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Article *in* Aesthetic Surgery Journal · July 2017

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Supplement

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Aesthetic Surgery Journal
2017, Vol 37(S3) S4–S8

DOI: 10.1093/asj/sjw270
www.aestheticsurgeryjournal.com
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Abstract

The clinical use of adipose-derived cells is being explored very actively around the world for various human diseases. Adipose tissue is an abundant tissue source that can be easily harvested using liposuction. Human lipoaspirates contain a significant amount of mesenchymal stromal cells, as well as other progenitors and terminally differentiated cell types. This review covers the isolation of adipose stromal vascular fraction (SVF), the quality control and safety analysis of freshly isolated cell suspensions. The comparison between freshly isolated stromal cells and culture expanded cells from adipose tissue samples is also highlighted. This article provides a brief but comprehensive review about SVF isolation in the clinical setting, cell characterization, and biological potency of freshly obtained adipose stromal cells.

Editorial Decision date: December 13, 2016.

ADIPOSE TISSUE AND STROMAL VASCULAR FRACTION

Mesenchymal stem cells (MSCs) are a potential tool for cell-based therapies in the regenerative medicine field. Many scientific studies have reported the capacity of these cells to exert anti-inflammatory and regenerative properties.

Adipose tissue and bone marrow are the two main sources commonly harvested for cell isolation which results in a heterogeneous mixture of autologous adult stem cells, and other stromal cell types with potential clinical merit. Both tissue sources have advantages and disadvantages as shown in Table 1.

Subcutaneous adipose tissue is becoming the first choice for cell isolation because it is easily accessible via liposuction, is relatively abundant in many patients, and contains a higher yield of MSCs compared to other tissue sources (Table 2).[1]

The SVF can be defined as a heterogeneous population of freshly isolated cells from adipose tissue after enzymatic dissociation and subsequent cell concentration by centrifugation. This cell population comprises many different cell types, such as mature endothelial cells, endothelial progenitors, pericytes, fibroblasts, mesenchymal stromal cells, macrophages, etc but excludes mature adipocytes.

Conversely, adipose stromal/stem cells (ASCs) are a more specific cell population derived from whole fresh SVF, which are adherent to plastic in culture, have the ability to differentiate into mesenchymal lineages, and express a very specific immunophenotype (CD45-, CD31-, CD73 + , CD90 + , CD105 +).[2]

In the last 15 years we have seen a huge increase in the number of manuscripts showing basic research and clinical studies using adipose-derived cells. A variety of different methods and point-of-care devices have been developed in the last decade to isolate ASCs from human lipoaspirate obtained through liposuction and the number of preclinical and clinical reports in favor of the safety and efficacy of freshly isolated SVF cells is steadily building.[3]

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Table 1. Comparison Between Bone Marrow and Adipose Tissue Sources for Cell Isolation

	Bone marrow	Adipose tissue sources
Processing time	Fast (15-20 min)	Moderate (60-90 min)
MSC content	Low yield	High yield
User-friendliness	Easy to process	Requires more refined method
Advantages	Easy extraction for orthopedic surgeons	Rich in stromal cells, abundant
Disadvantages	Peripheral blood admixture	Heterogeneous population

MSC, mesenchymal stem cells.

Table 2. Yield of MSCs for Different Tissue Sources. Adapted from Murphy et al.[1]

Tissue source	CFU-f concentration (range per mL)	MSC frequency (CFU per million nucleated cells)
Bone marrow aspirate	109-664	10-83
Adipose	2058-9650	205-51000
Peripheral blood	0	0-2

CFU, colony forming units; CFU-f, colony forming units fibroblastic; MSCs, mesenchymal stem cells.

OVERVIEW OF THE DIFFERENT METHODS AVAILABLE FOR SVF ISOLATION

SVF Cell Isolation Methods: Enzymatic vs Mechanical Isolation Methods

There are a variety of methods available for the isolation of SVF cells, but overall they fall into two general categories: those which use proteolytic enzymes to dissociate lipoaspirate (enzymatic methods) and those which do not (mechanical methods). Mechanical methods include techniques such as washing, shaking, vibrating, or centrifuging in order to separate stromal cell populations from lipoaspirate samples. Enzymatic methods combine washing and shaking with the use of proteolytic enzymes to assist in tissue dissociation. There are advantages and disadvantages to both methods.

Enzymatic methods yield significantly more nucleated cells from an equivalent weight of tissue than mechanical methods and tend to isolate a lower frequency of cells with hematopoietic origin and a higher frequency of stromal/stem cells.[4,5] The use of proteolytic enzymes, usually collagenase and/or neutral protease, can potentially introduce additional risk to the process, as either can potentially trigger an allergic reaction or unwanted tissue degradation in vivo if not adequately removed or neutralized during isolation.

Mechanical methods offer the advantage of being less expensive and time consuming than enzymatic methods because there is no need to purchase expensive good manufacturing practices (GMP) grade proteolytic enzymes and no need to include a digestion step in the isolation process. Mechanical methods typically take 20 to 40 minutes whereas enzymatic methods usually take 60 to 90 minutes. Mechanical methods can be cost-effective in the laboratory setting, where a large quantity of cells may not be required or cells will be cultured, but enzymatic methods tend to be ideal for the clinical setting due the higher yields and superior phenotypic composition of cells isolated.

Manual, Semi-Automated, and Automated Isolation Systems

There are a variety of isolation systems which are commercially available for SVF isolation. These systems simplify the process by providing all of the necessary supplies and reagents in a disposable, single-use kit for purchase. They offer the notable advantage of being closed systems, which reduces the risk of contamination during isolation and can eliminate the need for a biosafety hood. Commercially available systems tend to be less variable than traditional methods using laboratory equipment and glassware (ie, separatory funnel, beakers), as the processes and reagents are more controlled. This approach is better from a regulatory standpoint, as manufacturers may already have product safety and characterization profiles available for consumer use.

There are 3 main levels of commercial systems: manual, semi-automated, and fully automated. As the level of automation increases, so does the cost to operate, with manual methods being less expensive than automated or semi-automated systems; however, as automation increases, less skill is required by the operating technician. Manual methods require the use of standard laboratory equipment (ie, centrifuge, heated shaker, biosafety hood) and a well-trained technician to carry out every step of the process. Semi-automated systems require some user intervention during the isolation process, but the process is simplified by a specialized device which can carry out multiple steps of the process automatically.[6] Fully automated systems require almost no user intervention and contain all necessary equipment to conduct the complete isolation within a single closed system. Users are typically only required to insert lipoaspirate and tissue dissociation enzymes into the system and the device will conduct the entire isolation process. There are isolation systems available at a variety of price ranges to accommodate the needs and financial capability of different labortatories and clinicians; however, laboratories and clinics should be aware that

there can be significant variability between the outputs of different isolation systems.[7-9]

QUALITY CONTROL AND SAFETY ANALYSIS OF SVF CELL SUSPENSIONS

Infection Control

When conducting a clinical treatment using SVF cells, infection control is the most important quality control measure in regards to ensuring patient safety. The two main tests which need to be run in order to assess the sterility of the product for human administration are a Gram stain and aerobic/anaerobic cultures. Administration of the therapeutic product should not proceed until the results of the Gram stain are received and are negative. If a positive Gram stain result is achieved, meaning bacteria are observed, the procedure should not proceed. In the event of a positive aerobic or anaerobic culture, the subject should be monitored closely for signs of infection.

Bacterial Endotoxin Testing

Bacterial endotoxin testing is vital in terms of assessing the safety of the SVF product as well. Bacterial endotoxins are lipopolysaccharides present in the cell membrane of gram-negative bacteria which can potentially cause fever or disease at high enough levels. The maximum safe amount of endotoxins which can be present in a sample is known as the endotoxin limit. The endotoxin limit is determined based on the patient's weight, the sample volume, and the method of administration. United States Pharmacopeia (USP) General Chapter <85> provides complete guidelines for determining the endotoxin limit allowed by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) (USP <85>).[10] Elevated endotoxin levels can be an indicator of a major bacterial contamination of the sample during the isolation process. Additionally, SVF will have endotoxins present as a result of the use of proteolytic enzymes, as these are usually from bacterial origins. There are residual levels of endotoxin present in the final lyophilized product as a result of the manufacturing process. The leading assay used to assess bacterial endotoxin levels is the Limulus amoebocyte lysate (LAL) assay.[11]

Nucleated Cell Counting

Nucleated cell counting and viability assessments are crucial to proper dose preparation. The isolation of SVF cells is variable from isolation to isolation primarily due to patient variability. Setting lot release specifications is an essential part of a proper manufacturing process. The SVF cell isolation should be required to isolate a minimum amount of viable nucleated cells while also being above a certain cellular viability (usually ≥70%). If the isolation does not meet the predetermined lot release criteria, then the clinical treatment should not proceed. The nucleated cell count and cellular viability are indicators of the efficiency of the isolation process and will be used to ensure accurate dosing.[12] It is very important to know the mean cell yield (number of nucleated cells per gram of tissue), since different methods and/or systems can produce different yields.

Flow Cytometry

Product characterization is very important aspect of quality control. Flow cytometry allows for identification of the abundance of the various cell types present in the therapeutic product. For SVF, being that it is a heterogeneous population of cells, this is important as there is significant variation between SVF isolations. While flow cytometry is not included in lot release specifications, it should be well established prior to clinical initiation. The goal of flow cytometry is to identify the proportions of the different cell types contained in the SVF,[13] and most importantly the adipose-derived stem cell content (usually <2%), so that clinicians actually know what they are treating subjects with. To do this, a more targeted flow cytometry protocol is required. Typically, markers screened for are CD31, CD34, CD45, CD90, and CD105 with the target cell population being cells which are CD45-, CD31-, CD73+, CD90+, and CD105+[2].

Residual Proteolytic Enzymes

If the SVF cell isolation method employs the use of proteolytic enzymes, such as collagenase, there will be a risk of excess residual proteolytic enzymes in the final product. The toxicity of residual enzymes with SVF cells is not fully understood but in theory can result in allergic reaction or unwanted tissue degradation in vivo if not adequately removed.[14] The residual enzyme levels can be measured using a number of different assays, but the most common is the furylacryloyl-leucine-glycyl-propyl-alanine (FALGPA) assay. While this would not need to be conducted on every isolation, it is important to have demonstrated in a large enough sample size that the levels present in the final output are so low that they are not clinically significant and do not pose significant risk.

Colony Forming Unit-Fibroblast Assay

The colony forming unit-fibroblast (CFU-F) assay is a valuable tool and a definitive method for quantifying the number of adipose-derived stem cells in a sample of SVF cells. This assay will assess the number of colonies formed after culturing, which is an overall indicator of the frequency and growth properties of adipose-derived stem cells.

Culture conditions must be identical from assay to assay to be comparable from an analytical standpoint. This assay develops the identity of the therapeutic product. A CFU-F assay conducted in tandem with a 6-marker flow cytometry panel will give an accurate assessment of the composition of the SVF isolate once the data set is large enough. Hicok and Hendrick published a method for conducting a CFU-F assay on SVF cells.[15]

Fresh Versus Cultured Autologous Cell Therapies

The clinical use of autologous cell-based therapies using adipose tissue as a cell source can be conducted following two different approaches: use of culture expanded cells (ASCs) or freshly isolated SVF cells. Various factors affect the decision making process, but among the most important are the regulatory requirements needed, the availability of approved GMP facilities, the associated costs, cell dosage, and processing time.

The use of culture expanded ASCs allows the clinician to purify and amplify the number of progenitor cells overtime (several weeks) in culture, which generates a consistent and relatively homogeneous cell population. This procedure follows strictly controlled conditions in a GMP facility, but is costly and takes several weeks to have the cellular dose ready for injection.

The use of autologous freshly isolated SVF cells at the point of care in real time during the same surgical procedure has important advantages: less chance for contamination (especially when using closed processing devices), more rapid processing and clinical application, and fewer associated costs.

The pharmaceutical industry is highly involved in the cell therapy field and supports the clinical use of cultured cells. Conversely, the biotechnology companies that create or manufacture medical devices promote the use of fresh cells that are ready to use at the bedside.[16]

Both strategies have pros and cons, but surprisingly there are relatively few scientific or clinical studies comparing both cell types for the same disease or clinical indication.

There is uncertainty about which cell population would be more effective for different clinical conditions: the heterogeneous SVF or culture expanded ASCs.

Several studies have shown improved results with freshly obtained stromal cells compared with cultured cells. For example, Semon et al[17] reported that an intraperitoneal dose of 1 million SVF cells was more effective than ASCs inhibiting experimental autoimmune encephalomyelitis progression. Marx et al determined that autologous SVF cells also had a more evident effect (by improving the range of motion and pain) than allogeneic ASCs in a model of hip dysplasia in dogs.[18]

In another study, Jurgens et al showed better results of freshly isolated SVF cells compared with cultured ASCs in promoting cartilage and subchondral bone regeneration in a goat model.[19] All of these studies have shown safety and feasibility of point-of-care SVF cell therapies with no adverse effects related with the treatments reported.

In the most recent study, Wu et al in 2016 demonstrated that SVF was better than ASC forming new cartilage matrix when co-incubated with primary human chondrocytes.[20]

Many ongoing clinical trials for different diseases around the world utilize these two different cell populations extracted from adipose tissue. However, due to the lack of scientific and clinical data demonstrating the advantage of either cell type, new well-designed scientific studies are required to compare the efficacy of these cell populations for any given clinical indication. It is also important to highlight that several published manuscripts use the terms ASC when referring to SVF, which is absolutely wrong and must be avoided.[21]

DISCUSSION

This study and the findings included point out the importance of stablishing comparisons between different cell products for human clinical use. In particular, since most published results regarding the clinical efficacy of fresh adipose SVF vs cultured ASCs are comparable, or even better, when using SVF cells, we propose that the use of SVF cells might be more favorable for clinical use. This is supported by the fact that cell culture is time consuming, costly, and with an increased risk of contamination due to more handling.

The use of adipose freshly isolated stromal cells allows the possibility to use and concentrate native cells at the implantation site without changing the natural phenotypic cellular characteristics, as opposed to the use of cultured cells. These cells maintain many cell membrane markers and biological capabilities that might be very useful from a functional standpoint. This is the case of sialomucin CD34 marker expression, which is lost over time during in vitro culture, but stromal cells expressing this marker are key for angiogenesis and osteogenesis in vivo.[22,23]

Nevertheless, new scientific studies are necessary to support these statements, focused on cell quality and potency assays (in vitro and in vivo) of the different cell products applied to specific clinical indications.

CONCLUSIONS

Any clinical procedure involving the isolation and/or use of human adipose-derived cells must be supported by minimum quality and safety controls. It is also strongly recommended to perform cell count and viability analyses at the point of care for every clinical case using live cells.

It remains unclear whether significant functional differences between fresh SVF cells and cultured ASCs exist, and if it has relevance for specific clinical applications. Additional preclinical data (in vitro potency assays or animal studies) and human clinical trials comparing both cell types may help to clarify these questions.

Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

This supplement was funded by GID Europe Ltd (London, UK). The authors did not receive compensation for writing the manuscripts.

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