INTRODUCTION

Autologous fat grafting is a widely used technique for soft-tissue filling and augmentation in reconstructive and aesthetic applications, including in the face, buttocks, and breasts. However, optimization is needed to increase engraftment efficiency and achieve more predictable clinical outcomes, particularly improvement of long-term graft volume retention. Reports of volume loss range from 20% to 90% over the first few months, far from the desirable clinical outcome.

Reasons for inconsistent outcomes following fat grafting procedures are poorly understood. Possible contributing factors include the donor site, harvest procedures, processing methods, graft placement, and recipient site conditions. The processing method has been implicated as the most crucial, yet variable, factor affecting clinical outcome, as an appropriate method can ensure graft quality and more consistent results. Lipoaspiration procedures introduce some degree of damage to harvested tissue, leading to generation of contaminants such as free oils, blood cells, collapsed cell debris, stringy tissue, and excessive fluid; if those contaminants are not removed before fat transfer, it can lead to inflammation, tissue necrosis, volume loss, and poor injectability upon transplant.

Disclosure: Dr. Fang, Dr. Li, Dr. Huang, Ms. Connell, and Dr. Xu are employees of LifeCell Corporation, an Allergan Company. At the time the research was carried out, Mr. Patel, Ms. Wan, and Mr. Collins were employees of LifeCell Corporation, an Allergan Company.
Processing techniques are therefore designed to achieve removal of contaminants before transfer.

Centrifugation remains the most popular fat processing method, likely owing to ease of use and familiarity. However, there are concerns about the effects of high forces on fat cell viability and efficiency because of the cumbersome nature of processing large volumes of liposaprate. Decantation is an alternative method, circumventing force-induced damage to cells, but with a lower overall efficacy. Recently, filtration-based methods, including the REVOLVE System (LifeCell Corporation, an Allergan Company, Bridgewater, N.J.) and PureGraft (Cytori Therapeutics, Inc, San Diego, Calif.), have become commercially available. These are designed to provide optimal processing of liposaprate by preserving the quality and regenerative properties of the native tissue, while removing extraneous fluid and damaged tissue components.

Previous studies have evaluated volume composition of fat grafts processed by REVOLVE and PureGraft. Both systems were shown to yield a higher and more consistent fat tissue content, with significantly less extraneous fluid, free oil, and red blood cells, than that obtained using decantation and centrifugation. Despite these improvements in graft composition, a clinical study showed a long-term graft retention rate of only 41% with injected fat grafts processed by PureGraft. This suggests that factors beyond graft composition, potentially including viability and function of grafted adipocytes and adipose-derived stem cells, may contribute to long-term graft retention.

This study analyzed the physical and biologic characteristics of fat grafts processed via REVOLVE and PureGraft compared with standard centrifugation and decantation methods. Assessments included graft yield, quality, viability, and function of the processed fat tissue, as well as waste tissue removed by each filtration device.

MATERIALS AND METHODS

Autologous Fat Harvesting

Fresh liposaprate samples were collected from 12 healthy consenting donors at local clinics. Donor age ranged from 25 to 66 years (mean, 44 ± 13 years). Tissue samples were obtained from the abdomen, chest, flanks, and back and processed within 2 hours of harvest.

Fat Graft Preparation

Liposaprate samples from each donor were allocated for processing by each of the 4 methods, as follows.

Decantation and Centrifugation

An aliquot of liposaprate was either set aside and maintained at room temperature for 20 minutes for decantation or centrifuged at 1200g for 3 minutes as previously described, to allow phase separation. Free oil and aqueous layers were carefully aspirated, and the fat tissue layer was retained as the processed graft for further analysis.

REVOLVE and PureGraft

An aliquot of liposaprate was loaded into either the REVOLVE System or the PureGraft device and was processed according to the manufacturer’s instructions. The processed fat graft, filtered waste tissue, and tissue from the REVOLVE paddle were collected for further analysis. For all methods, graft yield was measured and graft composition was determined, as previously described.

Particle Size Analysis

Fat particle size in the processed graft or waste tissue was analyzed using the Horiba Laser Scattering Particle Size Distribution Analyzer (Horiba, Ltd, Kyoto, Japan) according to the manufacturer’s instructions. Briefly, 1–3 mL of tissue samples was loaded into the sample cup. After agitation and circulation in phosphate-buffered saline, particle sizes were measured. Data were plotted to obtain a composite accumulative histogram, showing the under size distribution of particle populations.

Adipocyte Analysis

Adipocyte Count

Fat graft samples were digested for 1 hour at 37°C with gentle agitation in 200-U/mL collagenase (Sigma-Aldrich, St. Louis, Mo.) at a fat graft:solution ratio of 1:4 (v/v). Fat cells were harvested as described previously and stained with a cell viability kit from Nexcelom (Nexcelom Bioscience LLC, Lawrence, Mass.). The number of live and dead cells was counted on a Cellometer K2 (Nexcelom).

Lipolysis Assay

Lipolysis activity in each processed fat graft was measured as described previously. All data were expressed as the measured value for each parameter per milliliter of graft/tissue material.

Results of each sample processed by different methods were normalized to the decantation graft and the normalized data (ratio) were compared among different processing methods.

Stromal Vascular Fraction Cell Analysis

Nucleated cells in fat graft samples were isolated as previously described, and resulting stromal vascular fraction cells were enumerated and used for the following assays.

Fluorescence-activated Cell-sorting Analysis

Harvested stromal vascular fraction cells were washed with phosphate-buffered saline containing 0.5% (weight/volume [w/v]) bovine serum albumin and stained for 30 minutes at 4°C with fluorescein isothiocyanate (FITC)-labeled anti-CD34, phycoerythrin (PE)-labeled anti-CD31, and peridinin-chlorophyll-protein-cyanine 5.5 (PerCP-Cy5.5)-labeled anti-CD45 (BD Biosciences, San Jose, Calif.). The CD45−/CD31+/CD34+ cell population was acquired using a fluorescence-activated cell-sorting Calibur flow cytometer (BD Biosciences) and analyzed with CellQuest software (BD Biosciences). The total number of this population per milliliter of fat graft was calculated.

Colony-forming Unit Culture

Isolated stromal vascular fraction nucleated cells were seeded in a T-25 flask for colony formation as described
previously.22 Colony-forming units were counted, and the total number of colony-forming units per milliliter of fat graft was calculated. Ratio of both CD45−/CD31−/CD34+ cell population and colony-forming unit count in each graft from each processing method to decantation was calculated and presented as described above for adipocyte analysis.

**Growth Factor Content Measurement**

Growth factors in fat grafts processed from one donor sample were extracted with tissue lysis buffer [1:1 (w/v) ratio] and measured with the Bio-Plex Pro Human Cancer Biomarker and Bio-Plex Pro Diabetes Assay kits (BioRad, Hercules, Calif.) according to the manufacturer’s instructions.

**Microscopic Evaluations**

**Macrostructure of Fat Tissue Particles**

Tissues were collected from the REVOLVE canister and waste container. The same amount of each sample was dispersed on a glass slide, and images were taken under a Nikon SMZ1000 Stereoscopic Zoom Microscope (Nikon, Melville, N.Y.).

**Histology Staining**

Tissues were fixed in 10% neutral-buffered formalin, embedded, sectioned, and stained with Masson’s tri-chrome, as described previously.23 Images were taken with a Nikon Eclipse 80i microscope (Nikon).

**Extrusion Force for Injectability of Processed Grafts**

Fat grafts were loaded into a 3-mL syringe and expressed through a 21-G needle. The extrusion force (N) required for injection at a rate of 1 mm/s was evaluated on an Instron Model 5865 materials tester (Instron Corporation, Norwood, Mass.). Mean extrusion forces over time for both the REVOLVE fat grafts (n = 3) and paddle-collected tissues (n = 3) were calculated.

**Statistical Methods**

The paired t test was used to compare the parameters of grafts from the same donor sample processed by any 2 of the 4 different processing techniques. Raw data from all parameters measured in this study were used for each paired t test. Overall statistical significance is defined as a P value of ≤0.05 (2-tailed), and the statistical significance levels for all tests are summarized.

**RESULTS**

**Yield and Composition of Processed Grafts**

Decantation produced the highest yield at 47.35% ± 5.65% among the 4 methods (Tables 1 and 2). However, decantation grafts contained the lowest percentage of fat tissue and highest percentage of combined oil and liquid, whereas REVOLVE grafts had the highest percentage of fat tissue and lowest percentage of oil and liquid. PureGraft showed results similar to those of REVOLVE, whereas centrifugation grafts contained the highest oil fraction (Table 1).

**Fat Tissue Particle Characterization**

Approximately 57% of tissue particles in the decantation sample were greater than 1000 μm in size, and approximately 24% of particles were smaller than 300 μm in size (Fig. 1A). Similar results were observed for the centrifugation graft (data not shown). More than 75% of fat tissue particles from REVOLVE and PureGraft grafts were

| Processing Method | Processing Yield (Average ± SEM %) | Composition of Processed Graft (Average ± SEM %) |
|-------------------|-----------------------------------|-----------------------------------------------|
| Decantation       | 47.35 ± 5.65                      | Fat Tissue Layer: 70.10 ± 1.99                |
|                   |                                   | Oil Layer: 7.55 ± 1.40                        |
|                   |                                   | Aqueous Infranatant Layer: 22.35 ± 1.11       |
| Centrifugation    | 34.71 ± 4.72                      | Fat Tissue Layer: 78.90 ± 3.30                |
|                   |                                   | Oil Layer: 9.37 ± 0.98                        |
|                   |                                   | Aqueous Infranatant Layer: 11.73 ± 3.00       |
| REVOLVE           | 28.49 ± 2.30                      | Fat Tissue Layer: 88.27 ± 1.47                |
|                   |                                   | Oil Layer: 2.10 ± 0.60                        |
|                   |                                   | Aqueous Infranatant Layer: 9.63 ± 1.23        |
| PureGraft         | 34.97 ± 4.80                      | Fat Tissue Layer: 84.13 ± 2.59                |
|                   |                                   | Oil Layer: 2.85 ± 0.67                        |
|                   |                                   | Aqueous Infranatant Layer: 13.01 ± 2.30       |

SEM, standard error of the mean.

**Table 2. Significance Level of Each Paired t Test (2 Sided)**

| Processing Method | Composition of Fat | Viable Adipocytes | Lipolysis Activity | CD45−/CD31−/CD34+ | CFU |
|-------------------|--------------------|-------------------|--------------------|-------------------|-----|
| REVOLVE versus decantation | 0.003              | 0.000             | 0.000              | 0.021             | 0.007 |
| REVOLVE versus centrifugation | 0.135              | 0.023             | 0.000              | 0.009             | 0.014 |
| REVOLVE versus PureGraft | 0.084              | 0.106             | 0.191              | 0.158             | 0.026 |
| Decantation versus centrifugation | 0.012              | 0.013             | 0.394              | 0.001             | 0.358 |
| Decantation versus PureGraft | 0.013              | 0.003             | 0.013              | 0.007             | 0.024 |
| PureGraft versus PureGraft | 0.957              | 0.264             | 0.000              | 0.883             | 0.783 |

CFU, colony-forming unit. Bold numbers indicate statistical significance (P ≤ 0.05).
>1000 μm, and only <9% of particles were smaller than 300 μm. In contrast, in both REVOLVE and PureGraft waste containers, most particles (>85%) were smaller than 300 μm. Grafts processed with REVOLVE demonstrated an intact lobule structure with clustered adipocytes and interconnecting mesenchyme (Fig. 1B), whereas tissues in the waste comprised single or small clusters of adipocytes, mainly tissue debris (Fig. 1C).

Viability of the Processed Fat Grafts

REVOLVE grafts showed a statistically higher number of viable adipocytes and significantly higher lipolysis activity (as measured in response to stimulation with a β-adrenergic agonist) than grafts processed by the other 3 methods (Table 2 and Fig. 2). The PureGraft grafts had statistically higher viable adipocytes and lipolysis activity than the decantation grafts, but displayed a similar number of adipocytes as shown by the centrifugation grafts (Table 2 and Fig. 2). Interestingly, the lipolysis activity in centrifugation grafts was not proportionally higher than in decantation grafts, although its processing included a step of concentration by centrifugation.

Flow cytometry measurements demonstrated that the REVOLVE grafts contained the highest number of CD45−/CD31− cells, indicating a lower level of vascular elements compared to the other methods (Table 2 and Fig. 3).

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**Fig. 1.** Fat tissue particle analysis. Fat graft was harvested after being processed with either decantation or the 2 filtration systems (REVOLVE System and PureGraft) and analyzed for fat particle size distributions and particle morphology as described in Materials and Methods section. A, Particle size distribution (accumulative frequency) from the different processed grafts indicated (upper). The data shown are from one representative lipoaspirate sample, and the characterization results for each processing method are listed in the table (lower). B and C, Images of fat tissues from the canister (B) and waste container (C) of the REVOLVE System. Upper row: 20×; lower row: 40×.

**Fig. 2.** Analysis of adipocytes in each processed graft. A, Ratio of viable adipocytes in grafts processed with centrifugation, REVOLVE System, and PureGraft when compared with decantation. Adipocytes were isolated from the graft samples processed by the 4 methods and analyzed for viable adipocytes as described in Materials and Methods section. The data are presented as average ratio ± SEM. The adipocyte content in the decantation sample, presented as average + SEM, was: 3.763 ± 1.288 x 10^5 cells/mL graft, N = 5. B, Ratio of adipocyte lipolysis activity. Lipolysis activity was measured in each graft after stimulation with 10-μM isoprenaline as described in Materials and Methods section. The glycerol concentration in the decantation sample after stimulation, presented as average ± SEM, was 38.224 ± 9.292 μg/mL graft, N = 6. SEM indicates standard error of the mean.
CD31−/CD34+ cells among the 4 methods but was only statistically higher than the decantation grafts (Table 2 and Fig. 3A). However, the REVOLVE grafts contained a statistically greater number of colony-forming stromal vascular fraction cells (Table 2 and Fig. 3B), indicating that REVOLVE preserved more viable and actively proliferating stromal vascular fraction cells than other methods. Similar to the adipocyte observations, centrifugation grafts showed no difference from decantation grafts in the CD45−/CD31−/CD34+ cell count and even a trend of lower colony-forming unit number, indicating that centrifugation may have damaged some stromal vascular fraction cells.

### Growth Factor Content

Owing to extensive washing steps used in REVOLVE and PureGraft processing, those grafts were analyzed for potential loss of growth factors, including basic fibroblast growth factor, hepatocyte growth factor, placental growth factor, vascular endothelial growth factor, insulin-like growth factor–binding protein, and leptin. Grafts from REVOLVE or PureGraft showed a similar level of growth factors as the grafts processed by centrifugation (decantation not included owing to heavy contamination of growth factors in the infranatant phase), indicating that the washing procedures did not reduce growth factors in processed grafts (data not shown).

### Tissues Removed during the Filtration Processing

Tissues from waste containers of both filtration systems were harvested and evaluated. Waste tissue from REVOLVE contained approximately 10 times fewer adipocytes \( (P < 0.031) \) (Fig. 4A) and up to 60 times fewer colony-forming unit numbers (Fig. 4B).
stromal vascular fraction cells ($P < 0.038$) (Fig. 4B) than those in fat grafts collected for injection. Similar trends were found for waste tissue from PureGraft (Fig. 4). Furthermore, tissue in the waste of REVOLVE and PureGraft had undetectable lipolysis activity (data not shown).

The REVOLVE system also traps some tissues on its rotating paddle. Histology revealed that those tissues contained a high amount of collagen bundles mingled with broken and ruptured vessel walls (Fig. 5A), whereas processed fat from the collection canister contained intact fat lobules with adipocytes and sporadic intact small vessels embedded in the interconnecting mesenchyme, without thick collagen bundles (Fig. 5B). Furthermore, an injectability test showed that higher forces were needed to extrude the stringy paddle tissue through a 21-G needle compared with the graft tissue (Fig. 6). In addition, the amount of tissue trapped on the paddles was only 6.4% of the total tissue volume in the REVOLVE canister (Table 3), and both viable adipocytes and proliferative stromal vascular fraction cells trapped were only a small fraction of the total number of cells in the REVOLVE canister (Table 3).

**DISCUSSION**

Previous studies showed that contaminants created by liposuction such as cellular debris may lead to inflammation and cell death, which contribute to variable, unpredictable graft retention rate, or even graft failure. Although clinical correlation remains to be evaluated between graft fat content and clinical outcomes, a nude rat study showed better and more predictable fat tissue retention using grafts with higher fat content. Here we demonstrated that filtration systems produced the highest percentage of fat tissue, consistent with previous studies. Decantation grafts had the lowest fat tissue percentage, but achieved the highest volume yield due to their high oil, fluid, and tissue debris contaminants. This was supported by the particle size analysis showing decantation grafts had the highest percentage of small particles ($<300\ \mu m$), whereas grafts processed with REVOLVE and PureGraft contained fewer small particles. Better removal of small particle debris along with free oil and bloody tumescent fluid with REVOLVE and PureGraft is likely due to active washing steps and efficient removal of the liquid phase (drainage across filter membrane by gravity (PureGraft) or constant removal of filtered liquid under vacuum (REVOLVE)).

Native adipose tissue contains various angiogenic growth factors, including vascular endothelial growth factor, hepatocyte growth factor, placental growth factor, angiopoietins, fibroblast growth factor, tumor necrosis factor-$\alpha$, plasminogen activator inhibitor-1, and metalloproteases to support and maintain vascularization for normal tissue homeostasis. Extensive washing in a filtration method raises concerns about removing such beneficial growth factors from native fat tissue. In this study,

| Samples             | Percentage of Total Tissue Volume | Viable Adipocytes ($\times 10^6$) | SVF Cells by CFU ($\times 10^3$) |
|---------------------|----------------------------------|----------------------------------|----------------------------------|
| REVOLVE graft       | 93.62 ± 1.59                     | 54.7 ± 6.2                       | 244.9 ± 19.8                     |
| Tissue on paddle    | 6.38 ± 1.77                      | 1.6 ± 0.5                        | 6.7 ± 3.3                        |

CFU, colony-forming unit; SVF, stromal vascular fraction.
The function of these cells in the centrifugation graft was demonstrated by cell and colony-forming unit counts, again indicating that activation of adipose-derived stem cells. Recently, Kato et al. found that significant quantities of angiogenic growth factors, such as basic fibroblast growth factor and vascular endothelial growth factor, are retained in fat tissue after centrifugation to separate blood components and free oil.

Although the mechanisms of fat graft survival remain poorly understood, there are 2 prevailing theories. The graft survival theory states that survival depends on the number of viable adipocytes after implantation in vivo, whereas the host replacement theory suggests that survival depends on dynamic remodeling of adipose tissue through activation of adipose-derived stem cells. Recently, Kato et al. further characterized the mechanism of graft survival in vivo and demonstrated that both survival and regenerating processes are present in the newly placed graft. Therefore, in addition to the host conditions at the graft recipient site, a quality fat graft that contains high number of viable adipocytes and proliferative stem cells can maximize graft survival. In the present study, processed fat grafts were further evaluated for the viability and lipolysis activity of adipocytes and proliferative capacity of adipose-derived stem cells by colony-forming unit formation. Interestingly, the grafts from REVOLVE displayed a statistically higher level of viable and active adipocytes than grafts processed by the other 3 methods. Furthermore, grafts processed with REVOLVE also contained the highest number of CD45−/CD31−/CD34+ cells and had significantly higher counts of colony-forming units than grafts processed with other methods. These data strongly indicate the impact of processing methods on graft quality, even between 2 filtration systems. Although both filtration devices have a similar efficiency in removing small tissue debris, the paddle of REVOLVE is more effective in removing nonviable tissue debris. Removal of this tissue debris is a superior advantage of filtration methods to increase the functionality of a processed graft because the tissue debris was reported to be associated with inflammation and volume loss upon autologous fat grafting.

Overall, this study demonstrated that different fat processing methods result in fat grafts with varying physical and biologic properties. The variability in fat processing, therefore, may contribute to fat graft viability and retention in vivo. Further studies are needed to correlate these differences with clinical outcomes. Understanding the contribution of various factors in fat processing and their effects may help standardize a clinical protocol for fat grafting in the future.

**CONCLUSIONS**

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Grafts processed with REVOLVE and PureGraft contained a similar level of growth factors as the graft processed by centrifugation, which did not include washing steps. Although our sample size was small, these results were consistent with those of a previous study by Zhu et al. Thus, we believe that the washing steps in these 2 filtration systems did not remove growth factors from the processed grafts. This is likely due to the fact that growth factors are fat tissue associated, either tightly bound to the matrix or localized inside the cells. Indeed, Pallua et al. found that significant quantities of angiogenic growth factors, such as basic fibroblast growth factor and vascular endothelial growth factor, are retained in fat tissue after centrifugation to separate blood components and free oil.

Although the mechanisms of fat graft survival remain poorly understood, there are 2 prevailing theories. The graft survival theory states that survival depends on the number of viable adipocytes after implantation in vivo, whereas the host replacement theory suggests that survival depends on dynamic remodeling of adipose tissue through activation of adipose-derived stem cells. Recently, Kato et al. further characterized the mechanism of graft survival in vivo and demonstrated that both survival and regenerating processes are present in the newly placed graft. Therefore, in addition to the host conditions at the graft recipient site, a quality fat graft that contains high number of viable adipocytes and proliferative stem cells can maximize graft survival. In the present study, processed fat grafts were further evaluated for the viability and lipolysis activity of adipocytes and proliferative capacity of adipose-derived stem cells by colony-forming unit formation. Interestingly, the grafts from REVOLVE displayed a statistically higher level of viable and active adipocytes than grafts processed by the other 3 methods. Furthermore, grafts processed with REVOLVE also contained the highest number of CD45−/CD31−/CD34+ cells and had significantly higher counts of colony-forming units than grafts processed with other methods. These data strongly indicate the impact of processing methods on graft quality, even between 2 filtration systems. Although both filtration devices have a similar efficiency in removing small tissue debris, the paddle of REVOLVE is more effective in removing nonviable tissue debris. Removal of this tissue debris is a superior advantage of filtration methods to increase the functionality of a processed graft because the tissue debris was reported to be associated with inflammation and volume loss upon autologous fat grafting.

There is loss of some fat tissues into the waste containers of both the REVOLVE and PureGraft systems, raising concerns for some surgeons, especially when processing liposagrams from thinner patients. In this study, fat tissue collected from waste containers of both devices contained only small clusters of fat tissue particles or single cells, which are vulnerable to damage and can easily burst into free oil. Furthermore, tissues from waste containers demonstrated a significantly lower number of viable adipocytes (P < 0.031) (Fig. 4A), as well as undetectable lipolysis activity (data not shown) and significantly lower number of colony-forming stromal vascular fraction cells (P < 0.038). (Fig. 4B), indicating that tissues filtered into waste are true nonviable tissue debris. Removal of this tissue debris is a superior advantage of filtration methods to increase the functionality of a processed graft because the tissue debris was reported to be associated with inflammation and volume loss upon autologous fat grafting. Furthermore, unlike PureGraft, the REVOLVE device contains rotating paddles within the filter basket. In addition to ensuring thorough but gentle washing of the fat tissue, the paddle was shown to entrap and remove stringy collagen bundle tissue and large pieces of vascular debris while rotating, which may reduce potential clogging of the syringe during graft injection albeit contributing additional small percentage of tissue loss (Table 3). These features make REVOLVE an attractive fat processing method in the operating room.

**CONCLUSIONS**

Overall, this study demonstrated that different fat processing methods result in fat grafts with varying physical and biologic properties. The variability in fat processing, therefore, may contribute to fat graft viability and retention in vivo. Further studies are needed to correlate these differences with clinical outcomes. Understanding the contribution of various factors in fat processing and their effects may help standardize a clinical protocol for fat grafting in the future.
ACKNOWLEDGMENTS

The authors thank Maryellen Sandor for her contributions in reviewing and editing the manuscript, Eric Stec for data collection, and Ming Luo for assistance in statistical analysis. Editorial assistance was provided to the authors by Katie Dean, PhD, and Susan Stuch, PharmD, of Evidence Scientific Solutions, Inc, Philadelphia, Pa, and funded by Allergan plc. All authors met International Committee of Medical Journal Editors authorship criteria. Neither honoraria nor payments were made for authorship.

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