

Enhancement of Viable Adipose-Derived Stem Cells in Lipoaspirate by Buffering Tumescent with Sodium Bicarbonate

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Background: Fat grafting is a growing field within plastic surgery. Adipose-derived stem cells (ASCs) and stromal vascular fraction (SVF) may have a role in fat graft survival. Our group previously demonstrated a detrimental effect on ASC survival by the lidocaine used in tumescent solution. Sodium bicarbonate (SB) buffers the acidity of lidocaine. The purpose of this study was to determine whether SB buffering is a practical method to reduce ASC and SVF apoptosis and necrosis seen with common lidocaine-containing tumescent solution.

Methods: Human patients undergoing bilateral liposuction for any indication were included in this study. An internally controlled, split-body design was utilized. Tumescent liposuction on one side of the body was conducted with tumescent containing lidocaine. On the opposite side, liposuction was conducted by adding SB to the tumescent. Tumescent solution and lipoaspirate pH were measured. Lipoaspirate from each side was processed for SVF isolation and ASC culture. The number of viable ASCs was counted and SVF apoptosis/necrosis was examined.

Results: The pH of the SB-buffered tumescent was significantly higher than that of the standard tumescent, an effect also seen in the lipoaspirate. Adipose-derived stem cell survival in the SB-buffered lipoaspirate was approximately 53% higher. However, there was no significant difference in SVF apoptosis and necrosis between the groups.

Conclusions: The acidic standard tumescent solution commonly used in liposuction diminishes ASC viability from lipoaspirates. Sodium bicarbonate buffering tumescent solution can enhance ASC viability, but does not affect SVF apoptosis and necrosis. We recommend buffering tumescent with SB to potentially improve fat graft take. Our findings advocate for further research investigating mechanisms and optimal harvest techniques that maximize SVF/ASC survival and the clinical effect on overall fat graft viability. (*Plast Reconstr Surg Glob Open* 2019;7:e2138; doi: 10.1097/GOX.0000000000002138; Published online 20 March 2019.)

INTRODUCTION

Liposuction is a popular procedure in plastic surgery. Tumescent anesthesia is widely accepted as a standard technique in large-volume liposuction.^{1,2} Tumescent an-

esthesia can be achieved by infusing large volumes of Ringer's solution containing dilute anesthetic such as lidocaine with epinephrine.³⁻⁶ However, commercially available Ringer's solution and lidocaine and epinephrine are acidic and can cause perioperative and postoperative pain. Recently, several studies have demonstrated the benefits of neutralization of tumescent solution. For example, Wallace et al⁷ reported that buffering of tumescent anesthetic solution with sodium bicarbonate (SB) is a simple, safe, inexpensive, and effective means to reduce perioperative and postoperative pain. Best et al⁸ also showed that buffering tumescent solution to neutral pH with SB is a practical method to mitigate the burning sensation.

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Human lipoaspirate harvested by liposuction is an ideal filler for reconstruction of soft-tissue defects.^{9,10} The main drawback of fat grafting historically cited was graft resorption, which has shown graft volume loss to vary between 20% and 90% at 1 year after transplantation.¹¹⁻¹³ However, more recently, large-volume fat grafting has been performed reliably with high retention rates by adhering to principles of fat grafting: optimizing recipient site capacity, graft harvest/preparation, and precision graft delivery.¹⁴ In recent years, fat grafting using autogenic lipoaspirate with enrichment of adipose-derived stem cells (ASCs) or stromal vascular fraction (SVF) has gained popularity in aesthetic surgery.¹⁵⁻²⁰ Although ASCs and SVF are a minor fraction of lipoaspirate, investigators have postulated that their high potential for self-renewal, multilineage differentiation, and higher yield may compensate for some of the graft volume loss and may improve graft take.^{19,20} A thorough literature search indicated that the effect of SB on the viability of ASC and SVF has not been studied. The purpose of the present study is to determine whether acidic tumescent solution used in liposuction is harmful to ASC and SVF viability, and whether SB buffering is a practical method to reduce ASC and SVF cell death.

METHODS

Experimental Protocol

Human lipoaspirate was harvested from adults undergoing outpatient, cosmetic or reconstructive liposuction procedures with associated fat grafting. All the participants were provided informed consent and agreed to inclusion into the study. The Institutional Review Board at the University Medical Center approved all the protocols involving human tissue and cells. Lipoaspirate was harvested via standard liposuction techniques by a single plastic surgeon (R.C.B.). In brief, through a 4-mm incision, wetting solution was infiltrated into the subcutaneous fat at a ratio of 1:1 (infiltrate volume: aspirate volume). The lipoaspirate was procured using a blunt-tipped 3.7-mm Mercedes cannula, machine suction, and was collected in a sterile canister for processing. For quality control, only individuals who underwent liposuction on bilateral body areas were included. Under general anesthesia, tumescent liposuction on one side of the body was conducted with the standard tumescent (1,000 mL of lactated Ringer's solution with 30 mL of 1% lidocaine and 1 µg/mL epinephrine) without SB. On the opposite side, liposuction with SB buffering was conducted by adding 7 mL of 8.4% SB to the standard tumescent. The pH in the tumescent solution and lipoaspirate were measured in each sample. The harvested lipoaspirate from each liposuction was processed for SVF isolation. The number of adherent ASCs, reflecting viability, was counted after 24 hours of SVF culture. Live, apoptotic, and necrotic of SVF cells were stained by Annexin V-fluorescein isothiocyanate (FITC)/Propidium iodide (PI) and quantitatively analyzed by flow cytometry.

Isolation of SVF

The method for SVF isolation has been previously described by our group.²⁰⁻²⁵ Briefly, 5 mL of lipoaspirate from

each liposuction side was processed. Lipoaspirate samples were then centrifuged at 430g for 10 minutes. After oil removal, the lipid phase of the lipoaspirate was harvested from the top of the conical tube and then diluted with an equal volume of collagenase digestion solution (final concentration: 0.3 U/mL; Collagenase NB 4G proved grade, Serva Electrophoresis, Heidelberg, Germany). After 30 minutes of incubation, an equal volume of Dulbecco's Modified Eagle Medium containing 20% fetal bovine serum (FBS) was added to end enzymatic digestion. The floating layer containing adipocytes and the pellet containing SVF were separated by centrifugation. The isolated SVF was filtered through a 100-µm nylon filter and processed for density gradient by centrifugation with Histopaque-1077 (Sigma-Aldrich, St. Louis, Mo.). The white band (mononuclear cells) remaining at the plasma interface was carefully aspirated and the total number of SVF cells was counted. Harvested SVF was then either cultured in nonhematopoietic expansion medium (Miltenyi Biotec, Auburn, Calif.) for ASC purification or stained by Annexin V-FITC/PI and analyzed by flow cytometry.

Purification of ASCs through SVF Culture

SVF is highly heterogeneous and contains many cell subsets including ASCs, endothelial cells, hematopoietic cells, etc. One of the characteristics of ASC is that they are adherent to the plastic surface. Therefore, isolation of ASC can be achieved through SVF culture. In brief, all the isolated SVF cells from the lipoaspirate sample were added into a 75 cm² cell culture flask containing 15 mL of prewarm nonhematopoietic expansion medium and 1% of Penicillin–Streptomycin. The flask was cultivated at 37°C, 5% CO₂, and 95% humidity. After 24 hours of culture, the nonadherent cells in the flask were removed by PBS washing. Trypsin/ethylenediaminetetraacetic acid (EDTA) 1 mL was added into the flask and incubated at 37°C for 10 minutes. After complete dissociation, the total number of adherent ASC was harvested and counted.

Detection of Apoptotic and Necrotic Cells in SVF Populations

The apoptosis and necrosis of SVF cells were detected by Annexin V-FITC/PI assay.²⁰⁻²⁵ Briefly, 1 × 10⁵ SVF cells were washed with 1 mL of binding buffer followed by centrifugation. After supernatant removal, SVF cells were suspended in 100 µL of binding buffer with 10 µL of Annexin V-FITC or without (unstained control). Following 15 minutes of incubation in the dark, SVF cells were washed again by 1 mL of binding buffer and then centrifugation. After supernatant removal, SVF cells were suspended in 500 µL of binding buffer with 5 µL of PI or without (unstained control). Two tubes were used to set up compensation and quadrants with: (1) unstained and (2) stained with Annexin V-FITC and PI. Necrosis was determined by PI and apoptosis was determined by Annexin V-FITC. Ten thousand SVF cells from each sample were scanned and analyzed by flow cytometer. Data acquisition and analysis were performed by flow cytometer with BD FACS Aria III software v6.1.3 (Becton Dickinson, San Jose, Calif.) using an excitation wavelength of 488 nm with an argon laser.

Statistical Analysis

We have analyzed the continuous response variable from matched pairs of study subjects in our previous studies.^{21–26} Our prior preliminary data based on 7 subjects indicate that the difference in the response of matched pairs is normally distributed with SD 386.7 and the difference between means is 514.3. If the true difference of ASC number in the mean response of matched pairs is 514.3, we will need to study 6–8 pairs of subjects to be able to reject the null hypothesis with probability (power) 0.9 (90%). The Type I error probability (*P* value) associated with this test of this null hypothesis is 0.05.

RESULTS

The participants (*n* = 7/group) were all female adults. There were no children, no pregnant woman, or prisoners. There were no exclusions based on gender, race/ethnicity, or medical conditions. The study completed after 10 months of participant accrual. The average age of the participants was 57 ± 5.0 years (\pm standard error of the mean; SEM) and the average body mass index was 30 ± 2.0 (\pm SEM). Liposuction sites included the flank and abdomen. The pH of the modified tumescent solution with SB (Fig. 1) was significantly higher than standard solution (7.06 ± 0.05 versus 6.17 ± 0.1 , $P = 0.001$). The pH of lipoaspirate treated with SB (Fig. 2) was significantly higher compared with nonbuffered lipoaspirate (6.81 ± 0.06 versus 6.67 ± 0.03 , $P = 0.043$). The average number of viable ASCs in the lipoaspirate treated with SB (Fig. 3) was approximately 53% higher ($377,214 \pm 125,505$ versus $245,643 \pm 81,971$, $P = 0.028$) than that in the lipoaspirate from standard tumescent. In the Annexin V-FITC/PI assay for SVF viability (Fig. 4), however, we found that SB did not show any significant effect on SVF cell survival, apoptosis, and necrosis. In the tumescent with SB group, the average percentage of live SVF cells was $68\% \pm 10\%$, apoptotic cells were $23\% \pm 7\%$, and necrotic cells were $9\% \pm 4\%$. In the tumescent without SB group, the average percentage of live cells was $79\% \pm 7\%$, apoptotic cells were $20\% \pm 6\%$, and necrotic cells was $8\% \pm 4\%$. There was no significant difference between SB-treated and SB-untreated groups.

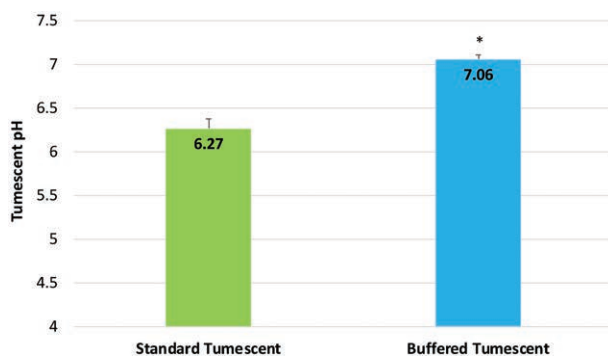


Fig. 1. The pH (\pm SEM) of the modified tumescent solution with SB (blue) was significantly higher than that of the standard tumescent solution (green; 7.06 ± 0.05 vs. 6.17 ± 0.1). * indicates statistically significant differences ($P = 0.001$) between tumescent with SB and tumescent without SB.

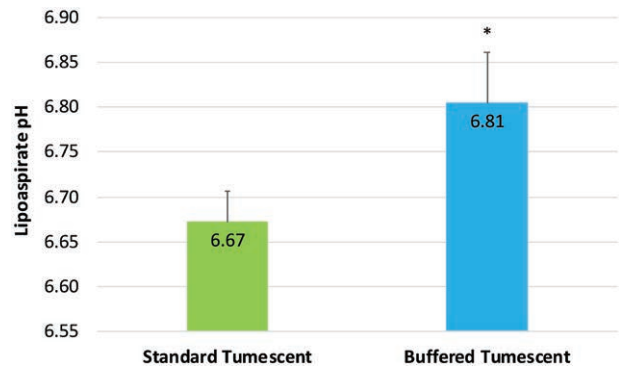


Fig. 2. The pH (\pm SEM) in the lipoaspirate treated with SB (blue) was significantly higher compared to that in the lipoaspirate treated with standard tumescent solution (green; 6.81 ± 0.06 vs. 6.67 ± 0.03). * indicates statistically significant differences ($P = 0.04$) between lipoaspirate with SB and lipoaspirate without SB.

Discussion

Several studies^{7,8} have shown that a tumescent-induced perioperative and postoperative burning sensation is related to the acidic pH of the solution. All the compositions in the standard tumescent solution, including lactated Ringer's solution, lidocaine, and epinephrine, are acidic. Data from other laboratories^{7,8} indicate that 1% lidocaine with epinephrine has a pH of 4.38, and even when diluted to 0.1%, the solution remains significantly below physiological pH at 6.32. Sodium bicarbonate is a chemical compound with the formula NaHCO_3 . It is a salt composed of sodium ions and bicarbonate ions. Postulating that the acidic nature of lidocaine contributed the pain on intradermal infiltration, multiple groups not only established the feasibility of using SB to neutralize lidocaine to a physiological pH, but also demonstrated pain attenuation.^{27,28} Since then, several reports^{29,30} have suggested that the neutralization of tumescent anesthetic solution with SB is a simple, safe, inexpensive, and effective means to reduce perioperative and postoperative pain.

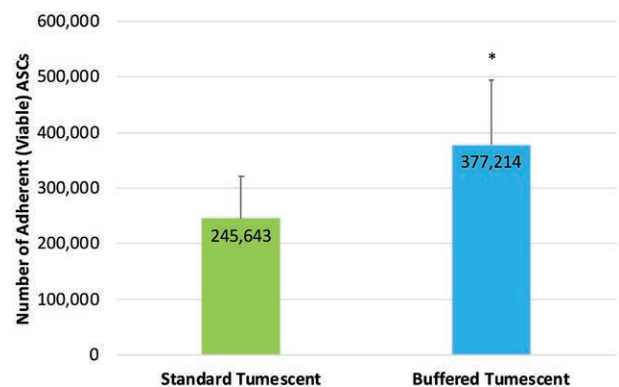


Fig. 3. Average number (\pm SEM) of adherent (viable) ASCs in the lipoaspirate treated with SB (blue) was approximately 53% higher ($377,214 \pm 125,505$ vs. $245,643 \pm 81,971$) than that in the lipoaspirate treated without SB (green). * indicates statistically significant differences ($P = 0.028$) between lipoaspirate with SB and lipoaspirate without SB.

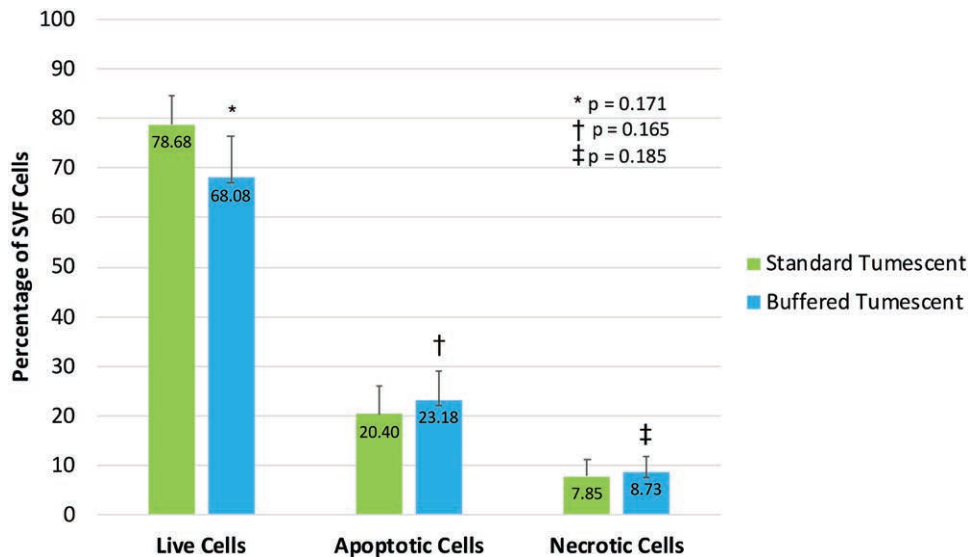


Fig. 4. In the Annexin V-FITC/PI assay for Please incorporate the following change: SVF viability, no significant difference was found on the average percentage (\pm SEM) of viable SVF cells between the buffered lipoaspirate and the lipoaspirate treated without SB. * $P = 0.171$, † $P = 0.165$, and ‡ $P = 0.185$.

Our previous studies^{21,26} have demonstrated that lidocaine has a negative impact on ASC survival. Removing lidocaine from tumescent solution significantly reduced SVF and ASC cells apoptosis from the standard tumescent liposuction with lidocaine. It is unclear if the toxicity of lidocaine to the cells is attributed to the acidic pH of tumescent solution. In the present study, we attempt to determine if the acidic tumescent solution used in liposuction is harmful to ASC and SVF viability, and if SB buffering is a practical method to reduce ASC and SVF cell death and apoptosis. Our results showed that adding 7 mL of 8.4% SB not only significantly neutralized tumescent solution to a physiological pH at 7.06 but also considerably increased lipoaspirate pH from 6.67 to 6.81. As a result of this pH modification in tumescent solution, the number of adherent ASC was significantly enhanced in the SB treated lipoaspirate, which was 53% higher than that in the untreated lipoaspirate.

In the present study, cell viability of the adherent ASCs was not measured. One of the unique characteristics of viable ASCs is that they adhere to plastic surfaces. This phenomenon is the foundation for current ongoing ASC research.^{31–33} Most nonadherent cells (either non-ASCs or dead ASCs) in the flask were removed by PBS washing after 24 hours culture of SVF cells. In our previous studies,^{21–26} we found that about 90% of adherent cells to the plastic surface after 24 hours culture of SVF were viable (negative for both Annexin V-FITC and PI) and the ethanol-treated dead ASCs were unable to adhere to the plastic surface in the culture flask. We believe that the dead ASCs may lose their ability to adhere and could have been removed by PBS washing or medium change. Therefore, the adherent cells from SVF culture can be defined not only as ASCs but also as viable ASCs.

Just like ASCs, SVF possesses similar potential in regenerative medicine and has far-reaching clinical implications.^{11–13,15–19} Some investigators have suggested that

SVF may even have an advantage over ASCs because of the presence of endothelial progenitor cells, pericytes, immune cells, and other stromal components along with the ASCs.¹¹ Stromal vascular fracture cells are relatively easy and quick to obtain in large quantities without the need for processing or cell culture; therefore, both liposuction and SVF transplantation procedures can be accomplished in the same day. Moreover, the clinical application of SVF is not currently restricted by the United States Food and Drug Administration (FDA). In recent years, liposuction followed by fat grafting with SVF enrichment has gained popularity in aesthetic surgery. Several studies^{15–18} have shown SVF to be effective as a regenerative cell therapy in treating chronic conditions ranging from arthritis, diabetes mellitus, chronic wounds, breast cancer, and radiation injuries. However, SVF viability in the lipoaspirate before fat grafting is not well described in the literature. The amount of SVF in the lipoaspirate can be variable among different populations such as overweight versus lean, aged versus young, diabetic versus healthy, different locations such as abdomen versus thigh, etc., and different liposuction techniques.^{34–36} To control these variances, we standardized the liposuction techniques with a single plastic surgeon and excluded individuals who underwent liposuction only on unilateral body areas. Therefore, the influence of the variances is largely diminished or controlled because both samples (with SB or without SB) came from the symmetrical pairs of the same individual.

One of the purposes of the present study was to determine whether acidic tumescent solution used in liposuction is harmful to SVF viability, and whether SB buffering can reduce SVF cell death and apoptosis in the lipoaspirate. Stromal vascular fracture apoptosis and necrosis were examined by Annexin V-FITC/PI staining with flow cytometry. Unfortunately, our results did not detect a significant difference in SVF apoptosis and necrosis between SB-treated and SB-untreated lipoaspirates. This is

probably due to the fact that SVF contains multiple cell populations, whereas ASCs are a single cell population, so cell numbers of certain types may change without affecting the total SVF. Buffering tumescent solution with SB may be sensitive to ASCs, but not sensitive to other cell populations of SVF. Nevertheless, further study is warranted to explore the mechanism.

Additionally, although our results demonstrate an improvement in ASC viability following bicarbonate buffering, it is unclear whether this effect translates to an enhanced outcome with respect to fat graft survival and volume retention over time. This question is important clinically and is an area of current investigation by our group.

CONCLUSIONS

The acidic standard tumescent solution used in liposuction adversely affects the cell viability of ASCs isolated from lipoaspirates. Buffering tumescent solution with SB can significantly enhance ASC viability. The implication of potentially improved fat graft take is promising and prompts our continued investigation.

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