effectiveness of a product prepared through manual mechanical fragmentation when used for mouse diabetic ulcers,[3] a limitation of the current study was the absence of an animal experiment, which we plan to perform in the future.

CONCLUSIONS

We investigated adipose tissue–derived products obtained through two mechanical processing techniques, squeeze and emulsification, both of which can be performed easily in an operating room without any concerns regarding regulatory issues. Our results demonstrated that the two methods not only micronize the adipose tissue into a particle size injectable through a small needle/cannula, but they can also condense the tissue by selective removal of adipocytes without damaging key components, such as adipose-derived stromal cells and endothelial cells. The results also suggested that sharp cutting (squeeze) of the tissue may be less traumatic to adipose-derived stromal cells than blunt cutting (emulsification). Such mechanical micronization techniques seem to be valuable to efficiently use adipose graft materials. Depending on the extent (slight or considerable) of adipocyte removal, the product may be useful for efficient tissue volumization or therapeutic revitalization/fertilization of pathologic conditions, such as during the treatment of irradiated fibrous tissue or critical ischemia.

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