

# Aesthetic Surgery Journal

<http://aes.sagepub.com/>

---

## **Acute Adipocyte Viability After Third-Generation Ultrasound-Assisted Liposuction**

Mark E. Schafer, Kevin C. Hicok, Daniel C. Mills, Steve R. Cohen and James J. Chao

*Aesthetic Surgery Journal* published online 29 May 2013

DOI: 10.1177/1090820X13485239

The online version of this article can be found at:

<http://aes.sagepub.com/content/early/2013/05/28/1090820X13485239>

---

Published by:



<http://www.sagepublications.com>

On behalf of:



[American Society for Aesthetic Plastic Surgery](http://www.asap.org)

**Additional services and information for *Aesthetic Surgery Journal* can be found at:**

**Email Alerts:** <http://aes.sagepub.com/cgi/alerts>

**Subscriptions:** <http://aes.sagepub.com/subscriptions>

**Reprints:** <http://www.sagepub.com/journalsReprints.nav>

**Permissions:** <http://www.sagepub.com/journalsPermissions.nav>

>> [OnlineFirst Version of Record](#) - May 29, 2013

[What is This?](#)

## Body Contouring

# Acute Adipocyte Viability After Third-Generation Ultrasound-Assisted Liposuction

Mark E. Schafer, PhD, FASA, FAIUM; Kevin C. Hicok, PhD; Daniel C. Mills, MD, FACS; Steve R. Cohen, MD, FACS; and James J. Chao, MD, FACS

Aesthetic Surgery Journal  
XX(X) 1–7  
© 2013 The American Society for  
Aesthetic Plastic Surgery, Inc.  
Reprints and permission:  
[http://www.sagepub.com/  
journalsPermissions.nav](http://www.sagepub.com/journalsPermissions.nav)  
DOI: 10.1177/1090820X13485239  
[www.aestheticsurgeryjournal.com](http://www.aestheticsurgeryjournal.com)



### Abstract

**Background:** Although clinical evidence of successful autologous fat transfer (AFT) using third-generation ultrasound-assisted liposuction (UAL) is readily available, no study has quantified adipocyte viability using standardized methods.

**Objectives:** The authors assess acute adipocyte viability following fat aspiration as a first step in determining the overall efficacy of using third-generation UAL for AFT.

**Methods:** Lipoaspirate samples were collected from patients who underwent elective liposuction procedures at multiple surgery centers. Patients with a history of bleeding disorders, diabetes, human immunodeficiency virus, or lipoatrophy disorders were excluded. The UAL system (VASER; Sound Surgical Technologies, Inc, Louisville, Colorado) was set at 60% amplitude in pulsed mode with vacuum aspiration of 15 in Hg or less. Laboratory analysis included free lipid volume, viability via lipolysis and propidium iodide staining, and cytological analysis, including cell surface protein examination and hematoxylin and eosin staining.

**Results:** The lipolysis assay revealed metabolically active adipocytes with a mean (SD) correlative viability of 85.1% (11%). Direct measures of acute viability via propidium iodide staining resulted in a mean (SD) viability measure of 88.7% (3.5%). Both mean values are within the historical range reported from syringe and vacuum-assisted lipoaspiration. Aqueous and lipid contents were favorably reduced after washing and filtering (Puregraft system; Cytori Therapeutics, Inc, San Diego, California). Cellular phenotypes identified were primarily white blood cells or vascular endothelial and vascular associated cells.

**Conclusions:** Adipose tissue acquired via third-generation UAL is viable at harvest and is potentially a suitable source for autologous fat grafts. These results confirm reported clinical successes utilizing third-generation ultrasound lipoaspirate for AFT.

### Keywords

body contouring, lipoaspirate, autologous fat transfer, AFT, third-generation ultrasound, liposuction, UAL, adipocyte viability

Accepted for publication January 11, 2013.

There is growing interest in the use of lipoaspirate for autologous fat transfer (AFT) and as a source material for adipose-derived regenerative cells. Autologous fat transfer is performed for both cosmetic and reconstructive purposes, as evidenced by an increasing number of articles and presentations on this topic.<sup>1-4</sup> Although patient safety is the primary concern,<sup>5</sup> the ultimate retention of grafted material is also an important measure of efficacy for both patients and surgeons. Graft retention is dependent on a number of factors, many of which are still not completely understood or quantified.<sup>6-8</sup> The ultimate success of the procedure depends on elements of the entire process: aspiration, processing/handling, and ultimately reinjection. Although a number of authors have examined the effects of different approaches on each step in the

process,<sup>9-11</sup> the foundation of fat grafting is built on the initial step—harvesting adipose tissue.

Dr Schafer is the Chief Technology Officer at Sound Surgical Technologies, LLC, Louisville, Colorado. Dr Hicok is a Senior Research Manager at Cytori Therapeutics, Inc, San Diego, California. Dr Mills is an Assistant Clinical Professor of Plastic Surgery at Loma Linda University, Loma Linda, California. Dr Cohen and Dr Chao are Clinical Professors of Plastic Surgery at the University of California in San Diego, California.

#### Corresponding Author:

Dr Mark E. Schafer, 23 Brookline Court, Ambler, PA 19002, USA.  
E-mail: [marks@sonictech.com](mailto:marks@sonictech.com)

In terms of harvesting adipose tissue, patients seeking elective body contouring have several options. Fifty-two percent of patients are subjected to suction-assisted lipoplasty (standard liposuction), 23% undergo power-assisted liposuction, and 4% undergo laser-assisted liposuction. The remaining 21% of patients undergo ultrasound-assisted liposuction (UAL); the majority of whom (comprising 16% of all body contouring patients) receive third-generation UAL.<sup>12</sup> VASER Lipo System (VAL) (Sound Surgical Technologies, Louisville, Colorado), the third-generation UAL system, has been in use for almost a decade for the safe and efficient removal of adipose tissue. VAL is an internal ultrasound system that incorporates significant design improvements from previous generations; small-diameter, solid, multiringed probes (rather than a hollow cannula) deliver power at only the levels necessary for targeting and disrupting fatty tissues. VAL is able to achieve good results at reduced power levels by operating in a pulsed mode and at a frequency of 36 kHz.

There is debate as to whether lipoaspirate from VAL procedures is viable at harvest. Although some literature indicates that VAL lipoaspirate includes viable adipose-derived stromal cells,<sup>13</sup> laboratory evidence is scant regarding the acute viability of adipose tissue. We assessed the acute viability of adipose tissue collected from VAL procedures from a functional and morphological standpoint and by utilizing spectrophotometry, histology, and immunohistochemistry techniques. This is the first step in the research cascade of *in vitro*, preclinical, and clinical evaluation for determining the adequacy of VAL lipoaspirate in ATF.

## METHODS

### Patient Selection

Aspirate samples were collected from 5 patients who underwent primary (nonrevisional) body contouring using VAL at 1 of 4 surgery clinics. The patients were 20 to 50 years old, were in good general health, and had a body mass index (BMI) of less than 30. Patients were excluded if they had a history of bleeding disorders, diabetes, human immunodeficiency virus, or lipoatrophy disorders (lupus, scleroderma, etc). Surgical sites were chosen based on patient requirements and included the abdomen, flanks, and thighs. A central independent review board approved this study (BIOMED Institutional Review Board, San Diego, California), and all subjects provided written informed consent for bench processing of their fat aspirate.

### Procedures and Settings

Patients received tumescent fluid infiltration of 1.5 or 2 mL for every 1 mL removed. The infiltration fluid was either Lactated Ringer's or saline solution with 1% or 2% lidocaine at 50 or 25 mL per liter, respectively, and 1 ampule

of epinephrine per liter. The solution was applied with a 14-gauge multihole infiltrator, with an infiltration rate of 350 mL/min. Ultrasound was applied with a 3.7-mm, 3-ring probe at an amplitude of 60% in pulsed mode (10 Hz). Aspiration occurred using atraumatic 3.0- to 3.7-mm vented cannulae and a vacuum level of 15 in Hg. All settings, infiltration amounts, start/stop times, and other relevant data were recorded. The manufacturer's recommended ultrasound application time was 1 minute per every 100 mL of infiltrate. A minimum of 500 mL of lipoaspirate was collected from each donor.

### Sample Processing

Lipoaspirate from all 5 patients was transported to a central laboratory facility (at Cytori Therapeutics, Inc, San Diego, California) immediately after aspiration for processing. Upon arrival, samples were gently mixed in large, sterile glass beakers and the lipoaspirate was allowed to settle for 10 minutes, to permit the wetting solution to separate from the tissue. Sample aliquots were then collected in Toomey syringes (Bard Medical, Covington, Georgia). Approximately 10 mL of the collected adipose tissue was used for lipolysis assessment. An additional 5 mL was set aside for histologic analysis.

To determine relative aqueous, lipid, and intact adipose content of lipoaspirate, approximately 30 mL of tissue was divided into 3 equal-volume aliquots and centrifuged for 5 minutes at 400 g. The volumes of aqueous content, free lipid, and tissue were measured and recorded.

### Adipocyte Viability Determination

A lipolysis assay was chosen to evaluate adipocyte viability because it indicates whether the adipocytes are alive and, more important, whether they can functionally metabolize lipid. Briefly, 300-mg samples of intact, washed adipose tissue were placed in triplicate into a 24-well assay plate and incubated in assay buffer, either alone or in the presence of an agonist of glycerol release, isoproterenol. Basal- and agonist-induced release of glycerol was measured using the Free Glycerol Determination Kit (Sigma Aldrich, St Louis, Missouri). Free glycerol content was determined by spectrophotometry at a wavelength of 540 nm. Results were normalized to mass and time. The amount of agonist-induced release of glycerol was linearly correlated to the amount of functional adipocytes present in the tissue and was used to establish the relative viability of the fat cells.<sup>14,15</sup>

The lipolysis assay chosen to evaluate the functional capabilities of harvested adipocytes in this study is uncommon in plastic surgery research. Therefore, to compare the lipolysis assay results with a more commonplace technique, a subsequent viability assessment was performed on 3 additional patients, using similar exclusion and inclusion criteria, save for a less restrictive age limit of 55 years old. Demographics and procedural information

for these patients were recorded separately. In this assessment, propidium iodide was used as a nuclear stain to indicate acute viability of the VAL-harvested fat. Sample processing began immediately following aspiration. Briefly, a standard collagenase digestion using collagenase type II at a concentration of 30 mg/10 g fat in 30 mL of Hank's balanced salt solution with 3.5% bovine serum albumin was performed on 3 separate 10-g samples from each patient's lipoaspirate. Samples were agitated at 120 rpm in a 37°C water bath for 30 to 40 minutes. The working solution produced from the digestion process was assessed for viability with an NC-100 NucleoCounter (ChemoMetec, Copenhagen, Denmark). Each of the 3 digested samples from each patient was tested in triplicate.

## Histologic Analysis

To assess the morphologic characteristics of the lipoaspirate, histology and cytology were performed on the samples from the first 5 subjects in this study. Adipose tissue was fixed, dehydrated, and paraffin-embedded according to standard histologic methods. Sample slides were prepared by cutting tissue into 5- $\mu$ m sections. Slides were then deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). Samples were microscopically examined for healthy morphology, in particular to assess adipocyte membrane appearance and whether membranes were intact.

Cytological assessment of the retained, "loosely adherent" fraction of cells (an indicator of the extent of tissue disruption) was performed by centrifuging lipoaspirate and preparing cytospin samples from the remaining cell pellet. Single cell surface marker protein profiling for CD31 (for platelets, monocytes, and neutrophils), CD34 (for lymph and T cells), CD45 (for all differentiated hematopoietic cells except erythrocytes and plasma cells), and CD68 (for monocytes, histocytes, giant cells, Kupffer cells, and osteoclasts) was performed on these samples using immunocytochemistry with biotin-labeled antibodies and streptavidin-conjugated substrate.

## Additional Sample Processing

Approximately 100 mL of tissue from the first 5 patients in this study was set aside, washed using the Puregraft system (Cytori Therapeutics, Inc, San Diego, California), and then analyzed for aqueous content along with free lipid and tissue volume. The Puregraft system is a US Food and Drug Administration–approved, sterile, closed-tissue washing and filtering system that enables processing of up to 250 mL of lipoaspirate within 15 minutes. The operator of the Puregraft system washed the tissue twice using an equal volume of Lactated Ringer's solution, according to the manufacturer's instructions. Processed material was retrieved from the system using a 60-mL Toomey syringe. Triplicate 10-mL aliquots of the tissue were analyzed after centrifugation for 5 minutes at 400 g.

**Table 1.** Patient Demographics and Procedural Data, Lipolysis Assay

| Patient/Procedure Data                            | Mean $\pm$ SD      |
|---|--------------------|
| Age, y  | 35.4 $\pm$ 9       |
| BMI, kg/m <sup>2</sup>                            | 25.5 $\pm$ 2.9     |
| Infiltration volume, mL                           | 1482.0 $\pm$ 669.0 |
| Aspirate volume, mL                               | 1230.0 $\pm$ 363.0 |
| US application time, min                          | 15.4 $\pm$ 9.7     |
| Recommended <sup>a</sup> US application time, min | 14.8 $\pm$ 6.7     |
| Ratio of applied to recommended                   | 1.0 $\pm$ 0.3      |

BMI, body mass index; US, ultrasound.

<sup>a</sup>Based on manufacturer recommendations of 1 min/100 mL infusate.

**Table 2.** Patient Demographics and Procedural Data, Propidium Iodide Staining

| Patient/Procedure Data                            | Mean $\pm$ SD       |
|---|---------------------|
| Age, y  | 39.7 $\pm$ 12.9     |
| BMI, kg/m <sup>2</sup>                            | 28.7 $\pm$ 6.6      |
| Infiltration volume, mL                           | 2026.0 $\pm$ 1147.0 |
| Aspirate volume, mL                               | 857.0 $\pm$ 211.0   |
| US application time, min                          | 20.0 $\pm$ 11.8     |
| Recommended <sup>a</sup> US application time, min | 20.3 $\pm$ 11.5     |
| Ratio of applied to recommended                   | 1.0 $\pm$ 0.4       |

BMI, body mass index; US, ultrasound.

<sup>a</sup>Based on manufacturer recommendations of 1 min/100 mL infusate.

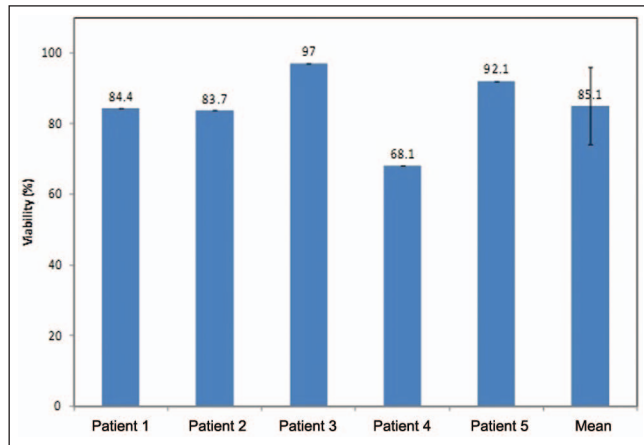
## RESULTS

### Patient Demographics

Table 1 displays patient demographics for the 5 subjects in this study whose lipoaspirate was examined by lipolysis assay and histology studies for cytology and aqueous content, as well as free lipid and tissue volume (both with and without washing and filtering processes of the Puregraft system). These patients were all women with a mean (SD) age of 35.4 (9) years (range, 24-45 years) and a mean (SD) BMI of 25.5 (2.9) kg/m<sup>2</sup>. Table 2 displays demographics of the 3 additional patients whose lipoaspirate was subjected to collagenase digestion and viability staining with propidium iodide. These patients were all women with a mean (SD) age of 39.7 (12.9) years (range, 29-54 years) and a mean (SD) BMI of 28.7 (6.6) kg/m<sup>2</sup>.

### Viability Studies

Figure 1 shows adipocyte viability of the unwashed/unfiltered samples from the lipolysis assay for each



**Figure 1.** Adipocyte viability as assessed by lipolysis assay.

patient. Mean (SD) viability was 85.1% (11%) from the 5 lipoaspirate samples. Figure 2 illustrates the presence of intact adipose on histologic examination, which is certainly a requirement for tissue viability. Figure 3 shows adipocyte viability of the samples processed via the NucleoCounter for each of the 3 additional patients. Mean (SD) viability was 88.7% (3.5%) from these lipoaspirate samples. Upon harvest, the unprocessed VAL aspirate of the 5 pilot patients had a mean (SD) aqueous content of 20.7% (2%) and a mean (SD) free lipid content of 5.29% (1.8%). After filtering and washing steps, the aqueous and free lipid contents were reduced to 16.5% (3.5%) and 0.63% (0.5%), respectively. The samples had an average of more than 32 000 “loosely adherent” cells per gram of tissue, which is a measure of the ancillary cells that accompany fat cells in the lipoaspirate (these cells are thought to be important in sustaining the tissue during and immediately after any reimplantation process).<sup>16,17</sup>

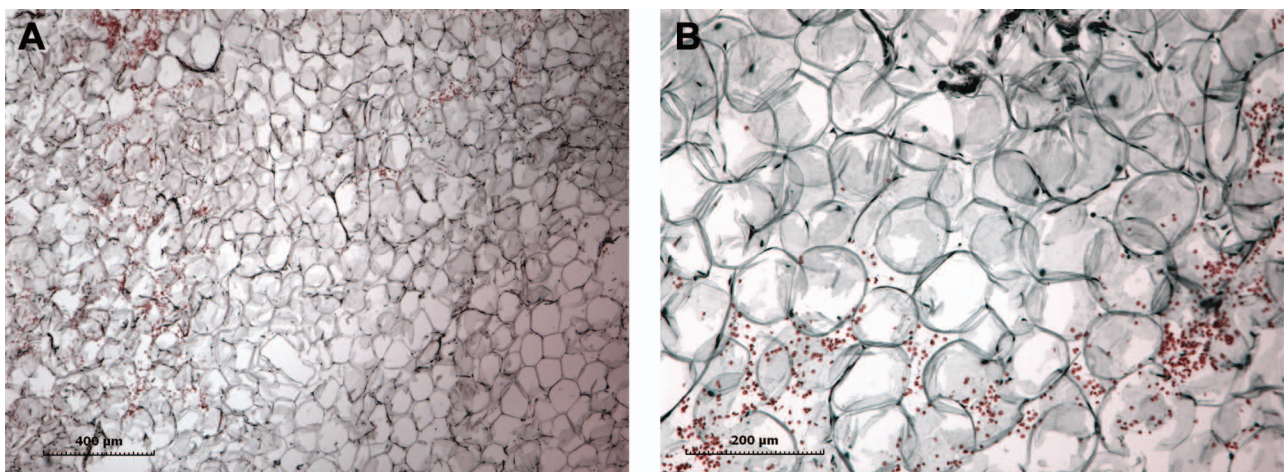
## Immunohistochemistry and Histology

The cells were primarily either white blood cells (WBC) or vascular endothelial and vascular-associated cells (Figure 4). Histologic evaluation of loosely adherent cell fraction from each patient demonstrated comparable levels of CD31 + and CD34 + microvasculature fragments, CD68 + tissue macrophages, and CD45 + WBC. Variable amounts of collagenous extracellular matrix were observed as well, but the amount of matrix did not appear to correlate to anatomical location of tissue harvest. The composition of loosely adherent cells obtained by centrifugation of ultrasound-treated tissue was not qualitatively different from what was observed in fat tissue obtained by other methods (ie, syringe and low-vacuum-assisted lipoaspiration alone).

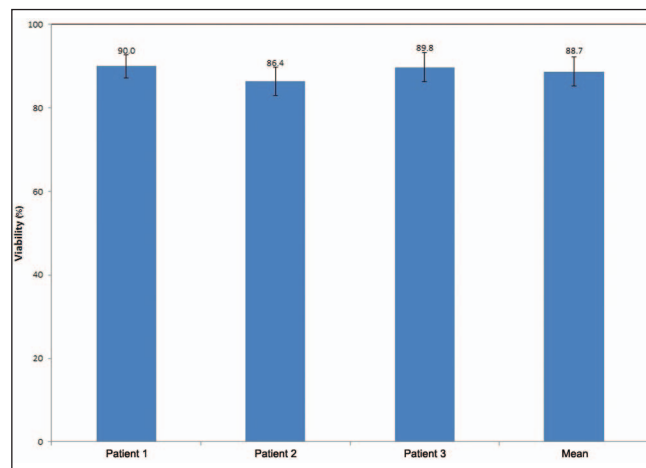
## DISCUSSION

An increasing number of physicians are exploring the use of AFT or fat grafting procedures to provide lasting, natural structural and contour changes postoperatively.<sup>18-21</sup> Enthusiasm for AFT has increased as the technique has become both more common and more reliable. For these fat transfers, the viability of the harvested tissue is of primary importance. Coleman<sup>8</sup> has advocated that appropriate technique in harvest and refinement of the autologous fat is critical to the efficacy of the procedure.

By traditional methods, fat harvested specifically for grafting is gently aspirated by handheld syringes with small blunt cannulae, as described by Coleman.<sup>8</sup> Once the lipoaspirate is obtained, the aqueous content is reduced to improve predictability in fat grafting.<sup>8</sup> Although most investigators do not report acute adipocyte viability values in terms of a percentage of viable adipose cells, Piasecki et al<sup>22</sup> did report such measures in an experimental study of murine adipose tissue. Their study utilized a



**Figure 2.** Microscopic evaluation of extracted fat cells at (A)  $\times 100$  and (B)  $\times 200$ . Mature unilocular adipocytes with intact membranes are evident.



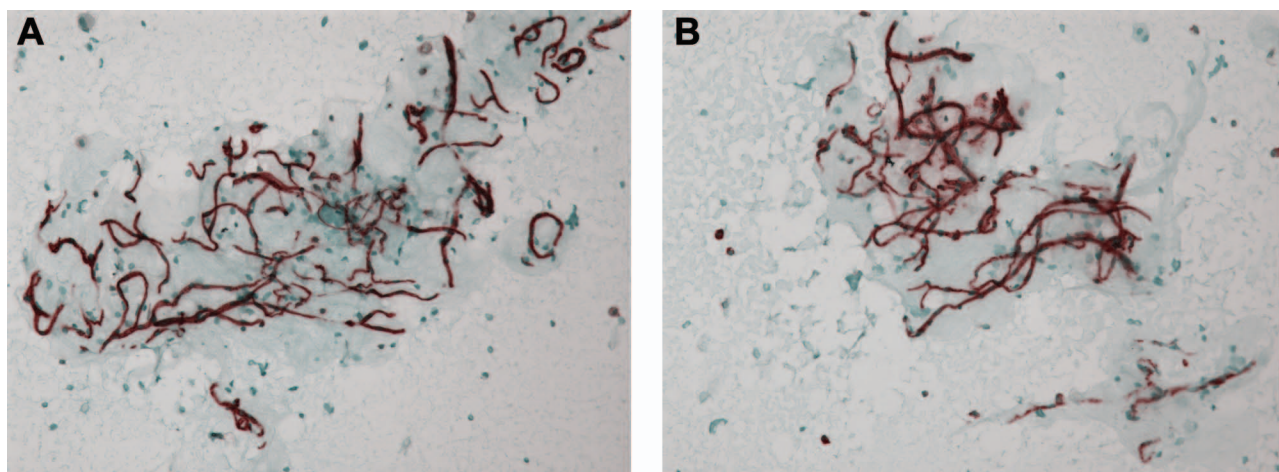
**Figure 3.** Adipocyte viability as assessed by propidium iodide staining.

hemocytometer to count live adipocyte cells and cell fragments stained with trypan blue. A digestion process was performed via collagenase to enable automated cell counting. Acute adipocyte viability, not influenced by processing techniques such as centrifugation or washing, resulted in roughly 73% viability for excised fat, 48% viability for fat harvested with an 18-gauge blunt cannula, and 35% viability for fat harvested by an 18-gauge sharp needle. Other authors have reported 98% to 100% fat viability based on morphology alone, as identified through H&E staining.<sup>23</sup> Our study yielded an acute viability rate of 85.1% based on the ability of the retrieved fat to functionally metabolize lipids and of 88.7% through propidium iodide staining of the adipocyte nuclei. Many other studies have focused on refinement or specific harvesting techniques, but these can be time-consuming and labor intensive.<sup>8,22,24</sup> In this series, we focused on the initial viability of adipose cells immediately after aspiration. The overall

viability of implanted fat depends on subsequent processing and reimplantation steps; however, assuming that these steps do not cause undue tissue damage, the high level of viability we achieved compares favorably with other methods for successful AFT and did not come at the expense of operative time or convenience.<sup>11,25</sup>

A crucial factor affecting the viability of adipose tissue is the manner in which the tissue is obtained. Third-generation UAL has been available since 2002 for lipoaspiration and has been associated with reduced blood loss as well as a decrease in the complication rates for UAL procedures.<sup>26-28</sup> The VASER system uses ultrasound energy at 36 kHz to separate the adipose cells from the tissue matrix through a combination of stable cavitation and acoustic streaming.<sup>29</sup> Although these acoustic phenomena could theoretically damage the fat cells of the lipoaspirate, the results reported here indicate that this technique (under the described conditions) does not result in large-scale cell lysis of adipocytes. Furthermore, it is our belief that the aforementioned acoustic phenomena separate the adipose tissue into smaller clumps in situ. These effects have been well demonstrated in biological media and in vivo via similar technology used for ultrasonic mixing and dispersion.<sup>30-35</sup> This procedure permits the use of relatively atraumatic, vented canulae and lower vacuum levels to obtain the lipoaspirate. The net effect is less tearing or shearing of the fat cells during aspiration. Finally, by maintaining the overall cellular distribution of the lipoaspirate (mature adipocytes and the “loosely adherent” cell population, eg, peri-adipocytes, stem cells, stromal cells, etc), the success rate of the implanted tissue matrix may be enhanced.<sup>36</sup>

A limitation of the quantitative data in this study is that long-term cell viability for soft tissue augmentation requires investigation in vivo in animal models. This should be addressed in future work. However, demonstrating cell viability at harvest is a necessary first step before proceeding to preclinical studies. The results of this study provide quantitative evidence that future preclinical work



**Figure 4.** Immunostaining of “loosely adherent” cells/components. (A) CD31 (vascular endothelial marker cell). (B) CD34 (endothelial and progenitor marker cells).

is warranted. Whether or not treatment with third-generation UAL has an impact on long-term survival of grafted fat remains to be seen, although it is fair to predict that the initial viability of adipose cells in the lipoaspirate is sufficiently high to ensure long-term success with VAL-mediated fat transfer. Additional comparative validations of graft quality in the clinical setting are under way. These data will be valuable in determining the effectiveness of third-generation UAL technology in harvesting adipose tissue for autologous fat grafting.

## CONCLUSIONS

We present evidence that third-generation UAL could provide an efficient means of harvesting adipose without sacrificing tissue viability. Future work should attempt to demonstrate graft retention capability and provide comparative evaluations between UAL-harvested grafts and those harvested using other liposuction techniques.

## Acknowledgment

The authors acknowledge the assistance of Drs Peter Rubin and Kacey Marra of the McGowan Institute for Regenerative Medicine at the University of Pittsburgh Medical Center for their helpful advice with regard to the collagenase digestion of lipoaspirate. The authors also acknowledge the assistance of Maurice Sherman and Judd Kirk for contributing to the collection of patient samples.

## Disclosures

Dr Schafer is an employee of Sound Surgical Technologies, LLC, a study sponsor and the manufacturer of products discussed in this article. Dr Hicok is an employee of Cytori Therapeutics, Inc, also a study sponsor and manufacturer of products discussed in the article. Dr Cohen is a part-time employee of Cytori Therapeutics, Inc and has received honoraria as a trainer/consultant for Sound Surgical Technologies. Dr Cohen is also an investigator and/or consultant for Lithera, Suneva, Stryker, Osteomed, Galderma, and Ulthera; receives fellowship support from KLS-Martin, and is a founder, shareholder, and member of the board of directors for LeonardoMD. Dr Mills is a consultant to Allergan, TouchMD, and Suneva. Dr Chao is a consultant to Novus Scientific and Boston Scientific Neuromodulation.

## Funding

This study was sponsored by Sound Surgical Technologies, LLC (Louisville, Colorado), the manufacturer of products discussed in the article. The sponsor provided funding for laboratory testing fees. Cytori Therapeutics, Inc (San Diego, California), also a manufacturer of products discussed in the article, was a co-sponsor of this study and provided technical support and analysis.

## REFERENCES

1. Saint-Cyr M, Rojas K, Colohan S, Brown S. The role of fat grafting in reconstructive and cosmetic breast surgery: a review of the literature. *J Reconstr Microsurg.* 2012;28(2):99-110.
2. Kamakura T, Ito K. Autologous cell-enriched fat grafting for breast augmentation. *Aesthetic Plast Surg.* 2011;35(6):1022-1030.
3. Sykes JM, Tapias V, Pu LL. Autologous fat grafting viability: lower third of the face. *Facial Plast Surg.* 2010;26(5):376-384.
4. Bucky LP, Percec I. The science of autologous fat grafting: views on current and future approaches to neoadipogenesis. *Aesthetic Surg J.* 2008;28(3):313-321.
5. Gutowski KA, ASPS Fat Graft Task Force. Current applications and safety of autologous fat grafts: a report of the ASPS Fat Graft Task Force. *Plast Reconstr Surg.* 2009;124(1):272-280.
6. Smith P, Adams WP, Lipschitz AH, et al. Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte graft survival. *Plast Reconstr Surg.* 2006;117:1836-1844.
7. Carpaneda CA. Study of aspirated adipose tissue. *Aesthetic Plast Surg.* 1996;20:399-402.
8. Coleman SR. Structural fat grafting: more than a permanent filler. *Plast Reconstr Surg.* 2006;118:108-120.
9. Tommaso A, Davide L, Alessandro P, et al. Wet and dry techniques for structural fat graft harvesting: histomorphometric and cell viability assessments of lipoaspirated samples. *Plast Reconstr Surg.* 2012;130(2):331e-339e.
10. Locke MB, de Chalain TM. Current practice in autologous fat transplantation: suggested clinical guidelines based on a review of recent literature. *Ann Plast Surg.* 2008;60(1):98-102.
11. Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. *Dermatol Surg.* 2000;26(12):1159-1166.
12. Ahmad J, Eaves FF III, Rohrich RJ, Kenkel JM. The American Society for Aesthetic Plastic Surgery (ASAPS) survey: current trends in liposuction. *Aesthetic Surg J.* 2011;31(2):214-224.
13. Panetta NJ, Gupta DM, Kwan MD, et al. Tissue harvest by means of suction-assisted or third-generation ultrasound-assisted lipoaspiration has no effect on osteogenic potential of human adipose-derived stromal cells. *Plast Reconstr Surg.* 2009;124(1):65-73.
14. Rodbell M. Metabolism of isolated fat cells, I: effects of hormones on glucose metabolism and lipolysis. *J Biol Chem.* 1964;239:375-380.
15. Hoffman BB, Chang H, Farahbakhsh Z, Reaven G. Inhibition of lipolysis by adenosine is potentiated with age. *J Clin Invest.* 1984;74:1750-1755.
16. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation.* 2004;109(10):1292-1298.

17. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. *Tissue Eng*. 2006;12(12):3375-3382.
18. Coleman SR. Structural fat grafts: the ideal filler? *Clin Plast Surg*. 2001;28:111-119.
19. Jackson IT, Simman R, Tholen R, et al. A successful long-term method of fat grafting: recontouring of a large subcutaneous postradiation thigh defect with autologous fat transplantation. *Aesthetic Plast Surg*. 2001;25:165-169.
20. Garcia-Olmo D, Garcia-Arranz M, Herreros D, et al. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum*. 2005;48:1416-1423.
21. Magalon G. Limb autologous adipose tissue reinjection: a series of 62 cases. Paper presented at: 30th Anniversary Course of the Foundation of G. Sanvenero Rosselli; September 16, 2005; Milan, Italy.
22. Piasecki JH, Gutowski KA, Lahvis GP, Moreno KI. An experimental model for improving fat graft viability and purity. *Plast Reconstr Surg*. 2007;119(5):1571-1583.
23. Shiffman MA, Mirrafati S. Fat transfer techniques: the effect of harvest and transfer methods on adipocyte viability and review of the literature. *Dermatol Surg*. 2001;27(9):819-826.
24. Pu LLQ, Cui X, Fink BF, Cibull ML, Gao D. The viability of fatty tissues within adipose aspirates after conventional liposuction—a comprehensive study. *Ann Plast Surg*. 2005;54(3):288-292.
25. Keck M, Janke J, Ueberreiter K. Viability of preadipocytes in vitro: the influence of local anesthetics and pH. *Dermatol Surg*. 2009;35(8):1251-1257.
26. Garcia O, Nathan N. Comparative analysis of blood loss in suction-assisted lipoplasty and third generation internal ultrasound-assisted lipoplasty. *Aesthetic Surg J*. 2008;28:430-435.
27. Jewell ML, Fodor PB, de Souza Pinto EB, Al Shammari MA. Clinical application of VASER-assisted lipoplasty: a pilot study. *Aesthetic Surg J*. 2002;22:131-146.
28. Nagy MW, Vanek PF. A multicenter, prospective, randomized, single-blind, controlled clinical trial comparing VASER-assisted lipoplasty and suction-assisted lipoplasty. *Plast Reconstr Surg*. 2012;129(4):681e-689e.
29. Schafer ME, Hicok K. Viability of harvesting stem cells from adipose tissue using an ultrasonically assisted method. Proceedings of 38th Northeast Bioengineering Conference (NEBEC). IEEE CFP12NEB, 2012:109-110.
30. Enslinger D. *Ultrasonics*. 2nd ed. New York, NY: Marcel Dekker; 1988.
31. Thompson LH, Doraiswamy LK. Sonochemistry: science and engineering. *Ind Eng Chem Res*. 1999;38(4):1215-1249.
32. Nyborg WL. Ultrasonic microstreaming and related phenomena. *Br J Cancer*. 1982;5(suppl):156-160.
33. Miller DL. A review of the ultrasonic bioeffects of microsonation, gas-body activation, and related cavitation-like phenomena. *Ultrasound Med Biol*. 1987;13(8):443-470.
34. Baker KG, Robertson VJ, Duck FA. A review of therapeutic ultrasound: biophysical effects. *Phys Ther*. 2001;81(7):1351-1358.
35. O'Brien WD. Ultrasound-biophysics mechanisms. *Prog Biophys Mol Biol*. 2007;93(1-3):212-255.
36. Wan DC, Lim AT, Longaker MT. Craniofacial autologous fat transfer. *J Craniofac Surg*. 2009;20(2):273-274.