Adipose Stromal Vascular Fraction Isolation

A Head-to-Head Comparison of 4 Cell Separation Systems #2

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Introduction: With stromal vascular fraction (SVF) cell and adipose-derived stem cell–based technologies translating into the clinical setting, numerous isolation systems have been developed for the point of care isolation of SVF cells from adipose tissue. A relative lack of performance data on these systems can make objective assessment difficult for prospective clinicians. This study compared the performance of 4 SVF cell isolation systems.

Methods: Four isolation systems were compared: the MultiStation by PNC International, the LipoKit by MediKhan, the GID SVF-2 platform by GID Europe Ltd, and the StemSource 900/MB system by Cytori Therapeutics, Inc. Identical lipoaspirate samples for 5 separate donors were used. Stromal vascular fraction output was compared in terms of nucleated cell yield, viability, residual collagenase activity, sterility of the output, colony-forming unit–fibroblast frequency, frequency of CD31−/CD34+/CD45− cells, and operating statistics.

Results: Mean process time ranged from 65.4 to 120.8 minutes. Mean nucleated cell yield per milliliter of tissue processed ranged from 1.01×10^5 cells/mL to 6.24×10^5 cells/mL. Mean cellular viability ranged from 50.3% to 84.02%. Residual collagenase activity was negligible across all systems. Observed colony-forming unit–fibroblast frequency ranged from 0.495% to 1.704%. No significant difference was observed in frequency of CD31−/CD34+/CD45− cells. Results of the anaerobic/aerobic cultures were mixed.

Conclusions: There was considerable variability between the outputs of each system. The system used by a clinician should be tailored to the individual needs of the practice. There is a range of cost options available. This study may help clinicians make more educated decisions when choosing an isolation system to meet their clinical needs.

Key Words: SVF, stromal vascular fraction, stromal vascular fraction isolation, ADSC, adipose-derived stem cells, StemSource 900/MB, Cytori, MediKhan, LipoKit, SVF-2, GID Europe, MultiStation, PNC International, SVF isolation

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The clinical use of stromal vascular fraction (SVF) cells and adipose-derived stem cells has been on the rise in recent years for a variety of clinical indications, both cosmetic and regenerative. Notable uses include the potential to improve the volume retention in autologous fat grafting, facilitating the healing of chronic wounds and treatment of osteoarthritis.[1–3] In an attempt to capitalize on the recent demand, numerous companies have designed various systems for point of care SVF isolation. As a result, several manual, semiautomated, and automated systems for SVF cell isolation are now commercially available or attempting to becoming so. The main goal of these systems was to ultimately provide a rapid, safe, and effective method of providing patients with autologous adipose-derived cellular therapies at the point of care.

It can be difficult for a clinician to assess the performance of the marketed separation systems because of a relative lack of unbiased, head-to-head comparison data between the systems. Additionally, it can be difficult to compare independently reported results between systems because the studies often differ on a significant amount of metrics and end-point assays.[4–8] Various clinically important parameters are needed to accurately compare the clinical utility of systems including process time, processing capacity, nucleated cell yield, cell viability, sterility of the final product, and population characterization of the final output. In this study, we compared 4 different isolation systems: the PNC MultiStation, the Cytori StemSource 900/MB system, the LipoKit by Medi-Khan, Inc, and the GID SVF-2 Platform from The GID Group, Inc.

PATIENTS AND METHODS

Patients and Harvest

Five patients undergoing liposuction procedures at Tower Surgical Center of Santa Monica donated excess lipoaspirate samples for use in this study. Table 1 summarizes the patient demographics. Lipoaspirate was harvested using a tumescent liposuction technique. Tissue was harvested from a variety of locations in each donor, but was aseptically homogenized before being distributed to remove any differences in the tissue quality between isolation systems. Following collection and homogenization, tissue samples were distributed to each system via sterile 60-mL syringes. Between 500 and 1000 mL of lipoaspirate was available for processing from each patient. An estimated 10 to 15 minutes elapsed between the time tissue was harvested and tissue processing began.

SVF Isolation Systems

There are 4 SVF isolation platforms compared in this study. The first system is the MultiStation (PNC International, Gyeonggido, Republic of Korea), an open, manual processing system composed of a heated shaker and centrifuge contained within a biosafety hood (Fig. 1). The second system is the Cytori StemSource 900/MB system (Cytori Therapeutics, Inc, San Diego, CA) which is a closed, automated processing system which requires minimal user intervention to operate (Fig. 2). The third system is the LipoKit platform (Medi-Khan, Inc, Irwindale, CA) which is a closed, manual processing system (Fig. 3). The fourth and final system compared is the GID SVF-2 platform (The GID Group, Inc, Louisville, CO) which is a completely disposable closed, manual processing system (Fig. 4). See Table 2 for a summary of device demographic information. All 4 systems use different tissue dissociation enzyme (TDE) blends to dissociate tissue during the isolation process.

Lipoaspirate Processing

Once aseptically homogenized and aliquoted in 60-mL syringes, tissue samples were distributed to technicians trained on the operation of each system. Varying volumes of tissue were given to each system based on the volumetric capacities of each system (Table 2). Processing was carried out by technicians trained on the operation of each specific system. All systems were physically located at the point of care in the surgical facility and all systems were operated simultaneously. Total processing time, volume of tissue given, volume of washed lipoaspirate, and final volume of SVF output were recorded for each system.

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TABLE 1. Summary of Patient Demographics

Patient #	Age, y	Sex (M/F)	BMI	Harvest Location(s)
1	25	F	22.4	Abdomen Flanks
2	37	F	21.8	Flanks Inner thighs Buttocks
3	27	F	28.3	Abdomen Flanks Buttocks
4	36	F	28.3	Arms Back
5	37	F	27.4	Abdomen Flanks Back
Average ± SD	32.4 ± 5.9	n/a	25.6 ± 3.3	n/a

SVF Analysis

There were 5 main analyses conducted on the output of each system. These 5 tests included the following: nucleated cell count and viability, bacterial detection, flow cytometry, colony-forming unit–fibroblast (CFU-F) assay, and residual collagenase levels.

Sterility Testing

Immediately after isolation was completed, a portion of each sample was aseptically inoculated into blood cultures to test for bacterial growth. One milliliter was inoculated into an aerobic culture bottle (BD BACTEC Plus Aerobic/F Culture Vial) and 1 mL was inoculated into an anaerobic culture bottle (BD BACTEC Lytic/10 Anaerobic/F Culture Vials). Inoculated culture bottles were then couriered to a local pathology laboratory for screening and culturing. Samples were cultured for a maximum of 5 days.

FIGURE 1. The PNC MultiStation.

FIGURE 2. The Cytori StemSource 900/MB system.

Nucleated Cell Count and Viability

Nucleated cell counts and viability measurements were determined using a Chemometec NC-200 cell counting system (ChemoMetec, Allerød Denmark). The total nucleated cell count of the resulting SVF product was determined for each isolation. Additionally, the nucleated cell yields were normalized by calculating the total nucleated cell yield per milliliter of lipoaspirate processed, to remove discrepancies resulting from variable amounts of tissue processed between systems and isolation. Volume digested for the LipoKit and MultiStation protocols was measured as the volume of lipoaspirate used for digestion after the respective washing steps were performed. The volume processed for the Cytori system was determined based on the readout of the machine before digestion. For the SVF-2 platform, it was determined by weighing the system and subtracting the weight of the equipment to

FIGURE 3. The LipoKit system.

FIGURE 4. The GID SVF-2 system.

obtain the weight of the dry adipose tissue contained within after washing was completed. The Cytori and LipoKit samples were passed through 100-μm cell strainers before counting, as the protocols did not include a straining step in their respective protocols.

Flow Cytometry

Cellular identification of the stromal population of cells contained in the final SVF output from each system was screened for analysis of the surface markers CD31, CD45, and CD34. Antibodies used were BD Pharmingen FITC mouse anti-human CD31, APC mouse anti-human CD34, and PE mouse anti-human CD45 (BD Biosciences. San Jose, CA). eBioscience fixable viability dye eFluor450 (eBioscience, Inc, San Diego, CA) was included to label dead cells during flow analysis. Flow cytometry samples were prepared after determining the nucleated cell density of each sample to normalize the number of cells analyzed. Samples were fixed in a 4% paraformaldehyde a run at the local flow cytometry core within a week of preparation. Samples were run on a Becton Dickinson LSRII. Thirty thousand events were recorded for each sample.

CFU-F Assay

Cells recovered from the different systems were plated in 6-well plates at 2 different cell densities per plate (1000 nucleated cells/well and 2500 nucleated cells/well) in standard adipose stromal cell growth media (Dulbecco Modified Eagle Medium: F12 with 10% fetal bovine serum, 1% penicillin/streptomycin and 1X GlutaMAX). Colonies were grown for 10 to 14 days and then fixed and stained with crystal violet for enumeration. Colonies were counted for all wells per plate, but the highest and lowest values per plate were discarded and the remaining 4 wells were averaged to generate a colony frequency for each sample.

Residual Collagenase Activity Assay

The residual collagenase activity was determined using the Collagenase Activity Colorimetric Assay kit (Biovision Incorporated, Milpitas, CA). The assay was conducted according to manufacturer's specifications. The assay reports activity levels in FALGPA units, but the final values in the paper were converted into Wunsch (PZ) units, a more common unit used to express collagenase activity. The conversion rate is 3.9 FALGPA units equals to 1 Wunsch unit.[9] One Wunsch unit catalyzes the hydrolysis of 1 μmol 4-phenylazobenzyloxycarbonyl- L-prolyl-leucyl-glycyl-L-prolyl-D-arginine per minute at 25°C, pH 7.[10]

Statistics

Nucleated cell yield, viability, CFU-F formation, flow cytometry results, and residual enzyme activity were each analyzed independently across systems using a 1-way analysis of variance. The Tukey–Kramer method was used to compare differences between systems. Statistical significance was defined as $P < 0.05$. Error bars in the figures represent the standard error. In graphs and tables, "Cytori" refers to the Cytori StemSource 900/MB system, "SVF-2" refers to the GID SVF-2 platform, "LipoKit" refers to the MediKhan LipoKit Platform, and "MultiStation" refers to the manual isolation method carried out using the PNC MultiStation.

RESULTS

Table 2 summarizes the device demographics and processing statistics recorded. It should be noted that during the fourth sample isolation, the LipoKit system was operated using an expired enzyme blend with little/no enzymatic activity. Therefore, data were not included from the fourth trial run for the LipoKit in terms of nucleated cell count, viability, residual collagenase, flow cytometry, or CFU-F frequency as these data would not accurately reflect the output from the system if run under normal processing conditions. Data were, however, included for infection control and processing demographics, as these would not be affected.

Nucleated Cell Count and Viability

The MultiStation yielded an average total nucleated cell count of 5.78×10^7 cells and an average of 5.35×10^5 nucleated cells per milliliter of lipoaspirate processed with an average viability of 82.02%. The LipoKit yielded an average total nucleated cell count of 4.90×10^7 cells and an average of 6.24×10^5 nucleated cells per milliliter of lipoaspirate processed with an average viability of 50.3%. The GID SVF-2 yielded an average total nucleated cell count of 1.44×10^7 cells and an average of 2.85×10^5 nucleated cells per milliliter of

TABLE 2. Summary of Processing Demographics

Platform	Processing Capacity, mL	Average Lipoaspirate Volume Given [Range], mL	Average Washed Lipoaspirate Processed [Range], mL	Average Processing Time [Range], min	Average Final Volume of SVF [Range], mL
MultiStation	50–800	156 [120–180]	105.6 [68–150]	65.4 [59–74]	12.2 [10–15]
LipoKit	25–400	140 [100–180]	71.4 [40–97]	120.8 [99–149]	20 [15–25]
GID SVF-2	20–120	102 [90–120]	53.2 [32–88]	71.4 [68–75]	7.2 [6–9]
Cytori	120–360	162 [120–180]	126 [90–150]	89.4 [85–93]	5 [5]

TABLE 3. Summary of Cell Counts and Viability

Platform (n)	Average Total Nucleated Cell Count [Range], cells	Average Nucleated Lipoaspirate [Range], cells/mL	Viability [Range], %
MultiStation (5)	57,790,000 [25,400,000–145,200,000]	535,516 [282,222–1,161,600]	82.02 [71.3–91.3]
LipoKit (4)	39,501,200 [41,040,000–58,500,000]	624,627 [538,660–678,462]	50.3 [30.7–72.6]
GID SVF-2 (5)	14,454,000 [10,080,000–21,910,000]	284,734 [114,545–466,875]	69.26 [54.3–82.3]
Cytori (5)	12,010,000 [6,700,000–15,750,000]	101,061 [49,630–175,000]	84.02 [80.5–88.6]

lipoaspirate processed with an average viability of 69.26%. The Cytori StemSource 900/MB system yielded an average total nucleated cell count of 1.20×10^7 cells and an average of 1.01×10^5 nucleated cells per milliliter of lipoaspirate processed with an average viability of 84.02%. Results are summarized in Table 3 and Figures 5A, B.

Residual Collagenase Activity

The SVF output from the MultiStation was shown to contain 3.21×10^{-3} total Wunsch units of collagenase activity and 2.87×10^{-4} Wunsch units per milliliter of SVF. The SVF output from the LipoKit was shown to contain 1.14×10^{-2} total Wunsch units of collagenase activity and 5.71×10^{-4} Wunsch Units per milliliter of SVF. The SVF output from the GID SVF-2 was shown to contain 4.78×10^{-3} total Wunsch units of collagenase activity and 6.46×10^{-4} Wunsch units per milliliter of SVF. The SVF output from the Cytori StemSource 900/MB system was shown to contain 1.49×10^{-3} total Wunsch units of collagenase activity and 2.97×10^{-4} Wunsch units per milliliter of SVF. Results are summarized in Table 4 and Figures 6A, B.

Sterility Testing

The SVF output from the MultiStation tested positive for bacterial growth in 4 of 5 samples. The SVF output from the LipoKit tested positive for bacterial growth in 4 of 5 samples. The SVF output from the GID SVF-2 tested positive for bacterial growth in 3 of 5 samples. The SVF output from the Cytori StemSource 900/MB system tested positive for bacterial growth in 3 of 5 samples. Results are summarized in Table 5. Contaminating organisms identified include *Staphylococcus capitis*, *Staphylococcus epidermidis*, and *Staphylococcus lugdunensis*.

Flow Cytometry

Analysis via flow cytometry showed the SVF output from the MultiStation contained an average CD31−/CD34+/CD45− cell frequency of 9.0%. Analysis via flow cytometry showed the SVF output from the LipoKit contained an average CD31−/CD34+/CD45− frequency of 7.15%. Analysis via flow cytometry showed the SVF output from the GID SVF-2 Platform contained an average CD31−/CD34+/CD45− frequency of 8.88%. Analysis via flow cytometry showed the

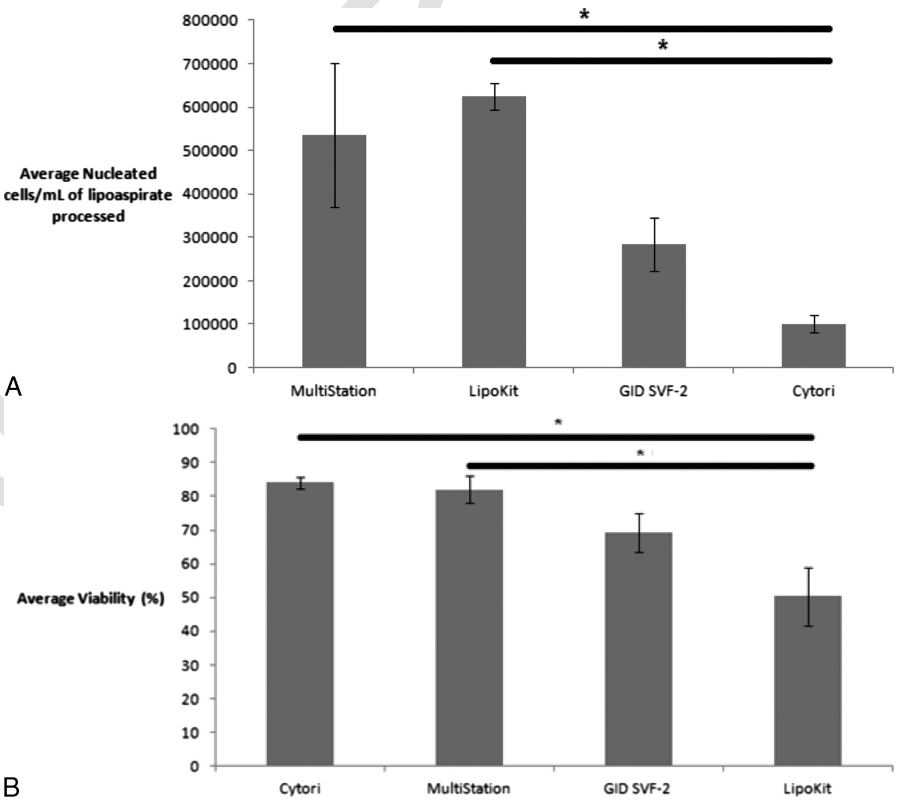

FIGURE 5. A, Average nucleated cells per milliliter of washed lipoaspirate processed. Vertical bars shown represent the standard error. Horizontal bars represent statistically significant differences ($0.05 > P$). B, Average nucleated cell viability. Vertical bars shown represent the standard error. Horizontal bars represent statistically significant differences ($0.05 > P$).

TABLE 4. Summary of Residual Collagenolytic Activity

Platform (n)	Wunsch Units per mL SVF [Range]	Total Wunsch Units [Range]
MultiStation (5)	0.000287 [0.000103–0.000538]	0.00321 [0.00154–0.00538]
LipoKit (4)	0.000571 [0.000436–0.000795]	0.0114 [0.0107–0.0122]
GID SVF-2 (5)	0.000646 [0.000436–0.000872]	0.00477 [0.00305–0.00785]
Cytori (5)	0.000297 [0.000128–0.000487]	0.00149 [0.000641–0.00244]

SVF output from the Cytori StemSource 900/MB system contained an average CD31−/CD34+/CD45− frequency of 10.68%. Results are summarized in Figure 7.

CFU-F Assay

The SVF output from the MultiStation resulted in an average CFU-F frequency of 0.680%. The SVF output from the LipoKit resulted in an average CFU-F frequency of 0.495%. The SVF output from the GID SVF-2 platform resulted in an average CFU-F frequency of 1.388. The SVF output from the Cytori StemSource 900/MB resulted in an average CFU-F frequency of 1.704%. Results are summarized in Figures 8A, B.

DISCUSSION

There is a growing need for unbiased characterization of the output of SVF isolation platforms. These data are vital to the proper evaluation of the growing body of clinical research using SVF cells as well as the generation of future data. When establishing a protocol for the clinical use of SVF cell–based therapies, it is important to set up quality standards for the final therapeutic product which will be

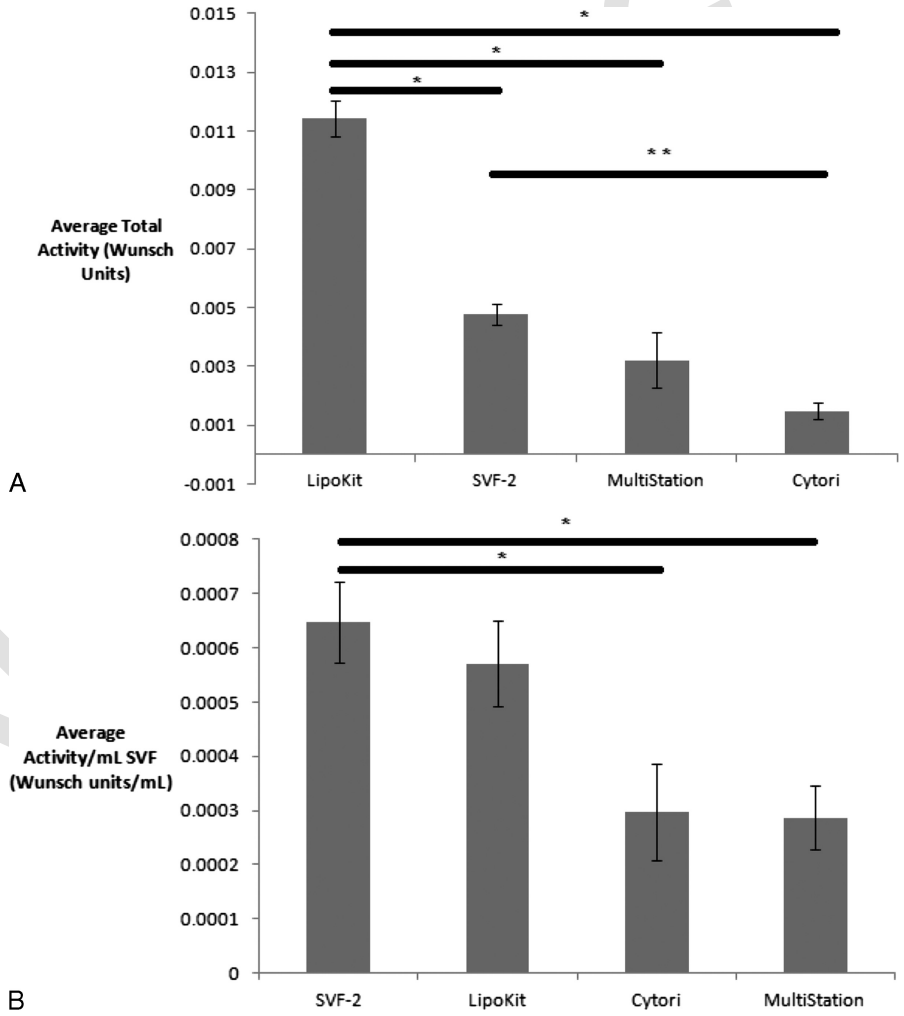

FIGURE 6. A, Average total collagenase activity in the final SVF output reported in terms of Wunsch units. Vertical bars shown represent the standard error. Horizontal bars represent statistically significant differences (0.05 > *P*). B, Average collagenase activity per milliliter of SVF output reported in terms of Wunsch units. Vertical bars shown represent the standard error. Horizontal bars represent statistically significant differences (0.05 > *P*).

TABLE 5. Summary of Anaerobic/Aerobic Culture Results

	Culture Result				
Platform	**Sample #1**	**Sample #2**	**Sample #3**	**Sample #4**	**Sample #5**
MultiStation	+	+	+	−	+
LipoKit	+	+	+	−	+
GID SVF-2	+	−	+	−	+
Cytori	+	+	+	−	−

+, Positive for bacterial growth.
−, Negative for bacterial growth.

used for treatment. This often involves controlling the number of cells injected, meeting infection control standards, having a well-characterized output and meeting minimum cellular viability requirements to proceed with a clinical procedure. Having a better idea of the expected yield and composition of the SVF output can allow clinicians to better judge which system is best suited for their clinical needs. Although the results here have shown various significant differences between the outputs from each platform, it does not necessarily make one system more superior than another because there are more practical aspects which need to be considered such as cost, processing time, and ease of operation.

The nucleated cell count is one of the primary factors used to judge and/or market a system, but a lower count does not necessarily mean an inferior output. Nucleated cell yield is important from a clinical standpoint because all Food and Drug Administration (FDA)–approved clinical trials in the United States and most of those abroad are required to administer a controlled dose, either a total number of cell injected or a density-based dosage such as number of cells per unit of area (cells/area), depending on the type of treatment being administered. The ability to isolate a large number of cells is vital to being able to prepare an adequate dose every time. It is also worth noting that there is no established dose versus effect relationship for most proposed clinical applications of SVF or cultured adipose-derived stem cell–based therapies.

Nucleated cells per milliliter of tissue processed are an important statistic to track because it is a gauge of the efficiency of the system. Being able to isolate more cells from a given volume of tissue allows a system to potentially process less tissue to achieve a similar yield as another system, which has important clinical implications because some patients do not always have excessive adipose tissue to submit for isolation. However, a lower average nucleated cell yield per milliliter of tissue can be offset by the ability process larger amounts of tissue. This will allow an adequate number of cells to still be isolated to hit the target dosage assuming adequate lipoaspirate is available. Looking at the Cytori StemSource 900/MB system, we see the lowest average cell counts between all of the systems, but the StemSource 900/MB system is able to process a large amount of tissue, up to 360 mL which can help offset a lower yield per milliliter of tissue. In summary, the nucleated cells per milliliter of tissue is not necessarily as important as long as you are able to process enough tissue to compensate and be able to isolate the required number of cells for the prespecified dosing scheme. It is worth noting that the nucleated cell yields observed from the StemSource 900/MB in this trial are lower than those we have typically observed in our personal experience with the device in both our published[11] and unpublished data, most likely due to the use of enzymes close to expiration. Additionally, although the GID SVF-2 platform was investigated here, GID also makes the GID SVF-1 platform which has a larger processing capacity of 100 to 350 mL of dry adipose tissue and has been reported to yield more nucleated cells, 7.19×10^5 nucleated cells/mL of tissue, using an almost identical processing method as the SVF-2.[7] It is unclear what may have led to the difference in reported nucleated cell yield between these 2 platforms.

In terms of cellular viability, there were some significant differences between platforms. The viability of the final output is a very important clinical factor because nonviable cells provide no therapeutic value and can potentially lead to excess localized inflammation in the treatment site due to excessive cellular debris. Viability standards in most clinical protocols have typically been set at greater than or equal to 70% in terms of lot release criteria. Although this number is arbitrary, it is a good standard to go by. Although all of the platforms measured were able to achieve single isolation viabilities greater than 70% viable, there were a notable number of isolations which were below this mark. The StemSource 900/MB had the highest average and most stable viability across all 5 isolations, with all of the isolations yielding greater than 80% viability. All 5 of the isolations using the MultiStation yielded greater than 70% viability as well. Only 1 of 4 isolations using the LipoKit was greater than 70% viability and 3 of 5 isolations were greater than 70% for the GID SVF-2 platform. There are a number of factors which can affect the cellular viability of the output including processing methodology, enzyme blend used, digestion time and more,

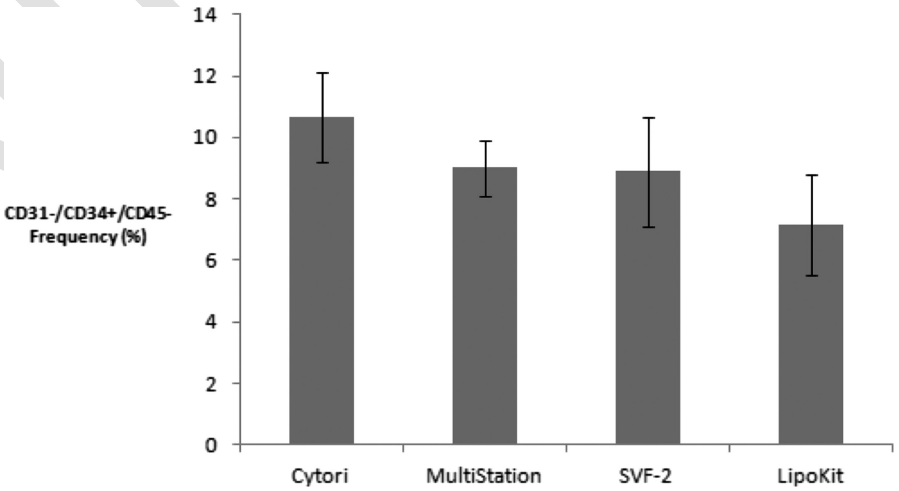

FIGURE 7. Comparison of CD31−/CD34+/CD45− frequency as measured by flow cytometry. Vertical bars shown represent the standard error. There were no statistically significant differences observed ($P > 0.05$).

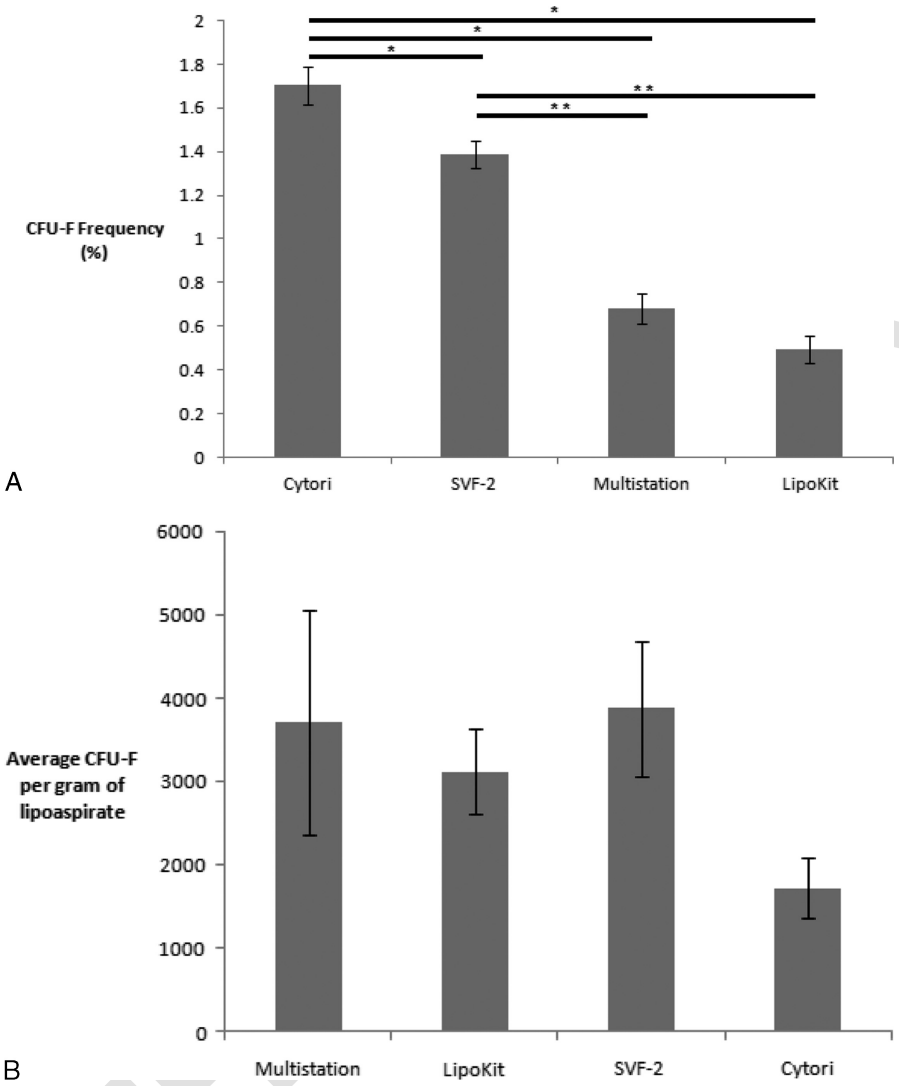

FIGURE 8. A, Average CFU-F frequency of the SVF output from each platform. Vertical bars shown represent the standard error. Horizontal bars represent statistically significant differences ($0.05 > P$). B, Average CFU-F per gram of lipoaspirate processed. Calculated based on comparison of CFU frequency and nucleated cells yield per gram of lipoaspirate processed. Vertical bars represent the standard error. No statistically significant differences were observed between any of the systems ($P > 0.05$).

but it would be difficult to assess the strongest contributing factor here, as these factors were not controlled across systems.

Establishing the level of residual collagenase activity for a platform has clinical merit as well. Focusing on the United States FDA, residual collagenase levels will be required to be established for any collagenase-based enzymatic isolation protocol. This is because of the theorized risks associated with the injection of proteolytic enzymes like collagenase. These risks include allergic reaction as well as unwanted

TABLE 6. Summary of Cost to Operate and TDE Mixtures

Platform	Disposable Cost (US$)	TDE Mixture	TDE Activity	Additional Equipment Needed
MultiStation	$250 per 100 mL of tissue processed	SERVA Collagenase NB6	≥100 Wunsch units per gram	MultiStation
LipoKit	$450 per 100 mL of tissue processed	Time Machine Accelerator (CSN-TMAX)	25 Wunsch units per vial	Centrifuge Celltibator
SVF-2	$1000 per 20–120 mL of tissue processed	GIDzyme-50	50,000 CDU* per vial	Heated Shaking Unit Centrifuge
Cytori	$2400 per 120–360 mL of tissue processed	Reagent A	Not disclosed	StemSource 900/MB system

*One Collagen Digestion Unit (CDU) liberates 1 μmol of L-leucine equivalents from collagen in 5 hours at 37°C, pH 7.5.

tissue dissociation in vivo. When looking at the results of the residual collagenase activity from all of the platforms examined in this study, it seems that all of the isolation protocols are able to effectively remove almost all of the enzymes. The levels of activity observed in this study are negligible and in our experience have not posed any significant risk in the clinical setting.[12] Compared to collagenase-based products already approved by the FDA in the United States such as Xiaflex (injectable) and Collagenase Santyl (topical),[13–20] we can see that these products contain much higher activity than those seen in the final SVF outputs of any of these systems and have still been deemed safe for human use with low risk of serious adverse events.

The flow cytometry and CFU-F assay can be looked at in tandem. These 2 parameters give us a better idea of the composition of the nucleated cells isolated and how many of them are actually stem and regenerative cells. Despite having lower overall nucleated cell counts, the Cytori and GID SVF-2 platforms had the highest CFU-F frequencies, suggesting that fewer blood cells are isolated using these methods. The stromal fraction, CD31−/CD34+/CD45− cells, typically falls between 5% and 15% for SVF from adipose tissue,[8] and all of the system outputs fell within that expected range. Although differences were observed in the average frequency of stromal fraction cells, none of these differences were deemed statistically significant. Proper characterization of the SVF output is important because it is reasonable to assume that the composition of the cellular population will affect efficacy of treatments; for example, an SVF output may have a high cell count, but a high frequency of blood leukocytes, it may not be as effective as an output with fewer total cells containing a lower frequency of blood leukocytes.

Infection control is a major requirement for safe clinical administration of SVF-based therapeutic agents. Although most of the samples tested in this study resulted in cultures positive for the presence of bacteria, this does not mean that they are not suitable for clinical use. From a clinical trial standpoint, a positive culture result would not necessarily be a factor for disqualifying a subject, but merely suggests that an antibiotic regimen might be required. On the basis of the microbial identification, we can see that the 3 species which were identified in any of the cultures are part of the normal skin microflora and could result from a number of factors. The presence of a positive culture does not necessarily mean that contamination occurred during processing, as the lipoaspirate may in fact contain these bacteria from the skin as a result of the harvesting. Although this study only took into account the results of a long-term culture, a full infection control scheme for a clinical trial in the United States using SVF would most likely be required to have 3 parts: a STAT gram stain which must be negative before treatment can occur, an anaerobic and aerobic culture, and a STAT endotoxin test showing acceptable levels of endotoxin before treatment. Were we to repeat this study, we would include both a Gram stain and endotoxin testing in addition to the aerobic/anaerobic culture.

Additional important factors from a clinical use standpoint include ease of use and cost to operate. The StemSource 900/MB system was the easiest system to operate. There is almost no user intervention required from the time tissue is introduced into the system until the time the final output is extracted. This reduces the variability in the processing steps by reducing errors which result from user intervention. In theory, this leads to a more regular and predictable output from the system. The largest clinical deterrent for the StemSource 900/MB system is the cost to operate, with a single disposable kit costing up to $2400 for the TDE mixture and disposable kit, according to our most recent quote (January 2015) directly from Cytori Therapeutics, Inc. The MultiStation and LipoKit protocols both require significant user intervention and would require a more skilled technician to operate to the potential seen in this study, but the tradeoff being that these have the lowest cost to operate. The MultiStation costs around $250 per 100 mL of tissue processed. The LipoKit costs $450 per 100 mL of tissue processed giving a range of $450 (≤100 mL) to $1800 (400 mL), depending on the volume of tissue processed. The GID SVF-2 platform required a moderate amount of user intervention, but overall was a very simple system to be trained on and operate with a low processing time. The cost for a single isolation using the SVF-2 platform is around $1000 for one vial of enzyme and 1 disposable kit. The SVF-2 platform is only limited by its small volume processing, with its maximum processing volume set at 120 mL, but as previously mentioned a larger model, the SVF-1, is available for larger isolations.

The processing time is another clinically relevant characteristic. In this study, there were variable processing times seen for processing similar volumes of tissue which ranged from 65.4 to 120.8 minutes. As we can see from the differences between the LipoKit and MultiStation, longer processing time is not directly associated with increased cellular yield. For a clinician, a shorter isolation time is ideal, allowing for less idle time during the procedure and also would allow for more procedures to be completed in a single day if desired. However, all of the isolation times reported in this study are still clinically relevant and would still be viable options for point-of-care processing.

All cost points for the StemSource 900/MB, GID SVF-2, and LipoKit disposable materials were received directly from the manufacturers and are subject to change. The cost of the MultiStation protocol was determined based on our own unpublished data. The cost points presented here do not include the cost of the systems or additional equipment required to operate the systems. The GID SVF-2 system requires a centrifuge and a heated shaker to carry out isolations. The LipoKit platform requires the use of a centrifuge as well as the Celltibator, a specialized incubation unit. Table 6 summarizes cost to operate.

CONCLUSIONS

Although the 4 systems compared in this trial differ in various aspects of the SVF output, the system a clinician uses depends on the needs of the practice. We feel that the information presented in this article will allow clinicians and potential clinicians to make more educated decisions when choosing a system to use in their practice. In addition, we hope the information presented here will help to facilitate proper clinical practices for new clinicians entering into the field of adipose-derived SVF-based cellular therapies.

REFERENCES

1. Matsumoto D, Sato K, Gonda K, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived stem cells for soft tissue augmentation with lipoinjection. *Tissue Eng*. 2006;12:3375–3383.
2. Marino G, Moraci M, Armenia E, et al. Therapy with autologous adipose-derived regenerative cells for the care of chronic ulcer of lower limbs in patients with peripheral arterial disease. *J Surg Res*. 2013;185:36–44.
3. Garza JR, Santa Maria D, Palomera T, et al. Use of autologous adipose-derived stomal vascular fraction to treat osteoarthritis of the knee: a feasibility and safety study. *J Regen Med*. 2015;4:1.
4. Wang L, Lu Y, Luo X, et al. Cell-assisted lipotransfer for breast augmentation: a report of 18 patients. *Zhonghua Zheng Xing Wai Ke Za Zhi*. 2012;28:1–6.
5. Lin K, Matsubara Y, Masuda Y, et al. Characterization of adipose tissue-derived cells isolated with the Celution system. *Cytotherapy*. 2008;10(4):417–426.
6. Fraser JK, Hicok KC, Shanahan R, et al. The Celution® System: automated processing of adipose- derived regenerative cells in a functionally closed system. *Adv Wound Care*. 2013;3:38–45.
7. Vilaboa SD, Navarro-Palou M, Llull R. Age influence on stromal vascular fraction cell yield obtained from human lipoaspirates. *Cytotherapy*. 2014;12:1092–1097.
8. Aronowitz JA, Lockhart RA, Hakakian CS. Mechanical versus enzymatic isolation of stromal vascular fraction cells from adipose tissue. *Springerplus*. 2015;4:713.
9. Optimize your tissue dissociation procedure. Roche. Accessed November 23, 2015. Available at: https://lifescience.roche.com/webapp/wcs/stores/servlet/PrintView?langId=−1&storeId=15016&articleId=68027.
10. Wünsch E, Heidrich HG. On the qualitative determination of collagenase. *Hoppe Seylers Z Physiol Chem*. 1963;333:149–151.
11. Aronowitz JA, Ellenhorn JD. Adipose stromal vascular fraction isolation: a head-to-head comparison of four commercial cell separation systems. *Plast Reconstr Surg*. 2013;132:932e–939e.

12. Aronowitz JA, Lockhart RA, Hakakian CS, et al. Clinical safety of stromal vascular fraction separation at the point of care. *Ann Plast Surg*. 2015;75:666–671.
13. Shi L, Carson D. Collagenase Santyl ointment: a selective agent for wound debridement. *J Wound Ostomy Continence Nurs*. 2009;36:512–516.
14. Smith & Nephew. Exploring Clinical Data for Collagenase Santyl Ointment. Available at: http://www.santyl.com/hcp/clinical-data. Accessed November 4, 2014.
15. AAC Briefing Document for Collagenase Clostridium Histolyticum (AA4500) September 2009. Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisDrugsAdvisoryCommittee/UCM182015.pdf. Accessed October 7, 2014.
16. Egui Rojo MA, Moncada Iribarren I, Carballido Rodriguez J, et al. Experience in the use of collagenase histolyticum in the management of Peyronie's disease: current data and future prospects. *Ther Adv Urol*. 2014;6:192–197.
17. Gelbard M, Goldstein I, Hellstrom WJ, et al. Clinical efficacy, safety and tolerability of collagenase clostridium histolyticum for the treatment of Peyronie disease in 2 large double-blind, randomized, placebo controlled phase 3 studies. *J Urol*. 2013;190:199–207.
18. Nunn AC, Schreuder FB. Dupuytren's contracture: emerging insight into a Viking's disease. *Hand Surg*. 2014;19:481–490.
19. Gilpin D, Coleman S, Hall S, et al. Injectable collagenase clostridium histolyticum: a new nonsurgical treatment for Dupuytren's disease. *J Hand Surg Am*. 2010;35:2027–2038.
20. Sood A, Therattil PJ, Paik AM, et al. Treatment of Dupuytren's contracture with injectable collagenase in a veteran population: a case series at the Department of Veterans Affairs New Jersey Health Care System. *Eplasty*. 2014;14:e13.