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ARTICLE

Factors affecting cell viability and the yield of adipose-derived stromal vascular fraction

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ABSTRACT

The stromal vascular fraction (SVF) is isolated from adipose tissue and has tremendous regenerative potential for proliferation and differentiation. This study aimed to investigate the factors affecting the cell yield and viability of the SVF to improve the outcomes of its clinical applications and enhance its clinical usage. We performed a retrospective analysis with 121 patients who underwent liposuction to harvest adipose-derived SVF. We recorded patient demographic and clinical characteristics, including age, sex, body mass index (BMI), blood type, medical comorbidities, and smoking and alcohol consumption. As for operative variables, we noted the amount of lipoaspirate and the donor areas, including the lower and entire abdomen. The viability and the cell count of SVF were documented. Sex was a statistically significant factor for viability rate ($p < 0.015$) and cell count ($p < 0.009$). Men had higher viability, while women had higher cell counts. We found a statistically significant difference in the presence of hypertension ($p = 0.024$) and alcohol consumption ($p = 0.024$). There was a statistically significant relationship between cell count and age ($p < 0.001$), BMI ($p = 0.006$), and amount of lipoaspirate ($p < 0.001$). Sex had significant associations with cell count and viability, while age, BMI, and lipoaspirate amount were significantly associated with cell count. Hypertension and alcohol consumption significantly affected cell count, which is the first such report of this association. Surgeons could apply this knowledge to patient selection for optimal treatment outcomes. Additionally, understanding these factors can help manage patient expectations.

Abbreviations: AD: adipose-derived; AT: adipose tissue; ADSCs: AD stem cells; BMI: body mass index; SVF: stromal vascular fraction

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Stromal vascular fraction; lipoaspirate amount; cell count; donor area

Introduction

The innovative idea of using adipose tissue (AT) as a grafting tool was first introduced by plastic surgeons [1]. Recently, AT transfer has become a powerful regenerative tool, primarily because of its cellular components as a stromal vascular fraction (SVF). AT transfer is used for various purposes, including breast reconstruction, repairing surface contour deformities, enhancing the aesthetic appearance, and enhancing tissue regeneration. The SVF is isolated from AT and has tremendous regenerative potential for proliferation and differentiation because it contains multipotent stem cells and stromal cells [2,3].

Adipose-derived (AD) SVF has been gaining popularity in recent years due to its easy isolation, therapeutic potential, and lack of ethical concerns tied to other sources of multipotent cells. SVF contains a heterogeneous cell population, including endothelial cells, fibroblasts, smooth muscle cells, leukocytes, pericytes, pre-adipocytes, and AD stem cells (ADSCs) [4]. Due to the potential differentiation of ADSCs into osteogenic, myogenic, adipogenic, and chondrogenic lineages [4,5], the clinical application of SVF has increased in plastic and reconstructive surgery and orthopedics practice, especially in wound treatment, autologous fat grafting, and osteoarthritis [6]. The success of an autologous fat graft depends in part on its total SVF [7]. SVF contains angiogenic and neovascularization mechanisms (i.e. paracrine function and involvement in forming new vessels) and has anti-inflammatory properties [8,9].

The ADSC specimens obtained *via* liposuction are the gold standard [10]. Liposuction has been conducted worldwide for years, increasing in popularity for fat removal and body contouring. The main advantages of harvesting ADSCs compared to bone marrow are ease of access, safety, abundant sources, and low mortality risk. Unlike the bone marrow, AT is very easy to collate and use for tissue engineering in large quantities without needing an *ex vivo* expansion step [11].

Despite the broad applications and usefulness of SVF, no study has identified factors that affect the SVF cell yield and viability. Therefore, this study aimed to identify factors affecting the cell yield and viability of SVF to improve clinical application outcomes and optimize its clinical use.

Materials and method

We conducted a retrospective chart review of 121 patients who underwent liposuction to harvest AD-SVF at our stem cell center from June 2017 to August 2021. The study included all patients who received the procedure at our institution. The institutional

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ethics committee approved the study in October 2021 (protocol no. 2021-20/31), and all participants provided written informed consent.

We reviewed electronic medical records retrospectively for demographic, clinical, and operative variables and outcomes. We collected clinical data, including age, sex, body mass index (BMI), and blood type, from the hospital database. We also recorded medical comorbidities such as diabetes, hypertension, coronary artery disease, and smoking and alcohol use. We recorded the amount of lipoaspirate and SVF harvest sites, including the entire abdomen and lower abdomen. We also document SVF viability and cell count.

At harvesting

All operations were performed under sedoanalgesia in a sterile operating room. We used the tumescent technique and harvested lipoaspirates from the abdominal region. The same plastic surgeon performed all operations. Umbilical and suprapubic areas were selected as entry sites. A tumescent solution containing ringer lactate with 1:500.000 units of epinephrine and 20 mg bupivacaine hydrochloride was infiltrated through the 3-mm incisions and allowed to rest for 10 min to achieve hemostasis. The volume of the tumescent solution injected was 150 cc.

Following the hemostasis, a blunt cannula (the "Fat Grater") with a 2-mm diameter, the tip of which has 10 holes measuring 1.5 mm in diameter, was used to harvest the adipose aspirate (Figure 1). The cannula attached to a 60-ml syringe and gently pulling pack on the syringe's plunger provided a light negative pressure while the cannula was advanced and retracted through the harvest site. After filling the syringe with harvested tissue, the cannula was removed from the syringe, and the lipoaspirate was transferred into sterile 50-ml Falcon tubes for further enzymatic processing.

Isolation of the SVF, production, and assessment of quality

All production processes were carried out following Good Manufacturing Practices. Fat tissue samples taken from the patient were placed in Falcon 50-ml Polypropylene Conical Tube (Avenida Industrial del Norte S/N, Parque Industry del Norte, Reynosa, Tamaulipas, Mexico). A sample was drawn from the tissue with an injector, and an input sample was given for microbiological quality control.

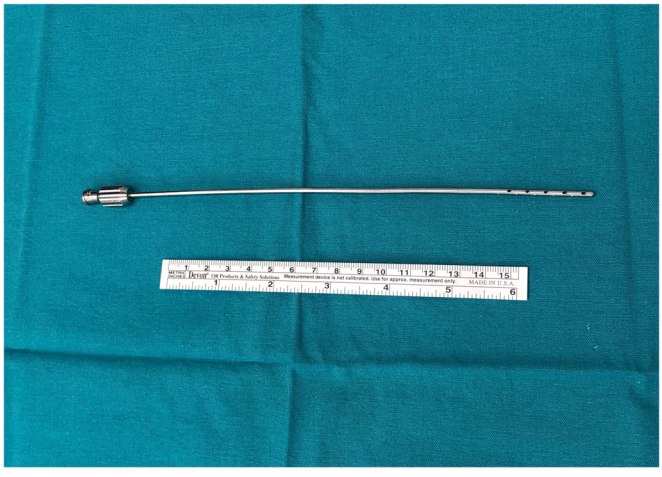

Figure 1. Fat grater with a 2-mm diameter and 10 holes in the tip (1.5-mm hole diameter).

The AT samples in the tubes were arranged in Falcon tubes, and collagenase enzyme (SIGMA-ALDRICH, St. Louis, MO, USA) was placed in each Falcon tube. The collagenase enzyme had been sterility controlled, and the endotoxin level was approved as less than 10 IU/ml before use. The caps of the tubes were tightly closed, and the tubes were placed in the shaker. The shaker was adjusted to 100 rpm, and the tubes were incubated for 60–90 min.

The tubes were placed directly in the centrifuge device and centrifuged at 500 × g for 10 min when the incubation was finished. After centrifugation, the supernatant was removed using a pipette, and the pellet containing the cells was manually vortexed.

The pellet was removed and collected in a single tube *via* pipette. Filtering was done into a new tube with Cell Strainer, and the volume was completed with Ringer Lactate Solution. The mix was spun in a centrifuge again for 10 min at 500 × g. Following the second round of spinning, the supernatant was removed, Ringer Lactate Solution was again added to the pellet in the desired amount, and the mixture was centrifuged for a third time at 500 x g for 10 min.

Quality control tests

To assure microbiological quality control after the final centrifugation, the sample was drawn from the supernatant with an injector and run through the BD BACTEC Automated Blood Culture System (BD, East Rutherford, NJ, USA) for aerobic and anaerobic cultures, and the fungus culture sample was obtained. Additionally, we analyzed endotoxin levels. Once the Gram staining was negative in the sampling, the product was released for microbiological culture results. Culture results were monitored for two weeks.

Statistical analysis

Patient data collected within the scope of the study were analyzed with IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp., Armonk, NY). Frequency and percentage were given for categorical data and median, minimum, and maximum descriptive values for continuous data. For comparisons between groups, we used the Mann-Whitney-*U* Test for two groups and the Kruskal-Wallis-*H* Test for three groups. The relationship between continuous variables was evaluated using Spearman's Correlation Test. The results were considered statistically significant when $p < 0.05$.

Results

Table 1 presents the study population's demographic data, BMI, blood type, and comorbidities. The study population consisted of 121 patients (90 women [74.4%] and 31 men [25.6%]). The median age of the study population was 57 years (ranging, from 18 to 85). The donor area for most patients ($n = 84$; 69.4%) was the lower abdominal region.

Table 2 presents the correlation analysis to assess the relationship between the viability rate, cell count, and patient factors. Cell count and age ($p < 0.001$; Figure 2), BMI ($p = 0.006$; Figure 3), and amount of lipoaspirate ($p < 0.001$; Figure 4) were significantly correlated. Age and BMI shared a low linear relationship with cell count, and the amount of lipoaspirate had a moderate linear relationship with cell count.

The viability and cell count were affected by several variables. Table 3 shows the results of the Mann Whitney-U and Kruskal Wallis-H tests to determine a significant difference between

demographic, clinical, and operative variables for viability and cell number. Sex had a statistically significant effect on viability ($p = 0.009$) and cell count ($p = 0.015$). The viability rate of men was significantly higher than women, while women had higher cell counts than men.

Patients with hypertension had prominently higher cell count (mean, 48×10^6; range 20.8 to 96×10^6) than subjects with normal blood pressure (mean, 41.5×10^6; range 10 to 90×10^6; $p = 0.024$). Patients who did not consume alcohol had significantly higher cell count (mean, 45.5×10^6; range, 10 to 96×10^6) than alcohol users (mean, 36×10^6; range, 12 to 52×10^6; $p = 0.024$). We found no statistically significant associations between other variables regarding cell viability ($p > 0.05$).

Table 1. Demographic, clinical, and operative data.

Characteristics ($N = 121$)	n (%) or Median (Range)
Sex	
Female	90 (74.4)
Male	31 (25.6)
Age (years)	57 (18–85)
BMI, kg/m^2	29 (18.6–57.8)
Blood type	
0+	37 (30.6)
0−	10 (8.3)
A+	45 (37.2)
A−	1 (0.8)
B+	18 (14.9)
AB+	8 (6.6)
AB−	2 (1.7)
Diabetes mellitus	26 (21.5)
Hypertension	41 (33.9)
Coronary artery disease	30 (24.8)
Smoking	26 (21.5)
Alcohol consumption	11 (9.1)
Donor zone	
Lower abdominal region	84 (69.4)
Entire abdominal region	37 (30.6)
Lipoaspirate amount (cc)	180 (25–300)
Cell count	45×10^6 ($107–96 \times 10^6$)

Abbreviation: BMI, body mass index.

Table 2. Evaluation of viability rate and cell count according to demographic and operative variables.0.006

Spearman's correlation		Age	BMI	Lipoaspirate amount	Cell count
Viability	Correlation coefficient	−0.074	−0.103	−0.006	−0.071
	P-value	0.418	0.261	0.946	0.438
Cell Count	Correlation coefficient	0.318	0.248	0.511	1.000
	P-value	**<0.001**	0.006	**<0.001**	–

Abbreviation: BMI, body mass index.

Discussion

SVF use in plastic surgeries is increasing due to the characteristics and the differentiation capacity of SVF cells [3]. SVF increases the survival of the fat graft by promoting neoangiogenesis [12,13] and can differentiate into AT, which leads to satisfactory long-term results [14]. Many cells are needed for optimal outcomes in rejuvenation, wrinkle reduction, wound healing, scar reduction, or breast augmentation [12,15–19]. This study aimed to determine the patient factors affecting the cell count and the cell viability in SVF. Surgeons could apply this knowledge to patient selection for optimal treatment outcomes. Additionally, understanding these factors can help manage patient expectations.

Previous studies have generally focused on the effects of sex, age, BMI, and donor area on the viability and cell count of SVF. Vilaboa et al. interpreted the effects of age on SVF yield in 52 women, reporting that age is a strong determinant of SVF yield in women [2]. Alaaeddine et al. reported that while age and sex did not affect the cell yield of SVF, BMI and harvest site had a significant impact [3]. Contrary to their report, we found a statistically significant relationship between cell count and age. We also found that cell count was significantly higher in women than men, but men had higher viability than women, indicating sex is a factor. We found a low linear relationship between cell count, age, and BMI. Tsekouras et al. published a study reporting that younger subjects had a higher differentiating capacity and efficiency in forming mature adipocytes than older subjects [20]. They also found that BMI significantly affected the quality of harvested tissue. In their study, ADSCs' proliferation capacity and functionality dramatically increased activity in some massively obese patients compared to lean controls. Likewise, we found higher cell counts in patients with high BMI; however, this relationship was low linear.

The donor area is another concern for SVF. Tsekouras et al. reported that the outer thigh region had the highest SVF yield

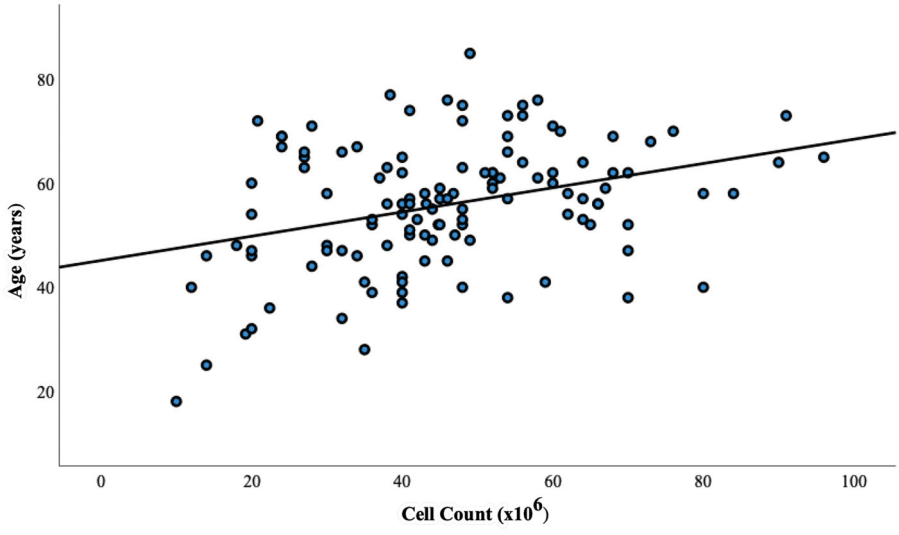

Figure 2. Correlation between age and cell count.

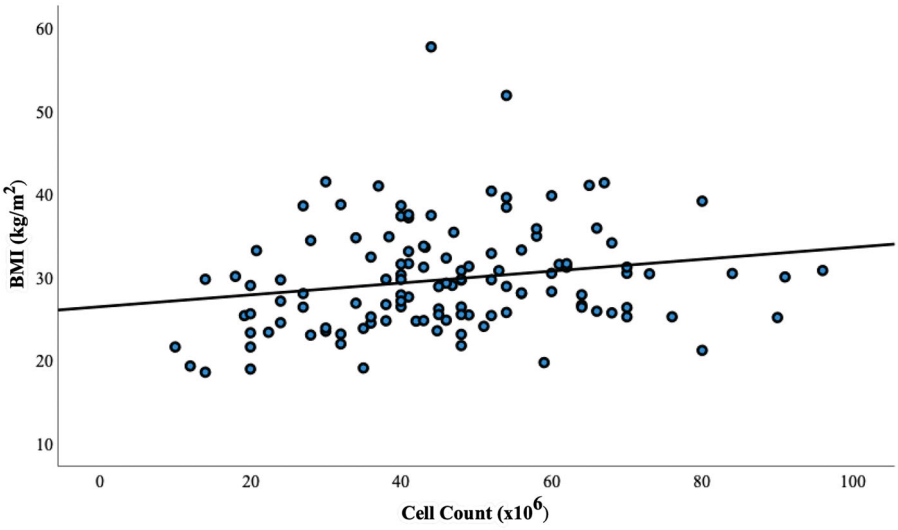

Figure 3. Correlation between body mass index and cell count.

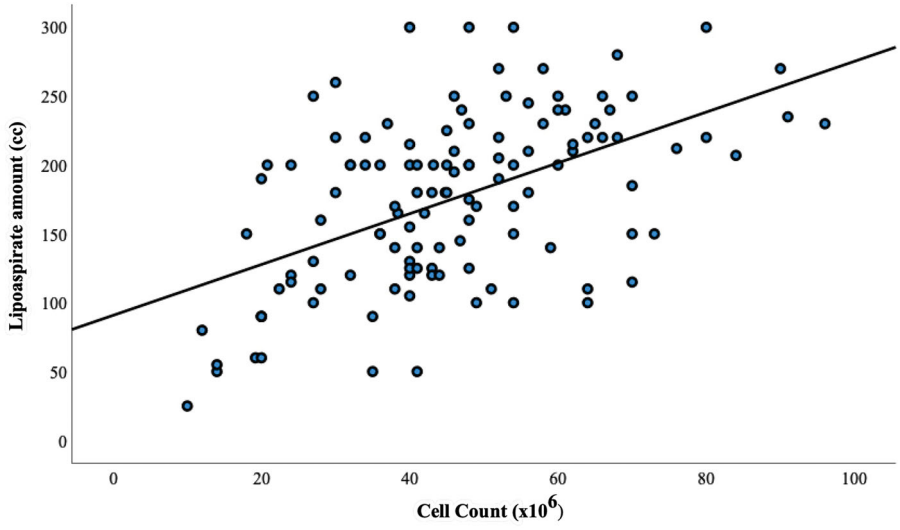

Figure 4. Correlation between lipoaspirate amount and cell count.

and the highest concentration of ADSCs compared to the other regions (e.g. abdomen, waist, and knee) [20]. However, Jurgens et al. found that the abdominal region was optimal for harvesting AT due to the higher ASCs yield [21]. Grasys et al. concluded that the abdomen might be a slight favorite over the inner thigh and knee because of its richer content of soluble factors such as matrix metalloproteinase-9 [22]. However, we found no relationship between donor sites and the cell count or cell viability of SVF. Likewise, in previous studies, AT from the abdomen, waist, thigh, and inner knee had no differences in cell viability [23,24].

Andjelkov et al. found that SVF viability was significantly negatively affected by age and smoking [25]. They have reported that increased physical activity deteriorated SVF cell viability [25]. Our results regarding age and viability were similar, but we found no statistical significance associated with smoking and viability.

The tumescent technique has been performed for local anesthesia to reduce surgical blood loss and the risks of general anesthesia [26]. We administered equal amounts of tumescent solution to all patients to eliminate bias regarding lipoaspirate volume. There was a statistically significant relationship between the cell count of lipoaspirate in this cohort.

We investigated the SVF cell count and viability using more comprehensive parameters than in previous studies. The effects of lipoaspirate amount, alcohol use, and comorbidities on SVF cell count and viability have not been studied before. No previous study has investigated the effects of hypertension and alcohol consumption on cell count. Also, our sample size ($n = 121$) was remarkably higher than in previous trials. Our results showed that hypertensive individuals had prominently higher cell counts than patients with normal blood pressure. This could be due to increased intraluminal pressure, which might have facilitated the cell yield. It is also possible that calcium channel blockers might deteriorate cellular attachment [27], thus increasing harvested cells. Patients who did not consume alcohol had significantly higher cell counts than alcohol users.

Sex was significantly associated with cell count and viability, while age, BMI, and lipoaspirate amount were associated with cell count. Hypertension and alcohol consumption were significant

Table 3. Distribution of viability rate and cell count by other characteristics.

Characteristics ($N = 121$)	Viability median (range)	*P*-Value	Cell count median (range)	*P*-Value
Sex		**0.009**		**0.015**
Female	**92 (85–97)**		**46,4 × 10^6 (12 × 10^6–96 × 10^6)**	
Male	**95 (88–100)**		**40 × 10^6 (10 × 10^6–80 × 10^6)**	
Blood type		0.659		0.577
0+	93 (88–100)		45 × 10^6 (12 × 10^6–91 × 10^6)	
0-	92 (85–100)		49 × 10^6 (14 × 10^6–80 × 10^6)	
A+	92 (85–99)		41 × 10^6 (14 × 10^6–96 × 10^6)	
A-	92 (92–92)		60 × 10^6 (60 × 10^6–60 × 10^6)	
B+	93 (90–97)		49 × 10^6 (10 × 10^6–84 × 10^6)	
AB+	92.5 (90–96)		41.5 × 10^6 (35 × 10^6–62 × 10^6)	
AB-	96.5 (95–98)		44.6 × 10^6 (19,2 × 10^6–70 × 10^6)	
Diabetes		0.939		0.432
No	92 (85–100)		44 × 10^6 (10 × 10^6–96 × 10^6)	
Yes	92.5 (85–97)		47.5 × 10^6 (24 × 10^6–91 × 10^6)	
Hypertension		0.560		**0.024**
No	93 (85–100)		**41.5 × 10^6 (10 × 10^6–90 × 10^6)**	
Yes	92 (85–97)		**48 × 10^6 (20,8 × 10^6–96 × 10^6)**	
Coronary artery disease		0.309		0,062
No	93 (85–100)		43.2 × 10^6 (10 × 10^6–96 × 10^6)	
Yes	92 (85–97)		52 × 10^6 (24 × 10^6–91 × 10^6)	
Smoking		0.714		0.198
No	93 (85–100)		45 × 10^6 (10 × 10^6–96 × 10^6)	
Yes	92 (86–100)		41 × 10^6 (14 × 10^6–84 × 10^6)	
Alcohol consumption		0.946		**0.024**
No	92 (85–100)		**45.5 × 10^6 (10 × 10^6–96 × 10^6)**	
Yes	93 (86–97)		**36 × 10^6 (12 × 10^6–52 × 10^6)**	
Donor zone		0.635		0.107
Lower abdomen	92 (85–99)		45.5 × 10^6 (12 × 10^6–96 × 10^6)	
Entire abdomen	93 (86–100)		41 × 10^6 (10 × 10^6–80 × 10^6)	

factors in cell count, which is the first such report of this relationship in the literature. Although this study had illuminating insights for medical teams regarding the potential success of treatments and helping to set patient expectations, we believe that randomized controlled trials incorporating a larger cohort of patients are warranted to verify our results.

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Disclosure statement

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