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THE ROLE OF LIPOSUCTION IN STEM CELL MOBILIZATION AND CIRCULATION

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ABSTRACT

Stem cells are of vital interest to the scientific and medical communities for their promising role in regenerative medicine, avoiding host rejection, and providing curative therapy for a bevy of pathophysiological conditions. However, being able to obtain enough high-quality, versatile stem cells, especially later on in life, is a pressing obstacle that needs to be addressed. It has been observed that certain nutraceuticals have the potential to increase the activation and proliferation of various stem cells. However, it thus far has not been examined whether abdominal liposuction involving physical alterations of adipose fat leads to similar stem cell activation. Specifically, it has not been probed as to whether internal stem cell mobilization occurs as a viable alternative to SVF treatment that exclusively works with the lipoaspirate. Such activity is expected to be the case as liposuction disturbs the interstitial space inhabited by stem cells thereby increasing the presence of stem cells in the peripheral blood following the procedure. To that end, an intensive literature analysis indicates that there are proven and accurate techniques by which to measure the titers of mesenchymal, hematopoietic, and endothelial progenitor stem cells in blood samples obtained from patients. This will rest largely on antibody staining and flow cytometry analysis for quantification purposes. A tentative methodology, supported by precedent, consists of analyzing blood samples periodically over two weeks to gauge maximal stem cell activation, and to support such a hypothesis, various cytokine, growth factor, and endothelial cell counts will also be examined to substantiate the presence of interstitial disruption. The results of this study, whether concordant or discordant with the hypothesis, will provide consequential insights into potential mechanisms to mobilize stem cells for clinical and therapeutic endeavors.

KEYWORDS: nutraceuticals, lipoaspirate, mesenchymal, cytometry.

CONCLUSION

A thorough analysis of the literature as it pertains to parts of this novel query gleans that this experimental design and methodology is supported with clear, actionable guidelines. There is strong histological and pathological evidence and precedent that adipose tissue can provide an ample supply of high-quality stem cells. Liposuction is already a researched pathway to obtain these cells, but work has singularly focused on the lipoaspirate and not ramifications on the systemic circulatory system after the procedure. Considering that there is significant mechanical disruption of the tissue where blood vessels are prevalent, it is worthy to investigate whether stem cell circulation increases. Analysis of prior literature indicates that there are established experimental parameters by which to obtain blood, on a structured schedule, and analyze the samples with antibody staining and flow cytometry for numerous cell types. The main determination factor is positive hits for cell type-specific surface antigen markers, of which there are plenty that are exclusive to certain cells. Mesenchymal stem cells and endothelial progenitor cells would provide highly versatile cells that could prove significant therapeutic value where stem cells are indicated as being beneficial. Endothelial cells can represent confirmation of blood vessel rupture, where increased circulation has been utilized as a proxy measurement to that end, and growth factors will further substantiate such a point. It is expected that liposuction will increase stem cell circulation, and the past examples noted in the literature suggest that this avenue of investigation is untested, worthy, and feasible.

Stem cells are a promising focus of recent medical research and advancements that have the possibility to revolutionize current approaches to treat a variety of conditions from cell-based therapies for cancer or tissue regeneration for organ loss (Kuehnle and Goodell 2002). By having the ability to differentiate and specialize into any cell line in the body, stem cells derived from a patient can be used without severe risk of host immunological rejection that conventional transplants have (Ryan et al. 2005). Sourcing and obtaining stem cells, however, has been a significant obstacle precluding their widespread implementation in routine medical care; specifically, totipotent and pluripotent stem cells originate during the developmental process in utero, and thus they are unable to be sourced from somatic cells without significant reprogramming techniques to undo the differentiation process (Zakrzewski et al. 2019). Multipotent stem cells, also commonly referred to as adult stem cells, are more limited in scope in that only certain cell lineages can be yielded. Nevertheless, such stem cells have the potential to still provide adequate supply of stem cells for treatment purposes (Mirzaei et al. 2018). Researching and fine-tuning multipotent stem cell procurement is a promising research avenue that stands to bolster and enhance stem cell therapeutics, and that will be the primary goal of this investigation.

One potential source of such stem cells is the Stromal Vascular Fraction (SVF) which is obtained from adipose tissue with blood, adipocytes, and connective tissue removed to leave behind a concoction of mesenchymal stem cells, immune cells such as macrophages, growth factors, and endothelial cells and their progenitor cells (Nguyen et al. 2016). SVF requires minimally invasive procedures: lipoaspirate that is obtained from a patient can be used and separated using collagenase and centrifugation (Veronesi et al. 2021). Such ease of access enables SVF to be a logistically feasible source of stem cells for therapeutic applications. The SVF yields a heterogeneous mixture of cells that has versatile utility in combatting a vast array of conditions due to the variety of cells included, but research into and applications of SVF are limited following lawsuits regarding FDA approval and court injunctions banning its use. Still, the mesenchymal stem cells that can be obtained from adipose tissue have the ability to differentiate into myocytes, osteoblasts, neurocytes, chondrocytes, and adipocytes, implicating it in treatments for a range of conditions such as diabetes mellitus, vascular lesions, hepatic diseases, and bone conditions (Miana and González 2018). As such, there is an impetus to center research on new and alternative ways of sourcing such stem cells without the SVF technique, and examining adipose tissue remains of key interest due to its relative ease of access to numerous high-quality cell populations. Even though there are other sources of such stem cells, such as the bone marrow, analyses have determined that the titer obtained from lipoaspirate is far in excess compared to quantities captured from bone marrow aspirate (Ugarte et al. 2003). The purpose of this paper, therefore, is to investigate a novel stem cell acquisition technique following adipose tissue liposuction; the conceptual rationale, existing literature, and detailed experimental outline will be provided in order to support the hypotheses of this query of maximal importance.

In order to elucidate the experimental procedures needed for this investigation, it is first imperative to describe the anatomical and histopathological structure of adipose tissue. The interstitial space between the adipocytes is dominated by numerous other cell types and structures including but not limited to endothelial cells, capillaries, mesenchymal stem cells, endothelial progenitor cells, and leukocytes (Tsuji et al. 2014). Liposuction is a technique by which adipose tissue is forcefully aspirated out from the body in areas where it is typically in excess, and the lipoaspirate that is obtained is used to obtain the SVF cocktail **(Francesco et al. 2019)**. However, while the use of the lipoaspirate is currently curtailed, there is still a potential avenue to access these prized stem cells, and that is the crux of this investigation's hypothesis. Liposuction leads to the breakdown of the interstitial space of the adipose tissue, and with the abundance of capillaries that are present, the mechanical interruption can lead to a rupture and release of the stem cells as well as the other cells present into the peripheral circulatory system. Such mechanical disruption of the adipose tissue is noted to occur as nearly all patients who undergo liposuction deal with ecchymosis for up to a week afterward, indicative of a rupture of blood vessels (Dixit and Waugh 2013). Furthermore, it is has been established that stem cells obtained from lipoaspirate tend to be in lower concentrations and, at times, quality due to loss and pressure experienced during the liposuction procedure; adipectomies, however, usually retain higher proportions of stem cells, indicating that liposuction leaves a considerable amount of stem cells behind either in the adipose tissue or released into circulation (Duscher et al. 2016). Quantitative analysis of the titer of these cells circulating in the blood before and after liposuction can indicate whether this is another, more feasible route of access to stimulated and accessible stem cells. The hypothesis is believed to stand in the affirmative, and it is believed that data obtained from consenting patients from blood analysis will corroborate this premise.

The presence and analysis of stem cells in the circulatory system is by no means an untested experiment in the scientific literature. For instance, there are numerous substances that are marketed and sold as being supplements that can boost stem cell proliferation naturally. Neutraceuticals are one such class of substances that have been promoted as having the ability to bolster stem cell mobilization—the movement of stem cells into the bloodstream and around the body (Mikirova et al. 2010). It has also been studied extensively how tissue damage can elicit stem cell mobilization of mesenchymal stem cells, hematopoietic stem cells, or endothelial progenitor cells from the bone marrow to assist in tissue healing and repair (Rennert et al. 2012). Thus, such a phenomenon of stem cell mobilization in the circulatory system has been investigated before, but the question of how liposuction affects the degree of such stem cell activation has not been probed as of yet.

Therefore, the principle experimental aim of this investigation is to quantify the amount of circulating stem cells following liposuction, which will be indicative of their mobilization and activation. One of the most significant confounding variables will be, however, how long such mobilization will persist and when samples should be taken to see any differences. The paper examining neutraceuticals, for instance, observed maximal stem cell measurements around 7 days post treatment initiation; however, while there is reason to believe abdominal stem cell activation could take a similar amount of time, it could be much longer or much shorter (Mikirova et al. 2010). To that end, the blood samples of approximately 25-30 willing and consenting patients will be examined over the span of two weeks to appropriately assess any changes in circulation concentration, no matter the degree of the change. Two groups will be established—control and experimental—in which one will undergo abdominal liposuction and the other will not; experimenters involved in hematological assays and assessments will be blinded to ensure the scientific rigor of the study and reduce any biases. Those receiving liposuction will be receiving it in the same general area of the body, such as the torso, buttocks, or breasts (the area that is chosen will be based on the patient pool that is readily accessible). Around 8 mLs of blood will be taken and assessed on days 0 (to establish a baseline comparative control per individual), 1, 2, 7, and 14 to surveil the patients over the timeframe of the study. 10 mLs may be needed, however, in case certain assays need to be run again or mishaps occur along the way. Strict eligibility requirements will be implemented and kept consistent to ensure that the patient pool is similar in most regards to reduce the influence of potentially confounding variables. For instance, patients will be between the ages of 20-50, will not have any deleterious or serious unrelated medical condition, and would be assessed to not be clinically compromised in any way following the liposuction procedure. As such, only patients who sought out liposuction for personal and aesthetic reasons, not medical, will be included in the groups. Diets and lifestyles between the participants will be kept as similar as possible to ensure that any differences noted in stem cell circulation can be attributed to the liposuction procedure alone.

The most important cell type to be examined in the peripheral blood supply is the mesenchymal stem cell, which, as aforementioned, is able to differentiate and specialize into numerous cell lineages that can play a significant role in modulating a whole host of varying conditions. Methodologies regarding mesenchymal stem cell analysis have been described extensively in the current corpus of medical literature, and such precedent will be referred to when establishing the testing protocol for this paper. Specifically, surface antigen testing will be done to identify which cells are mesenchymal stem cells and use that information to then obtain a quantitative categorization of the number that are present. For mesenchymal stem cells, the surface antigens that will be examined are a presence of CD73, CD90, CD44, CD105 and a lack thereof of HLA-DR, CD45, CD14, and CD34 (Camilleri et al. 2016). A negative result for the last marker, CD34, is vital as it is a common immunological glycoprotein that populates the surface of hematopoietic stem cells; therefore, testing positive for CD34 would suggest negating that result and removing it from the data for mesenchymal stem cells (AbuSamra et al. 2017, Kobolack et al. 2016). To substantiate the notion that mesenchymal stem profiling is well studied and described, several strict parameters have been adopted to clearly demonstrate that data indicate a mesenchymal stem cell profile: greater than 95% of the sample must report positively for the aforementioned surface antigen markers, and less than 2% can have expression of the markers listed as needing to be negative (Lin et al. 2013). Continuing, listing out as many potential identification markers is important since many cells can share similar surface antigen markers, complicating identification efforts.

There are established experimental protocols when it comes to analyzing and isolating mesenchymal stem cells from blood samples. Density centrifugation, a staple first step, is done to obtain a cell pellet that is then cultured in a flask for maintenance; immunoprofiling, also known as identifying, can be done using antibody staining against the specific surface antigen markers expected on mesenchymal stem cells; lastly, flow cytometry, can be done using a FACSCalibur machine with CellQuest Pro software, common laboratory equipment, to definitively determine the quantity of mesenchymal stem cells present in the sample (Ouryazdanpanah et al. 2018). It is important to note that this straightforward protocol will be implement for all other cell types that need to be assessed for the purposes of this experiment; the main difference lies in which antibodies specific to the cell surface antigens are used for immunoprofiling as the other steps of cell culture and flow cytometry remain largely the same. To assess for the quality of the derived cells, meaning their ability to differentiate into desired cell lines for therapeutics, the isolated cells can be cultured with specific differentiation mediums such as for osteogenic or adipogenic cells; a retained ability to form such cells indicate that liposuction neither irreparably damages the cells nor yields insufficient and unstable cells for medical purposes. Such analyses will be carefully considered as it may be outside the streamlined scope and hypothesis of this singular investigation.

Another cell type that will be investigated and compared with and without liposuction will be hematopoietic stem cells, which typically reside in the bone marrow and can yield numerous cell lineages with hematological and immunological purposes. Such cells are identified as those being CD34, CD3e, B220, CD11b, CD48, CD150 positive and negative for markers such as CD38 (Frisch and Calvi 2014). Staining with these antibodies and analysis with flow cytometry can be undertaken to determine the quantity of hematopoietic stem cells in circulation following liposuction (Frisch and Calvi 2014). An alternative approach can be done using an ATP-focused HALO assay with hematopoietic stem cell growth factors, and this has also been shown to positively quantify stem cell counts in samples (Mikirova et al. 2010).

For endothelial progenitor cells, which hold appreciable power in yielding endothelial cells that line the surfaces of numerous organs and the vasculature of the circulatory system, are known to populate adipose tissue and thus their concentrations need to be examined for the purposes of this investigation. In order to quantify these cells, a similar protocol will be called upon except the surface antigens that will be pinpointed will be different. For endothelial progenitor cells, they are expected to positively stain for CD31, CD144, CD34, and KDR such that it can be qualitatively assessed using flow cytometry (Farkas et al. 2020).

Another cell population that is hypothesized to be disrupted and released during liposuction is endothelial cells. This is based on the premise that some degree of vasculature rupture will occur, and being that endothelial cells line the vessels, they will be released. Thus, increases in their concentration will support the notion that mechanical ruptures of the adipose tissue have occurred, providing a telling rationale for why the other aforementioned stem cell populations increased as well, if they do as they are hypothesized to. The prior literature suggests that surface antigen CD146 is expected to stain positively with antibodies on endothelial cells whereas they are expected to be negative for 7-AAD and CD45 (Elshal et al. 2009). Flow cytometry, as with the other cell types, can be used to obtain quantitative results, and this is considered the standard experimental protocol for cell quantity determination.

The last factor that will be investigated for the purposes of this study is cytokines that could potentially be released following liposuction based on the same premise of there being mechanical disruption of the adipose tissue. In particular, PDGF, TGF-beta, and VEGF will be assessed in the blood samples to see if there are any observable quantitative differences in the circulation concentrations (Czarkowska-Paczek et al. 2006, Nishimoto et al. 2015). There are clear and established guidelines for how such proteins will be assessed, and using an ELISA with the specific antibody following standard blood sample processing is deemed the most appropriate (Szymkowiak et al. 1995).

Altogether, the above that is detailed and listed describes the bulwark of the experimental procedure that is needed for this experiment, and much of the anticipated steps are reinforced and corroborated by numerous examples populating the literature for examining these cell types. Increases in the cell types would support the hypothesis that liposuction causes mechanical disruption that can lead to increased stem cell activation and mobilization. Such findings would be consequential as the need for stem cells only continues to grow as their potential to significantly shape the future of medical treatments is clearer. While liposuction, specifically lipoaspirate, has already been examined extensively, issues regarding SVF hamper its utility in medicine. Finding alternatives is imperative, and this paper seeks to provide evidence to support the notion that stem cell mobilization into the circulatory system occurs.

One limitation of this study is that examining the quality of the stem cells—their degree of stimulation, in other words—is not within the scope of the experiments as currently anticipated. If the hypothesis is corroborated, then probing such questions will be appropriate for another study. Nonetheless, any findings on the concentration of circulating cells, whether there is an increase or no change, will contribute to the growing collective knowledge regarding these highly prized and valuable cell types. Such results can inform future medical interventions and provide new opportunities and alternatives to patients that are currently unavailable.

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