Background: Laser-assisted liposuction (LAL) has been used to maximize viable adipocyte yields in lipoaspirates, although optimizing tissue processing methods is still a challenge. A high-quality lipoaspirate has been a key factor for extended graft longevity.

Objective: To assess the viability and potency of stromal vascular fraction (SVF) cells and adipose-derived stem cells (ASCs) in fat samples from lipoaspirates harvested with a novel 1470-nm diode, radial emitting LAL platform. Two processing methods, enzymatic and nonenzymatic, were compared.

Methods: Laser-assisted liposuction lipoaspirates harvested from 10 subjects were examined for cell viability after processing by enzymatic or nonenzymatic methods. Isolated SVF cells were cultured with an ASC-permissive medium to assess their viability and proliferation capacity by cell proliferation assay. Flow cytometric analysis with ASC-specific markers, gene expression levels, and immunofluorescence for ASC transcription factors were also conducted.

Results: Lipoaspirates showed high SVF cell viability of 97% ± 0.02% and 98% ± 0.01%, averaged SVF cell count of 8.7 × 10^6 ± 3.9 × 10^5 and 9.4 × 10^6 ± 4.2 × 10^5 cells per mL, and averaged ASC count of 1 × 10^5 ± 2.2 × 10^5 and 1.2 × 10^6 ± 5 × 10^5 cells per mL in enzymatic and enzymatic methods, respectively. The ASC-specific markers, gene expression levels, and immunofluorescence for ASC transcription factors confirmed the adipose origin of the cells.

Conclusions: The laser liposuction aspirates provide a high yield of viable and potent SVF cells and ASCs through both enzymatic and enzymatic processes. Improved purity of the harvested lipoaspirate and high ASC content are expected to result in extended graft longevity. Furthermore, eliminating enzymatic digestion may provide advantages, such as reducing processing time, cost, and regulatory constraints.

Key Words: laser liposuction, liposuction, adipose-derived stem cells, stromal vascular fraction, autologous fat transfer

Liposuction is the second most popular aesthetic procedure performed in the United States. It is commonly assisted by mechanical suction, also known as suction-assisted liposuction, power-assisted liposuction, ultrasound, or laser energy. Laser-assisted liposuction (LAL) is considered safe and tolerable and has been associated with a lower touchup rate and fewer complications compared with conventional liposuction techniques. One of the main concerns in autologous fat grafting is the unpredictable resorption after transplantation. In fact, the rate of resorption over time in the grafted site may range from 20% to 90% of the filled volume. This could be attributed to the fact that the aspirated fat is poor in quality and has low number of adipose-derived stem cells (ASCs). Therefore, optimizing methods of harvesting adipose tissue is of maximal importance as it can have a significant impact on the yield and viability of isolated cells.

Stromal vascular fraction (SVF) cells are a heterogeneous population of cells including endothelial cells, erythrocytes, fibroblasts, lymphocytes, monocytes/macrophages, and most importantly ASCs. Adipose-derived stem cells can differentiate into a range of mesenchymal tissues and their enrichment in the lipoaspirate has become important in aesthetic surgery recently.

To isolate ASCs from liposuction aspirates, disruption of the adipose tissue by enzymatic or nonenzymatic manipulation of liposuctions is necessary, followed by centrifugation to obtain the SVF cell pellet. Enzymatic digestion is the most commonly used method for SVF isolation, providing high nucleated cells per mL of liposipuate. However, it is associated with regulatory concerns and limitations. Enzymatic methods, although known to produce significantly lower yields of ASCs, are appealing as they are simple, fast, and associated with less regulatory issues. Therefore, researchers are exploring nonenzymatic methods of separation using protocols that involve pressure, shear, and centrifugal force.

We conducted an open-label, prospective clinical study using a novel 1470-nm radial emitting LAL (Alma Lasers, BeauFill by LipoLife) to assess the viability and differentiation potential of SVF cells and ASCs extracted from the collected liposutures. Moreover, we compared nonenzymatic and enzymatic processing methods of these liposutures.

MATERIALS AND METHODS

Donors
The study included 10 consecutive subjects (1 man and 9 women) with a mean age of 47.7 ± 12 years and body mass index (BMI) of 27.7 ± 4.4 kg/m². All subjects provided informed consent for bench processing of their fat aspirate in accordance with the Shamir Medical Center Institutional Review Board. Helsinki approval number 0095-17-ASF. The average volume obtained from the abdomen and thighs was 1844 mL. The maximum aspirated material was 3620 mL, and the minimum was 400 mL (Table 1).

Surgical Procedure
Liposuction with LipoLife (Alma Lasers, BeauFill by LipoLife) involves the simultaneous action of laser and suction. The LipoLife system consists of a 1470-nm diode laser and LipoFlow system (Alma Lasers, Ltd.), which provides vacuum for liposuction and enables infiltration.

High-Quality Lipoaspirate Following 1470-nm Radial Emitting Laser-Assisted Liposuction

Eyal Shapira, MD,a Lori Plonski, MD,a Shaked Menashe, MD,a Andre Ofek, MD,a Adaya Rosenthal, MD,a Massimiliano Brambilla, MD,b Gary Goldenberg, MD,c Sahar Haimowitz, Msc,d and Lior Heller, MDa

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From the *Department of Plastic Surgery, Shamir Medical Center, Zerifin, Israel; 1Department of the Health of the Woman, Child and Neonate, Fondazione IRCCS Ospedale Maggiore, Milan, Italy; 2Department of Dermatology, Icahn School of Medicine at Mount Sinai Hospital, Mount Sinai, NY; and 3Prostate Cancer Research Laboratory, Department of Urology, Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. Conflicts of interest and sources of funding: None declared.

Reprints: Shapira Eyal, MD, Department of Plastic Surgery, Shamir Medical Center, Zerifin, Israel. E-mail: shapiraeyal@gmail.com

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The procedures were performed under general anesthesia. Before liposuction, the subjects were prepped with betadine. Tumescent solution (400 mg lidocaine and 1 mg adrenaline per liter saline) was introduced into the treated area using Lipoflow with a ratio of injected liquid (tumescent) to aspirated material of 2:1. For fat aspiration, a 4-mm cannula specially designed with a swivel handle (Alma Lasers, BeautiFill by LipoLife) was used. The 1470-nm, 600-micron, radial emitting laser fiber (Alma Lasers, Ltd.) was maneuvered and positioned at the center of the distal opening of the cannula. The harvested lipoaspirate was collected in a sterile canister and filtered through a canister mesh to separate the adipocytes from the tumescent fluid, blood, cell debris, and free oil.

### Adipose Tissue Harvesting

Enzymatic or nonenzymatic manipulation processing began within 1 to 2 hours of harvesting. Lipoaspirate samples (100 mL) were washed extensively 3 times with sterile Dulbecco phosphate-buffered saline (PBS) without calcium and magnesium (Biological Industries, Beit HaEmek, Israel) to dispose of tissue debris and blood residuals. Before the processing methods, small samples of lipoaspirate were taken for further RNA analysis (Fig. 1).

### Isolation of ASCs

Lipoaspirate processing by enzymatic or nonenzymatic manipulation for isolation of SVF cells.

#### Enzymatic Preparation

Fifteen milliliters of washed lipoaspirate sample was transferred to a 50-mL tube containing 30 mL of 0.1% collagenase IV (Sigma-Aldrich cat. no. C2139) and 5% BSA in PBS (Biological Industries). The sample was incubated for 45 minutes at 37°C and 5% CO₂ at 100 rpm. Stromal vascular fraction was obtained by centrifuging the sample at 1200 rpm for 5 minutes and discarding the collagenase solution without disturbing the SVF cell pellet. This preparation is based on the protocol of Aronowitz et al.30,32

#### Nonenzymatic Preparation

Fifteen milliliters of washed lipoaspirate sample was transferred to a 50-mL tube containing 30 mL PBS (Biological Industries). The sample was vortexed gently for 6 minutes at 600 rpm to disrupt the tissue and release the cells. Then, the cells were centrifuged at 1600 rpm for 6 minutes to obtain the SVF. The supernatant was removed without disturbing the SVF cell pellet.

The SVF cell pellets containing ASCs were resuspended in MSC NutriStem XF Medium supplemented with MSC NutriStem XF, Male AB Human Serum 2.5%, and 0.1% penicillin/streptomycin (Biological Industries) and seeded on precoated cell culture plates with MSC attachment solution (Biological Industries) and grown for 7 days at 37°C and 5% CO₂ before further analysis. The remaining floating cells and debris were aspirated after 72 hours. Using this permissive medium resulted in more ASCs being isolated.

| Subject Number | Weight (kg) | BMI   | Age | Sex | Liposuction Area | Total Lipoaspirate Volume (mL) |
|----------------|-------------|-------|-----|-----|-----------------|-------------------------------|
| 1              | 72.5        | 29.21 | 44  | Female | Abdomen              | 900                           |
| 2              | 99          | 30.56 | 22  | Male  | Abdomen              | 1900                          |
| 3              | 51          | 18.73 | 51  | Female | Abdomen              | 400                           |
| 4              | 81.4        | 30.49 | 55  | Female | Abdomen              | 2050                          |
| 5              | 68          | 27.94 | 58  | Female | Abdomen              | 500                           |
| 6              | 93.8        | 35.3  | 47  | Female | Thighs               | 3620                          |
| 7              | 73          | 26.81 | 45  | Female | Thighs               | 1870                          |
| 8              | 63.5        | 24.01 | 37  | Female | Thighs               | 2500                          |
| 9              | 70          | 25.71 | 65  | Female | Thighs               | 2500                          |
| 10             | 71.8        | 28.44 | 53  | Female | Thighs               | 2200                          |
| Average        | 74.4        | 27.7  | 47.7|       |                  | 1844                          |
| SEM            | 4.4         | 1.4   | 3.8 |       |                  | 316                           |

**FIGURE 1.** Isolation and characterization of SVF cells and ASCs from laser-assisted lipoaspirate.
in a pure homogenous ASC culture characterized by typical morphology of spindle-shaped cells as described by others27,29,34 (bright-field images in Fig. 3). PromoCell commercial human mesenchymal stem cells (hMSCs) from adipose tissue were defined as a positive control (Biological Industries).

SVF Cell Viability Count
To determine the number of viable cells present in the SVF cell pellet, the cell suspension was diluted 1:1 with 0.4% trypan blue dye (Sigma-Aldrich). The viable cells were counted with a hemocytometer and calculated to determine the number of viable cells per mL.

Live Staining of ASCs
Cell culture medium was replaced with fresh medium containing the primary antibody CD90 2.5 μg/mL (eBioscience, San Diego, CA). Adipose-derived stem cells were incubated in 5% CO2 at 37°C for 30 minutes. The antibody-containing medium was gently removed, and cells were washed 3 times with PBS (Biological Industries). Fresh cell culture medium was added, and cells were immediately examined by fluorescence microscopy with appropriate filters.

FACS
Samples were analyzed using an 8-color 3-laser FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ) using a 100-μm nozzle. Forward-side scatter dot plots were used to exclude debris and cell aggregates.

The following fluorochrome-conjugated monoclonal antibodies were used: CD45-eFluor 450, CD90-FITC, CD34-APC, CD105-PE, and CD73-PerCP-eFluor 710. All antibodies were purchased from eBioscience. Data were analyzed using BD FACSDiva software (BD Biosciences).

XTT Cell Proliferation Assay
Adipose-derived stem cells were seeded in 96-well plates (300 cells/well) for cell proliferation assays using a 3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT) kit (BD Biosciences). The absorbance of the samples was measured using an ELISA reader at 680 (Bio-Rad, Hercules, CA) at a wavelength of 450 nm subtracted by 655 nm. All experiments were performed in duplicate.

Immunofluorescence Staining and Microscopic Analysis
Adipose-derived stem cells were plated into 6-well plates with 13-mm-diameter cover glasses. After incubation, the cells were fixed and permeabilized with cold PHEMO buffer, 3.7% formaldehyde, 0.05% glutaraldehyde, and 0.5% Triton X-100 for 10 minutes. Briefly, blocking was performed in 1% BSA and 10% normal donkey serum in PBS (Biological Industries). Fresh cell culture medium was added, and cells were immediately examined by fluorescence microscopy with appropriate filters.

Quantitative Real-Time PCR Analysis
Total RNA was extracted from cells using a NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. One microgram of total RNA was reverse transcribed into cDNA using ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA). Quantitative real-time PCR analyses were performed to determine the expression of the target genes: vascular endothelial growth factor (VEGF), von-Willebrand factor (v-WF), and CD31. β-actin was selected as a housekeeping gene for mRNA normalization. Specific primers for the genes v-WF and CD31 were designed using the GenScript primer design tool (https://www.genscript.com/tools/real-time-pcr-taqman-primer-design-tool). Primers for VEGF and β-actin were designed as previously described.35 Quantitative real-time PCR was conducted in duplicate sets for each sample using SyGreen Blue Mix Hi-ROX (PCR Biosystems, London, United Kingdom) in a StepOnePlus Real-Time PCR System (Applied Biosystems).
Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Student’s t-test was used to calculate P values, and a P value of 0.05 or less was considered statistically significant.

RESULTS

Adipose tissue laser liposuction was performed in all 10 subjects. The average age of the subjects was 47.7 ± 3.8 years, and the average BMI was 27.7 ± 1.4. Liposuction sites included the abdomen and thighs, and the average liposuption volume was 1844 ± 316 mL (Table 1). After liposuction with LipoLife, harvested liposuaspirate was either enzymatically digested (collagenase) or nonenzymatically treated (vortex). Then, fat samples were centrifuged to isolate the SVF. The SVF cell pellet was cultured, and the adherent ASCs were further analyzed to confirm stem cell markers (Fig. 1).

Viability of SVF Cells and ASCs in Lipoaspirates Obtained With Laser

The average SVF cell viability measured with trypan blue was \(8.7 \times 10^6 \pm 3.9 \times 10^6\) and \(9.4 \times 10^6 \pm 4.2 \times 10^6\) cells per mL of liposuaspirate and reached high SVF viability values of 97% ± 0.02% and 98% ± 0.01% in nonenzymatic and enzymatic manipulations, respectively. After

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**FIGURE 3.** Live Staining of ASCs Derived from LAL (CD90 Marker). Analysis of live ASCs was performed using a fluorescence microscope. ASCs isolated using enzymatic and nonenzymatic processing methods highly express CD90. Representative data of subjects 4 and 5 are shown.
FIGURE 4. SVF and ASC yield per mL of lipoaspirates harvested from the thighs with or without laser. These are representative results of ASCs isolated from an enzymatically processed laser and nonlaser lipoaspirates of subject 9. A, Table showing SVF viability and ASC count in samples derived from abdominal and thigh lipoaspirates following enzymatic and nonenzymatic processing methods. B, Summary of the data presented in panel A. The corresponding SEM values from 2 individuals are shown. A significant difference (P < 0.001) was found between the laser and mechanical liposuction in the enzymatic processing method. *Significant difference between liposuction with and without laser was found only in the enzymatic processing method (n = 2, P < 0.05). (C) Fluorescent staining of ASCs derived from the enzymatic processing method. Higher expression of CD90 was detected during laser harvesting. hMSC was used as a positive control, and the PC-3 cell line was used as a negative control (microscope magnification, 20×). SVF and ASCs of lipoaspirates obtained from 2 subjects with or without laser liposuction.

FIGURE 5. FACS analysis of ASCs. A, Expression of surface stem cell markers, positive (CD90, CD73, and CD105) and negative (CD34 and CD45) detected by flow cytometric analysis of cultured ASCs. Data show a representative set of dot plot from subject 5 compared with the positive control (hMSCs) and negative control (unstained hMSCs). B, Summary of the immunophenotypic characteristics of ASCs. For the enzymatic processing method, data are representative of the analysis of 9 individuals, and for the nonenzymatic processing method, data are representative of the analysis of 6 individuals compared with the positive control (hMSCs) and negative control (PC-3 cells). ***Significant difference between enzymatic and negative control was found for CD90 and CD73 (n=9, P<0.001). A significant difference between nonenzymatic and negative control was found for CD90 and CD73 (n=6, P<0.001).
7 days of culture, the isolated ASC average counts were $1 \times 10^6 \pm 2.2 \times 10^5$ and $1.2 \times 10^6 \pm 5 \times 10^5$ cells per mL in nonenzymatic and enzymatic manipulations, respectively (Figs. 2A–B).

**Live Staining of ASCs Derived From LAL**

Live staining of ASCs revealed intense and homogenous CD90 staining in ASCs isolated by the 2 processing methods (Fig. 3). This demonstrates that the isolated ASCs expressed the necessary mesenchymal marker, verifying them as stem cells. This marker was missing in the negative control (prostate carcinoma (PC-3) cell line).

**Viability of SVF Cells and ASCs in Lipoaspirates Obtained With or Without Laser**

Preliminary analysis of the yield obtained in 2 subjects revealed 3.6-fold SVF cells levels in the enzymatic manipulation of lipoaspirates obtained with the laser ($2.5 \times 10^7$) compared with mechanical liposuction ($6.9 \times 10^6$). This difference was found to be highly significant ($P < 0.001$) (Figs. 4A–B). CD90 expression levels in the isolated ASCs were as high as those in the positive control hMSC (Fig. 4C).

**FACS of ASCs Derived From Laser-Assisted Aspirates**

Stromal vascular fraction cells contained a large number of erythrocytes; therefore, erythrocytes and other debris were excluded from the analysis by gating them out by cell size. Consequently, only nucleated cells were analyzed. Compared with commercial hMSCs, samples of isolated cells contained similar percentages of cells positive markers CD90, CD105, and CD73 and similar percentages for negative markers CD34 and CD45, suggesting that isolated cells from samples contained a large number of ASCs (Figs. 5A–B).

**Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction Analysis of Laser Lipoaspirates and ASC mRNA**

Quantitative real-time Polymerase Chain Reaction (PCR) was performed to evaluate the gene expression levels of the endothelial cell markers, endothelial growth factor (VEGF-A), CD31, and v-WF. All laser lipoaspirates showed significantly high expression of VEGF-A ($**$, $P < 0.01$), CD31 ($***$, $P < 0.001$), and v-WF ($***$, $P < 0.001$) compared with positive control hMSCs. All ASC samples showed significantly high expression of endothelial markers CD31 ($*$, $P < 0.05$) and v-WF ($***$, $P < 0.001$) compared with positive control hMSCs.

**DISCUSSION**

This study aimed to assess the viability and proliferative potency of SVF and ASCs isolated from harvested laser liposapirates. Achieving high cell viability and high cell count in nonenzymatic manipulation is a huge advantage.

Human nonembryonic adult MSCs, including blood, bone marrow, and ASCs, represent important cell resources and hold great promise for cell-based therapies. Bone marrow-derived MSCs are considered the main source of MSCs for clinical applications. In comparison, adipose tissue also contains a large number of MSCs and is easier to isolate. Fat tissue consists of mature adipocytes, SVF cells, blood vessels, lymph nodes, and nerves. The SVF contains ASCs, preadipocytes, endothelial cells, pericytes, macrophages, and fibroblasts. Adipose-derived stem cells from enzymatic and nonenzymatic processing methods were found to similarly express these 2 transcription factors 7 days after seeding (Fig. 7).

ASCs Proliferation Capacity

The XTT cell proliferation assay was performed after 1, 4, 8, 12, 16, and 20 days (Fig. 8). Two growth curves for the 2 processing methods are presented with higher variability noted in the last 2 time points. In both processing methods, ASCs continue to proliferate on day 12 and reach the highest proliferating rate on day 16, followed by a subsequent decline.
protocols. Within heterogeneous SVF cells, the subgroup of ASCs is usually isolated through plastic adherence in culture conditions.

Using biological enzymes to disrupt the tissue has raised safety concerns where some countries do not even permit its use for fat grafting. In fact, the FDA considers cell populations produced by enzymatic manipulation “more than minimally manipulated” and demands heavy regulation. Thus, alternative processing techniques need to be developed. Achieving high cell viability and cell count in nonenzymatic manipulation would definitely be an advantage.

In this study, we report high yields of SVF cells and ASCs from both enzymatically and nonenzymatically processed lipospires. Other studies have reported lower yields from mechanical processing compared with that from enzymatic methods, probably due to the tightly bound cells in adipose connective tissue, which is not efficiently disrupted by mechanical action alone. We suggest that our reported similar cell numbers, using both preparation methods, were caused by laser assistance in liposuction even though lasers are known to hinder the quality of isolated adipocytes in laser liposuction. Furthermore, histology data of the 1470-nm laser liposuction showed no effect on the quality of the adipocytes (data not shown). The lipospires were gently harvested due to the unique radial design of the fiber, which results in a less aggressive treatment due to the low energy density. This could be also attributed to the fact that the 1470-nm diode laser emits light that is preferentially absorbed by water and collagen, rendering it ideal for gentle fat tissue collection.

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Strengthening our findings, Levenberg et al reported that abdominal fat samples harvested with the LipoLife were more homogeneous, demonstrated higher viable adipocyte counts, and contained fewer fibrous and blood contaminants than those collected via mechanical liposuction.
In accordance with the criteria defined by the International Society for Cell Therapy for ASC identification, the FACS analysis and live staining showed specific profile markers for ASCs, which were found to be similar to the commercial human positive control ASCs. The controversial CD34 marker was found to be positive in subjects 3 and 4, similar to the positive control ASCs. This marker in ASCs has been the subject of dispute for many years. Its presence depends on the culture conditions, such as seeding density or type of culture medium.

High gene expression levels of VEGF-A, CD31, and v-WF in the ASCs and their high expression of NANOG and OCT4 transcription factors confirmed the cells adipose tissue-derived origin and their pluripotency, consistent with the findings of Domenis et al.

Another major issue with fat grafting is its absence of reproducibility. This could be attributed to variability in fat harvesting and processing methods and patient factors. These can include decreased proliferation and differentiation potential of ASCs with age, BMI, diabetes mellitus, and exposure to radiotherapy and tamoxifen. Our results show highly reproducible values in terms of the viability percentage and number of cells. The negligible variability observed in 10 different patients, with 2 different body areas and performed with 4 different surgeons, makes our clinical protocol highly reliable.

Comparing our findings to different processing methods is hard due to the use of different methodologies. Therefore, comparative investigations to other liposuction methods (eg, ultrasound-assisted, power-assisted, and mechanical) are essential. The lack of comparison to other liposuction methods, and the viability of these fat cells once they are grafted into the patient are limitations of the current study. Further studies should use the mechanically isolated fat cells as grafts and follow graft viability and longevity. In addition, future studies should examine a larger cohort with a larger number of males and a wider range of ages and BMIs. Another interesting direction will be to isolate spherical ASCs from LAL and examine their viability. The ASCs used in the study are slightly differentiated stem cells since they are incubated with serum and adhere to the cell culture plate, as compared with the spherical ASCs, which are less differentiated and therefore exhibit higher regenerative properties.

An important consideration going forward is regulatory approval. Although autologous fat grafting, consisting of removing autologous cells from an individual and reimplanting them without intervening processing steps beyond rinsing, cleansing, sizing, or shaping does not require approval, adding cell expansion steps using serum will necessitate regulatory approval. Developing a protocol in which SVF cells and ASCs are isolated and prepared for implantation without adding external factors, will make this procedure easily clinically applicable.

**CONCLUSIONS**

The quality of harvested SVF cells and ASCs from liposuctions is of exceptional importance in the field of fat grafting and reconstruction surgery. It is vital to have a large number of stem cells that are in a state of potency for subsequent transfer.

We present an improved clinical and processing protocol for the harvesting of high-quality SVF cells and ASCs. The 1470-nm radial emitting laser fiber with a specialized cannula that enables simultaneous lasing and suction, thus keeping the adipose tissue intact, with nonenzymatic processing of the harvested lipoaspirates, demonstrated excellent cell count numbers and high-quality adipose stem cells, compared with the enzymatic processing. Clinically, this study shows that LAL extracts viable SVF cells and ASCs, and thus provide a source of cells for fat grafting. Furthermore, achieving high viability levels using LAL and mechanical isolation makes cell isolation simpler and safer, and does not require regulatory approval.

The improved purity of the harvested liposapirate and high ASC content are expected to result in extended graft longevity.

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