

Comparison of Harvest and Processing Techniques for Fat Grafting and Adipose Stem Cell Isolation

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Background: Variability in harvest and processing technique may impact the success of fat grafting. This study compared properties of fat grafts produced by differing methods and assessed volume retention of the grafted tissue in a nude mouse model.

Methods: In phase I, fat was harvested by either suction-assisted lipoaspiration or ultrasound-assisted lipoaspiration and then filtered using two different pore sizes. Graft material was analyzed for average parcel size; relative oil, fat, and aqueous fractions; and stromal vascular fraction yield. Filtrands and filtrates were injected into athymic nude mice. In phase II, lipoaspirate harvested by suction-assisted lipoaspiration only was processed by centrifugation, cotton gauze rolling, or filtration, and then studied in a similar manner.

Results: Fat harvested by ultrasound- and suction-assisted lipoaspiration had comparable stromal vascular fraction counts and graft retention in vivo. Ultrasound-assisted lipoaspiration released only slightly more oil than suction-assisted lipoaspiration; filtering with either 500- or 800- μ m pore size effectively removed fluid and oil. Centrifugation, cotton-gauze rolling, and filtration also effectively removed fluid and oil. In vivo graft retention and stromal vascular fraction yield was highest with the cotton gauze method. Histologic analysis of all explants showed intact adipose tissue.

Conclusions: Ultrasound- and suction-assisted lipoaspiration yielded similar retention of fat grafts in a xenograft model. Processing with cotton gauze rolling may be best suited for grafting cosmetically sensitive areas of the body in which optimal retention is critical and lower total graft volumes are needed. Filtration and centrifugation both effectively removed fluid fractions and resulted in comparable graft retention, and are more feasible when larger volumes are required. (*Plast. Reconstr. Surg.* 132: 351, 2013.)

Over 100 years have passed since the first report of lipotransfer in the plastic surgery literature.^{1,2} Following the advent of liposuction, subsequent efforts by surgeons such as Coleman to systematize fat harvest, processing, and injection were pursued to produce reliable clinical results. A major focus was the use of centrifugation or other methods to separate the aqueous and oil components before injection.³

Interest in fat grafting has increased dramatically in recent years, with more reported uses for breast and buttock applications. As small-volume fat graft procedures are becoming routine in aesthetic practice and large-volume applications are becoming more popular, strong data are needed concerning processing methods to determine their impact on graft retention. Indeed,

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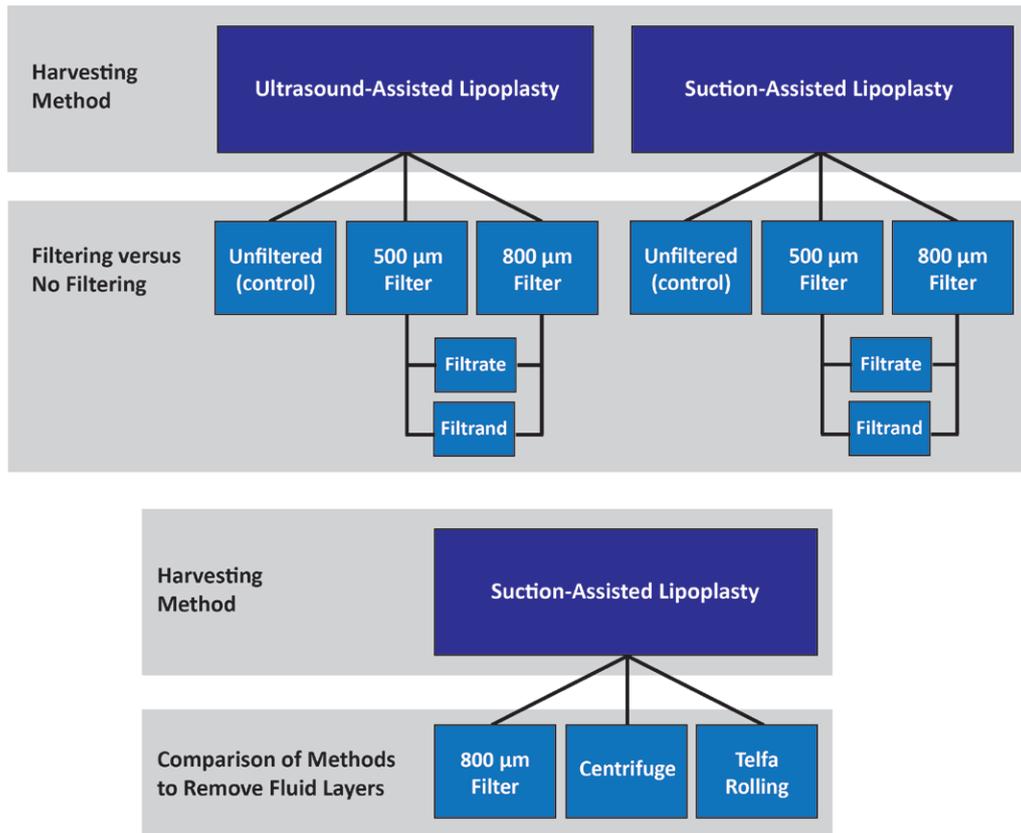


Fig. 1. Schematic representation of the study: (above) phase I and (below) phase II.

unpredictable resorption remains a major clinical issue. In addition, fat grafting for reconstruction after traumatic injuries, such as those caused by vehicular accidents or battlefield trauma, has been increasingly popular. Currently, pretransplant processing methods vary widely in clinical practice,⁴⁻⁷ and there is no general agreement on the optimal techniques for graft preparation. In addition, methods suitable for small-volume grafting may not be practical for large-volume uses.⁸⁻¹²

Although postharvest processing techniques vary widely, so do methods used for fat extraction. These include manual or machine-driven standard suction-assisted lipoaspiration, ultrasound-assisted lipoaspiration, and a host of newer energy-based technologies. In particular, ultrasound-assisted lipoaspiration is an established and widely used technology in body contouring.¹³ However, the retention of grafted fat extracted by ultrasound-assisted lipoaspiration, and the suitability of this technology for fat graft harvest, has been questioned. Although ultrasound-assisted lipoaspiration has been shown to preserve regenerative cell function,¹⁴ the ability of fat harvested by ultrasound-assisted lipoaspiration to retain its volume when grafted in vivo has not been well established in a scientific model.

Thus, the first aim of this study, executed in phase I, was to compare properties of fat tissue after ultrasound-assisted lipoaspiration and conventional suction-assisted lipoaspiration harvest, including a functional assay of graft retention in a nude mouse model. There has also been interest in filtration systems for rapid collection and separation of fluid fractions, with speculation that adipose stem cell-rich material (stromal vascular fraction) may be present in the aqueous layer, may pass through a filter, and may be inadvertently discarded. To answer this question, fat harvested by ultrasound- or suction-assisted lipoaspiration was collected in a filtration system (500- and 800-μm pore size) and both filtrand and filtrate were analyzed for composition, including stromal vascular fraction yield, and grafted into an animal model to assess retention.

Our second aim, carried out in phase II, was to compare three common processing techniques after suction-assisted lipoaspiration harvest only: filtration, cotton gauze rolling, and centrifugation (Coleman method). Similar analyses were performed, including determination of graft composition and retention in a nude mouse model.

MATERIALS AND METHODS

This two-phase study (Fig. 1) was conducted in accordance with the regulations of the University of Pittsburgh Institutional Review Board. Each phase was conducted using aspirated fat from a single patient, thus eliminating intersubject variability in adipose tissue characteristics. Both patients were women, ages 42 and 57 years, respectively, undergoing elective surgery. Tissue was harvested from the flank in phase I and the thigh in phase II by the same surgeon (J.P.R.).

Phase I: Comparison of Ultrasound- and Suction-Assisted Lipoaspiration Harvested Fat Using a Filtered Collection System

In the first phase of the study, fat was extracted by tumescent liposuction at a maximum vacuum of 430 mmHg, using a Shippert Biplane Cannula (Shippert Medical Technologies Corp., Centennial, Colo.) with a 3-mm outer diameter and a 30-cm length with 24 blunt-edged round holes, with each hole measuring 0.086-inch diameter. Large-diameter collection tubing ($\frac{5}{8}$ -inch inside diameter) of a relatively short (6 feet) length was used,¹⁵ and aspirate was collected in two versions of an inline Tissu-Trans Filtron canister (Shippert Medical), with filter pore sizes of 500 and 800 μm , respectively. In the same patient, separate but adjacent anatomical regions treated with Vibration Amplification of Sound Energy at Resonance (VASER; Sound Surgical Technologies, Louisville, Colo.) energy, through a 2.9-mm cannula at a power level of 60 percent in pulsed mode, were aspirated with the same type of cannula, suction system, and filter canisters. Application of ultrasound energy and aspiration was multiplanar and multidirectional.

The filtrand, or graft material captured by the filter, was removed in a sterile fashion. The filtrate, representing all fluid and solid material passing through the filter, was also collected in a sterile manner. Control specimens were collected in the Tissu-Trans Mega 1500 canister (Shippert Medical), which has no filter (Fig. 2), and allowed to decant for 30 minutes before further analysis or injection into the animal model.

Material captured in the two different filters (filtrand) and material that passed through the filters (filtrate), in addition to unfiltered control suction-assisted lipoaspiration specimens, were analyzed for (1) average fat parcel size; (2) percentage composition of oil, fat, and aqueous material; (3) stromal vascular fraction cell count after collagenase digestion; and (4) graft retention after injection in a nude mouse model.



Fig. 2. Tissu-Trans Filtron inline lipoaspirate filtration canister.

1. Average fat parcel size: After processing, filtrands were transferred to a petri dish and suspended in saline at a 1:10 ratio. Fat suspensions were imaged under light microscopy at 20 \times magnification. Images were analyzed visually by blinded observers for parcel size and range of sizes. Average fat parcel diameter was determined using ImageJ Software (National Institutes of Health, Bethesda, Md.). Confocal microscopic imaging with AdipoRed (Lonza Walkersville, Inc., Walkersville, Md.), 4',6-diamidino-2-phenylindole, and phalloidin green staining were used to confirm fat parcel structural appearance and composition. Filtrates, which have a high aqueous content, were centrifuged at 1200 g for 3 minutes and the resultant pellets were similarly resuspended in saline before analysis.
2. Percentage composition of oil, fat, and aqueous material: Filtrands and filtrates were transferred separately to 15-ml conical tubes and centrifuged at 1200 g (3000 rpm) for 3 minutes (Sorvall Legend RT; Thermo Fisher Scientific, Inc., Waltham, Mass.). Relative volumes of the resulting three layers were determined.
3. Stromal vascular fraction cell count: Stromal vascular fraction was isolated from filtrands and filtrates by means of collagenase digestion as described previously in the literature.^{16,17} The stromal vascular fraction cell counts were measured in triplicate by

hemocytometry, with trypan dye used to exclude nonviable cells, and indexed to the weight of the original tissue specimen (yield of viable cells per gram of tissue).

4. **In vivo studies:** All animal studies were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Fat graft retention in vivo was quantified volumetrically.^{18,19} Female athymic nude mice ($n = 25$, five per group) aged 6 weeks (Harlan Laboratories, Inc., Indianapolis, Ind.) were anesthetized intraperitoneally with xylazine (Vedco, Inc., Saint Joseph, Mo.), 12 mg/kg, and ketamine (Butler Animal Health Supply, Dublin, Ohio), 80 mg/kg, dissolved in phosphate-buffered saline. Bilateral graft injections (fan-style with multiple passes) to the subcutaneous tissues on the dorsal aspect of nude mice were performed using a 1-ml syringe and standardized blunt-tipped 14-gauge infiltration cannula (Fig. 3). Postoperative analgesia consisted of intramuscular injections of ketoprofen (Fort Dodge Animal Health, Fort Dodge, Iowa) following the procedure and again after 24 hours if animals displayed signs of pain or discomfort. At 6 weeks after injection, mice were killed and grafts excised from both injection sites in each animal. Explants were assessed for mass on a standard laboratory balance in triplicate and volume was measured using a gas pycnometer (Accupyc II 1340 Pycnometer; Micrometrics Instrument Corp., Norcross, Ga.).

Phase II: Comparison of the Three Processing Methods

In phase II of the experiment, fat was harvested from a second patient undergoing elective



Fig. 3. Photograph of a mouse with human adipose tissue injected.

liposuction and fat grafting, and processed by either filtering, cotton-gauze rolling, or the Coleman centrifugation method.

1. **Filtration group:** Fat was collected by suction-assisted liposuction at 430 mmHg using the Shippert Biplane cannula. The 800- μ m Tissu-Trans Filtron filter unit was used to separate the extracted fat into filtrand and filtrate. In addition, unfiltered control fat specimens were allowed to decant and were analyzed for composition.
2. **Centrifugation group:** A standard Coleman technique was used to extract and process fat grafts in this group to best exemplify a commonly used technique. Fat was collected by handheld lipoaspiration by means of a 15-cm Coleman bucket-handle cannula (Coleman ASPI; Byron Medical, Inc., Tucson, Ariz.) into a 10-ml syringe. The syringes were then placed into a centrifuge and spun at 1200 g (3000 rpm) for 3 minutes. Oil was decanted and the aqueous layer drained by gravity through the Luer outlet of the syringe. To further remove oil, an absorbent $\frac{1}{2} \times 3$ -inch Codman Surgical Pattie (Johnson & Johnson, Raynham, Mass.) was placed onto the top of each fat column in the syringe for 5 minutes. The process was repeated until no additional oil was absorbed into the patty. Fat was then loaded into 1-ml syringes for injection. For consistency, the most dependent 2 ml of fat in each 10-cc syringe centrifuged was used in this part of the study for analysis and grafting.
3. **Cotton-gauze rolling:** Fat was collected by handheld lipoaspiration through a 15-cm Coleman bucket-handle cannula (COL ASP) into a 10-ml syringe. The aspirate was poured from each syringe onto large (3×8 -inch) pieces of Telfa nonadherent dressing (Covidien, Mansfield, Mass.). The fat was gently rolled and kneaded along the gauze using a sterile scalpel handle for 5 minutes. The fat was then loaded by small spatula into 10-ml syringes and transferred by Luer lock adapter into 1-ml syringes for injection.

Fat grafts processed by each of the above methods were then analyzed for (1) percentage composition of oil, fat, and aqueous material; (2) stromal vascular fraction cell count after collagenase digestion; and (3) retention of injected graft in the nude mouse model. Methods of analysis were identical to those described for phase I. In

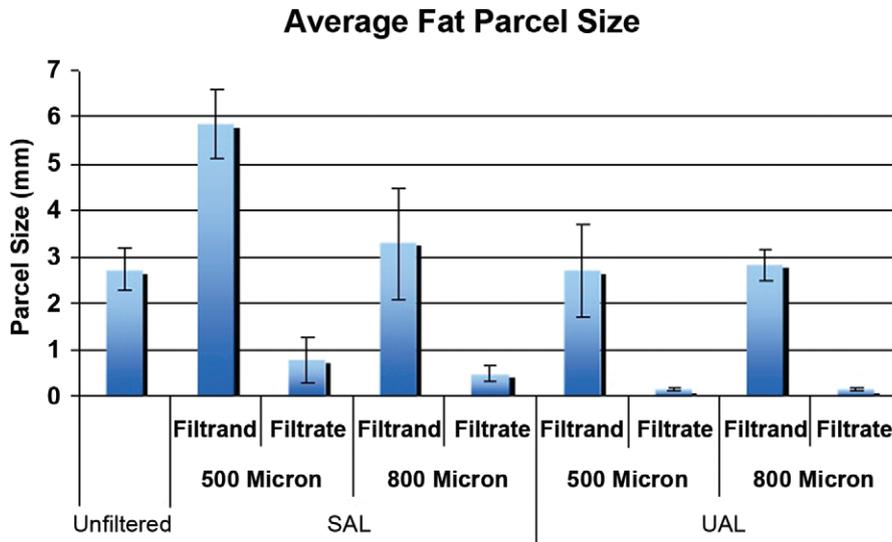


Fig. 4. Average fat parcel size when harvested with a 3-mm-diameter cannula with round blunt-edged holes. SAL, suction-assisted lipoaspiration; UAL, ultrasound-assisted lipoaspiration.

addition, immunohistochemistry was performed by staining for CD31 (ab7388; Abcam, Cambridge, Mass.), a marker for endothelial cells. Vascularity of the grafts was quantified by evaluating the number of blood vessels in three high-power fields per fat graft (center and each edge), as assessed by three blinded observers.

Statistical Analysis

Statistical analysis was performed using JMP Version 10 (SAS Institute, Inc., Cary, N.C.). Data were subjected to normality testing and compared with the *t* test for cases where two groups were present. Multiple comparisons were subjected to one-way analysis of variance, followed by the use of a Tukey-Kramer honestly significant difference test when indicated. An alpha level of 0.05 was set for all comparisons and results are presented as mean \pm SD.

RESULTS

Comparison of Fat Harvest Methods and Filter Pore Sizes

Lipoaspirate harvested by each technique, ultrasound-assisted lipoaspiration and conventional suction-assisted lipoaspiration, was found to contain parcel sizes less than 6 mm with similar overall architecture, using the Shipert biplane cannula described above (Figs. 4 through 6). Filtration effectively removed the aqueous and oil portions of lipoaspirate and yielded an injectable filtrand that was virtually

pure fat (Fig. 7). Both ultrasound- and suction-assisted lipoaspiration harvest followed by filtering yielded very low fractions of oil, with 2.2 percent for ultrasound-assisted lipoaspiration and 1.1 percent for suction-assisted lipoaspiration. As expected, the filtrates (the material that passed through the filter to be discarded) were composed mostly of aqueous material, with only small amounts of fat. Stromal vascular fraction counts of the resulting ultrasound- and suction-assisted lipoaspiration filtrands did not differ significantly (1.95×10^6 cells/g for ultrasound-assisted lipoaspiration versus 2.08×10^6 cells/g for suction-assisted lipoaspiration). The stromal vascular fraction cell yields extracted from the solid material in the filtrates were minimal and mixed with extensive cellular debris.

Fat grafts implanted in the mouse model after filtering demonstrated a high retention of 78 percent at 6 weeks compared with only 31 percent for the unfiltered decanted control specimens ($p < 0.0001$) (Fig. 8). Pore size had no effect on graft retention ($p = 0.826$, *t* test result after confirming normality of data). Importantly, there was no significant difference in graft retention between fat harvested with ultrasound-assisted lipoaspiration and fat harvested with suction-assisted lipoaspiration ($p = 0.928$, *t* test result after confirming normality of data). Finally, the small volume of fat that passed through the filter (filtrate) had negligible graft retention (<2 percent) during a 6-week survival in the murine model.

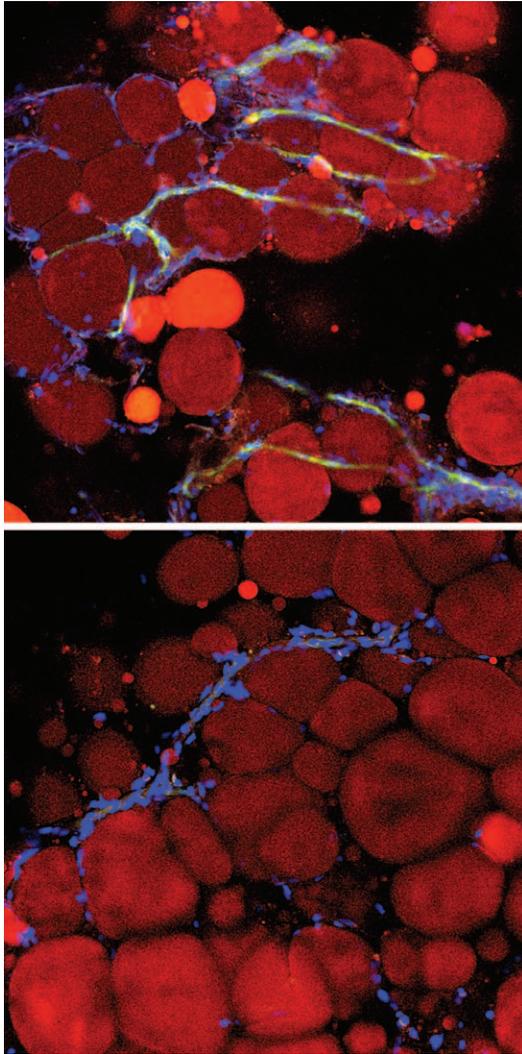


Fig. 5. Unfiltered ultrasound-assisted lipoaspirate (*above*) and unfiltered conventional suction-assisted lipoaspirate (*below*). Discrete parcels are visualized with intact stroma and vascular structures. Several small free oil droplets are visible.

Comparison of Three Processing Methods: Centrifugation, Filtration, and Cotton Gauze Rolling

When each group of processed fat (filtered, cotton gauze-rolled, centrifuged, or gravity separated) was subjected to progressively increasing centrifugation forces to determine residual oil and aqueous fractions, cotton gauze rolling was noted to have no detectable residual oil, compared with 4 percent and 1 percent for the centrifugation method and filtering, respectively. In addition, although all three methods were very effective in removing aqueous fluid, cotton gauze rolling nearly completely removed the aqueous fraction, with nearly no detectable fluid noted. After collagenase digestion of processed fat grafts,

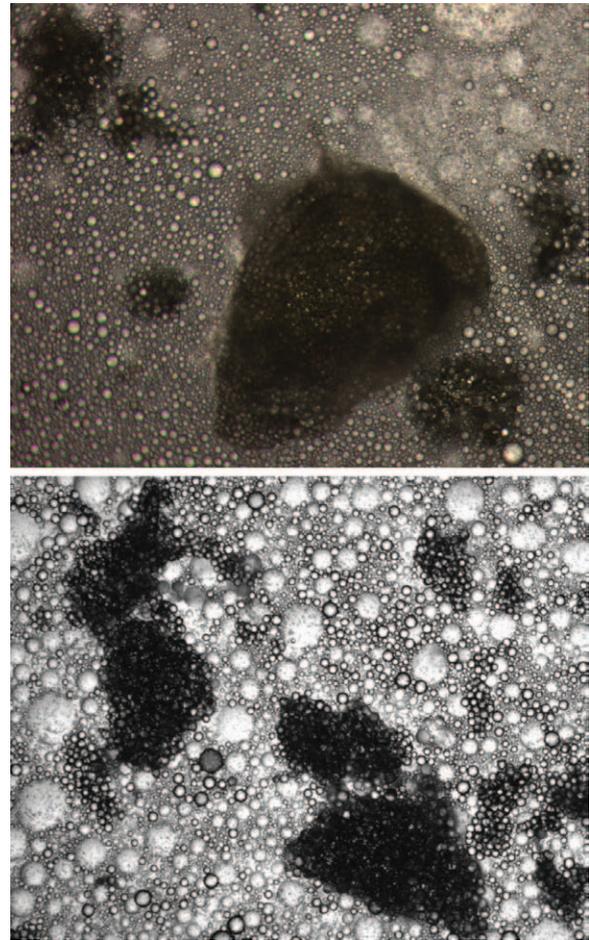


Fig. 6. Discrete, intact parcels are visualized in the unfiltered (*above*) and filtered (*below*) lipoaspirate collected by ultrasound, seen by light microscopy (original magnification, $\times 20$).

gauze-rolled fat had the highest stromal vascular fraction cell count per gram of fat tissue compared with the centrifugation method, 6.3×10^5 and 2.3×10^5 , respectively ($p = 0.0191$, t test result) (Fig. 9).

All three methods resulted in healed grafts *in vivo*, with fat grafts processed by the cotton gauze method showing improved fat graft retention compared with either filtration or the centrifugation method (70, 58, and 47 percent volume retention, respectively). An analysis of variance for retained graft volume between the three study groups demonstrated a statistical difference ($p = 0.019$). A Tukey-Kramer honestly significant difference test revealed a statistically significant difference in graft volume retention between the cotton gauze method compared with the centrifugation method ($p = 0.0159$). No other statistically significant differences were found between the centrifugation method and the filtered grafts or the filtered grafts and the cotton gauze grafts for volume retention (Fig. 10). Explanted fat grafts from all three groups demonstrated intact adipose architecture (Fig. 11)

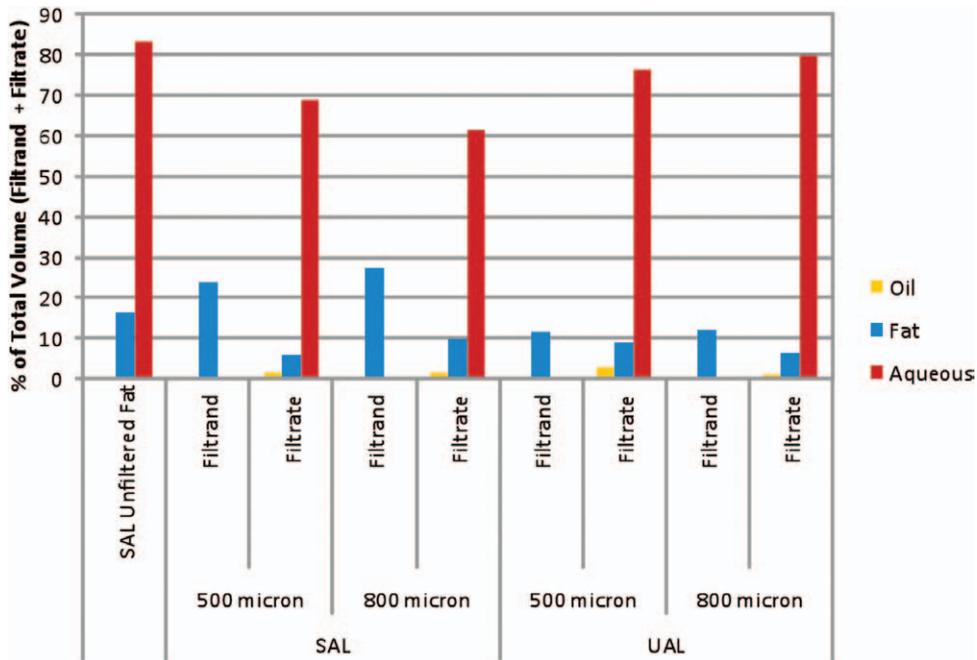


Fig. 7. Composition of lipoaspirate after filtration. There are no significant differences caused by spin rate. SAL, suction-assisted lipoaspiration; UAL, ultrasound-assisted lipoaspiration.

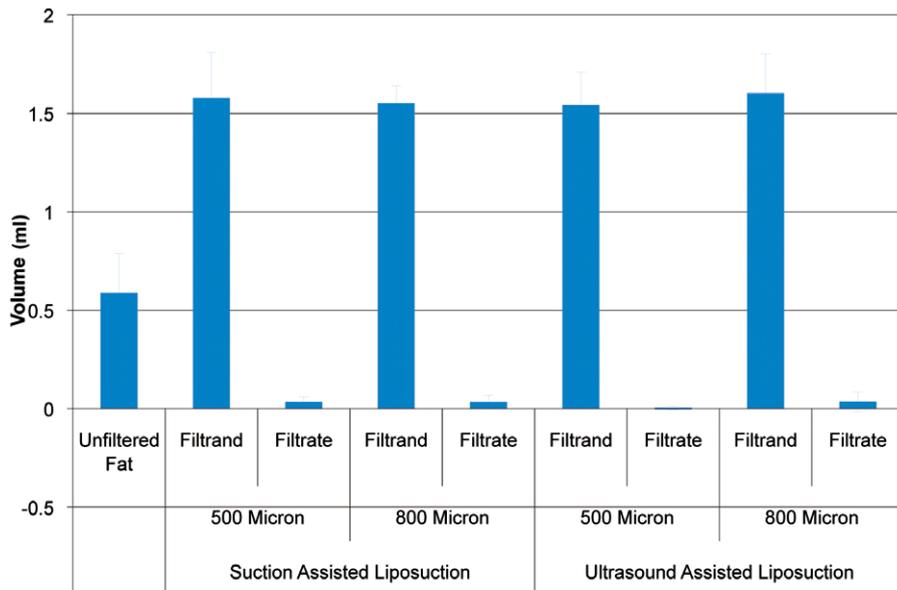


Fig. 8. Study phase I. Average graft volume at 6 weeks per 2-ml injection. Fat grafts (filtrands) processed by means of filtration had significantly higher retention at 6 weeks compared with unfiltered fat (average retention, 79 percent versus 30 percent, respectively; $p < 0.05$).

and equivalent vascularity as assessed by CD31 staining (Fig. 12).

DISCUSSION

Ultrasound-assisted lipoaspiration has gained considerable attention, but its role in fat transfer

is unclear. There have been questions about whether ultrasound-assisted lipoaspiration may damage the aspirated fat, potentially making it less likely to survive and incorporate in a new anatomical location. We compared the two harvest techniques of ultrasound- and suction-assisted lipoaspiration in this study (Fig. 1). Although

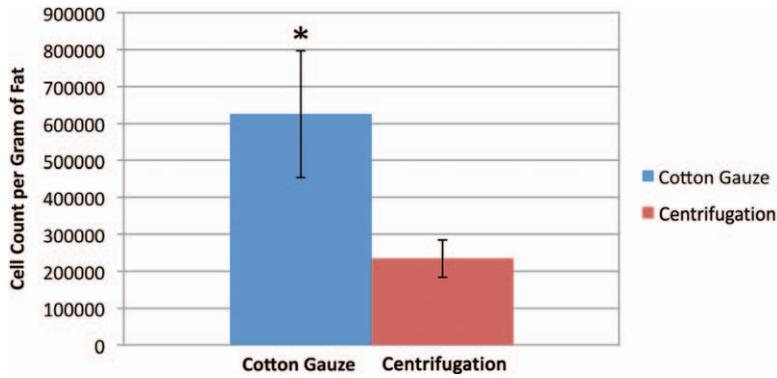


Fig. 9. Study phase II. Stromal vascular fraction cell count per 1 ml of fat. After collagenase digestion of processed fat grafts, gauze-rolled fat had a higher stromal vascular fraction cell count compared with centrifugation (6.3×10^5 versus 2.3×10^5 , respectively) ($*p < 0.01$).

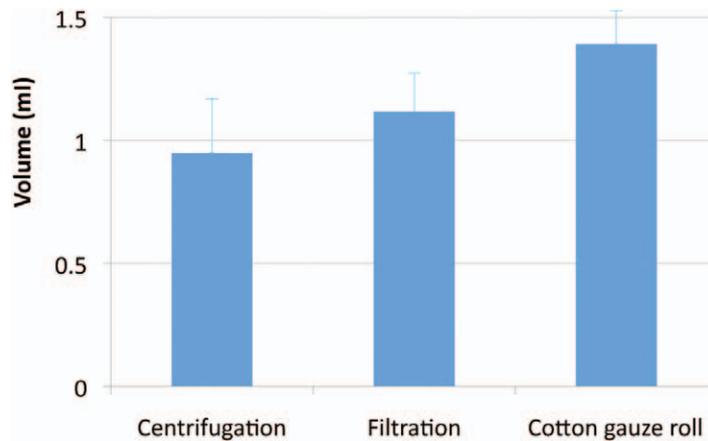


Fig. 10. Study phase II. Average graft volume at 6 weeks per 2-ml injection. Fat grafts processed by the cotton gauze method had improved fat graft retention compared with either filtration or centrifugation ($p < 0.0159$ for the single comparison).

ultrasound-assisted lipoaspiration released slightly more oil than suction-assisted lipoaspiration during the lipoaspiration process, both groups had similar low (<2 percent) percentage composition of oil when assessed after filter collection. Ultrasound treatment may release slightly more free lipids because of the additional round of blunt trauma to the adipose tissues during pretunneling with the ultrasound cannula. If the ultrasound energy itself were lysing fat cells en masse, we would expect a much higher relative oil volume in the ultrasound-assisted lipoaspiration group. Confocal imaging of ultrasound- and suction-assisted lipoaspiration samples revealed parcels with normal cellular architecture and intact stromal vascular structures. This finding is in agreement with several other studies analyzing the effect of ultrasound-assisted collection methods on fat tissues.

These studies showed that ultrasound releases adipose cells from the tissue matrix with minimal cell lysis and has no effect on graft viability in vivo.^{14,20,21} This is confirmed by the findings of our study.

Collagenase digestion of the processed fat results in a stromal vascular fraction that is rich in adipose-derived stem cells.²² It has become apparent through research over the past decade that adipose-derived stem cells are important for fat graft survival, largely because of their contributions to revascularization of the graft.^{23,24} The findings of our study demonstrate that harvest with ultrasound- and suction-assisted lipoaspiration yields fat grafts that contain equivalent amounts of these valuable stromal vascular fraction cells.

In this study, filtering the ultrasound- and suction-assisted lipoaspirates effectively captured viable parcels of fat, and filtered ultrasound- and

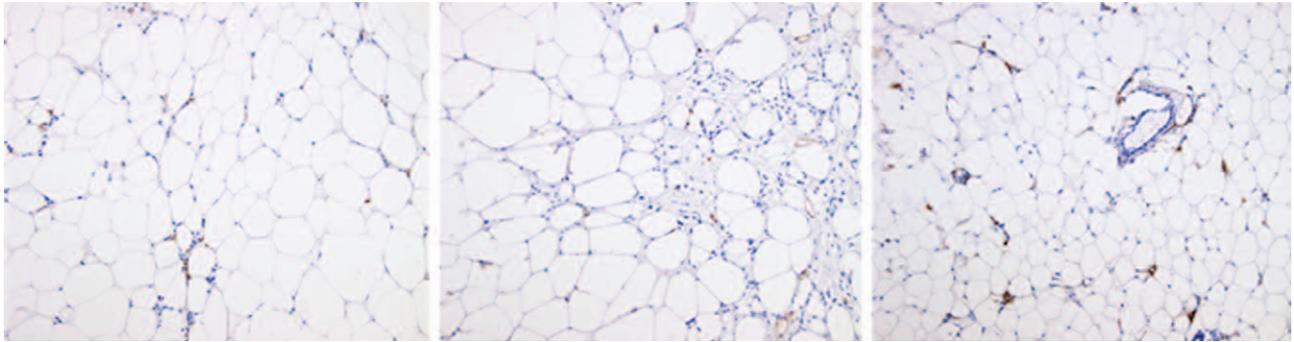


Fig. 11. Images of fat grafts explanted at 6 weeks and stained for the endothelial cell marker CD31. Processing by means of centrifugation (*left*), filtration (*center*), and cotton gauze rolling (*right*) yielded fat grafts with intact architecture.

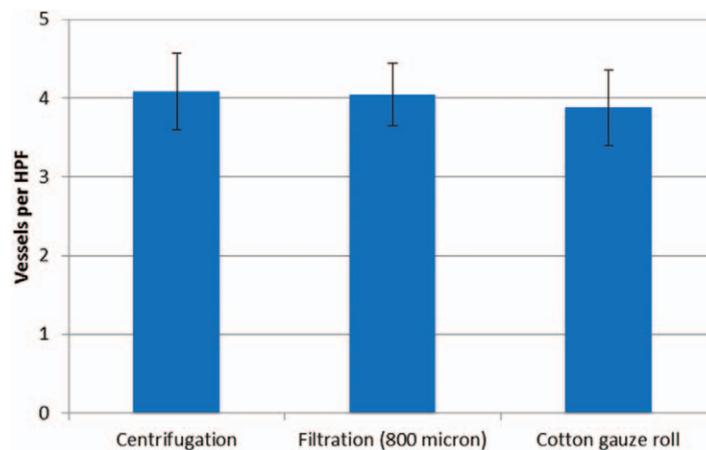


Fig. 12. Counts of CD31⁺ blood vessels per high-power field (HPF) are equivalent between fat grafts processed using centrifugation, filtration, and cotton gauze rolling.

suction-assisted lipoaspiration fat grafts were retained equally well in vivo. Clinically, the filtrate materials that pass through the filters are discarded. This study confirms that the discarded filtrate contains mostly valueless aqueous fraction and only minimal amounts of fat and stromal vascular fraction cells. The small volumes of fatty solids that do pass through both the 500- and 800- μ m filters do not survive when they are grafted in vivo, regardless of harvest type, and are thus not valuable graft material.

With regard to fat graft processing techniques, the literature contains conflicting reports as to whether any one method is superior.^{9,11,18,21} Both cotton gauze rolling and centrifugation have become established methods for removing unwanted oil and aqueous fluid from the lipoaspirate to purify the fat before grafting. We sought to compare these traditional methods of fat graft preparation to the less commonly used filtration approach. The use of an in-line filter device is attractive because of the potential to process large volumes of lipoaspirate quickly and efficiently.

Moreover, the closed system is considered superior by some clinicians, although there are no data to suggest that air exposure increases infection rates or reduces graft viability by oxidation or desiccation.

The cotton gauze–derived technique of Telfa-rolling proved to be the most efficient in removing the oil fraction, with filtration a close second. Filtration removed less aqueous fraction, but perhaps this can be improved on by leaving the suction on longer. Ultimately, all three techniques yielded equivalent volumes of pure fat fraction, with volumes that were significantly higher than those obtained by gravity separation (regardless of harvest technique). This indicates that centrifugation, cotton gauze, and filtration techniques are equivalent in their efficiency to concentrate the pure fat fraction, and that harvest technique (suction-assisted lipoaspiration versus handheld) does not make a difference in yield.

Processing by all three methods resulted in fat that was well incorporated into the new anatomical location in vivo. In all cases, vascularized adipose

tissue with intact architecture was confirmed histologically. However, gauze-rolled fat had the least reabsorption over time. The superiority of the cotton gauze technique may be explained by differences in stromal vascular fraction cell count. In our study, gauze-rolled fat had a significantly higher stromal vascular fraction yield compared with filtration or centrifugation. Adipose stem cells within the stromal vascular fraction have the ability to proliferate and differentiate into mature adipocytes and therefore provide a source for regenerating adipose tissue. It should be noted that, in phase I, the percentage retention of fat in vivo was 75 percent, but in phase II, it was lower (56 percent). This most likely reflects population variation, as the two phases were conducted with fat from two different patients. Indeed, unpublished work from our laboratory has shown that fat from different patients can differ significantly in its adipose stem cell content, adipose-derived stem cell proliferative and differentiation capacities, and the ability to secrete angiogenic growth factors. The effect of patient characteristics (e.g., body mass index, sex, and age) on adipose stem cell content and functionality, and on the long-term retention of transplanted fat, needs to be elucidated further, and these studies are underway.

When large volumes of fat grafts are needed, the cotton gauze rolling and centrifugation methods are less practical because of time and personnel constraints in the operating room. In these cases, filtration may provide an acceptable alternative: the resultant fat product is comparable to that obtained with the centrifugation method in terms of both stromal vascular fraction count and graft retention. Filtration with either pore size used in this study resulted in equal retention of graft material in our animal model, with decanted fat showing a much lower retention rate. The material that passes through the filter (filtrate) contains negligible amounts of stromal vascular fraction and fat, and is poorly retained in vivo.

CONCLUSIONS

Fat harvested using ultrasound-assisted lipoaspiration has the same in vivo retention rate as that from conventional suction-assisted lipoaspiration when injected into a murine model after filtration processing, and both filter pore sizes (500 and 800 μm) had equivalent graft survival. The stromal vascular fraction cell counts of fat harvested by ultrasound- and suction-assisted lipoaspiration are also not significantly different. Processing suction-assisted lipoaspiration

harvested fat with Telfa cotton gauze best optimizes graft retention and stromal vascular fraction yield. This technique may be most appropriate for grafting cosmetically sensitive areas of the body such as the face, in which optimal retention is critical and lower total graft volumes are needed. Processing by means of filtration is a reasonable alternative when there is a need for large volumes of fat for grafting to anatomical regions such as the breast and buttocks. In this study, graft retention of filtered fat was comparable to the commonly used Coleman centrifugation method. When filtration is selected, a filter pore size of either 500 or 800 μm can be used with equal efficacy.

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