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Stromal Vascular Fraction Obtained From Subcutaneous Adipose Tissue: Ex-Obese and Older Population as Main Clinical Targets

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ARTICLE INFO

Article history:
Received 5 January 2022
Received in revised form
12 October 2022
Accepted 4 November 2022
Available online 26 November 2022

Keywords:
Adipose-derived stem cells
CD90
Regenerative medicine
Stromal vascular fraction

ABSTRACT

Introduction: Human adipose tissue contains a heterogeneous and synergistic mixture of cells called stromal vascular fraction (SVF) with highly proliferative and angiogenic properties, conferring promising applicability in the field of regenerative medicine. This study aims to investigate if age, body mass index (BMI), history of obesity and massive weight loss, and harvest site are related to SVF cell marker expression.

Methods: A total of 26 samples of subcutaneous adipose tissue were harvested from patients admitted to the Plastic and Reconstructive department in University Hospital Center of São João, Porto, Portugal, for body contouring surgery. The percentage of cells expressing CD31, CD34, CD45, CD73, CD90, and CD105 was assessed and compared with patient's age, BMI, history of obesity and massive weight loss (ex-obese group), and harvest site.

Results: In the ex-obese group, a significantly higher number of cells expressing CD90 ($P = 0.002$) was found. BMI, harvest site, and age appear to have no association with SVF subpopulations.

Conclusions: This study suggests that ex-obese patients have a higher percentage of SVF cells expressing CD90, which correlates with higher proliferative and angiogenic rates. The effect of former obesity and massive weight loss on the expression of CD90 is a new and relevant finding because it makes this population a suitable candidate for reconstructive and aesthetic surgery and other fields of regenerative medicine. The use of SVF appears also promising in older patients because no negative correlation between increasing age and different cell markers expression was found.

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https://doi.org/10.1016/j.jss.2022.11.012

Introduction

Human adipose tissue is an important and easily accessible source of mesenchymal stem cells and has been recognized by its properties as a relevant therapeutic weapon in regenerative medicine.[1] Adipose depots contain a heterogeneous population of adipocytes and stromal vascular fraction (SVF) cells. SVF cells include adipose-derived stem cells (ASCs), preadipocytes, fibroblasts, vascular endothelial cells, and a variety of hematopoietic cells.[1-3] Due to this composition, the SVF of adipose tissue is considered highly angiogenic, containing the potential to spontaneously create a capillary-like network *in vitro*.[4] On the other hand, ASCs are thought to display a higher rate of proliferation than other stem cells and are as well extracted from more accessible donor sites.[5]

The potential plasticity and therapeutic utility of ASCs isolated from adult adipose tissue offers new approaches in regenerative medicine and in tissue and organ reconstruction surgery. Actually, in recent years, ASC-based autologous cellular therapies have been tested in several Plastic Surgery clinical settings, namely scars, hemifacial atrophy, breast reconstruction and augmentation, and wound healing.[6] Due to their plasticity, capacity of differentiation, ability to promote neoangiogenesis, and their paracrine and trophic effects, they are also a potential target in other medical and surgical fields, as noted by the increasing interest in therapies for osteoarthritis, Alzheimer, heart defects, bone and peripheral nerve regeneration, and many other conditions.[7,8]

Although there has been much investigation regarding ASCs as a form of cell-based therapy in regenerative medicine, the need of extensive culturing periods and regulatory hurdles limits the clinical translatability of cultured ASCs. Therefore, scientists and clinicians have begun to explore the regenerative capabilities of the SVF because it is a more readily available, heterogeneous, and synergistic mixture of cells.[9]

The aim of the present study is to evaluate whether age, body mass index (BMI), harvest site, and ex-obesity are related to the different cell subpopulations expressed in the SVF. This would allow us to establish which features can be associated with higher proliferative and angiogenic properties. In clinical practice, the surgeons would then be able to predict if a patient has a favorable profile for the use of SVF cells in the management of the many conditions stated previously.

Methods

Study design and participants

Our work consists of a scientific research using human adipose tissue samples for SVF isolation that were consecutively collected from healthy patients undergoing elective body contouring surgery. After local ethics committee's approval, a written informed and voluntary consent was given accordingly. Anonymization of all patients and respective adipose tissue samples was ensured. All procedures took place at Centro Hospitalar de São João in the departments of Plastic and Reconstructive Surgery during a 14-month period. The surgeons who collected the samples informed the patients about the aim of their utilization, also mentioning that collecting them would not interfere in their treatment.

Eligibility criteria and study size

The researcher did not have any contact with the patients or with the surgeons who collected the samples. The sample was eligible for inclusion if it was obtained from a dermolipectomy procedure, a technique that removes skin and excess fat from different anatomic locations. A sample was excluded if it showed infection, malignancy, or trauma affecting cutaneous and subcutaneous tissue quality. This was assessed based on the macroscopic appearance of the samples. Samples with bacterial or fungal infection usually present a hazy preservation medium or whitish, milky surface with bad odor. Malignancy would be hypothesized if an uncharacteristic mass was observed in the tissues. In this case, the medical team providing the sample would be warned and the tissue preserved for further analysis. Trauma would be hypothesized in the presence of fibrotic tissue, not so uncommon due to previous surgeries. A total of 35 samples were collected resulting in a sample size of 26 patients after exclusion criteria were applied. All 26 participants were female patients, with ages between 25 and 60 y.

Harvest, processing, and isolation of stromal vascular fraction cells

Samples of human adipose tissue were harvested from two different anatomic regions: arms and abdomen, corresponding to dermolipectomy procedures: brachioplasty or abdominoplasty, respectively. The samples were immediately placed in preservation solutions consisting of Phosphate Buffered Saline (PBS) with 10% antibiotic/antimycotic (penicillin, streptomycin, and amphotericin) and kept at 4°C until further processing. Then, the samples were processed for isolation of the SVF of the adipose tissue; processing was always performed within 24 h of harvesting. In a first phase, samples were washed extensively with PBS to remove blood and loose tissue debris. The subcutaneous fat tissue was then chopped into small pieces and manually separated from the dermis using blades or scissors. Afterwards, tissue digestion was induced with a collagenase solution at 37°C for 30 min, under agitation. The collagenase solution used in the study was at a concentration of 0.05% (w/v) of collagenase type II sourced from Sigma Aldrich, catalog number C6885. The solution was prepared fresh, as per the manufacturer's instructions. Digested tissue was percolated through a 100-μm nylon mesh strainer and washed and centrifuged several times at 4°C, for 10 min. The obtained SVF was incubated with a red blood cell lysis buffer (154 mM of ammonium chloride, 10 mM of potassium bicarbonate, and 0.1 mM of ethylenediaminetetraacetic acid) and a crystal violet solution was used on cells in a hemocytometer to count nucleated cells only.

Flow cytometry analysis

Flow cytometry was performed to further characterize SVF subpopulations, as per the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy.[10]

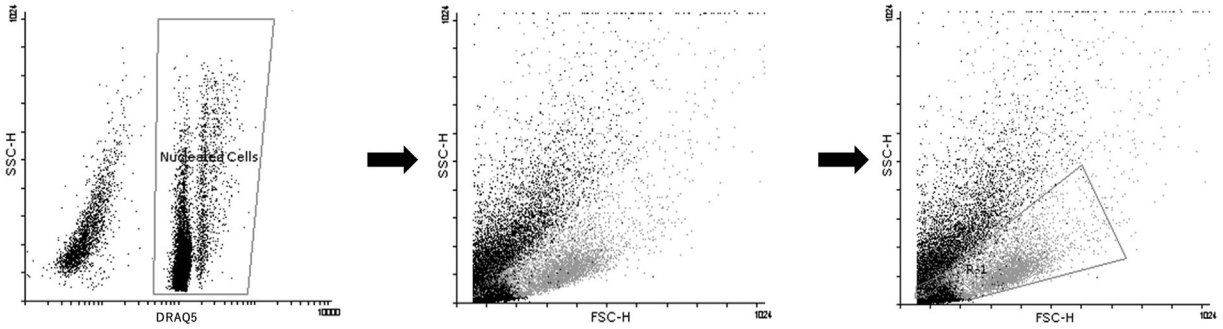

Fig. 1 – Gating strategy for nucleated cells in the SVF population by using the membrane permeant dye DRAQ5. It allowed a clear distinction between cells of interest and undesired debris or remaining erythrocytes.

Markers used for the characterization of SVF were CD105–FITC (BioRad, the Netherlands), CD73–PE (BD Biosciences, Germany), CD90–APC (BD Biosciences, Germany), CD45–FITC (BD Biosciences, Germany), CD34–PE (BD Biosciences, Germany), and CD31–APC (R&D Systems). Furthermore, cells were stained with DRAQ5 (eBioscience) for nuclear staining to discern the cells of interest from any remaining erythrocytes and tissue debris. Each of these fluorochrome conjugated antibodies was added to 1 × 105 cells, incubated during 20 min, washed thoroughly with PBS, and resuspended in PBS with 1% of formaldehyde. Twenty thousand events were acquired for each tube (Fig. 1).

Variables

The dependent variables considered were the percentage of positive cells for the markers—CD31, CD34, CD45, CD73, CD90, and CD105—found in the adipose tissue of each patient. The independent variables were assumed as continuous (age and BMI) or categorical (harvest site and ex-obesity). The ex-obesity variable included patients who had a BMI more than 40 or 35 associated with comorbidities (arterial hypertension, diabetes melittus, obstructive sleep apnea, and dislipedemia), performed an obesity surgery, and have subsequently lost at least 50% of the weight in excess, that is, 50% of the kilograms that were part of the weight more than BMI 30.

Statistical analysis

All continuous variables were assessed for normality and described accordingly using Shapiro–Wilk's test and Lilliefors' test. All the dependent variables were non-normal, and nonparametric tests were used. The relation between harvest site, ex-obesity, and SVF cell markers was accessed using the Mann–Whitney *U*-test. Spearman's correlation was used to test the relationship of two continuous variables (age and BMI) and the number of cells expressing specific markers in the SVF.

Statistical significance was assumed for a P value < 0.05. Statistical analysis was performed using SPSS software.

Table 1 – The correlation between harvest-site and SVF. The Mann–Whitney *U*-test performed did not show any statistically significant correlation between the different harvest sites and the SVF cells, except for the CD34 marker ($N_{abdomen} = 22$, $N_{arm} = 4$) = 13.00, $z = -2.203$, $P = 0.028$.

Cell marker	Harvest site	*N*	Percentiles			*P* value
			25	50	75	
CD31	Abdomen	22	34.34	43.57	51.54	0.395
	Arm	4	27.34	34.18	52.78	
CD34	Abdomen	22	45.88	66.52	75.43	0.028
	Arm	4	75.42	76.96	84.16	
CD45	Abdomen	22	19.22	25.40	31.80	0.102
	Arm	4	9.40	16.37	19.19	
CD73	Abdomen	22	16.09	43.11	56.64	0.256
	Arm	4	42.58	51.80	66.71	
CD90	Abdomen	22	60.61	82.08	86.14	0.136
	Arm	4	81.84	86.87	92.79	
CD105	Abdomen	22	10.32	16.08	20.56	0.831
	Arm	4	13.47	14.61	24.90	

Fig. 2 – Box plots graphic showing a statistically significant difference between the two harvest sites (abdomen or arm) and the percentage of SVF cells expressing CD34.

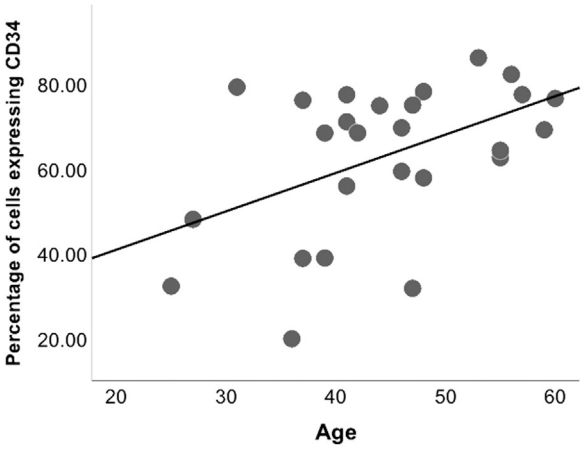

Fig. 3 – Scatter plot graphic showing a statistically significant positive correlation between age and the percentage of SFV cells CD34.

Results

The influence of harvest site in cell markers of stromal vascular fraction

The variable harvest site was dichotomized in two groups: abdominal and arm anatomical regions. The Mann–Whitney *U*-test performed did not show any statistically significant difference between the two harvest sites and the SVF cells marker expression in flow cytometry, except for the CD34 marker ($N_{abdomen} = 22$, $N_{arm} = 4$) = 13.00, z = −2.203, $P = 0.028$ (Table 1 and Fig. 2).

The influence of age and body mass index in cell markers of stromal vascular fraction

The Spearman's correlation was performed to determine the relationship between age and SVF cells expressing specific markers and showed a strong positive correlation between age and CD105 (= 0.637, $n = 26$, $P = 0.0005$) and a positive correlation between age and CD34 (= 0.424, $n = 26$, $P = 0.031$). No other statistically significant difference was found (Table 2, Figs. 3 and 4). When it comes to BMI, the Spearman's correlation test did not show any statistically significant correlation (Table 2).

Table 2 – The correlation between BMI and SVF and between age and SVF. Strong positive correlation between age and CD105 (= 0.637, $n = 26$, $P = 0.0005$) and a positive correlation between age and CD34 (=0.424, $n = 26$, $P = 0.031$). When it comes to BMI, the Spearman's correlation performed did not show any statistically significant correlation.

Cell marker	BMI		Age	
	Correlation coefficient	P value	Correlation coefficient	P value
CD31	−0.341	0.088	−0.128	0.534
CD34	0.286	0.161	0.424	0.031
CD45	0.107	0.604	−0.014	0.947
CD73	0.079	0.701	0.257	0.205
CD90	0.196	0.337	0.141	0.491
CD105	0.105	0.611	0.637	0.000

The influence of ex-obesity in cell markers of stromal vascular fraction

The variable ex-obesity was also treated as a binary categoric variable with two groups: ex-obese patients and nonex-obese patients. Comparison between the two groups through Mann–Whitney *U*-test showed that ex-obese patients had a higher number of cells expressing CD90 (Mdn = 84.69) compared with the nonex-obese group (Mdn = 54.26). This

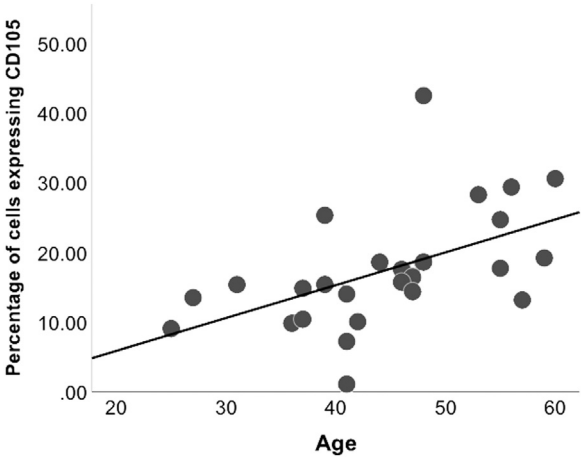

Fig. 4 – Scatter plot graphics showing a statistically significant positive correlation between age and the percentage of SFV cells CD105.

Table 3 – The correlation between ex-obesity and SVF. The CD90 marker in ex-obese patients (Mdn = 84.69) was expressed in higher quantity than in nonex-obese patients (Mdn = 54.26). A Mann–Whitney test indicated that this difference was statistically significant ($N_{ex\text{-}obese} = 20$, $N_{nonex\text{-}obese} = 6$) = 10.00, z = −3.043, P = 0.002.

Cell marker	Ex-obesity	N	Percentiles			P value
			25	50	75	
CD31	No	6	28.47	37.66	44.15	0.162
	Yes	20	34.02	44.12	53.46	
CD34	No	6	32.25	59.98	64.47	0.088
	Yes	20	63.18	70.49	77.37	
CD45	No	6	16.97	22.98	27.30	0.584
	Yes	20	16.96	24.13	31.89	
CD73	No	6	3.84	17.88	46.80	0.068
	Yes	20	29.09	47.91	56.75	
CD90	No	6	44.98	54.26	59.48	0.002
	Yes	20	81.10	84.69	86.73	
CD105	No	6	8.61	15.90	17.55	0.394
	Yes	20	13.26	15.57	25.14	

difference was statistically significant ($N_{ex\text{-}obese} = 20$, $N_{nonex\text{-}obese} = 6$) = 10.00, z = −3.043, P = 0.002 (Table 3 and Fig. 5).

Discussion

The SVF, when compared with the bone marrow mononucleated fraction, contains a higher percentage of stromal elements: endothelial, hematopoietic, and pericytic lineages represent 10%-20%, 25%-45%, and 3%-5%, respectively, of the total nucleated cells.[11] Although there is no single marker to identify SVF cell subpopulations, a combination of negative and positive markers is used to investigate SVF cellular composition. CD45 (leukocyte common antigen) is the classic marker to identify cells of hematopoietic origin; CD31 (PECAM-1) is a classic marker for endothelial cells and their progenitors, although it is also detected on platelets and leukocytes; CD34 expression is shared by hematopoietic stem/progenitor cells and endothelial cells; CD73 marker is highly expressed on the CD45-CD31-CD34+ cell population and may help to identify stromal cells better; CD90 (Thy-1) and CD105 (Endoglin) are also expressed by the endothelial population, being CD90 also a marker present in ASC.[11,12]

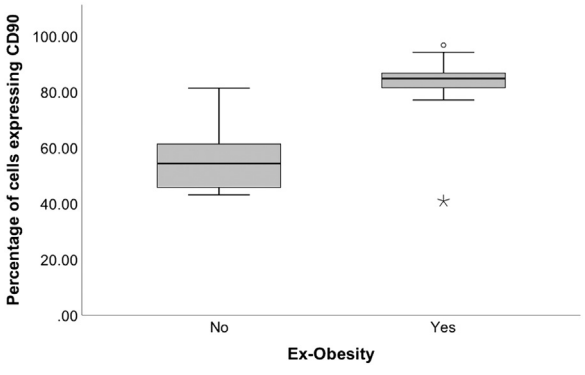

Fig. 5 – Box plots graphic showing a statistically significant difference between the ex-obesity and nonex-obesity groups and the percentage of SVF cells expressing CD90.

Regarding the current knowledge about the influence of age, gender, BMI, and harvest site over SVF, further investigation is needed to obtain more accurate information. Some studies suggest that age does not significantly affect the cell yield from SVF,[7,13,14] while others suggest that it does.[15] The impact of BMI and the harvest site are also still controversial.[7,13,14,16]

In our work, as the statistical results did not show any correlation between BMI and the SVF cell subpopulations, we assumed that BMI plays little or no role in the SVF marker expression.

Regarding the age variable, the number of cells positive for CD34 and CD105 was significantly increased in older people. Although there is still a major lack of references in the literature about its precise role,[17] CD34 seems to be essentially expressed by endothelial cells actively participating in angiogenic processes.[18,19] Also, the expression levels of endothelial progenitor markers seem to be higher in CD34+ cells and CD34+ ASCs seem to have a greater replicative capacity.[20] However, the percentage of cells that are positive for CD34 can vary depending on the method of adipose tissue harvest, the degree of vascular hemorrhage, and the subsequent digestion and isolation techniques.[11]

When it comes to CD105, a correlation has also been noted between this marker levels and other markers of cell proliferation, being Endoglin also involved in the angiogenic process.[21,22]

Although the observed increased percentage of cells expressing CD34 and CD105 in the SVF does not have a clear meaning as of yet, we have assumed, as per our results and the literature, that an increase in age does not have a negative correlation with the number of cells positive for the studied markers and may even increase the CD34 and CD105 positive

cells. The bigger expression of cells containing the CD34 and the CD105 markers in the older patients is a new finding. As ASC therapy is easy to use and can be applied in the treatment of difficult-to-heal wounds, especially common in the older population, this can play a very important role in the clinical management of pain, improvement of tissue healing, and patient quality of life,[23] in a more advanced age group.

Regarding the harvest site, we also found a statistically significant correlation, with the percentage of cells expressing CD34 being higher in the adipose tissue extracted from the arm than from the abdomen. As mentioned above, it is difficult to establish significance to this finding. However, it has been proven that the arm depots demonstrate more lipolytic activity and maintain high lipolytic activity regardless of patient age, when compared to abdominal, deep abdominal and thigh tight depots, and that PPAR- γ-2 levels, a protein required for adipogenesis, are highest in the arm subcutaneous tissue.[24] Although CD34 is expressed in a wide variety of hematopoietic stem cells and nonhematopoietic cells, such as vascular endothelial cells and soft tissue neoplasms, CD34+ progenitor cells have also been extensively characterized as adipocytes progenitors[20]—CD34 is a marker of cell proliferation and of adipocytes progenitors. For this reason, the CD34 overexpression in SVF cells from the arm depots might not only be correlated with higher lipolytic und lipogenic characteristics, already described in the literature, but also with higher proliferative capacity of SVF cells extracted from the subcutaneous adipose tissue in the arm region.

We have also studied the variable ex-obesity, defined as former obese patients who underwent weight reducing surgery in the past, with subsequent massive weight loss. It is well established that hypoxia triggers blood vessel formation and adipose tissue endothelial cells promote preadipocyte differentiation, making obesity a state of high angiogenesis.[25] Baptista *et al.*[26] concluded that the blood vessels that were formed during the development of obesity do not regress with weight loss—in his study it was demonstrated that the area of adipose tissue occupied by vessels was up to twofold higher than in control patients. Our findings were in favor to the previously described because ex-obese patients had a significant higher number of cells expressing CD90—a marker expressed in endothelial and ASCs—and in this case, the higher vascularity is hypothesized indirectly by the angiogenic marker CD90. Despite the correlation between CD90 and angiogenesis is yet not well established, there are studies suggesting its importance in this process. Angiogenesis is under tight control by a balance of angiogenesis inducers and inhibitors and based on the literature, Thy-1 is thought to operate in the modulation or stimulation of endothelial cell proliferation.[27,28] Wen *et al.*[28] suggested that Thy-1 is not only a marker of adult new blood vessels but also an indicator for newly formed blood vessels in the adult. Also Kawamoto *et al.*[29] stated that the vascular forming ability of some types of cells can be predicted in advance by measuring the ratio of CD31+ to CD90+ cells, suggesting CD90's importance in the angiogenesis process. Nonetheless, CD90 appears to be also involved in cellular growth and differentiation. Thy-1 is thought to empower ASCs with high potential in proliferation, mitotic clonal expansion, and adipocyte differentiation; CD90 is essential for AKT activation and CyclinD1 upregulation, which may promote the proliferation and stemness via G1-S phase transition, increasing the mitotic clonal expansion of ASCs.[30] This marker has also an important contribution in the adipogenesis process: animal studies demonstrated that mice that do not express CD90 have increased weight gain, increased levels of key adipogenic genes, and increased serum resistin levels.[31,32]

Although CD90 is a known marker of adult new blood vessels and of high mitotic expansion, and being this marker expressed in a higher percentage of SVF cells in the ex-obese population of our study, we reached two important positive correlations: a positive correlation between the ex-obese population and angiogenic potential of the SVF and a positive correlation between the ex-obese population and proliferation/mitotic capacity of the SVF.

When it comes to the potential bias of our study, the fact that only healthy patients undergoing elective plastic surgery were selected may lead to a different result than if subjects were to be randomly selected from the entire population. Furthermore, the number of samples is reasonable for a stem cell study but still small for the generalization of the results, compromising the external validity. The last bias consists on the fact that all the samples came from female patients. Although this would make us question the generalization of the results to the male gender, the few studies that investigated the influence of sex on the ASC/SVF did not find any statistically significant result: as per Harris *et al.*,[33] gender did not significantly diminish stem cell availability; Reumann *et al.*[34] concluded that gender did not play an important role when it comes to ASC differentiation; and citing Yang *et al.*,[35] neither osteogenic nor adipogenic differentiation in ASC was affected by donor gender.

Despite the theoretical basis of our work, we believe that the possibility of daily clinical usage of SVF cells extracted from SC adipose tissue in regenerative medicine will guide many further clinical studies—an example is the usage of stromal cells in animal studies for the repairment of Achilles tendon.[36] By studying the characteristics that can be associated with SVF with greater angiogenic and proliferative properties, we are tracing a very important path in the application of regenerative therapies and enabling the tracing of the most appropriate patient profile. Although further investigation is needed to obtain more accurate and generalizable data, these results are already a big step in understanding the SVF cells present in SC adipose tissue.

Conclusions

Recently, there has been an increased interest in the use of the SVF of human adipose tissue as an alternative cell source in vascularization strategies and tissue healing therapies, mainly due to its heterogeneous cellular composition and easily accessible anatomic localization. The results from our study constitute a new window of opportunity because we describe for the first time the relation between ex-obesity and higher SVF proliferative capacity; the possibility of patients from an increased age benefiting more than the younger population from SVF-based therapy and the potential of the arm as harvest site to obtain SVF cells. We expect our work to be a support for future research on this matter.

Author Contributions

Francisca Frias analyzed and interpreted the data, wrote the manuscript, and revised the manuscript. Beatriz Matos analyzed and interpreted the data and wrote the manuscript. Mariana Jarnalo collected the data and provided critical revisions. Sara Freitas-Ribeiro collected the data. Rui L. Reis collected the data. Rogério P. Pirraco conceived and designed the study, provided critical revisions, and revised the manuscript. Ricardo Horta provided critical revisions and approved the final version.

Disclosure

None declared.

Funding

None.

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