Title
Phenotypic Analysis of Stromal Vascular Fraction after Mechanical Shear Reveals Stress-Induced Progenitor Populations.

Journal
Plastic and reconstructive surgery, 138(2)

ISSN
0032-1052

Authors
Banyard, Derek A
Sarantopoulos, Christos N
Borovikova, Anna A
et al.

Publication Date
2016-08-01

DOI
10.1097/prs.0000000000002356

Peer reviewed

Published in final edited form as:

Plast Reconstr Surg. 2016 August ; 138(2): 237e–247e. doi:10.1097/PRS.0000000000002356.

Phenotypic Analysis of Stromal Vascular Fraction After Mechanical Shear Reveals Stress-induced Progenitor Populations*

Derek A. Banyard, MD, MBA[1], **Christos N. Sarantopoulos**[1], **Anna A. Borovikova, MD**[1], **Xiaolong Qiu, BS**[2], **Garrett A. Wirth, MD, MS**[1], **Keyianoosh Z. Paydar, MD**[1], **Jered B. Haun, PhD**[2,3,4], **Gregory R.D. Evans, MD**[1,2], and **Alan D. Widgerow, MBBCh, MMed**[1]

[1]Center for Tissue Engineering, Department of Plastic Surgery, University of California, Irvine

[2]Department of Biomedical Engineering, University of California Irvine, Irvine, CA 92697

[3]Department of Chemical Engineering and Materials Science, University of California Irvine, Irvine, CA 92697

[4]Chao Family Comprehensive Cancer Center, University of California Irvine, Irvine, CA 92697

INTRODUCTION

The discovery of adipose-derived stem cells (ADSCs) in human adipose tissue has introduced a new and exciting area of regenerative medicine with potential applications far beyond the scope of plastic surgery.[1–3] For aesthetic and reconstructive purposes, ADSCs and stromal vascular fraction (SVF), the starting material from which ADSCs are derived, have been used as adjuncts to more traditional fat grafting techniques, leading to the concept of cell-assisted lipotransfer (CAL).[4–8] The addition of mesenchymal stem cells (MSCs) to injectable autologous fat tissue has shown to increase the vascularity of transplanted tissue and thus promote fat graft survival.[5] Recently, attention has shifted towards the use of SVF for its comparable regenerative properties and ease of collection when compared to traditional ADSCs.[9–12]

*Presented at International Federation for Adipose Therapeutics and Science Annual Meeting, New Orleans, LA, 2015; European Plastic Surgery Research Council Annual Congress, Hamburg, Germany, 2015; British Association of Plastic Reconstructive and Aesthetic Surgeons and Royal Belgian Society for Plastic Surgery Combined Summer Scientific Meeting, Concertgebouw, Bruges, Belgium, 2015; American Society of Plastic Surgeons Aesthetica Super Symposium, Las Vegas, NV, 2015.

Corresponding Author: Alan D. Widgerow, MBBCh, MMed, FCS, FACS, Clinical Professor, Director, Center for Tissue Engineering, Department of Plastic Surgery, 200 S. Manchester Avenue, Suite 650, Orange, CA 92868, Phone: (714) 456-3482, Fax: (714) 456-7718, awidgero@uci.edu.

Financial Disclosure and Products

There are no other relevant financial disclosures.

Author's contributions

DB participated in conception, experimental design, acquisition of data, analysis and interpretation of data, drafting and revision of the final manuscript. CS and AA participated in acquisition of data, analysis and interpretation of data, drafting and revision of the final manuscript. XQ and JH participated in experimental design, acquisition of data, analysis and interpretation of data, drafting and revision of the final manuscript. GW and KP participated in acquisition of data and revision of the final manuscript. GE and AW conceived of the study and participated in its design, analysis and interpretation of data, and final editing of the manuscript. All authors read and approved the final draft.

In 2013 Tonnard *et al.* first reported on 'nanofat grafting,' a technique where autologous fat is utilized as a superficial injectable to improve skin quality in delicate areas.[13] As described in the original article, *nanofat* is obtained by repeatedly shuffling lipoaspirate (LA) between two interconnected syringes. The resulting emulsion is filtered and injected in appropriate anatomic areas with a small-bore needle. The authors reported good clinical results using this technique and demonstrated the ability to culture ADSCs capable of adipocyte differentiation from this mix. Ironically, while this group did not examine the various cellular components of *nanofat*, they revealed that this mix is actually devoid of adipoctyes.[13]

The former study has raised an interesting question since mechanical stress is an inherent part of any fat grafting technique. Mechanical forces are at play via negative pressure at the time of harvesting and positive pressure at the time of injection, with shear stress affecting the LA as it moves through the cannula in either direction. Traditionally, the success of fat grafting in plastic surgery has been linked to the number of viable adipocytes in the transplanted tissue, thus many authors have compared fat processing techniques in terms of adipocyte viability. While most of these studies have found an inverse relationship between the degree of applied force and number of intact adipocytes,[14–16] only a few have looked at the concentration and characteristics of the stem cells in the processed lipoaspirate. Bianchi *et al.* demonstrated that mild mechanical force increases the number of pericytes, pericyte-like elements, and MSCs in processed lipoaspirate.[17] Similarly, Conde-Green *et al.* found that the more aggressive the processing protocol, e.g. decantation vs. washing vs. centrifugation, the higher the concentration of stem cells found in the resulting product.[14,15]

Recently, a new population of mesenchymal stem cells has been described: multilineage differentiating stress-enduring (Muse) cells. The adipose-derived variant of these cells was first defined as a population isolated under conditions of extreme stress,[18] but have since been shown to also exist in native tissue.[19,20] These cells are of particular interest in regenerative medicine due to a presence of numerous pluripotency markers,[18] low telomerase activity,[20] and transplants involving these cells lack teratoma formation[20] and have been shown to significantly enhance wound healing in a murine model of diabetic ulcers.[19]

We hypothesized that the mechanical stress conveyed by *nanofat* processing results in modulation of its progenitor populations. In this study, we compare the cellular and phenotypic profiles of SVF recovered from standard LA to that of fat that was mechanically emulsified. Additionally, we explore the relationship between adipose tissue viscosity and the factors inherent to the common tools employed in the various forms of fat grafting.

MATERIALS AND METHODS

Lipoaspirate Collection & Processing

This study was conducted in accordance with the regulations of the University of California, Irvine Institutional Review Board (IRB# 2011-8236/2015-2181). Standard vacuum-assisted liposuction was used to obtain lipoaspirate from the abdomen and flanks of patients ($n = 10$) undergoing routine elective procedures at UCI Medical Center. The LA was transferred to a

large Erlenmeyer flask and washed with sterile phosphate-buffered saline (PBS) multiple times until golden in color. The LA was then divided into 2 samples. One sample was left unprocessed (control) while the second was processed as previously described *nanofat*.[13] Briefly, 10 mL of LA was loaded into a syringe coupled to a second syringe via a female-to-female luer lock connector. The lipoaspirate was then transferred vigorously between the two syringes for 30 passes at a rate of approximately 20 mL/s. These steps were repeated until all the fat from the experimental group was mechanically processed. Prior to enzymatic processing for the collection of the SVF, viscosity measurements were taken from both samples.

Flow and Shear Force Properties

The kinematic viscosity of fat samples was measured before and after emulsification using a Brookfield #2 dip viscosity cup (Middleboro, MA). The expected type of fluid flow passing between the syringes was determined by calculating the Reynolds number (Re) as follows:

$$Re=\frac{2Q}{\pi\nu R} \quad (1)$$

where Q is the volumetric flow rate, ν is the kinematic viscosity, and R is the radius of the luer coupler joining the syringes. Due to the high viscosity of the fat samples, we determined that fluid flow would be laminar under all conditions. For fully developed laminar flow within a tube, the fluid velocity (v) depends on radial position r, with a maximum fluid velocity (v_{max}) at the center of the coupler given by:

$$v_{max}=\frac{2Q}{\pi R^2} \quad (2)$$

Finally, shear force magnitude was quantified by calculating the wall shear stress (τ_w) as follows:

$$\tau_w=\frac{4\mu Q}{\pi R^3} \quad (3)$$

where μ is the dynamic viscosity of the fluid. We estimated μ by multiplying ν by the density of water. We assume that our fat samples will have a lower density than water, so this estimate would represent an upper bound for μ. Viscosity measurements are reported in units of centistokes (cSt).

SVF Isolation

The control and emulsified lipoaspirate were processed for SVF as previously described.[21] Briefly, a 0.1% enzymatic digestion solution was prepared by combining type I collagenase (Sigma-Aldrich Co., St. Louis, MO) with PBS which was then sterilized using a 0.22 μm vacuum filter (Millipore Corp., Billerica, MA). A 1:1 volume of collagenase solution to LA

was incubated in a water bath at 37^{O}C for 30 minutes, swirling intermittently. An equal volume of control medium (Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, 500 IU Penicillin, 500 μg Streptomycin) was then added to neutralize enzymatic activity and the mixture was allowed to separate for at least 10 minutes. The liquid infranatant layer containing the SVF was isolated, filtered through a 100 μm cell strainer (Corning Inc., Durham, NC), and centrifuged at 1800 rpm for 8 minutes. Each pellet was then resuspended in red blood cell lysis buffer (15.5 mM NH_4Cl, 1 mM $KHCO_3$, and 0.01 mM EDTA) for 5 minutes to minimize erythrocyte contamination. After the addition of 5 mL control medium, the suspensions were centrifuged one final time. The aqueous portions were removed by aspiration after each centrifugation step. The resulting pellets were then resuspended in control media and subjected staining and analysis.

SVF Analysis

A portion of the freshly isolated SVF obtained from each sample was subjected to acridine orange/propidium iodide staining (AO/PI, Logos Biosystems Inc., Annandale, VA) and quantified using a dual fluorescence automated cell counter (Logos Biosystems Inc., Annandale, VA) which delineates live versus dead and nucleated versus non-nucleated cells. Single-cell suspensions were then aliquoted into polystyrene tubes and maintained on ice for flow cytometry staining. For mesenchymal stem and stromal vascular fraction characterization (Table 1), cells were simultaneously stained with 5 μL each of the following monocolonal mouse anti-human antibodies: CD45-VioBlue, CD31-FITC, CD34-PerCP, CD146-APC, CD13-APC-Vio770, CD73-PE (Miltenyi Biotec Inc., San Diego, CA). For Muse cell quantification, cells ($n = 6$) were stained concurrently with 5 μL of CD13-APC-Vio770 and rat anti-human SSEA-3-PE (Stemgent, Lexington, MA). CD13 was used instead of CD105 due to its increased stability as a known MSC marker.[22] A portion of each sample was also stained with PI (Miltenyi Biotec Inc., San Diego, CA). Samples were fixed with 2% paraformaldehyde and multi-parameter data files were acquired on a three-laser MACSQuant analyzer (Miltenyi Biotec Inc., San Diego, CA) at a maximum flow rate of 25 μL/minute. Threshold and initial gating were set on PI fluorescence independently using the absolute cell count function of the cytometer to exclude subcellular debris and up to 2 million events were acquired per sample. Initially, regions were set with the appropriate isotype controls. Analysis was performed using MACSQuantify software (Miltenyi Biotec Inc., Bergisch Gladbach, Germany).

Statistical Analysis

Summarized results are given as arithmetic means ± SEM to indicate their precision. Mean values of cell count/viability, individual phenotypic marker expression, and subpopulation analysis were compared between groups using an unpaired t test, with values of $p < 0.05$ indicating significance.

RESULTS

Flow and Shear Force Properties

Kinematic viscosity was first measured to estimate the hydrodynamic flow and shear force properties experienced by samples over the course of fat emulsification. We found values of

86.9 ± 7.3 and 47.5 ± 1.5 cSt for samples prior to and after treatment, respectively (figure 1). Estimating the processing flow rate at 20 mL/s and the luer coupler radius at 0.074 in, we calculated Reynolds numbers of 78 and 143 using Equation 1. These values are well within the expected range for laminar flow. The maximum flow velocity at the center of the luer coupler was determined using Equation 2, which was 3.6 m/s for all conditions. Finally, wall shear stresses were calculated using Equation 3 to be 3326 and 1818 dynes/cm^2 for control and *nanofat* samples, respectively (figure 1). For comparison, the wall shear stress within the blood stream ranges from approximately 1 in post-capillary venules to 10 dynes/cm^2 in large arteries.

Stromal Vascular Cell Viability & Phenotypic Analysis

The average number of cells recovered from the SVF of standard lipoaspirate did not differ from that of the mechanically emulsified fat (siSVF) when quantified via a dual fluorescence cell counter (2.40×10^6 vs 2.25×10^6, respectively, p = 0.814). Interestingly, we observed a statistically significant reduction in the percentage of viable cells after analysis on the flow cytometer using the absolute cell count function (82.0% vs 48.1%, respectively, p = 0.004) (figure 2).

Stress-induced SVF (siSVF) demonstrated significantly higher expression of all phenotypic markers investigated when compared to the SVF of standard LA (mean ± SEM, SVF vs. siSVF). Most impressive was the nearly 3-fold upregulation of CD34 in siSVF, a universal marker of stem cell activity[23] (6.61% ± 1.4 vs. 17.14% ± 2.3, p = 0.001) (figure 3). When comparing MSC markers,[12] we observed a ~3-fold increase in CD13 (4.89% ± 1.3 vs. 13.5% ± 2.1, p = 0.003), a >2-fold rise in CD73 (4.79% ± 0.9 vs. 10.1% ± 1.3, p = 0.003), and a 2-fold increase in CD146 (7.70% ± 1.7 vs. 16.3% ± 3.4, p = 0.03) (figure 3). Interestingly, siSVF also exhibited a 2-fold increase in the expression of the hematopoietic marker CD45 (2.92% ± 0.26 vs. 5.30% ± 0.61, p = 0.004), as well increased expression of the endothelial marker CD31 (2.03% ± 0.15 vs. 4.14% ± 0.73, p = 0.018) (figure 3).

Finally, we looked at subpopulations to detect the presence of specific progenitor cells as well as multipotent MSCs and pluripotent Muse cells. Concurrent with the upregulation of CD34, we witnessed a >3-fold increase in this CD34$^+$/CD45$^-$ population (4.36% ± 1.5 vs. 13.8% ± 2.6, p = 0.006) (figure 4). Further subtype analysis of this population revealed ~3-fold increases in endothelial progenitor cells (EPCs: CD45$^-$/CD34$^+$/CD31$^+$/CD146$^+$, 2.20% ± 1.0 vs. 6.94% ± 1.6, p = 0.025) and ADSCs (CD45$^-$/CD31$^-$/CD13$^+$/CD73$^+$, 1.01% ± 0.3 vs. 3.11% ± 0.8, p = 0.024). There was no statistical difference in the expression of pre-adipocytes (CD45$^-$/CD31$^-$/CD34$^+$/CD146$^-$), transitional cells (CD45$^-$/CD31$^-$/CD34$^+$/CD146$^+$) or pericytes (CD45$^-$/CD31$^-$/CD34$^-$/CD146$^+$, figure 5). Interestingly, we also observed a near 3-fold increase in Muse cells (CD13$^+$/SSEA-3, 2.99% ± 1.3 vs. 8.70% ± 2.1, p = 0.046) (figure 6).

DISCUSSION

The shuffling of standard lipoaspirate, or “intersyringe processing,” is a common technique employed throughout the world for the purposes of lipofilling. This technique results in mechanically refined adipose tissue that can be freely injected through smaller cannulas and

syringes.[24] Similarly, *nanofat* processing is an extreme version of this that has been demonstrated effective for the correction of superficial rhytides and pigmentation.[13] While both techniques appear to be safe and effective, little is known with regards to the effect these various techniques have on the different components of the tissue and its cellular phenotypes.

Osinga *et al.* studied intersyringe processing at a flow rate of 10 mL/s which is roughly half of that used to create *nanofat*.[24] The key variable in their study involved a luer stopcock with a 90-degree bend and an internal diameter of 2 mm which is smaller than our luer fitting (3.76 mm). The combined effect of the lower flow rate and smaller stopcock diameter essentially cancel each other out with respect to Re (Equation 1), so we would still expect laminar flow if the samples had similar viscosities. Under these conditions, we do not believe that the 90-degree bend of their stopcock would introduce a significant effect on flow properties. Maximum flow velocity (Equation 2) and wall shear stress (Equation 3) are more strongly affected by diameter, and thus would be 1.8- and 3.3-fold higher, respectively. Interestingly, Osinga's group didn't detect any decline in the number or viability of cells recovered, and were able to generate ADSCs from this tissue that exhibited typical multipotentiality.[24]

Conversely, Tonnard *et al.* shuffled adipose tissue at a flow rate of 20 mL/s to create *nanofat*.[13] This group used calcein AM/PI staining to recognize that this technique results in the destruction of the majority of the adipocytes, rendering the term 'nanofat' a misnomer. Interestingly, they recovered a larger fraction of $CD34^+$ cells from the SVF of standard LA than from the SVF of *nanofat*, which is in contrast to our findings. Ultimately, this group found no difference in the morphology or culture characteristics of the cells cultured from SVF of any of the treatment groups. And while this group did not observe any difference in the ability of either population to differentiate into adipocytes, they did not go any further to characterize the individual components of the SVF, the raw material of which they actually injected to achieve the favorable cosmetic results observed.

During our creation of emulsified fat using the *nanofat* technique, we noted some significant findings. Most notably, this method results in the increased presence of EPCs, multipotent MSCs including ADSCs, and pluripotent Muse cells. And though the associated physical trauma resulted in a decreased percentage of viable cells, the surviving populations described above are associated with an increased regenerative capacity that may account for the favorable aesthetic results reported by Dr. Tonnard *et al.* Unlike the *nanofat* study, we digested the emulsified fat without employing a filtration step. It is likely that the mechanical shear stress liberates many of the SVF cells from the adipose matrix inherent to fat. However, internal experiments in our lab have revealed that some SVF cells are retained in the filtrand, and we wanted to capture all potential cells for complete analysis.

From the standpoint of stress, standard lipoaspirate is ideal for the mechanical force generation of increased progenitor cell populations in SVF (figure 1). Lipoaspirate has a viscosity that is twice that of *nanofat*, but still results in laminar flow when passed between two syringes (Equations 1 & 2). Based on the equation for wall shear stress (equation 3), the smallest diameter of a given processing system has the greatest impact on shear stress, and

thus the most logical variable to manipulate when exploring the effects that shear has on progenitor phenotype modulation and the mechanical disruption of cells and connective tissue.

Moving forward, we hypothesize that there exist a continuum whereby the amount of stress applied to immature progenitor cells is directly proportional to the multi and/or pluripotentiality induced. The question remains whether this phenotypic advantage translates to improved *in vitro* performance and ultimately a clinical benefit for the patient. Heneidi *et al.* originally demonstrated that Muse cells are superior to ADSCs in their efficient differentiation into cells of mesenchymal, endodermal and ectodermal lineages.[18] Allied to this regenerative potential, Kinoshita *et al.* showed that these cells significantly enhance wound healing in a murine model of diabetic ulcers.[19]

Curiously, Tonnard's group observed a higher ratio of $CD34^+$ cells in the SVF of standard lipoaspirate when compared to *nanofat*.[13] This phenomenon is in contrast to our findings and might be explained by the proportion of stem cells left behind in the filtrand that was never included in their final analysis. Indeed, CD34 is emerging as one of the most important/common markers of multipotentiality.[23] Our unique combination of phenotypic markers for the identification of ADSCs, EPCs, pre-adipocytes and pericytes (Table 1) is based on the findings of Zimmerlin et al.[25] and those most commonly cited in the literature.[11,12] Currently, we are exploring various potential mechanisms of regenerative action including the contributions that autophagy,[26] microparticles and extracellular matrix play in the induction of these progenitor populations. Ultimately, we will assess whether siSVF leads to improved fat graft retention in an animal model of cell-assisted lipotransfer.

The most significant shortcoming of our study involves the mechanical processing of lipoaspirate. When manually shuffling fat between syringes, there were often times where connective tissue would clog the coupler, and a greater force was needed to overcome this blockage resulting in a flow rate much greater than 20 mL/s. Additionally, the manual processing used to generate emulsified tissue is inherently variable, therefore our group is developing a device to automate this process and standardize the tissue obtained for further *in vitro* and *in vivo* studies.

CONCLUSION

Mechanical emulsification of standard lipoaspirate in the form of intersyringe and *nanofat* processing is not an inert process that simply breaks down adipose tissue for easy grafting. Mechanical shear stress is always created in these techniques which may lead to the upregulation of multipotent and pluripotent makers that connote a regenerative capacity. It is still unclear whether this phenotypic advantage alone translates to a clinical benefit; therefore further studies are needed to elucidate the mechanisms at play.

Acknowledgments

This work was supported in part by the California Institute for Regenerative Medicine training grant stipend TG2-012252 which was awarded to Dr. Banyard from the University of California, Irvine. Additional funding was provided by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health: Grant UL1 TR00015. The sponsors did not have any role in the study

design, collection of information, analysis, or interpretation of the data nor did they participate in the writing of the manuscript or the decision to submit the manuscript for publication.

References

1. Gutierrez-Fernandez M, Otero-Ortega L, Ramos-Cejudo J, Rodriguez-Frutos B, Fuentes B, Diez-Tejedor E. Adipose tissue-derived mesenchymal stem cells as a strategy to improve recovery after stroke. Expert Opin Biol Ther. 2015; 15(6):873–881. [PubMed: 25959243]
2. Richardson SM, Kalamegam G, Pushparaj PN, et al. Mesenchymal Stem Cells in Regenerative Medicine: Focus on Articular Cartilage and Intervertebral Disc Regeneration. Methods. 2015
3. Suzuki E, Fujita D, Takahashi M, Oba S, Nishimatsu H. Adipose tissue-derived stem cells as a therapeutic tool for cardiovascular disease. World J Cardiol. 2015; 7(8):454–465. [PubMed: 26322185]
4. Kolle SF, Fischer-Nielsen A, Mathiasen AB, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. Lancet. 2013; 382(9898):1113–1120. [PubMed: 24075051]
5. Matsumoto D, Sato K, Gonda K, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue engineering. 2006; 12(12): 3375–3382. [PubMed: 17518674]
6. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. Aesthetic Plast Surg. 2008; 32(1):48–55. discussion 56–47. [PubMed: 17763894]
7. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for facial lipoatrophy: efficacy of clinical use of adipose-derived stem cells. Dermatol Surg. 2008; 34(9):1178–1185. [PubMed: 18513295]
8. Tocco I, Widgerow AD, Lalezari S, Banyard D, Shaterian A, Evans GR. Lipotransfer: the potential from bench to bedside. Ann Plast Surg. 2014; 72(5):599–609. [PubMed: 24732078]
9. Charles-de-Sa L, Gontijo-de-Amorim NF, Maeda Takiya C, et al. Antiaging treatment of the facial skin by fat graft and adipose-derived stem cells. Plastic and reconstructive surgery. 2015; 135(4): 999–1009. [PubMed: 25811565]
10. Paik KJ, Zielins ER, Atashroo DA, et al. Studies in Fat Grafting: Part V. Cell-Assisted Lipotransfer to Enhance Fat Graft Retention Is Dose Dependent. Plastic and reconstructive surgery. 2015; 136(1):67–75.
11. Nguyen A, Guo J, Banyard DA, et al. Stromal vascular fraction: A regenerative reality? Part 1: Current concepts and review of the literature. J Plast Reconstr Aesthet Surg. 2015
12. Guo J, Nguyen A, Banyard DA, et al. Stromal vascular fraction: A regenerative reality? Part 2: Mechanisms of regenerative action. J Plast Reconstr Aesthet Surg. 2015
13. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H. Nanofat grafting: basic research and clinical applications. Plastic and reconstructive surgery. 2013; 132(4):1017–1026. [PubMed: 23783059]
14. Conde-Green A, Baptista LS, de Amorin NF, et al. Effects of centrifugation on cell composition and viability of aspirated adipose tissue processed for transplantation. Aesthet Surg J. 2010; 30(2): 249–255. [PubMed: 20442104]
15. Conde-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg. 2010; 63(8):1375–1381. [PubMed: 19679523]
16. Hoareau L, Bencharif K, Girard AC, et al. Effect of centrifugation and washing on adipose graft viability: a new method to improve graft efficiency. J Plast Reconstr Aesthet Surg. 2013; 66(5): 712–719. [PubMed: 23357708]
17. Bianchi F, Maioli M, Leonardi E, et al. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell transplantation. 2013; 22(11):2063–2077. [PubMed: 23051701]

18. Heneidi S, Simerman AA, Keller E, et al. Awakened by cellular stress: isolation and characterization of a novel population of pluripotent stem cells derived from human adipose tissue. PloS one. 2013; 8(6):e64752. [PubMed: 23755141]

19. Kinoshita K, Kuno S, Ishimine H, et al. Therapeutic Potential of Adipose-Derived SSEA-3-Positive Muse Cells for Treating Diabetic Skin Ulcers. Stem Cells Transl Med. 2015; 4(2):146–155. [PubMed: 25561682]

20. Ogura F, Wakao S, Kuroda Y, et al. Human adipose tissue possesses a unique population of pluripotent stem cells with nontumorigenic and low telomerase activities: potential implications in regenerative medicine. Stem Cells Dev. 2014; 23(7):717–728. [PubMed: 24256547]

21. Banyard DA, Salibian AA, Widgerow AD, Evans GR. Implications for human adipose-derived stem cells in plastic surgery. Journal of cellular and molecular medicine. 2015; 19(1):21–30. [PubMed: 25425096]

22. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013; 15(6):641–648. [PubMed: 23570660]

23. Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cells. 2014; 32(6):1380–1389. [PubMed: 24497003]

24. Osinga R, Menzi NR, Tchang LA, et al. Effects of intersyringe processing on adipose tissue and its cellular components: implications in autologous fat grafting. Plastic and reconstructive surgery. 2015; 135(6):1618–1628. [PubMed: 26017597]

25. Zimmerlin L, Donnenberg VS, Rubin JP, Donnenberg AD. Mesenchymal markers on human adipose stem/progenitor cells. Cytometry. Part A: the journal of the International Society for Analytical Cytology. 2013; 83(1):134–140. [PubMed: 23184564]

26. Guo J, Widgerow AD, Banyard D, et al. Strategic sequences in fat graft survival. Ann Plast Surg. 2015; 74(3):376–382. [PubMed: 25643185]

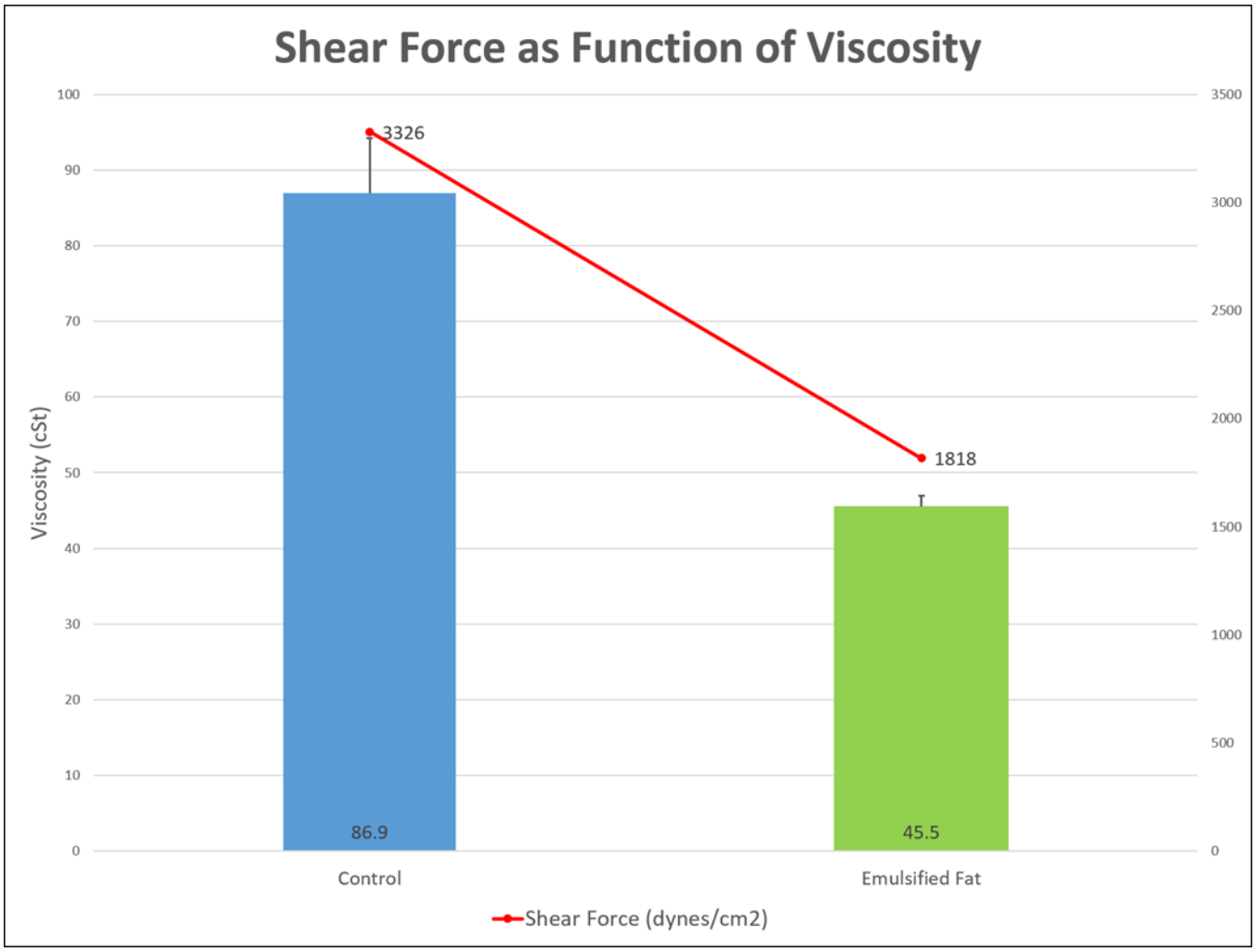

Fig. 1.

Relationship of kinematic viscosity (*bars*) and shear force (*line*) anticipated during the emulsification process of standard lipoaspirate into *nanofat*. As viscosity decreases, so does the ability to generate shear stress.

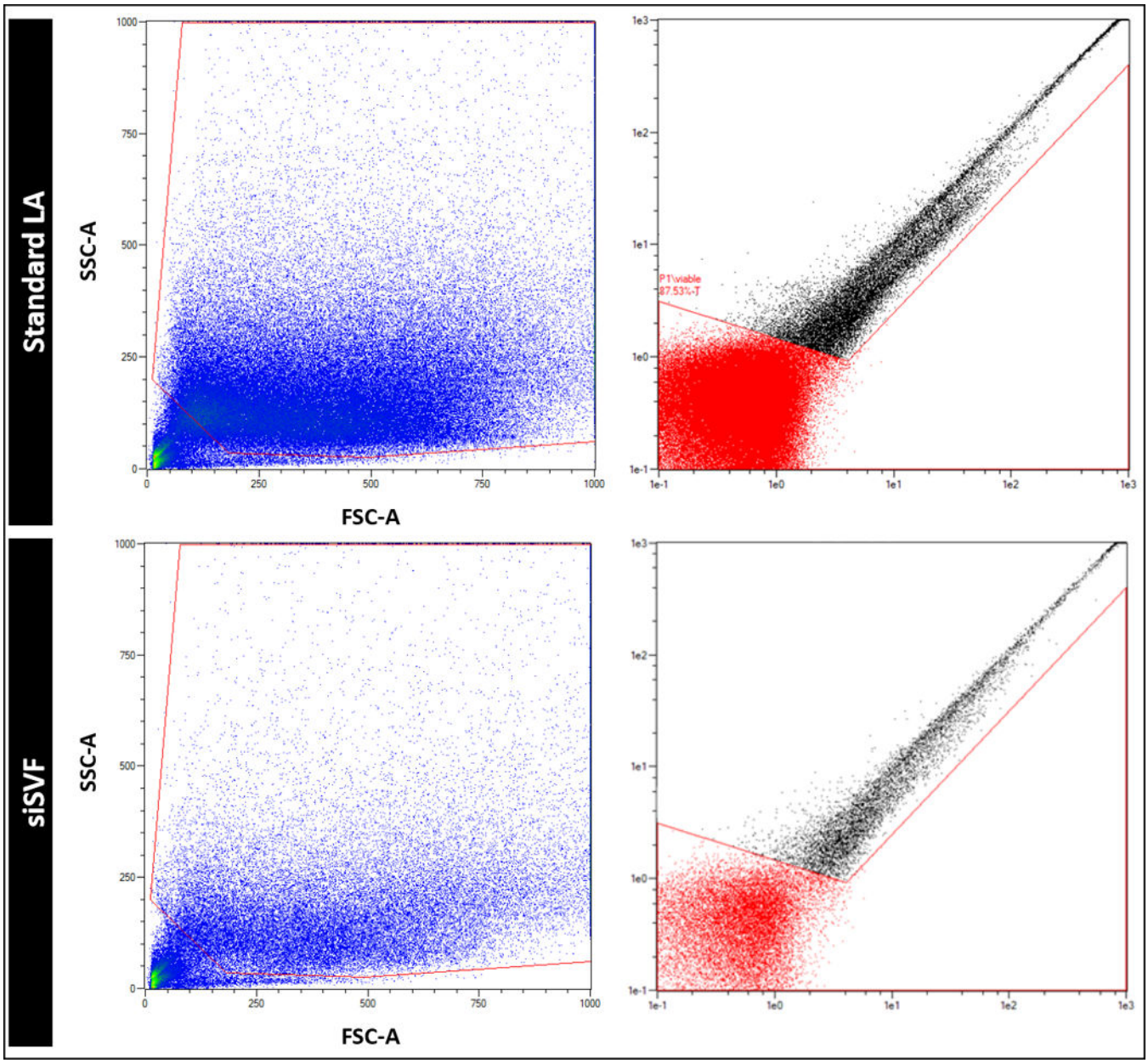

Fig. 2.

Gating strategy and viability analysis. (*Left*) A wide gating strategy was employed to capture data on all cells of the stromal vascular fraction by using FSC (forward scatter, representing cell size) and SSC (side scatter representing granularity). (*Right*) Absolute cell count (propidium iodide labeling) revealed significant decrease in viable cells recovered from lipoaspirate processed with mechanical shear ($p = 0.004$).

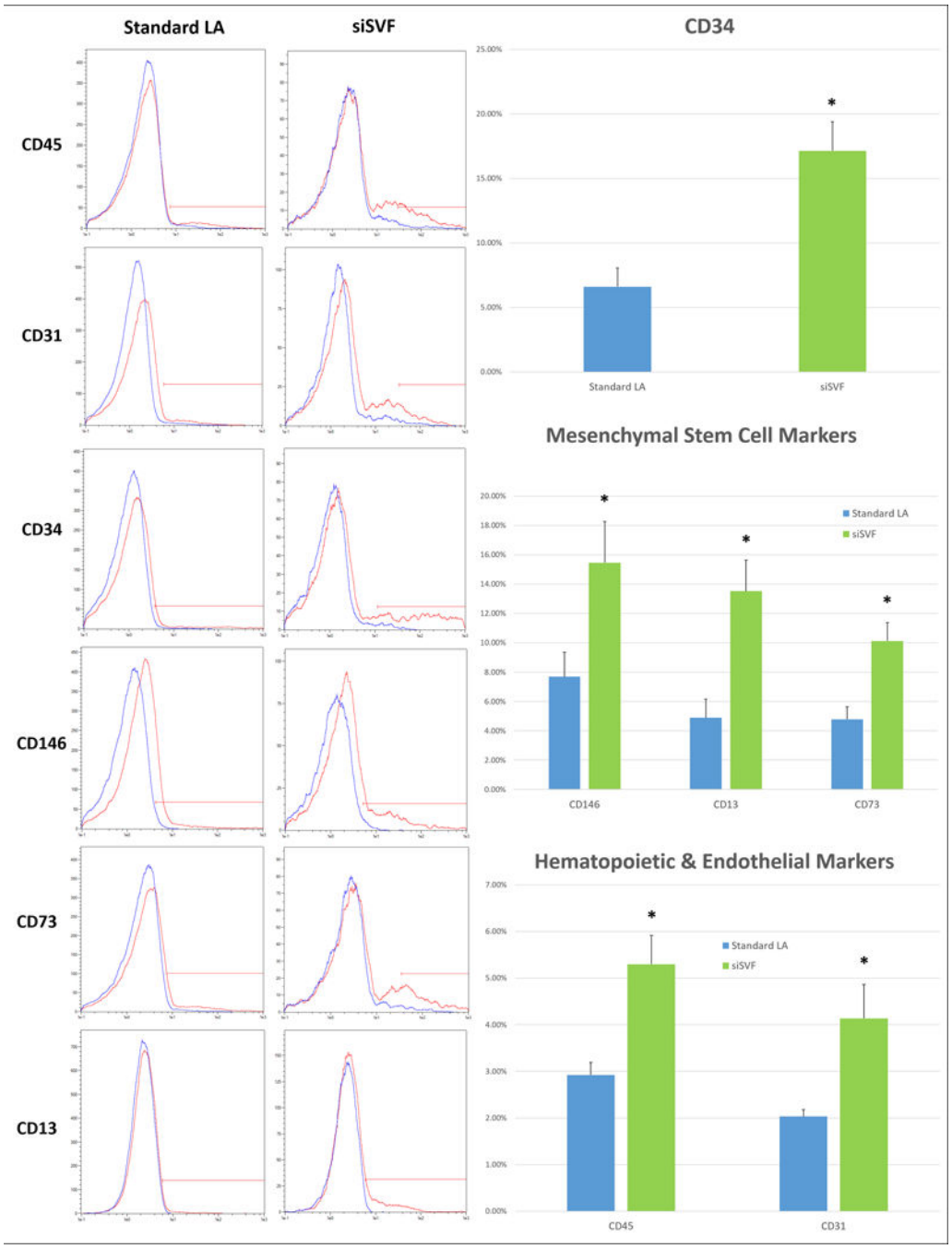

Fig. 3.

Flow cytometric comparison of cell phenotypes and summarized data. Histograms representing phenotypic analysis of stromal vascular fraction (SVF) from standard lipoaspirate (*Left column*) and stress-induced SVF (*right column*). (*Top graph*) CD34, a universal stem cell marker, found to be significantly greater in siSVF compared to control ($p = 0.001$). (*Middle graph*) Mesenchymal stem cell markers CD13 ($p = 0.003$), CD73 ($p = 0.003$) and CD146 ($p = 0.03$) all found to be greater in siSVF compared to control. (*Bottom*

graph) CD45 ($p = 0.004$) and CD31 ($p = 0.018$) were also found to be significantly upregulated in siSVF compared to control.

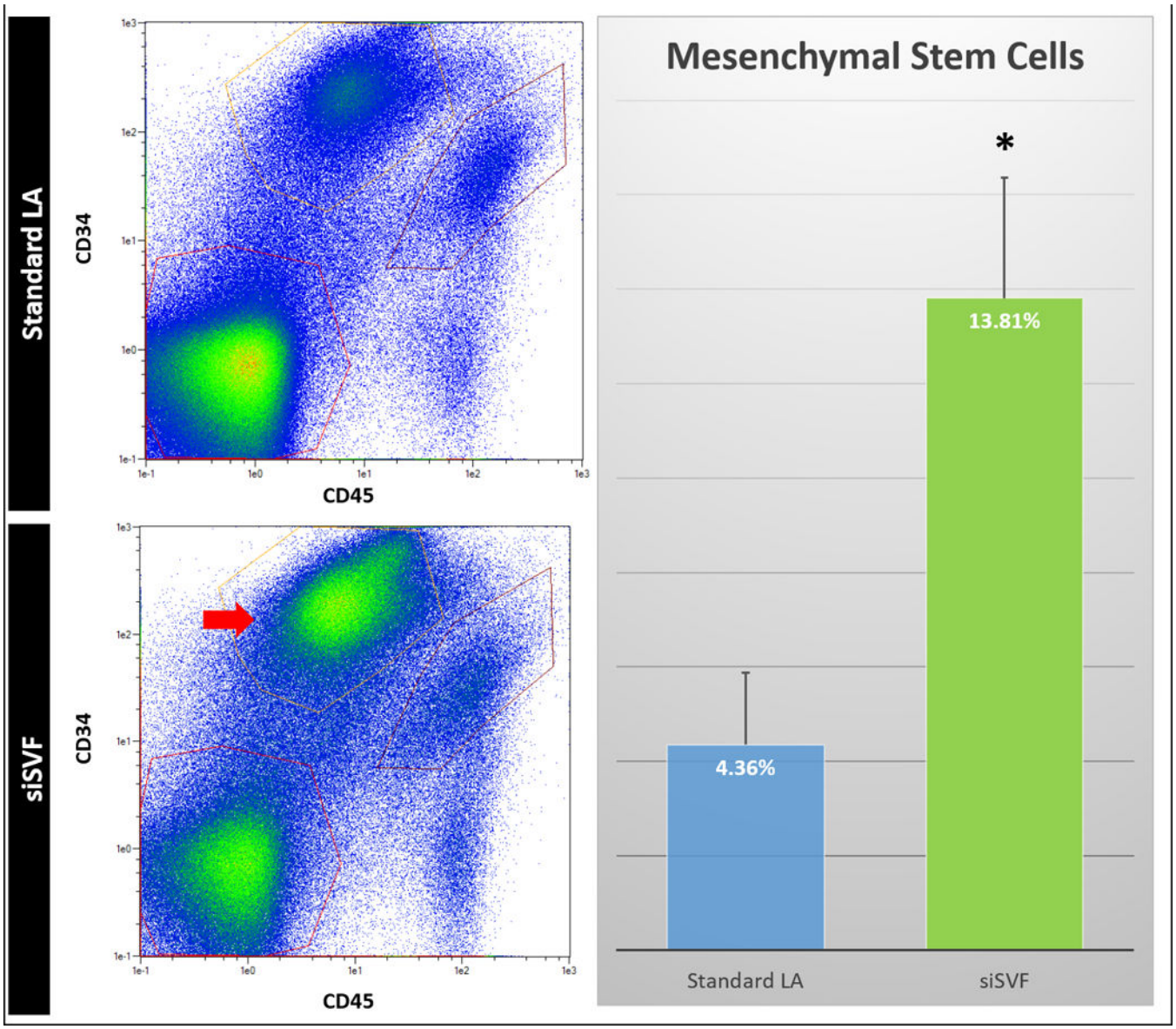

Fig. 4.

Flow cytometric comparison of mesenchymal stem cells ($CD45^{-}/CD34^{+}$) and summarized data. (*left*) Dot plot histogram representing significant upregulation of MSC population in stress-induced SVF (siSVF) when compared to control (red arrow). (*Right*) Summarized data representing almost 3-fold increase in MSC population ($p = 0.006$)

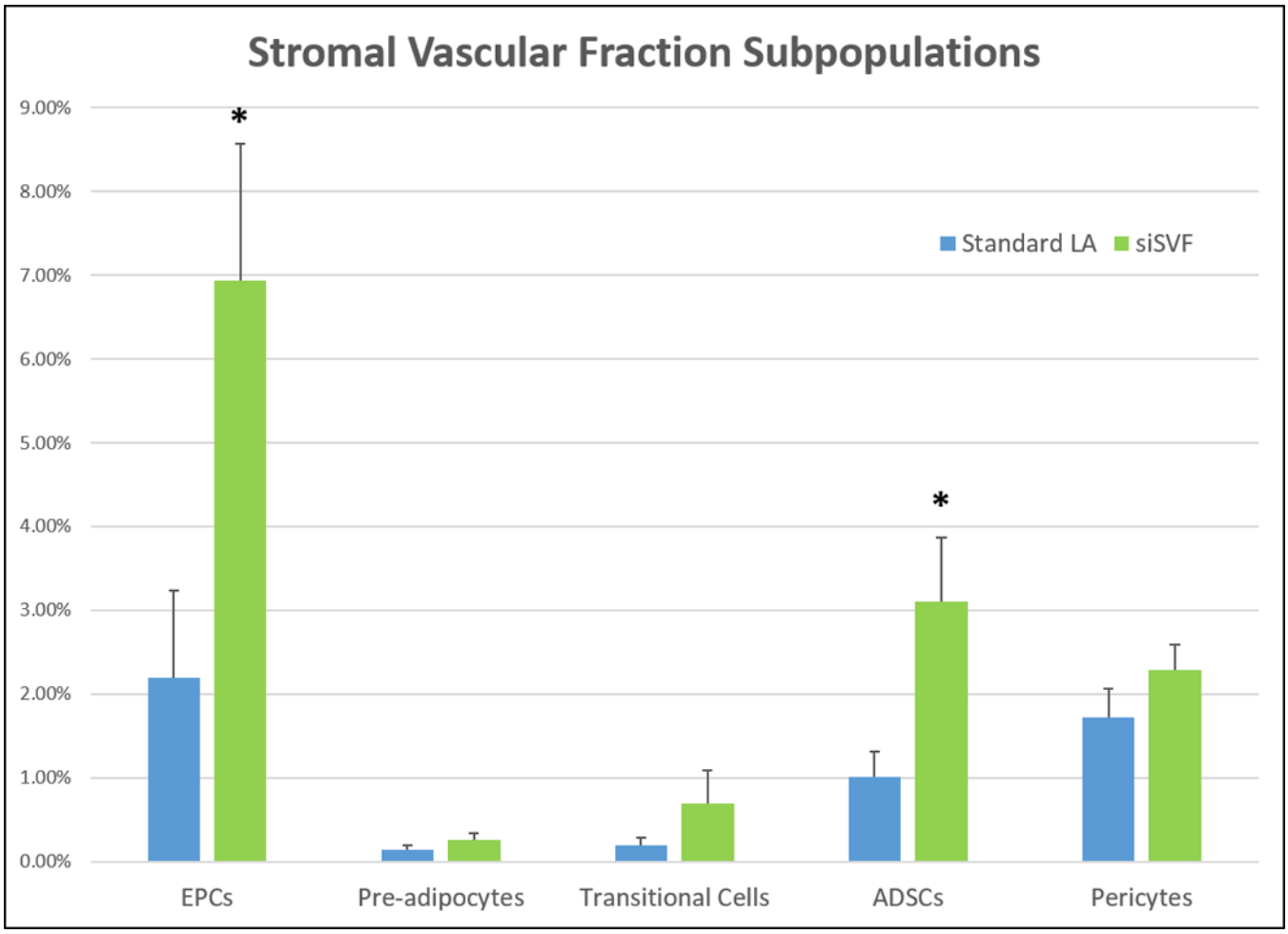

Fig. 5.

Flow cytometric comparison of stromal vascular fraction subpopulations in standard lipoaspirate: endothelial progenitor cells (EPCs, p = 0.025), pre-adipocytes (p = 0.205), transitional cells (p = 0.237), adipose-derived stem cells (ADSCs, p = 0.024), and pericytes (p = 0.230).

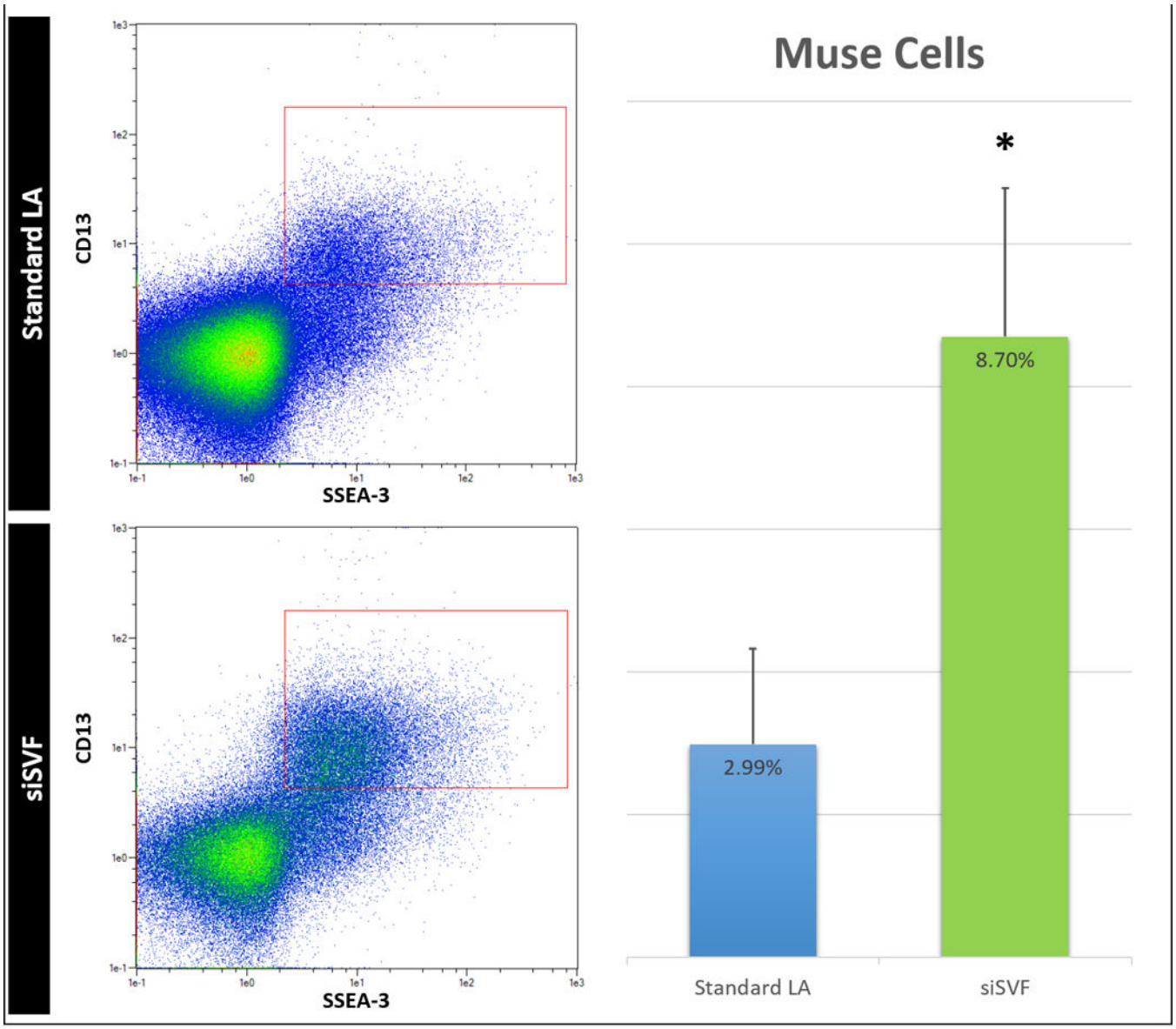

Fig. 6.

Flow cytometric comparison of Muse cells ($CD13^+/SSEA\text{-}3^+$) and summarized data. (*left*) Dot plot histogram representing upregulation of Muse population (red box). (*Right*) Summarized data representing ~3-fold increase in Muse population ($p = 0.046$).

Table 1

Accepted phenotypic markers used to characterize endothelial progenitor cells, adipose-derived stem cells, transitional cells, pericytes and pre-adipoctyes.

Cell Type	Clusters of Differentiation (CD)
Endothelial Progenitor Cells	$CD45^{-}/CD31^{+}/CD34^{+}/CD146^{+}$
Adipose-derived Stem Cells	$CD45^{-}/CD31^{-}/CD13^{+}/CD73^{+}$
Transitional Cells	$CD45^{-}/CD31^{-}/CD34^{+}/CD146^{+}$
Pericytes	$CD45^{-}/CD31^{-}/CD34^{-}/CD146^{+}$
Pre-adipocytes	$CD45^{-}/CD31^{-}/CD34^{+}/CD146^{-}$