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In Vitro Characterization of Fat Grafts Processed Using the REVOLVE ENVI System versus Decantation

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Background: This preclinical study evaluated benchtop/in vitro properties and fat viability and activity of grafts processed using the REVOLVE ENVI 600 system compared with decantation and evaluated properties of REVOLVE ENVI waste.
Methods: Lipoaspirate from six donors was processed using REVOLVE ENVI or decantation. The composition of each graft, hematocrit/red blood cell content, fat particle size/macrostructure, viable adipocyte count, and adipocyte activity were analyzed. Stromal vascular fraction was analyzed for viable progenitor cell count and colony-forming units.
Results: REVOLVE ENVI grafts had a higher mean (±SD) fat content at 85.6% ± 6.1% than decanted grafts at 72.1% ± 4.0% ($P < 0.001$), with negligible free oil (0.4% ± 1.1%) and cellular debris (<0.1%), whereas REVOLVE ENVI waste contained primarily aqueous fluid (91.0% ± 2.2%) with negligible viable fat. REVOLVE ENVI grafts had significantly lower hematocrit levels ($P < 0.001$) and contained significantly more large fat globules ($P < 0.001$) than decanted grafts or REVOLVE ENVI waste. The percentage of tissue particles of more than 1000 µm was highest for REVOLVE ENVI grafts at 61.6% ± 9.2% (decantation: 52.5% ± 13.4%; REVOLVE ENVI waste: 0.49% ± 1.50%), and the percentage of particles less than 200 µm was lowest for REVOLVE ENVI grafts at 15.7% ± 2.6% (decantation: 32.2% ± 8.9%; REVOLVE ENVI waste: 97.9% ± 4.5%). REVOLVE ENVI grafts contained 145.2% ± 36.0% more viable adipocytes, 145.7% ± 46.2% greater activity, 195.5% ± 104.2% more progenitors in SVF, and 363.5% ± 161.2% more SVF colony-forming units than decanted grafts.
Conclusion: Fat grafts processed using REVOLVE ENVI demonstrated greater viability and activity than decanted grafts in vitro. *(Plast Reconstr Surg Glob Open 2024; 12:e5615; doi: 10.1097/GOX.0000000000005615; Published online 8 February 2024.)*

INTRODUCTION

Autologous fat grafting is commonly used for a variety of reconstructive and aesthetic surgery procedures in the face, buttocks, and breasts.[1–5] The American Society of Plastic Surgeons reported that more than 155,000 cosmetic procedures involving fat grafting were performed in 2020.[6] In a clinical setting, one of the major challenges of autologous fat grafting is poor graft retention, ranging from 25% to 90% overall volume loss over time.[7,8] Liposuction procedures also typically generate contaminants, such as free oil, extraneous aqueous fluid, blood cells, and other cellular debris, in resulting fat grafts.[9,10] The presence of these contaminants has been associated with inflammation, oil cyst formation, tissue necrosis, suboptimal volume retention, and poor injectability, all of which may lead to unfavorable clinical outcomes and require additional interventions.[9,11–13] Processing lipoaspirate enhances fat graft quality, including fat viability and activity, by improving particle size population and reducing contaminants[9,10,14]; therefore, an optimized processing method would potentially be beneficial for patient outcomes.

The method used to process lipoaspirate for autologous fat grafting is a crucial factor in determining fat graft viability and retention, thereby impacting clinical decision-making.[9,12–15] Several fat-processing methods, including decantation, centrifugation, and active filtration, have been used in preparation for fat-grafting procedures; however, few comparative studies have evaluated these different methods.

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The REVOLVE ENVI 600 system (REVOLVE ENVI) is an active filtration-based fat-processing device with a 200-µm pore size mesh filter that can process up to 600 mL of lipoaspirate in preparation for fat grafting. The purpose of this preclinical study was to evaluate the benchtop/in vitro properties and tissue quality (fat viability and activity) of donated lipoaspirate tissue after processing with the REVOLVE ENVI system compared with those processed via standard decantation and to evaluate the properties of REVOLVE ENVI waste products.

Takeaways

Question: What are the key differences between fat grafts processed using the REVOLVE ENVI 600 system versus decantation?

Findings: This comparative preclinical benchtop/in vitro study showed that REVOLVE ENVI grafts had higher fat content with greater quality and lower contaminants than decanted grafts, whereas REVOLVE ENVI waste contained primarily aqueous fluid with negligible fat. Compared with decanted grafts, REVOLVE ENVI grafts had fewer red blood cells and more large-particle-size fat globules, viable adipocytes, adipocyte activity, and stromal vascular fraction colony-forming units per cubic centimeter of graft, which are rich in progenitor cells.

Meaning: REVOLVE ENVI improves fat graft quality compared with decantation in vitro.

MATERIALS AND METHODS

Lipoaspirate Preparation

Fresh lipoaspirate samples were collected with informed consent from six donors who were undergoing liposuction at one of the two surgical centers and who were capable of donating more than 1000 mL of lipoaspirate, based on body mass and desired aesthetic outcome. Donated lipoaspirate was collected from the abdomen, arms, and legs using a 3- to 4-mm cannula. Rather than being discarded, harvested lipoaspirate was re-warmed to 37°C, gently mixed to reach a uniform suspension, and then divided into two portions for in vitro testing, with one portion undergoing processing via the REVOLVE ENVI system and the other portion undergoing decantation. Processed fat grafts were used for in vitro testing only and were not re-injected/injected into patients.

Fat Harvesting

REVOLVE ENVI Filtration

Lipoaspirate samples were processed according to the REVOLVE ENVI instructions for use[16] and tested at maximum device fill volume of 600 mL lipoaspirate, with three to four separate washes of lactated Ringer's solution for a final 1:3 total tissue-to-wash solution volume ratio (Fig. 1).[17] Wash solution was filtered through the mesh basket and evacuated from the device into waste containers. Fat grafts were allowed to concentrate further within the mesh basket for 90 seconds and were then extracted from the device using a 60-mL catheter-tip syringe via the extraction port. Overall processing time for lipoaspirate with REVOLVE ENVI was approximately 10 minutes.

REVOLVE ENVI Waste

Containers used to collect waste generated from REVOLVE ENVI fat processing were maintained at room temperature until fluid and oil layers separated (≈5 min). After removal of the fluid and oil layers, the remaining adipose tissue layer was mixed well before sampling for particle size analysis and adipocyte counts.

Decantation

Lipoaspirate (150 mL) was maintained at room temperature for at least 20 minutes to allow for phase separation. The visible aqueous fluid and free oil layers were then removed by aspiration, and the remaining adipose tissue layer was mixed well and used for testing.

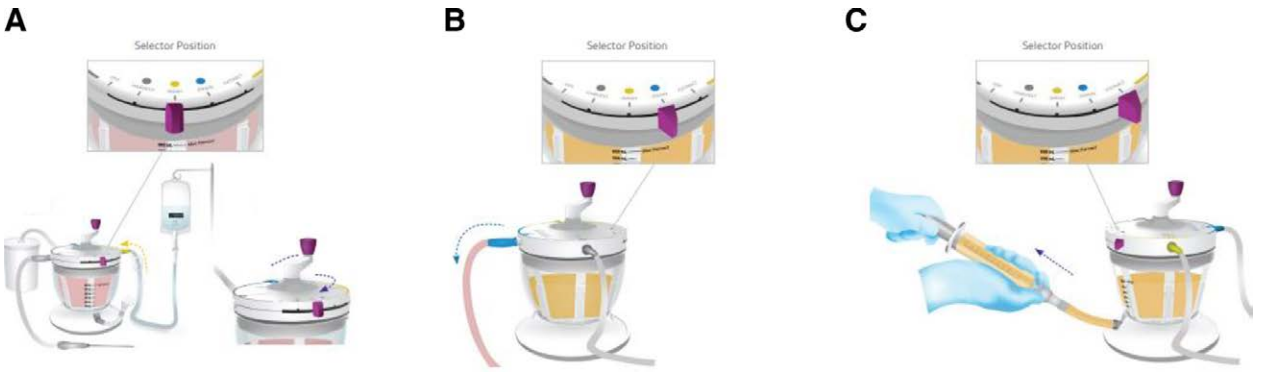


Fig. 1. Schematic of REVOLVE ENVI system for processing lipoaspirate for fat grafts. A, Wash step. Lipoaspirate entering the REVOLVE ENVI device is washed with warm lactated Ringer's solution. B, Drain step. Fluid is filtered through the porous mesh basket and suctioned from the canister. As the fat graft material becomes more concentrated within the mesh basket, the wash-drain sequence is repeated two additional times. C, Extract step. A catheter or Toomey syringe is inserted into the extraction port to harvest the processed tissue. The schematic was modified from the REVOLVE ENVI quick reference guide.[17]

In Vitro Testing of Processed Fat Grafts

Volume Composition Analysis

Triplicate aliquots (10 mL each) from REVOLVE ENVI–processed grafts, decanted grafts, and REVOLVE ENVI waste fraction were centrifuged at 300*g* for 10 minutes. After centrifugation, the volume of each resulting layer (free oil, fat, aqueous fluid, cellular debris) was replaced with deionized water and weighed, and the volume percentage of each layer was calculated based on the sum volume of all layers.

Hematocrit Content

The cellular pellet and aqueous fraction collected from the volume composition analysis were further centrifuged. The pellet was then resuspended and incubated in ammonium-chloride-potassium lysis buffer for 5 minutes at room temperature. The lysis reaction was terminated using phosphate-buffered saline (PBS), and serial dilutions were prepared. Optical density (OD) was measured at 400 nm using a spectrophotometer.

Fat Particle Size Analysis and Macrostructure

Fat graft particle size was analyzed using the Horiba Laser Scattering Particle Size Distribution Analyzer (Horiba, Ltd, Kyoto, Japan). Briefly, 1–3 mL aliquots of REVOLVE ENVI–processed grafts, decanted grafts, and REVOLVE ENVI waste fractions were used to measure mean particle size and size distribution. Testing was performed in triplicate for each sample, and data were plotted to obtain a composite histogram showing the size distribution of particle populations. For macrostructure imaging, a similar quantity of REVOLVE ENVI grafts, decanted grafts, and REVOLVE ENVI waste samples were dispersed into a glass petri dish, and images obtained using a Nikon SMZ1000 Stereoscopic Zoom Microscope (Nikon, Melville, N.Y.).

Viable Adipocyte Count

Each processed fat graft (10 mL) was digested for 1 hour at 37°C with 250 U/mL collagenase from *Clostridium histolyticum* (Sigma-Aldrich, St. Louis, Mo.) at a 1:4 fat graft to collagenase solution ratio. The adipocytes were isolated using centrifugation and stained for 30–60 minutes at 37°C with a live-dead cell-staining solution containing calcein acetoxymethyl (Invitrogen, Waltham, Mass.) and propidium iodide (Nexcelom Bioscience, Lawrence, Mass.) in PBS. The stained adipocyte suspensions were diluted and placed onto SD300 slides (Nexcelom Bioscience), and the adipocytes were counted with a Cellometer K2 (Nexcelom Bioscience).

Adipocyte Activity

A free glycerol determination kit (ZenBio kit, ZenBio Inc, Durham, N.C.) was used to measure free glycerol release from the processed fat graft samples after stimulation. Briefly, fat graft aliquots were placed into 24-well culture plates containing either nonstimulation medium (assay medium only) or stimulation medium [assay medium with 1 μM isoproterenol hydrochloride (Sigma-Aldrich)] and incubated for 3.5 hours at 37°C. Glycerol release (lipolysis) from samples and a glycerol standard solution (Sigma-Aldrich) were determined spectrophotometrically at a wavelength of 540 nm.

Fluorescence-activated Cell Sorting Analysis of Stromal Vascular Fraction

To isolate stromal vascular fraction (SVF) cells, 10 mL each of REVOLVE ENVI–processed and decanted grafts were digested with 250 U/mL collagenase from *Clostridium histolyticum* (Sigma-Aldrich) at a 1:4 fat graft to collagenase solution ratio for 1 hour at 37°C with agitation. The digested suspension was then centrifuged at 200*g* for 10 minutes. The supernatant was removed, and the red blood cells in the pellets were lysed with ammonium-chloride-potassium lysis buffer (Fisher) for 3–5 minutes. After lysis, the remaining cells were washed with PBS and pelleted by centrifugation. The cellular pellet was resuspended in PBS, then used for SVF fluorescence-activated cell sorting (FACS) and colony-forming unit (CFU) culture analysis.

FACS analysis was performed as previously described.[9] Briefly, SVF cells were washed in PBS containing 0.5% (weight/volume) bovine serum albumin and stained for 30 minutes at 4°C with fluorescence probe-labeled anti-CD34, anti-CD31, and anti-CD45 antibodies (BD Biosciences, San Jose, Calif.). The $CD45^-/CD31^-/CD34^+$ progenitor cell population was identified using a FACSCalibur flow cytometer (BD Biosciences) and analyzed with CellQuest software (BD Biosciences).

CFU Culture of SVF

Isolated SVF cells were counted using a Cellometer K2 and SD100 slides (Nexcelom Biosciences). Cells were seeded at densities of 100, 200, and 400 cells per cm^2 in a T-25 flask and cultured in low glucose Dulbecco's Modified Eagle Medium solution supplemented with 10% fetal bovine serum (VWR, Radnor, Pa.) and 1% antibiotic-antimycotic cocktail (Thermo Fisher Scientific, Waltham, Mass.). The culture medium was refreshed every 3 days and maintained for 14 days. Cells were washed in PBS, fixed in 10% formalin, then stained with CFU staining solution containing 0.1% toluidine blue in 2% paraformaldehyde. CFUs were counted and reported as CFU per cubic centimeter of graft.

Statistical Analyses

The linear mixed effects model was used to perform statistical analysis for each of the output variables. Because each sample had multiple observations across test arms, the observations within a distinct lipoaspirate sample are correlated. To account for this dependence among observations, the linear mixed model included lipoaspirate lot as a random effect, and the heterogeneity across test arms was evaluated by specifying different variances for each test arm. Two output variables (hematocrit content and CFU) exhibited nonnormality, and hence, the variables were log-transformed. The goodness of fit of the model was assessed using residual plots (quantile-quantile plots, histograms and residual versus predicted plots). Statistical analyses were performed using SAS software (SAS Institute, Cary, N.C.).

RESULTS

Volume Composition Analysis

REVOLVE ENVI grafts had significantly higher mean (±SD) fat volume composition than decanted grafts and REVOLVE ENVI waste (85.6 ± 6.1% versus 72.1 ± 4.0% and <0.1%, respectively, $P < 0.001$). REVOLVE ENVI grafts also contained less aqueous fluid than decanted grafts and REVOLVE ENVI waste (14.1 ± 6.2% versus 21.3 ± 6.2% and 91.0% ± 2.2%, $P < 0.001$), negligible free oil (0.4 ± 1.1% versus 6.2 ± 3.6% and 8.7 ± 2.1%, $P < 0.001$), and little to no cellular debris (<0.1% versus 0.4 ± 0.4% and 0.3 ± 0.3%, respectively, $P < 0.001$; Fig. 2).

Hematocrit Content

The hematocrit content (OD ± SD) was lower for REVOLVE ENVI grafts at 0.22 ± 0.38 OD compared with decanted grafts and REVOLVE ENVI waste at 4.39 ± 5.88 OD and 2.70 ± 2.53 OD, respectively. Log-transformed OD values revealed significantly lower hematocrit/red blood cells in REVOLVE ENVI grafts than decanted grafts and REVOLVE ENVI waste ($P < 0.001$). Figure 3 shows the mean ratio of hematocrit relative to decanted grafts.

Fat Particle Size Analysis and Macrostructure

REVOLVE ENVI grafts contained a greater frequency of larger fat globules than decanted grafts or REVOLVE ENVI waste (Fig. 4A). Specifically, REVOLVE ENVI grafts contained more fat globules (>1000 μm) than decanted grafts (61.6 ± 9.2% versus 52.5 ± 13.4%; $P < 0.001$) or REVOLVE ENVI waste (0.5 ± 1.5%), as well as less particles (<200 μm), with REVOLVE ENVI grafts at 15.7 ± 2.7% versus decanted grafts at 32.2 ± 8.9% ($P < 0.001$) and REVOLVE ENVI waste at 97.9 ± 4.5% (Fig. 4B). The average (±SD) adipose globule size was significantly larger for REVOLVE ENVI grafts at 1310.8 ± 185.7 μm compared with 1031.0 ± 287.7 μm for decanted grafts ($P < 0.001$; Fig. 4B). Both REVOLVE ENVI–processed and decanted grafts demonstrated intact adipose globule structures with clustered adipocytes, whereas REVOLVE ENVI waste consisted of small clusters of or single adipocytes, along with substantial tissue debris (Fig. 5A).

Viable Adipocyte Count and Adipocyte Activity

REVOLVE ENVI grafts demonstrated significantly more viable adipocytes than decanted grafts ($6.8 \times 10^5 \pm 1.7 \times 10^5$ cells/mL versus $5.0 \times 10^5 \pm 2.2 \times 10^5$ cells/mL, respectively, $P < 0.001$) with significantly higher glycerol release in response to isoproterenol stimulation, indicating greater adipocyte activity/lipolysis (152.6 ± 104.0 μg glycerol/mL processed fat versus 101.2 ± 63.4 μg glycerol/mL processed fat, $P = 0.012$).

FACS Analysis of SVF and CFU Culture of SVF

Flow cytometry analysis demonstrated that the SVF from REVOLVE ENVI–processed grafts contained numerically more $CD45^-/CD31^-/CD34^+$ cells/mL of graft than the SVF obtained from decanted grafts but did not reach statistical significance (22,335 ± 11,886 cells versus 13,923 ± 7990 cells, respectively, $P = 0.24$). REVOLVE

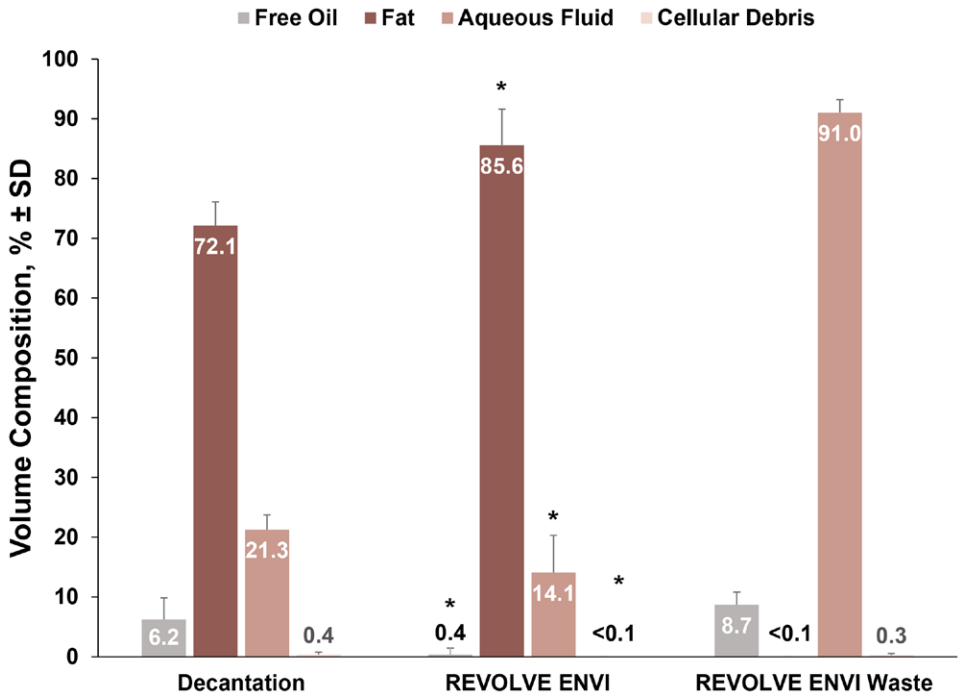


Fig. 2. Mean percentage volume composition ±SD of grafts processed using decantation and REVOLVE ENVI, as well as REVOLVE ENVI waste. N = 6 per group. *$P < 0.001$ vs decantation and REVOLVE ENVI waste.

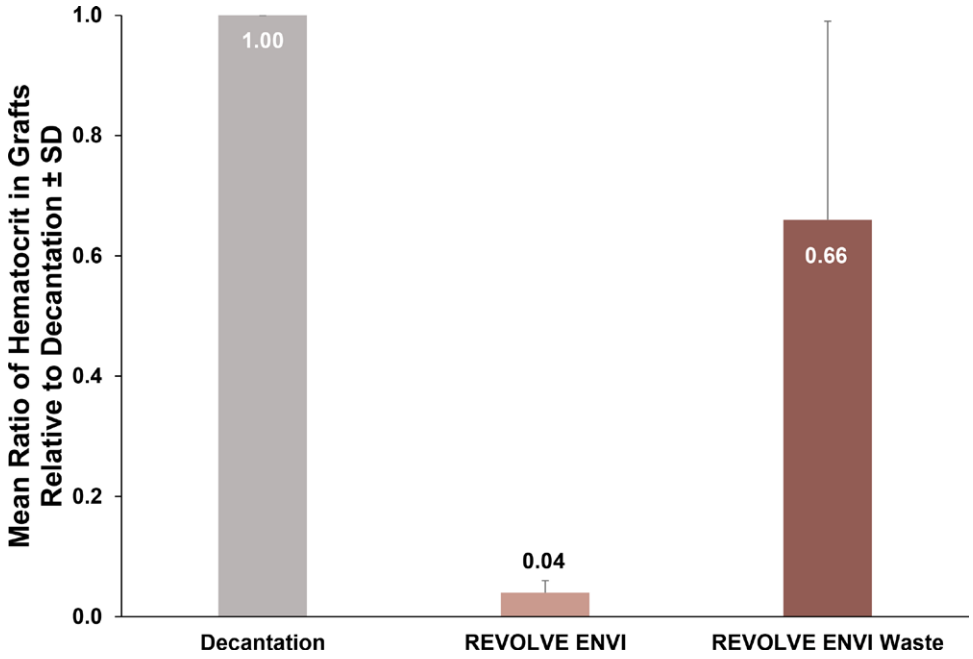
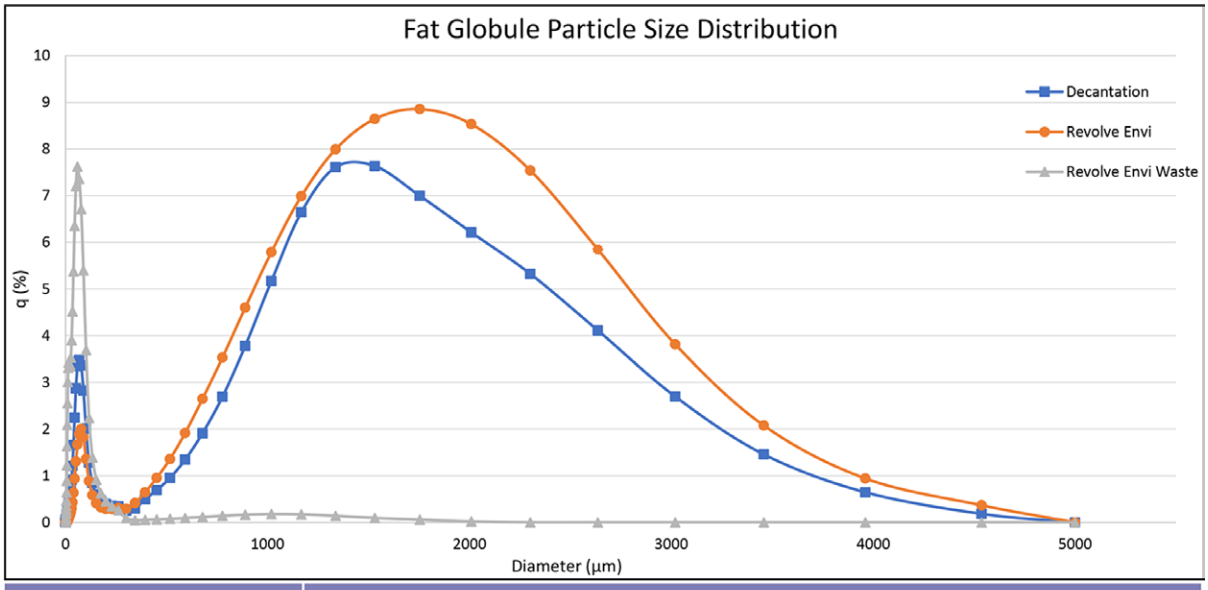


Fig. 3. Mean ratio of hematocrit ±SD in grafts relative to decantation as measured using a spectrophotometric method (OD at 400 nm) in grafts processed using decantation and REVOLVE ENVI, as well as REVOLVE ENVI waste. N = 6 per group.

A



B

Sample	Particle Size		
	Mean±SD (µm)	>1000 µm±SD (%)	<200 µm±SD (%)
Decantation	1031.0±287.7	52.5±13.4	32.2±8.9
REVOLVE ENVI	1310.8±185.7*	61.6±9.2*	15.7±2.7*
REVOLVE ENVI Waste	58.9±36.7	0.5±1.5	97.9±4.5

Fig. 4. Particle size distribution for fat grafts processed using decantation and REVOLVE ENVI, as well as REVOLVE ENVI waste. A, Average fat globule particle size distribution. B, Summary table for particle size analysis. N = 6 per group. *$P < 0.001$ vs decantation and REVOLVE ENVI waste.

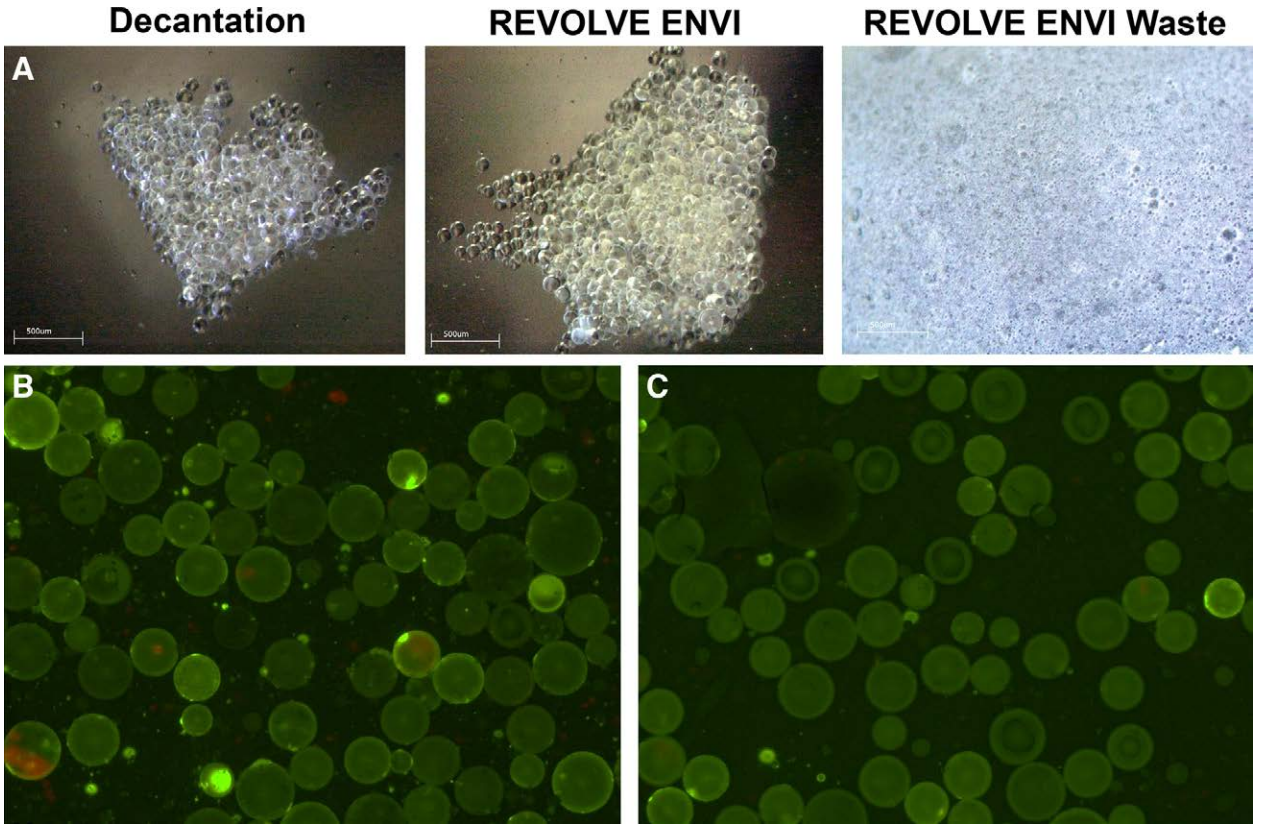


Fig. 5. A, Brightfield micrographs of fat tissues from grafts processed by decantation (left) and REVOLVE ENVI (center), as well as REVOLVE ENVI waste (right). B, Representative images of calcein acetoxymethyl/propidium iodide (live/dead) fluorescently labeled adipocytes after processing by decantation and C, REVOLVE ENVI. Aliquots shown had been equivalently concentrated and selected for viable adipocytes.

Table 1. Processed Graft Viability Measurement Summary

Measure	Decantation	REVOLVE ENVI	Mean % Relative to Decantation*	*P*
Viable Adipocytes				
Mean × 10^5 cells/mL (SD)	5.0 (2.2)	6.8 (1.7)	145.2 (36.0)	<0.001
Adipocyte Activity				
Mean µg glycerol/mL processed fat (SD)	101.2 (63.4)	152.6 (104.0)	145.7 (46.2)	0.012
Progenitors in SVF				
Mean number of cells/mL processed graft (SD)	13,923.0 (7990.8)	22,335.0 (11,886.5)	195.5 (104.2)	0.24
CFU in SVF				
Mean SVF CFU culture/mL processed graft (SD)	1151 (1364)	3554 (5655)	363.5 (161.2)	<0.001†

*To account for donor variability, the percentage values for REVOLVE ENVI relative to decantation were calculated for each donor, which are then used to calculate the overall mean percentage relative to decantation.
†Statistics were performed on log-transformed data due to nonnormality.

ENVI–processed grafts also contained more CFU in the SVF than decanted grafts (3554 ± 5655 CFU/mL versus 1151 ± 1364 CFU/mL, respectively). Log-transformed values revealed significantly more CFU in the SVF in REVOLVE ENVI–processed grafts than decanted grafts ($P < 0.001$). Table 1 summarizes these results and shows the mean percentage change in viable adipocyte count, adipocyte activity, progenitor cells in SVF, and CFU in the SVF of REVOLVE ENVI–processed grafts relative to decanted grafts. Figure 5B, C shows representative images of fluorescently labeled adipocytes.

DISCUSSION

Poor fat graft retention and the presence of graft contaminants are important concerns in reconstructive and aesthetic surgery because these factors are associated with unfavorable clinical outcomes and may lead to the need for additional interventions.[9,11,14,18] Processing of lipoaspirate enhances fat graft quality by preserving graft viability while reducing contaminants[9]; however, the relative benefits of various lipoaspirate processing methods are not well established. Data from this preclinical benchtop/in vitro study provide comparative

information on key distinguishing factors between two distinct methods.

Compared with decanted grafts, REVOLVE ENVI–processed grafts contained a larger fraction of concentrated graftable fat, high in large-particle fat globules, with less waste contaminants (free oil, aqueous fluid, and cellular debris). The reduction in cellular debris and hematocrit isolated from REVOLVE ENVI–processed grafts are a result of the multiple washing steps and active filtration/removal of the liquid phase across the porous membrane.[9] Because free oils released from ruptured adipocytes may be scavenged by tissue phagocytes and induce an inflammatory response, the comparatively greater removal of free oil and other cellular debris may contribute to better graft retention.[9,10,19] In addition, minimal adipocyte content was lost to the REVOLVE ENVI waste product, which may help ameliorate clinician concerns, especially when processing lipoaspirate from thinner patients with limited available fat tissues.[9] Compared with decanted grafts, REVOLVE ENVI grafts also had more SVF CFU/mL of graft. The SVF, which is rich in progenitor cells with high proliferative capacities,[9] releases growth factors, such as vascular endothelial growth factor, HGF, and transforming growth factor-beta (TGF-β), which have been shown to contribute to stem cell differentiation, angiogenesis, and tissue remodeling.[20–22] The use of fat grafts containing a high percentage of viable adipocytes and proliferative stem cells is perceived to contribute to increased graft survival.[9,11,12,14]

The current results are consistent with previous studies that have demonstrated enhanced performance of filtration-based systems.[9–11,13,23] The REVOLVE ENVI system evaluated in this study yielded fat grafts containing higher percentages of viable, active cells compared with grafts processed by decantation, but similar percentages of viable graft content (86%) compared with grafts processed by the original REVOLVE system (88%).[9] Similar to the REVOLVE ENVI system evaluated in the current study, the original REVOLVE system also produced grafts with a higher content of viable and active adipocytes (~1.5×) compared with decanted grafts, although at a lower overall processed graft volume as per device specifications. In clinical studies, fat processed using the REVOLVE system has also been shown to have comparable safety[24] with fewer complications (eg, oil cysts and fat necrosis) than other fat-processing methods in patients undergoing breast reconstruction, and may therefore offer an alternative to other systems for large-volume fat processing.[13,23]

Limitations of the Study

Compared with previous studies,[9,10] the current preclinical benchtop/in vitro study had a smaller sample size (ie, lipoaspirate from six patients versus 12–22 patients in other studies). Growth factor content was also not specifically assessed; however, a previous study[9] evaluating filtration systems demonstrated similar levels of growth factor retention between the original REVOLVE filtration-based system and other methods, such as centrifugation. This observation negates the potential concern that extensive washing used with filtration-based systems may remove beneficial growth factors.[9]

The current study did not evaluate processed fat grafts in an in vivo animal model or in patients. Future studies should evaluate the quality of the fat grafts processed from REVOLVE ENVI versus other methods (eg, centrifugation) or other filtration-based devices to address safety, efficacy, and cost efficiency. In vivo animal models[11] may be used to investigate graft retention, and potential clinical trials[25] could be performed to assess performance features, such as operational time factors, cost efficiency (ie, costs versus time required to process fat grafts), and clinical outcomes (eg, complications, proportion of patients undergoing reoperations). These are important factors to consider when processing large-volume fat grafts and enhancing fat graft survival.[18] Future in vivo preclinical and clinical studies that evaluate safety, efficacy, and cost efficiency can guide clinicians in choosing the processing method that is most appropriate for their patients to ultimately improve clinical outomes.[18,26]

CONCLUSIONS

The preclinical benchtop/in vitro study findings suggest that fat grafts processed using REVOLVE ENVI demonstrate greater viability and activity than grafts processed by decantation. Because of the inherent limitations of benchtop/in vitro studies, further in vivo preclinical and clinical studies are warranted.

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DISCLOSURES

Dr. Gabriel is a consultant for AbbVie and KCI. Mr. Kabaria, Dr. Fang, Mr. Lombardi, Mr. Stec, Dr. Huang, Dr. Li, and Dr. Sandor are employed by and may own stock in Allergan Aesthetics, an AbbVie Company. Allergan Aesthetics, an AbbVie Company, funded this study and participated in the study design, research, analysis, data collection, interpretation of data, reviewing, and approval of the publication. All authors had access to relevant data and participated in the drafting, review, and approval of this publication. No honoraria or payments were made for authorship.

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