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Supporting Literature

Study/Article Name	Publication	Date	Author	Key Points
Study of Mesenchymal Stromal Cell Yield from a Commercial Lipoaspirate Processing System	*White Paper*	2013	John R. Chapman, PhD	• Using the StromaCell for centrifugation of either whole lipoaspirate or only the lipoaspirate fluid fraction are rich sources for preparing cell concentrates containing MSC. • Clinical relevance of MSC yield was demonstrated by comparison to bone marrow aspirate which has been successfully used for regenerative medicine. There were greater than 30 fold more MSC present on a per ml basis with the StromaCell output compared to bone marrow aspirate.
Characterization of freshly isolated and cultured cells derived from fatty and fluid portions of liposuction aspirates	Journal of Cellular Physiology	2006	Kotaro Yoshimura, MD	• Fluid fraction of lipoaspirate contains a significant amount of adipose-derived adherent stromal cells (ASC). • The release of ASC into the fluid is thought to be due to mechanical injury during liposuction or released by proteases released during surgery
Isolating adipose-derived mesenchymal stem cells from lipoaspirate blood and saline fraction	Organogenesis	2010	Michael P. Francis, PhD	• Presents an enhanced method for isolating and quickly expanding a robust population of mesenchymal stem cells (MSCs) derived from lipoaspirate in less than 30 minutes. • MSCs have differentiation potential, characteristic cell surface markers, and proliferative lifespan indistinguishable from MSCs extracted from bone marrow or conventionally processed using collagenase.
A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells	Cytotherapy	2013	Jeffrey M. Gimble, MD, PhD	• A non-enzymatic, washing method for isolating ASCs is an alternative to collagenase, particularly when processing large volumes for autologous use. • Average ASC yield after culture for 6 days was 19 fold less for washing method than observed with collagenase.
Power assisted liposuction to obtain adipose-derived stem cells: Impact on viability and differentiation to adipocytes in comparison to manual aspiration	British Journal of Plastic Surgery	2013	Maike Keck, MD	"The quantity and quality of PAL-harvested ASC is similar or even better, respectively, compared to ASCs harvested by manual aspiration."

Study of Mesenchymal Stromal Cell Yield from a Commercial Lipoaspirate Processing System

John R. Chapman, Kayla Horton, Megan Showalter, Vanessa Hull.

Investigation Performed at California State University, Sacramento,
Department of Biology, Sacramento, California

Abstract

In clinical settings, control of technical procedures according to well defined operating procedures and materials are required. There is also an unmet need for a rapid and simple method to prepare cell concentrates containing mesenchymal stromal cells (MSC) at the intra-operative point of care using minimal manipulation methods. The purpose of this study was to evaluate cell concentrates prepared using a commercial lipoaspirate processing system (StromaCell, Microaire, Charlottesville, VA) which serves as both a suction canister and centrifuge based cell separator. In this report, we evaluated the StromaCell for its utility in salvaging a blood fraction from lipoaspirate as a means to prepare an MSC concentrate as determined by an *in vitro* adherent limiting dilution colony forming unit assay. After aspirating the lipoaspirate into the canister, the canister was centrifuged (1,000xg, 10 min) and the blood fraction recovered by syringe in a functionally closed manner. In addition to red cells, platelets and leukocytes, the lipoaspirate derived blood fraction contains cells liberated from the adipose tissue during the liposuction procedure. We employed the colony forming unit–fibroblast (CFU-F) assay so as to measure only viable MSC cells with intact cell proliferation capability. The results from testing 23 lipoaspirate samples demonstrate that harvesting the blood fraction of lipoaspirate using the StromaCell canister provides a rich source of MSC. Further, the results demonstrate that centrifugation of either whole lipoaspirate (adipose tissue fragments and fluid fraction) or only the lipoaspirate fluid fraction (without adipose tissue fragments) are rich sources for preparing cell concentrates containing MSC.

Background

There are three publications of particular importance for the processing of lipoaspirate under minimal manipulation conditions as a means to recover substantial quantities of MSC. These papers are summarized below in chronological order of publication.

Kotaro Yoshimura *et al.*, 2006, were the first to show that the liquid fraction of tumescent fluid contained adipose-derived adherent stromal cells (ASC). In their study, they isolated cells from the fluid portion of liposuction aspirates by centrifugation and designated the cells as liposuction aspirate fluid (LAF) cells. The investigators stated motivation was to pursue treating patients

with minimally manipulated fresh cells likely to have higher safety and efficacy because a sufficient number can be obtained directly by processing liposuction aspirates of large volume and avoiding in vitro cell culture. LAF cells were prepared from the fluid portions of the liposuction aspirates by centrifugation (400g, 10 min). The nucleated cells were further purified for research studies by using Ficoll density gradient. The LAF cells were compared to cells prepared by digestion with collagenase (designated as PLA cells). The LAF cells had a greater number of blood leukocytes than the PLA. The PLA and LAF had equivalent ability to differentiate into adipocytes, chondrocytes and osteoblasts. The immunophenotypic profile of the cultured cells had the same profile consistent with their being MSC. Culture of the PLA and LAF revealed that the adherent cells had the same fibroblast morphology and proliferated with the same doubling times. The number of adherent cells was lower in the LAF than the PLA. After one week of culture, the PLA cells were approximately three fold greater in number than the LAF. The yield of cells varied among the patients and was affected by many factors including donor site and storage duration, method of processing including duration of collagenase digestion. The authors concluded that a significant amount of ASC could be harvested from the liquid portion of the lipoaspirate. The release of the ASC into the liquid fraction was thought by the authors to be due to mechanical injury during liposuction procedure or released by proteases during surgery or subsequent storage periods or a combination thereof. The extent of mechanical injury is known to vary among patients because it can be affected by the size of the suction cannula, vacuum pressure strength during liposuction and suction procedure (powered vs manual) and other factors.

Michael Francis *et al.*, 2010, presented an enhanced method for isolating and quickly expanding a robust population of mesenchymal stem cells (MSCs) derived from lipoaspirate in less than 30 minutes. This isolation process yields an abundant population of adipose-derived stem cells (ASCs) (~100,000 cells per 100 ml of blood/saline collected from sonicated lipoaspirate) with differentiation potential, characteristic cell surface markers, and proliferative lifespan indistinguishable from MSCs extracted from bone marrow (BMSCs) or conventionally processed adipose using collagenase. The authors stated that the method is suitable for the straightforward establishment of patient-specific ASCs for regenerative medicine.

Forum Shah *et al.*, 2010, demonstrated a simple method of washing adipose tissue can be used to isolate adipose stroma/stem cells (ASC). The author characterized the cells and compared their method with the enzymatic procedure in terms of processing time, stem cell yield, differentiation potential and immunophenotype. In this work, the authors were focused on recovering ASC from the fat tissue fragments in the lipoaspirate by centrifugation without the use of collagenase. Independent of the isolation procedure, the resulting passaged ASCs were comparable based on immunophenotype and adipogenic and osteogenic differentiation potential. The average ASC yield after culture for about 6 days was 19 fold less for the washing method than observed with the traditional collagenase method. The authors concluded that an alternative, non enzymatic

method can have utility for isolation of ASCs, particularly when processing large volumes for autologous use.

Study Purpose

The StromaCell System (Microaire, Charlottesville, VA) is a sterile, single-use canister and accessories kit used for the collection of autologous adipose tissue in the clinical laboratory, or intraoperatively at the point of care, utilizing a centrifuge for the preparation of a cell concentrate. The product enables the operator to conveniently isolate and harvest the blood fraction present in lipoaspirate. The purpose of this study was to quantitatively characterize the StromaCell concentrate for the presence of MSC.

Methods

Human Adipose Tissue Samples

Aspirated subcutaneous adipose tissue was obtained from patients undergoing liposuction surgery under informed consent. The liposuction was performed using the PAL® System (MicroAire, Charlottesville, VA). The liposuction technique was essentially equally divided between super-wet technique and tumescent technique. In some cases, only the denser fluid portion (tumescent fluid) of the lipoaspirate was centrifuged in the StromaCell without tissue fragments being present. A method of preparing tumescent fluid fraction essentially depleted of tissue fragments is the use of a Lipofilter (Microaire, Charlottesville, VA) which is designed for preparing adipose tissue for fat grafting.

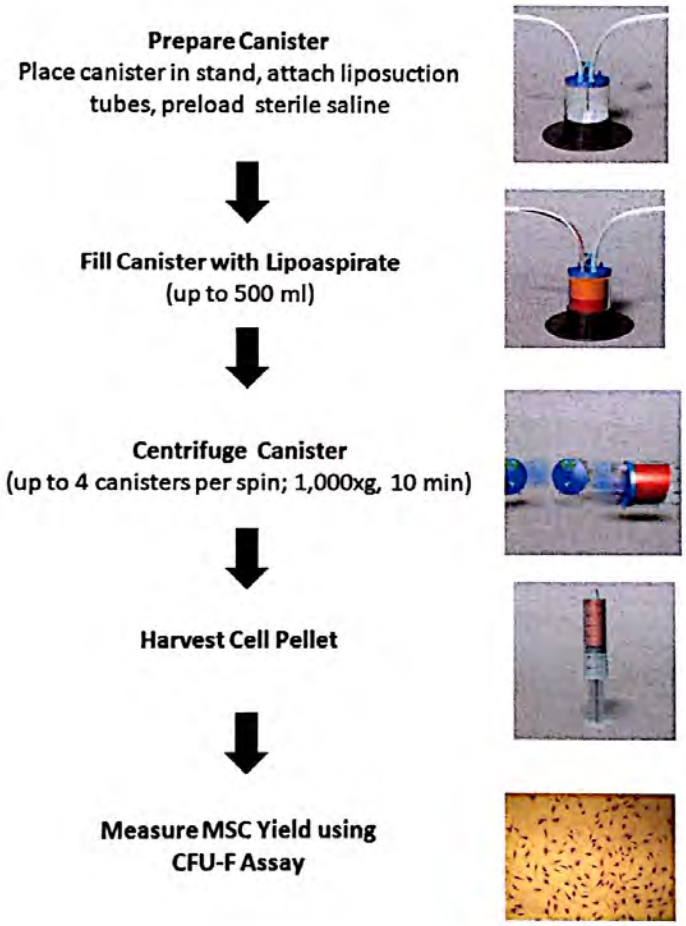
A total of 62 lipoaspirate donors were included in the present study having an average age of 46 years (range of 27-68) and an average body mass index (BMI) of 29 (range 23-37). Greater than 95% of the donors were female with only two male subjects in the study.

Tissue Processing

The lipoaspirate was stored at room temperature and processed within 24 hour of receiving the lipoaspirate. The lipoaspirate was processed using the StromaCell **(Figure 1)** according to the Instructions for Use provided by the manufacture (**Figure 2**). Canisters were filled with up to 500 ml of lipoaspirate or lipoaspirate fraction (e.g., tumescent fluid liquid fraction) using suctions ports in the canister lid. The filled canister was next centrifuged for 10 minutes at 1,000xg during which time the cells having a density of greater than 1.03 g/cm^3 transit out of a first reservoir chamber (500 ml capacity) into a second bottom chamber (15 ml capacity). After centrifugation, a 30 mL syringe was used to resuspend and aspirate the 15 mL lipoaspirate blood fraction cell concentrate in the bottom chamber. The process can be completed within 15 minutes of filling of the canister with lipoaspirate and the system remains functionally closed throughout the process.

Figure 1. StromaCell Suction Canister/Cell Separation Apparatus

Figure 2. Summary of Procedure



Cell Culture Method for Quantifying MSC (CFU-F) in Cell Suspensions

The MSC concentration present in the lipoaspirate derived blood fraction was measured by cell culture using a limiting dilution colony forming unit-fibroblast (CFU-F) assay. The isolated blood fraction concentrate from the StromaCell device were cultured in 24 well plates after performing serial two fold dilutions. The culture dish employed was Corning 3526 Costar 24 well, flat bottom cell culture plates. Media employed was Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% heat shocked fetal bovine serum and Pen-Strep as antibiotics. We also observed similar results when using MesenCult™-SF Culture Kit (Stem Cell Technologies, Vancouver, CA). To quantitate the concentration of mesenchymal stromal cells in the cell pellet recovered from the StromaCell, a limiting dilution assay was employed. First a preliminary dilution of 1/50 was made for each cell suspension to be tested using culture media. Next, each of the 24 wells in a culture dish was filled with 1 ml of media. Next 1 ml of the 1/50 diluted cell suspension was added to the first well to bring the volume to 2 ml in that first well so as to achieve a 1/100 dilution of the cell suspension. Serial two fold dilutions were then made for at least 12 wells (e.g., the 12^{th} well achieved a 1/102,400 dilution of the cell suspension being tested). Cultures were maintained for 10 days in a cell incubator having a normoxic humidified atmosphere with 5% CO_2 at 37°C. On the last day of culture, cells were fixed with methanol and then stained with Giemsa. Next, the number of CFU ($\geq$5 cells in a clear cluster constituting a colony) were counted under microscopic inspection. Samples were tested in duplicate and an average calculated for the CFU-F/ml at the endpoint dilution. The concentration of CFU-F per ml of cell suspension was next calculated by multiplying the dilution for the endpoint well multiplied by the number of CFU-F in that well and the results of duplicate tests averaged to for the CFU-F/ml concentration of cell suspension for each sample. To normalize the sample to the amount of lipoaspirate added to the canister, the MSC concentration per ml of lipoaspirate processed was calculated by dividing the total number of MSC present in the 15 ml of the cell suspension collected from the StromaCell after centrifugation and dividing it by the volume of lipoaspirate processed in that canister.

Flow Cytometric Method for Characterizing StromaCell Output and Expanded Cells

Flow cytometric analysis was performed on four female donors on the StromaCell recovered blood fraction using a Becton Dickinson Accuri C-6 system. Cell concentrates were aliquoted in 50 microliter 1.5 mL Eppendorf tubes and washed with 1 .3 mL of Cell Staining Buffer and re-supsended in staining buffer. The washed cells were stained with a panel of antibodies at recommended concentrations by the manufacturer (Becton Dickinson) with the antibody panel being selected for their specificity to both blood, and tissue derived cells. The cells were stained for 20 minutes in the dark on wet ice and then treated with a combination red cell lysis buffer/cell fixative (eBiosciences) for 30 minutes. Subsequently the cells were washed using two times with Cell Staining Buffer and stored at 4°C prior to flow analysis which occurred within 48 hours of staining. Using the BD Accuri C6 software, cell debris was eliminated by gating on intact cells based on dot plots of forward scatter versus side scatter. Fluorescence analyses in the

form of histograms were analyzed to determine the percentage of cells staining positive and negative for each monoclonal antibody employed. Controls included unstained cells and cells stained with isotype-matched controls conjugated with FITC and Phyoerythrin (PE).

In 2006, an International Society for Cellular Therapy (ISCT) proposed a cell surface marker panel for the minimal identification of human multipotent mesenchymal stromal (MSC) cells. Under this recommendation, MSCs should be positive CD73, CD90, and CD105 but be negative CD34, CD45, CD11b, CD14, CD19 and HLA-DR. In this study, we employed the panel that was proposed by the ISCT by using the BD Human MSC analysis kit (Product No. 562245) to analyze the cells growing in the tissue culture dishes after 3 passages. The procedure provided by the manufacturer for staining and analyzing the samples was followed using the BD Accuri C6 flow cytometer and analysis software.

Results

Tissue Processing Time

The elapsed time from point of filling the canister with lipoaspirate to having the syringe containing the MSC cell concentrate prepared was 15 minutes. The hands on time for the process is less than 5 minutes.

MSC Yield

The findings from analysis of 62 lipoaspirate donor samples processed with the StromaCell are presented in the Table 1 below. On average the volume of lipoaspirate processed was 272 ml yielding 14.7 ml of cell concentrate. Each ml of the cell concentrate was found to contain about 25,000 MSC per ml for a total 350,000 total MSC. Although the capacity of the StromaCell is 500 ml, the present experimental studies were only using about two-thirds of the capacity. For each 100 ml of lipoaspirate processed, we observed that there were 130,000 MSC. This is somewhat greater than the observation of Francis et al. who reported 100,000 MSC per each 100 ml of lipoaspirate processed. We did not observe reproducible differences in MSC yield on those occasions where more than one lipoaspirate sample was collected from body site including abdomen, flanks, arms, breast, and thighs.

Table 1. Recovery of Mesenchymal Stromal Cells from Lipoaspirate using StromaCell.

Lipoaspirate Volume Input (mL)	Stromacell Harvest Volume (mL)	MSC (CFU-F)/ml	Total MSC (CFU-F) Yield	MSC (CFU-F)/100 ml of Lipoaspirate Processed
272 ± 18	14.7 ± 0.2	$2.5x10^4$ ± $7.5x10^3$	$3.5x10^5$ ± $4.8x10^4$	$1.3x10^5$ ± 332

To understand whether the number of MSC recovered from the StromaCell were sufficient to be of clinical relevance, we compared the MSC content of bone marrow aspirate with the concentration of MSC present in the blood fraction recovered from the StromaCell. The results of this comparative study are presented in Figure 3 and Table 2. The data demonstrate that on average there is >30 fold the number of MSC in the StromaCell cell product. Therefore, it would be necessary to obtain >300 ml of bone marrow aspirate to match the MSC yield in the 15 ml of the Stromacell output.

Figure 3. Concentration of MSC in Bone Marrow Aspirate as compared to the MSC concentration observed in the blood fraction recovered from the StromaCell.

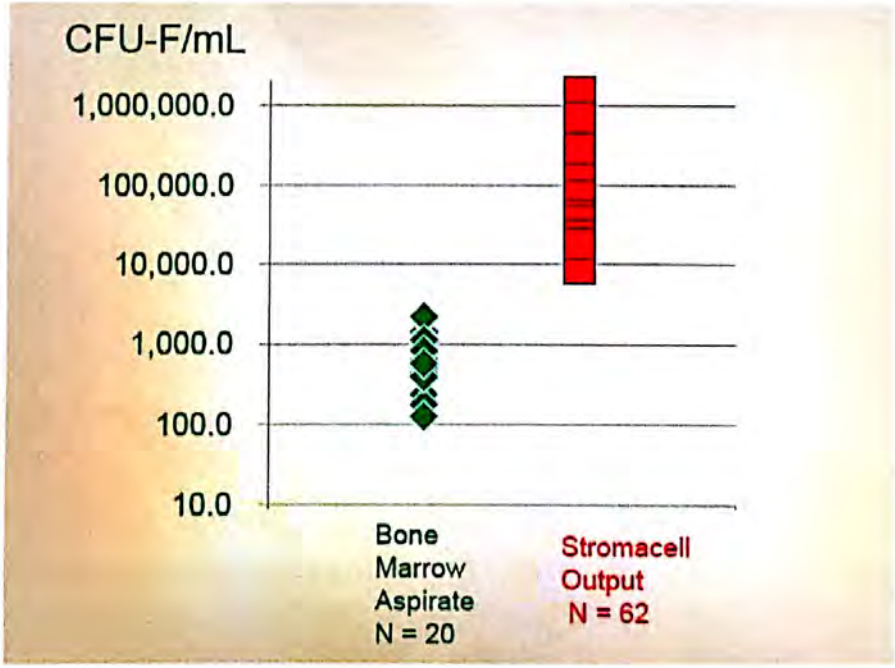


Table 2. Concentration of MSC in Bone Marrow Aspirate as compared to the MSC concentration observed in the blood fraction recovered from the StromaCell.

MSC Source	Number of Samples	MSC (CFU-F)/ml
Bone Marrow Aspirate	20	1,133 ± 261
StromaCell Output	62	25,488 ± 7,548

It was evident that there was significant variation from donor to donor on MSC concentration of the output of the StromaCell. To help understand what potential variables are operative, we analyzed the data in 42 lipoaspirate donations in regard to the following parameters: lipoplasty technique of the surgeon and the characteristics of the donor of the lipoaspirate including donor age, and body mass index. The results of this analysis are shown in Figures 4-6 below.

Figure 4. Plot of total MSC yield according to Lipoplasty Technique. No difference was observed between super-wet and tumescent methods of liposuction.

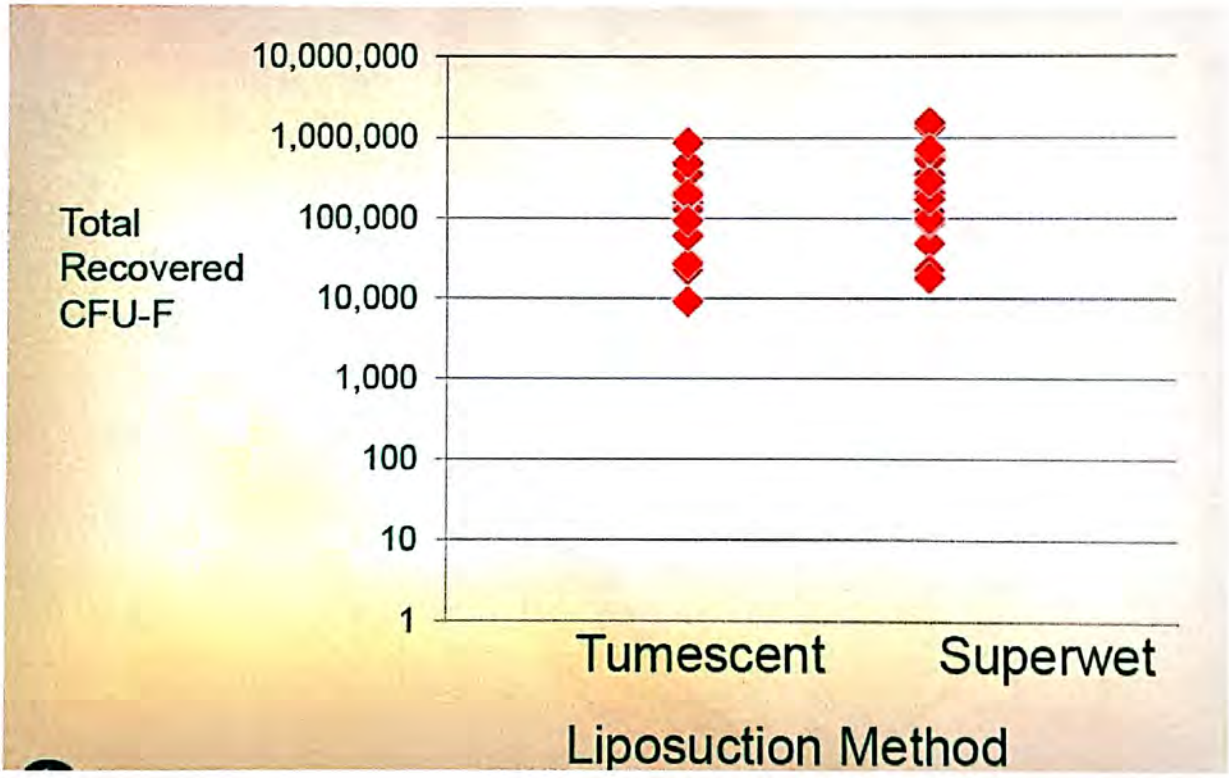


Figure 5. Plot of MSC yield (CFU-F/100 ml of Lipoaspirate processed) versus Donor Age. No correlation was observed.

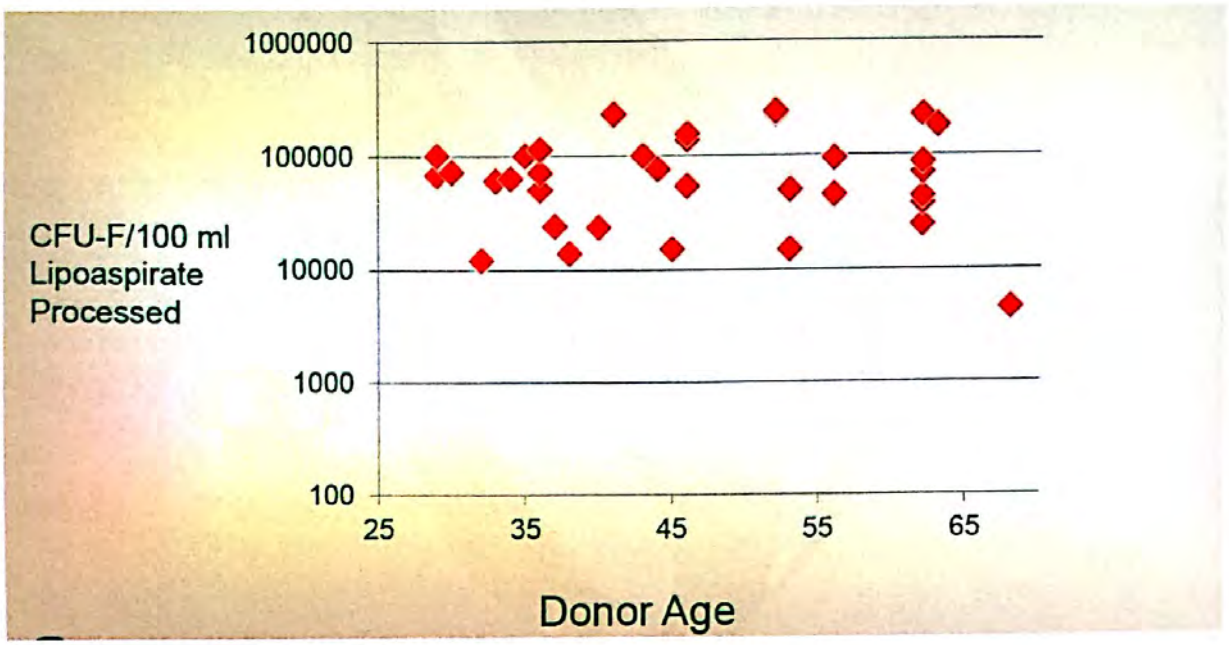
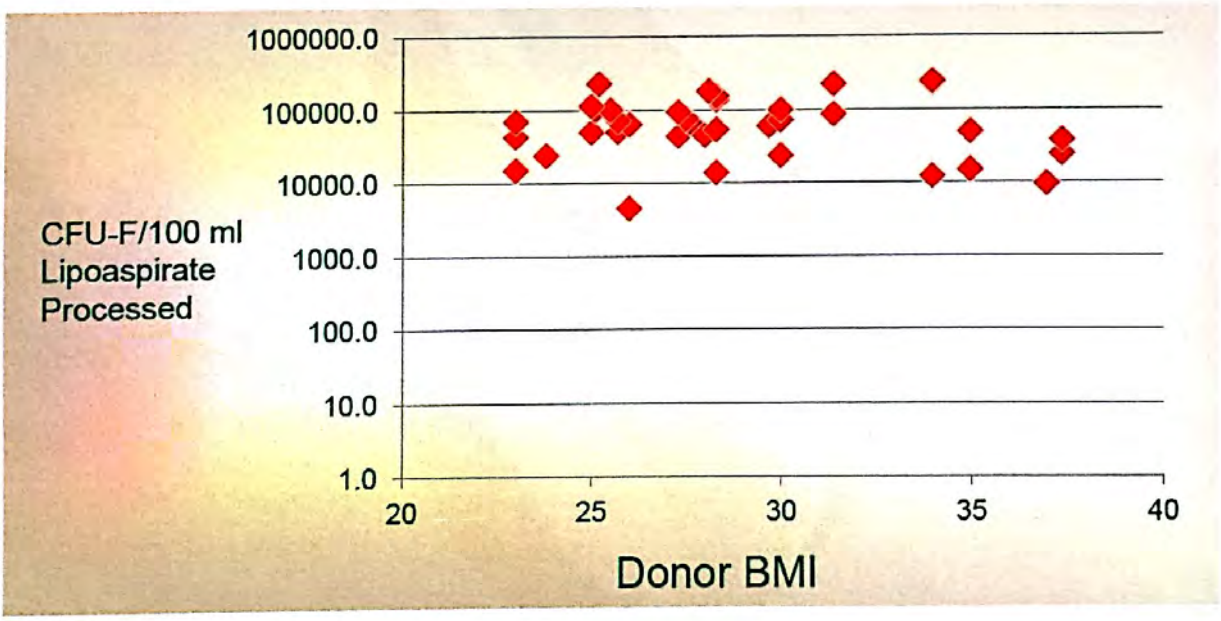


Figure 6. Plot of MSC yield (CFU-F/100 ml of Lipoaspirate processed) versus Donor Body Mass Index (BMI). No correlation was observed.

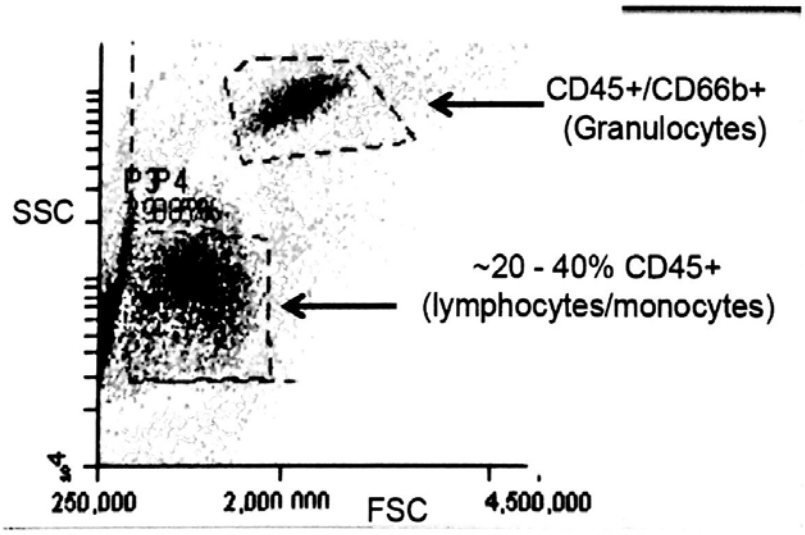


In an additional experiment set, we compared the tumescent fluid without tissue fragments versus whole lipoaspirate (adipose tissue fragments and tumescent fluid). To achieve this object, 400 ml of each donor lipoaspirate was processed as either a 50/50 mixture of adipose tissue and tumescent fluid or as only tumescent fluid. A total of 10 donor lipoaspirate samples were processed with the StromaCell for this data set. Canisters filled with the liquid fraction only of the lipoaspirate (no adipose tissue) as compared to canisters filled with a 50/50 with adipose tissue and liquid fraction were determined to have a MSC yield per 100 ml of lipoaspirate processed of 61,300 $\pm$ 21,400 versus 80,100 $\pm$ 29200 (CFU-F, mean $\pm$SEM), respectively. These results are not significantly different, supporting the conclusion that either the liquid fraction or whole lipoaspirate is a suitable material for processing in the StromaCell canister. The hematocrit of the sample is variable donor to donor and depended on the liposuction methodology employed. The hematocrit was 10 to 24% when the canister was filled with only the liquid phase (no fat) and was less than 7% when the canister was filled with the 50/50 mixture of adipose tissue and liquid fraction (whole lipoaspirate). These levels of red cell are comparable or below those observed in platelet rich plasma buffy coat preparations employed to promote wound healing without issue.

Characterization of Cell Content of StromaCell Output

Flow cytometric analysis of the blood fraction harvested from the StromaCell output demonstrated that a diverse population of cells beyond blood derived are present in the output. Inspection of the forward scatter versus side scatter plot of the flow cytometer revealed that two general population of cells were present in the blood fraction of the Stromacell output as shown below in Figure 7. The cells having greatest side scatter (cell granularity) and forward scatter (size) were identified using CD45 FITC and CD166b PE staining to be granulocytes.

Figure 7. Flow cytometric analysis of StromaCell Ouput reveals two general cell populations are present.



To identify the cell populations present in the low light scatter (forward and side scatter), a panel of antibodies was employed as shown in Figure 8.A. and Figure 8.B.

Figure 8A. Flow Cytometric Analysis of Cells in the Stromal Cell Output for CD33, CD14 (both monocytes markers) versus negative control (Isotype Controls). Monocytes and macrophages were not identified to be present in the StromaCell output based upon these stains.

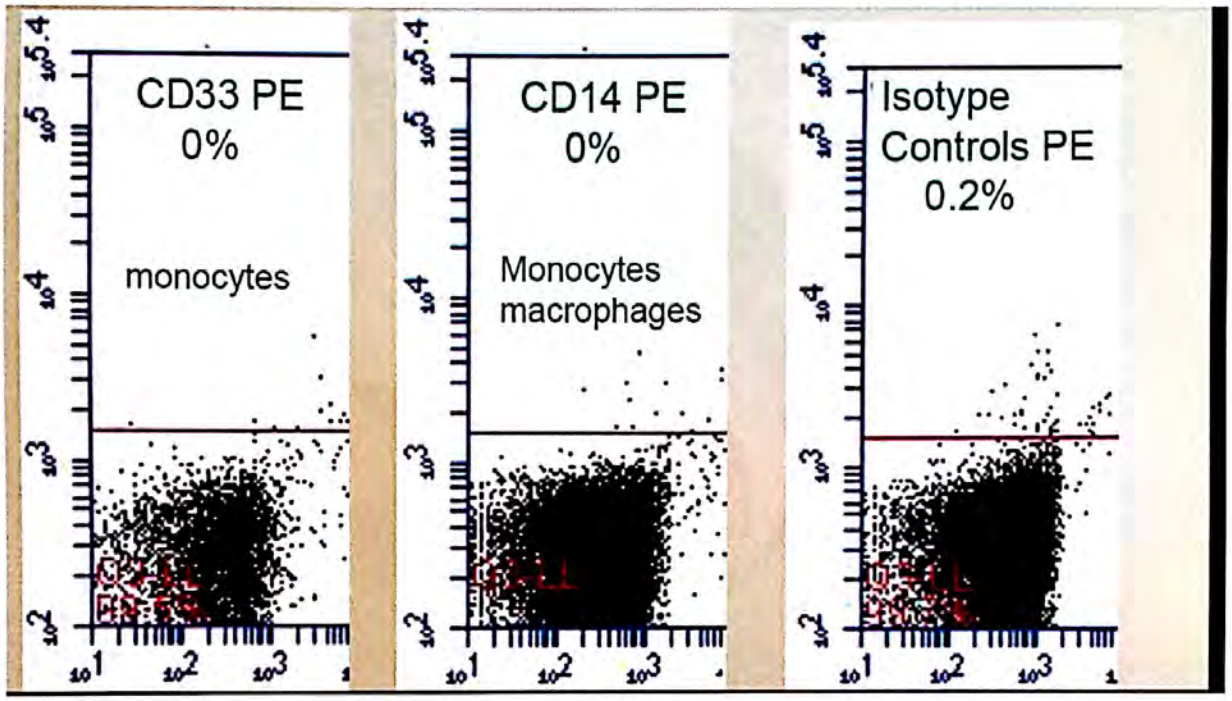
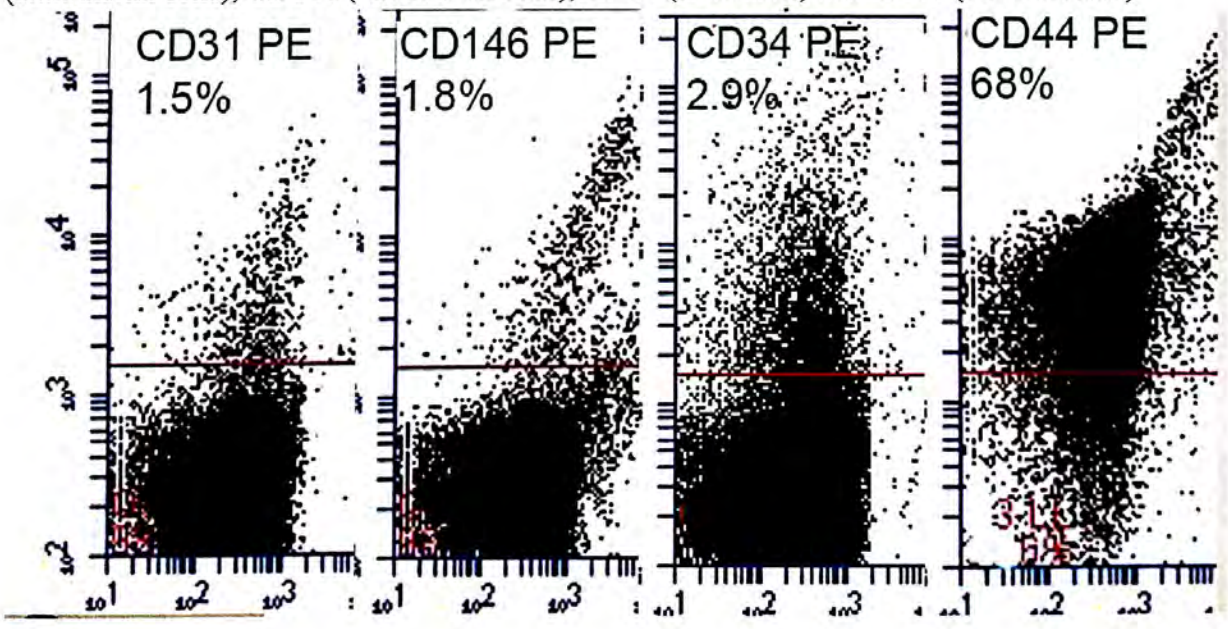


Figure 8B. Flow Cytometric Analysis of CD45- Cells in the Stromal Cell Output for CD31 (endothelial cells), CD146 (endothelial cells), CD34 (stem cells) and CD44 (MSC marker)



This data demonstrate that endothelial cells, stem cell and MSC-related cells are present in the Stromacell output. All of these cell populations are known to be biologically active in wound repair.

Confirmation of Identity of CFU-F cells as being Mesenchymal Stromal Cells.

CFU-F cells were allowed to continue to grow in culture for three passages so as to have sufficient cells numbers for flow cytometric analysis using the BD Human MSC Analysis kit. The results presented in Table 9 demonstrated that complete compliance with all the criteria for the cells to be identified as mesenchymal stromal cells.

Table 9. Results of Flow Cytometric Analysis of StromaCell Derived CFU Expanded in Tissue Culture with BD Human MSC Analysis Kit.

Antibody Stains	Observed Staining
Positive Staining Expected for MSC: CD90, CD44, CD73, CD105	>95% Cells Positive
Negative Staining Cocktail expected for MSC	<2% Cells Positive
Positive Staining Cocktail expected for MSC	>95% Positive

Discussion

In the present study, we evaluated the ability of a commercial lipoaspirate processing device to prepare blood fractions derived from lipoaspirate that contain cells derived from the adipose tissue. The majority of the MSC appear to be released from the adipose tissue during the harvest as the fluid fraction contains substantially the same number of MSC as does a mixture of adipose tissue and fluid fraction. A unique aspect of the present study was the use of a limiting dilution assay to estimate the concentration of MSC present in the processed cell concentrates. We selected this method as being preferable to estimating cell numbers harvested by previous authors which employed counting the total number of cells grown after the a fixed period in tissue culture. Our method requires that the MSC be viable and competent to replicate in order for it to be scored as CFU-F. A disadvantage of the limiting dilution method is that it could underestimate the number of CFU-F present in the sample due to their not being a feeder layer to support the cell growth. Exploratory studies however have shown that performance of CFU-F with a conventional method of CFU-F/10,000 cells cultured provided comparable estimates of MSC in the cell preparation (data not shown). We prefer the limiting dilution assay due to it being a rapid technique and when performed in duplicate provides reproducible data.

The question of how many MSC are required to achieve a clinically meaningful effect is not well established. In a quantitative dose response study conducted by Hernigou for non-union bone healing, the authors observed that a minimum of 50,000 bone marrow derived MSC needed to delivered to achieve a change in clinical outcome. This number of MSC can be readily obtained from less than 100 ml of lipoaspirate as was demonstrated in the present study.

Beyond bone repair, the potential of MSC to promote angiogenesis is a promising approach for improving surgical wound healing. The development of devices such as the StromaCell that makes it convenient to prepare a MSC concentrate from lipoaspirate creates a new opportunity for advancing regenerative medicine. From a regulatory perspective, it is noteworthy that the cell concentrate harvested from the StromaCell canister can be autologous, prepared and used in the same surgical procedure, employ only processing methods clearly defined to be minimal manipulation (centrifugation) by the FDA without additives and can be used for homologous purposes. These attributes are consistent with the cell concentrate being used under the practice of medicine according to the surgeon's considered judgment (Ittleman, 2012).

Conclusions

- We confirmed the findings of Yoshimura et al. (2006) and Francis at al. (2010) that significant numbers of MSC can be isolated from liposuction aspirates by centrifugation.
- MSC recovery was independent of donor age, BMI, or liposuction technique.
- Clinical relevance of MSC yield was demonstrated by comparison to bone marrow aspirate which has been successfully used for regenerative medicine. There were greater than 30 fold more MSC present on a per ml basis with the StromaCell output compared to bone marrow aspirate.
- Flow cytometric studies demonstrated for other cell types beyond MSC that are present in the StromaCell output that have potential therapeutic utility.
- Flow cytometric analysis of cells confirmed the presence of a diverse population of cells being present in the StromaCell output beyond blood derived cells and further demonstrated conclusively that cells being scored as CFU-F have the phenotypic markers of mesenchymal stromal cells.
- StromaCell System provides a simple and rapid means to harvest the MSC rich blood fraction of lipoaspirate in a sterile, functionally closed system.

Disclosure of Interest

JRC is an Adjunct Professor at CSUS and President of Stem Cell Partners LLC which is a biotech company focusing on medical devices for preparing autologous tissue at the point of care for regenerative medicine. The StromaCell is exclusively licensed by Stem Cell Partners to Microaire Aesthetics which provided funding for the study. No interests are declared by other authors.

References

Yoshimura K. *et al.*, (2006): Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. Journal of Cellular Physiology 208:64-76.

Francis M. *et al.*, (2010): Isolating adipose-derived mesenchymal stem cells from lipoaspirate blood and saline fraction. Organogenesis 6:11-14.Shah F. *et al.*, (2013): A non-enzymatic method for isolating human adipose tissue-derived stromal stem cells. Cytotherapy 15:979-985.

Hernigous P. *et al.*, (2005): Percutaneous autologous bone-marrow grafting for non-unions. Journal of Bone and Joint Surgery 87:1430-1437.

Ittleman A. (2012): Enforcement Discretion: How can FDA more reasonably regulate autologous stem cell procedures? Food and Drug Policy Forum 2:1-18.