

Revisiting Fat Graft Harvesting and Processing Technique to Optimize Its Regenerative Potential

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Background: The use of fat grafting has expanded to include cell and tissue regeneration, necessitating investigations to ensure the viability of stromal and adipose-derived mesenchymal stem cells (ASCs) within the transferred fat parcels. This study explored the impact of harvesting technique and centrifugation on the viability of stromal cells and ASCs in lipoaspirate.

Methods: Fat was harvested from patients undergoing fat grafting using 2 types of liposuction cannula: (A) a 3-mm blunt tip cannula with 3 smooth holes and (B) a 2.4-mm, sharp point port, multihole blunt tip cannula. Fat from cannula B underwent different processing methods: no centrifugation, 300g, 600g, and 900g centrifugation. Stromal cells were isolated, quantified, and evaluated for viability. ASCs were cultured from these samples to confirm survival.

Results: Lipoaspirates from 21 patients were analyzed. The mean stromal cell counts were $0.937 \times 10^9 \pm 0.346 \times 10^9/\text{mL}$ for cannula A and $0.734 \times 10^9 \pm 0.266 \times 10^9/\text{mL}$ for cannula B ($P = 0.684$), with viabilities of 98.79% and 98.22% ($P = 0.631$), respectively. ASCs isolated and after 2-passage culture were also higher for cannula A. Stromal cell quantification and viability were lowest in the noncentrifuged group ($P < 0.05$) and highest in the 600g centrifugation group.

Conclusions: Fat harvesting using cannulas A and B showed no significant difference in stromal cell yield or viability. Handheld syringe liposuction preserved stromal vascular fraction cell and ASC viability. Centrifugation at different speeds did not significantly affect stromal cell viability. (*Plast Reconstr Surg Glob Open* 2025; 13:e6420; doi: [10.1097/GOX.00000000000006420](https://doi.org/10.1097/GOX.00000000000006420); Published online 10 January 2025.)

INTRODUCTION

Fat grafting has been used for reconstruction and aesthetic purposes ever since its first introduction in 1893.¹ The assured biocompatibility of autologous fat grafting, the abundant source of fat, and the relative ease of harvesting have made autologous fat grafting

one of the most common procedures performed in plastic surgery.² As it grows in popularity, the techniques of autologous fat grafting become more complex and varied.^{1,3} Furthermore, the recent finding of multipotent adipose tissue-derived mesenchymal stem cells (ASCs) in subcutaneous adipose tissue has made it even more popular.⁴

Because ASCs can be isolated from stromal vascular fraction (SVF) of enzyme-digested adipose tissue, the utilization of autologous fat grafting has expanded. It is used not only for filling soft tissue deficiency but also for its regenerative potential.⁴ Improvement of various medical conditions have been reported in several studies, such as facial scars,⁵ radiation-induced injuries,⁶⁻⁸ and burn scars.⁹⁻¹¹ These studies have shown that autologous fat grafting is able to support tissue regeneration. It also enhances the wound healing process with the ability to reduce inflammation, fibrosis, hypertrophic scarring, and wound healing time. Most of these benefits rely on the ASCs present in the adipose tissue. It is believed that the ASCs have the ability to differentiate and replace the defective cells or to accelerate tissue repair by secreting various paracrine factors.⁴

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To preserve the regenerative potential of autologous fat graft, its harvesting and processing technique must be optimized. High-quality adipose tissue must be harvested to obtain the highest amount of stem cells.¹² Numerous previously reported studies have explored various fat graft harvesting and processing techniques to optimize the viability of fat cells. Several manufacturers have introduced fat harvesting cannulas specifically designed for stem cell or SVF harvesting, often referred to as “cell-friendly” cannulas. Other manufacturers have developed cannulas dedicated to preserving fat cells for fat grafting procedures. However, no prior studies have directly compared the impact of these cannulas on stromal cell yield and quality.^{3,13–18} There are still limited studies investigating fat graft harvesting and processing technique that ensure the viability of the stromal cells and ASCs.¹⁹ Therefore, this study aimed to explore the effects of harvesting technique and centrifugation on the viability of stromal cells and ASCs.

MATERIALS AND METHODS

Patients

Twenty-one consecutive elective surgery patients undergoing fat grafting procedures in our center from 2019 to 2021 were included in this study. Seven male patients and fourteen female patients participated in this study. The mean age of all patients was 52.4 ± 13.8 years old. The mean body mass index of the patients was 25.76 kg/m^2 . All the patients underwent a fat grafting procedure for aesthetic purpose. All patients were healthy and had no scar from previous surgery in the abdomen. Exclusion criteria for this study were patients with diabetes. Ethical approval was obtained from Health Research Ethics Committee of University of Indonesia and Cipto Mangunkusumo Hospital (HREC-FMUI/CMH) (No. 0249/UN2.F1/ETIK/2018). This study complied with the Declaration of Helsinki. Informed consents were obtained from each patient before the fat grafting procedure.

Takeaways

Question: How do harvesting techniques and centrifugation affect the viability of stromal cells and adipose-derived mesenchymal stem cells (ASCs) in lipoaspirates?

Findings: Differences in cell numbers and viability between the 2 lipoaspiration cannulas were not statistically significant, and centrifugation did not impact cell viability.

Meaning: Handheld syringe fat harvesting and lipoaspirate centrifugation are reliable techniques for preserving the regenerative capacity of fat grafts.

Fat Harvesting Procedure

The donor site for fat harvesting was the abdomen in all subjects. Stab incision was performed at the inferior side of the umbilicus. The donor area was infiltrated by using tumescent solution (lidocaine 0.04% and epinephrine 1: 1,000,000 in normal saline solution). The fat from each patient was harvested using the handheld technique with 2 different liposuction cannulas. Cannula A was a 3-mm blunt tip cannula with 3 smooth holes (MAFT-Gun; Dermato Plastica Beauty Co. Ltd., Taiwan), whereas cannula B was a 2.4-mm, sharp point port, multihole, blunt tip cannula (Tulip Tonnard Fat Harvester; Tulip Medical Products). The lipoaspiration procedure was performed manually with a 50-cc handheld syringe (Omnifix; B-Braun Medical Indonesia, Jakarta, Indonesia) to get volume-matched samples. During the decantation process, the lipoaspirate was separated into a fatty portion and a liquid portion. We ensured that the proportion of the fatty and liquid portions was equal for each sample to facilitate a fair comparison. Thirty-five milliliters of aspirate from each cannula, consisting of 25 mL of fatty portion and 10 mL of liquid portion, was further processed for stromal cell and ASC quantification. The remaining lipoaspirate was used for the fat grafting procedure (Figs. 1, 2).

SVF Processing and Centrifugation Procedure

The lipoaspirate collected from both cannulas was further processed using the enzymatic method with

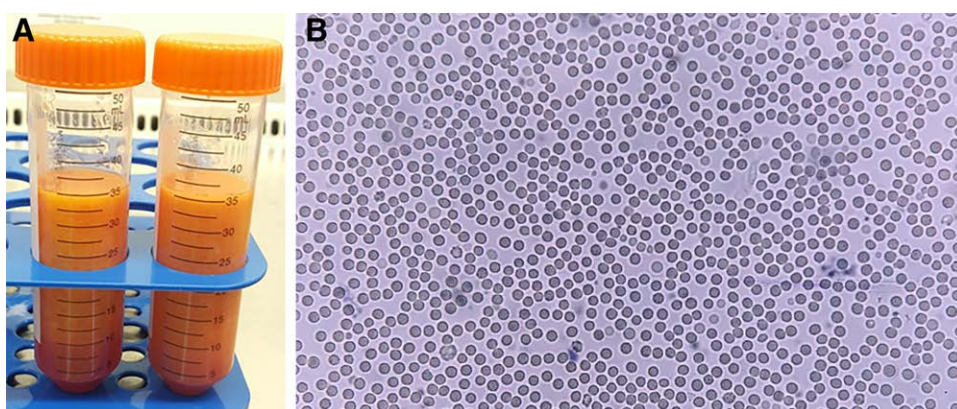


Fig. 1. Lipoaspirate samples and stromal cell microscopic evaluation. A, Thirty-five milliliters of lipoaspirate. B, Cell count view with Trypan blue staining from 10× magnification microscope.

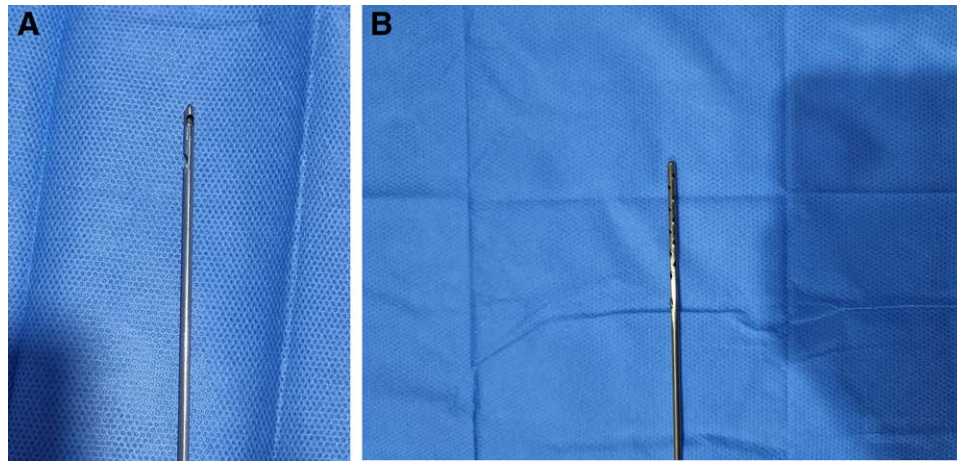


Fig. 2. The comparison of harvesting cannulas. A, Cannula A: a 3-mm blunt tip cannula with 3 smooth holes (MAFT-Gun). B, Cannula B: a 2.4-mm, sharp point port, multihole blunt tip cannula (Tulip Tonnard Fat Harvester).

H-Remedy recombinant enzyme (Hayandra Inspira Medica Ltd., Jakarta, Indonesia) and incubated at 37°C for 1 hour in a 50-mL tube for sedimentation. After 1 hour, inactivation of the enzyme was accomplished by using the growth medium of low glucose (1 g/L) Dulbecco's Modified Eagle's Medium and 4 mM L-glutamine (Gibco). All samples underwent 600g centrifugation for 5 minutes, and the supernatant was discarded. The obtained pellet (SVF) was then diluted in saline solution.^{20–22}

To study the effect of centrifugation, every sample collected with cannula B underwent 4 different interventions: (1) no centrifugation (gravity separation), (2) 300g centrifugation, (3) 600g centrifugation, and (4) 900g centrifugation. Afterward, the samples underwent identical standard procedures as mentioned earlier.

Trypan blue staining was used for counting of cell number and viability per 10 mL of adipose tissue digested. The number of cells was calculated using the following formula:

$$\text{Number of cells} = \frac{\text{Average live or dead cells}}{4 \text{ (chambers)}} \times Df \times 10^4$$

where Df is the dilution factor.

As for the cell viability, the following formula was used:

$$\text{Cell viability} = \frac{\text{Average live cells}}{\text{Average live + dead cells}}$$

Adipose-derived Mesenchymal Stem Cells Two-passag e Culture

The ASCs in the SVF were cultured using basic growth medium Dulbecco's Modified Eagle's Medium low glucose (1 g/L) with L-glutamine (Gibco), 10% fetal bovine serum, and 1× antibiotic-antimycotic (Gibco). The cell culture was then incubated at 37°C, 5% CO₂ with the medium being changed every 2–3 days. The cells isolated with this technique had been characterized in our

previous publication.¹⁹ The cultured cells have met the criteria of ASCs in accordance with the International Society of Cellular Therapy with the expression of CD73, CD90, and CD105, with less or without the expression of CD34/CD45/CD11b/CD19/HLA-DR. Subculture and expansion of the cells to passage 2 was done after reaching 80%–90% confluency.

Statistical Analysis

Statistical analysis was done using IBM SPSS statistics version 24.0 (IBM Corp., Armonk, NY) (RRID:SCR_002865). The Mann-Whitney U test and independent *t* test were used to analyze the effect of different cannulas on the cell yield, the viability of SVF cells, and the viability of mesenchymal stem cells in the first and second passage cultures. The Kruskal-Wallis test was used to analyze the effect of centrifugal forces on the cells yield and the viability of SVF cells. Statistical significance was determined by a 2-tailed *P* value of less than 0.05.

RESULTS

Effect of Different Cannulas on Yield and Viability of SVF Cells

Comparison of the cell yield and viability of SVF cells with 2 different cannulas (cannula A and cannula B) is shown in Table 1. Cannula A was found to yield more cells and showed slightly higher viability of SVF cells than cannula B. However, the statistical analysis showed no significant difference (*P* > 0.05).

Effect of Centrifugal Forces on Yield and Viability of SVF Cells

Analysis on the effect of 4 different centrifugation speeds on the yield and viability of SVF cells is illustrated in Figures 3 and 4, respectively. The yield of SVF cells was found to be higher with centrifugation (300g, 600g, and 900g) than without centrifugation. Statistical analysis also

showed a statistically significant difference between the yield of SVF cells with centrifugation and without centrifugation (no centrifugation versus 300g, $P = 0.038$; no centrifugation versus 600g, $P = 0.013$; no centrifugation versus 900g, $P = 0.009$). As for the viability of SVF cells, slightly lower viability was found in the group without centrifugation compared with the centrifugation groups (300g, 600g, and 900g). This difference was not statistically significant ($P > 0.05$).

Two-pass Culture of Adipose-derived Mesenchymal Stem Cells Isolated From SVF With Two Different Cannulas

Comparison of the 2-pass culture of mesenchymal stem cells isolated from the SVF with 2 different cannulas is shown in Table 2. Both the first passage and second passage cultures were observed to yield more ASCs and higher viability in the cannula A group, although statistical analysis showed no significant difference.

DISCUSSION

The theory about fat graft survival has evolved over time. The latest theory suggests that the retention of fat graft relies not only on the neovascularization at the recipient site but also on the work of ASCs to regenerate the fat tissue.²³ Therefore, the survival of ASCs is also essential, especially when fat grafting is performed for regeneration purposes, such as in chronic skin ulcers.^{24,25} There were previous reports about the harvesting and processing technique to maximize the viability of fat

cells during fat grafting. However, there was no previous study that explore whether the ideal fat grafting technique was well related with the viability of stromal cells and ASCs.

In this study, we found that the harvesting cannula affected the cell yield of SVF cells and ASCs. Cannula B enabled a more rapid fat tissue aspiration, especially in the area with dense fibrous tissue. This was because the cannula had multiple sharp point ports that helped releasing the fat tissue from the adjacent fibrous tissue. However, cannula A, which had a larger diameter and entry port, yielded more SVF cells and ASCs than cannula B, even though this was not statistically significant.^{26–28} The possible explanation for this finding is that the large cannula allowed more stromal tissue surrounding the fat tissue to be aspirated. More stromal tissue resulted in a higher SVF cell count that positively correlated with the cultured ASC count.

Regarding the SVF cell viability, there was not much difference between the 2 cannulas. Previous studies suggested that cannulas with smaller diameters possibly produce shear stress to the fat cells and negatively affect their viability.^{26–29} However, our finding showed that the viability of the SVF cells was not significantly affected by the harvesting cannula size (around 98%). The ASCs cultured from the SVF samples also had satisfactory viability rates (above 90%). A study by Wang et al³⁰ reported that the viability of adipocytes and SVF obtained from a liposuction procedure was around 79% and 66% subsequently. The viability of the ASCs was ranging from 30% to 78%.

Table 1. Yield and Viability of SVF Cells Collected With 2 Different Cannulas

	Cannula A	Cannula B	P
Yield of SVF cells (cells/mL) (mean \pm SD)	937,775,000 \pm 346,431,680	734,625,000 \pm 266,380,117	0.684
Viability of SVF cells (%)	98.79	98.22	0.631

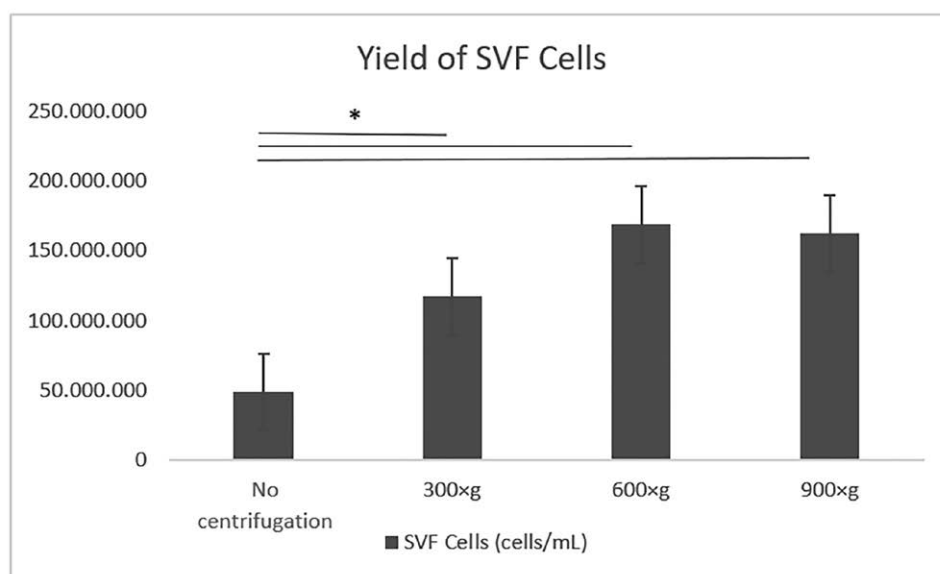


Fig. 3. Yield of SVF cells with 4 different centrifugation speeds (* $P < 0.05$).

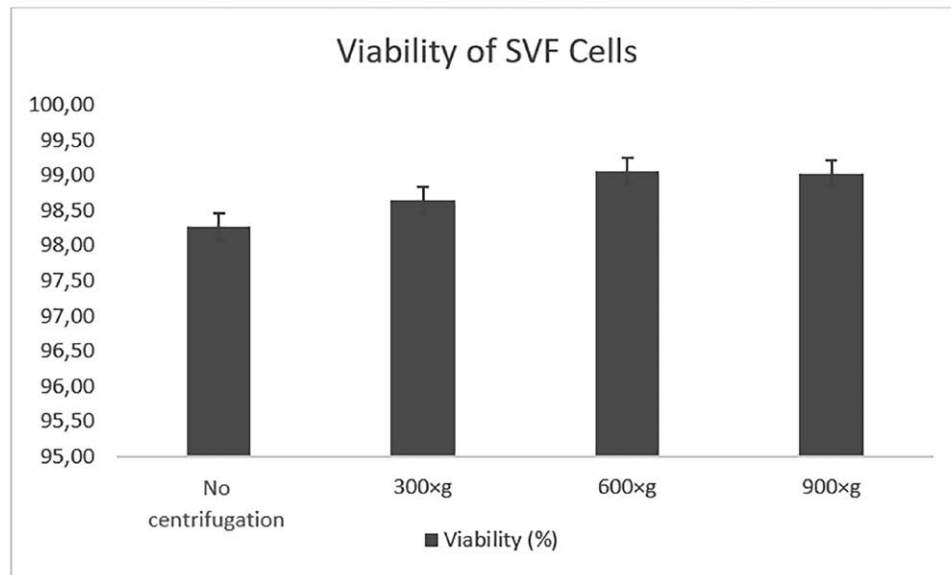


Fig. 4. Viability of SVF cells with 4 different centrifugation speeds.

Table 2. Two-pass Culture of Mesenchymal Stem Cells Isolated From SVF With 2 Different Cannulas

		Cannula A	Cannula B	P
P1	ASCs (mean \pm SD)	444,583 \pm 81,154	423,333 \pm 75,162	0.852
	Viability (%)	94.2012	90.5234	0.423
P2	ASCs (mean \pm SD)	345,100 \pm 62,584	319,333 \pm 46,905	0.916
	Viability (%)	94.02	90.07	0.818

P1: passage 1; P2: passage 2.

This finding differed considerably with our results. This might be caused by the difference in the staining technique to assess cell viability. The other possibility is the differences in the lipoaspiration technique. Wang et al did not specifically explain the lipoaspiration technique that they performed. We believe that the manual lipoaspiration by using a handheld syringe is more favorable to cell viability, including ASCs, than device-assisted liposuction, as also reported by several previous studies.^{31,32}

Manual lipoaspiration using a handheld syringe is preferable to device-assisted liposuction due to its higher stem cell yield, growth capacity, and functional properties. A review by Raposio and Bertozzi¹² showed that ultrasound-assisted liposuction had lower growth capacity and functional properties compared with manual lipoaspiration. Additionally, laser-assisted liposuction exhibited significantly lower ASC yield, viability, and proliferative capacity compared with manual lipoaspiration. The use of device-assisted liposuction results in less favorable outcomes due to the additional application of thermal energy, which damages the adipose tissue.¹²

We found that the yield of SVF cells was significantly higher with centrifugation than without centrifugation. A possible explanation for this finding is that centrifugation force was able to concentrate the stromal cells better than the gravitational separation method. Therefore, more SVF cells were found in the centrifugation groups. A previous study by Conde-Green et al³³ also reported similar

outcomes and hypothesis on the identical outcomes, as more SVF cells were found in the pellet (the fourth layer) of the centrifuged lipoaspirate.

Currently, there were mixed reports regarding the effect of centrifugation on fat cells viability. Some studies reported that the centrifugation force might induce damage to the fat cells, whereas some others demonstrated that the centrifugation procedure had no significant effect to fat cell viability.^{3,11,34} A study on a rat model favored centrifugation because centrifugation produced a compact fat tissue by removing nonviable components of oil, blood, and infiltration fluid; however, the quality of the tissue in histology after 3 months was less viable than the washed and the cell-assisted lipograft, as that middle layer of the centrifuged lipoaspirate contained fewer SVF cells.³⁵ However, there was only limited evidence that explored the centrifugation effect on SVF cells viability during fat grafting procedure. In our study, there was no significant difference of SVF cells viability between the groups with various centrifugation speeds and the non-centrifuged group. This result was similar to the study conducted by Kurita et al.³⁶ Because the viability of SVF cells was not affected, it was expected that the viability of ASCs would be unaffected as well. Thus, centrifugation during fat graft preparation most likely would not alter its regenerative potential.

There were several clinical studies that utilized fat grafting for its regenerative ability. The harvesting and

Table 3. Summary of Existing Clinical Studies Exploring the Regenerative Effect of Fat Graft

Author (Year)	Problems	Cannula Diameter	Liposuction Technique	Centrifugation	Outcome	Reference
Rigotti (2007)	Radiotherapy side effects	2 mm	Handheld syringe	2700rpm for 15 min	LENT-SOMA score and clinical improvement	⁷
Klinger (2008)	Burn scar and keloid	Not mentioned	Not mentioned	Not mentioned	Clinical and histologic improvement	¹¹
Panettiere (2009)	Irradiated breast	3 mm	Handheld syringe	No centrifugation	LENT-SOMA score and clinical improvement	⁸
Klinger (2013)	Scar	Not mentioned	Handheld syringe	Yes (speed was not mentioned)	POSAS score improvement	⁹
Bruno (2013)	Burn scar	3 mm	Handheld syringe	1250 G for 3 min	Clinical and histologic improvement	¹⁰
Pallua (2014)	Scar	2 mm	Handheld syringe	3000 rpm for 3 min	POSAS score improvement	⁵

LENT-SOMA, late effects normal tissue task force subjective, objective, management and analytic; POSAS, patient and observer scar assessment scale.

processing technique varied between studies, as summarized in Table 3.^{5,7–11} Almost all of the studies used the handheld syringe to perform the liposuction. Variation in liposuction cannula and centrifugation protocols existed among the studies. Nevertheless, all of the studies reported favorable results. Our findings supported the idea that the handheld syringe liposuction technique produces excellent SVF cell and ASC viability despite differences in the liposuction cannula size. The results of those studies also aligned with our findings regarding the effect of centrifugation on cell viability. Different centrifugation protocols in those studies could produce desirable clinical or histological changes.

There were several limitations in this study. There were many variables regarding the harvesting and processing technique of fat graft that could not be covered by this study. The fat graft injection technique is also one of the variables that could influence the final outcome. The injection technique and the clinical outcome were not observed in this in vitro study. A high cell count and excellent viability rate would not guarantee the desired tissue regeneration effect because the cells need to survive and function in the recipient site (in vivo) as well. We believe that future clinical studies need to be performed to explore the association of harvesting procedure, processing protocol, injection technique, and the clinical outcome. The findings in our study also need to be confirmed by further studies with a larger sample size and experiments on animals to investigate the effects in vivo.

CONCLUSIONS

To summarize, fat harvesting between a larger harvesting cannula (3mm) with smooth holes and a smaller (2.4mm) multihole cannula with handheld syringe technique showed no significant difference in stromal cell yield and viability. Liposuction with a handheld syringe preserves the viability of SVF cells and ASCs contained in the fat graft. Centrifugation during fat graft processing does not affect the viability of SVF and ASCs. Further

studies with larger sample size are needed to confirm our findings and associate these findings with the clinical outcome.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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