# Adipose Stromal Vascular Fraction Isolation: A Head-to-Head Comparison of Four Commercial Cell Separation Systems

Joel A. Aronowitz, M.D.
Joshua D. I. Ellenhorn, M.D.

*Los Angeles, Calif.*

**Background:** Supplementation of fat grafts with stromal vascular fraction cells is an emerging technique used to improve graft reliability. A variety of systems for isolating stromal vascular fraction cells are commercially available. The lack of performance data obtained operating the systems in a standardized environment prevents objective assessment of performance. This prospective, blinded study compared performance of four commercially available stromal vascular fraction isolation systems when operated in a clinical outpatient surgery environment.

**Methods:** Four different systems were compared: (1) PNC's Multi Station, (2) CHA Biotech Cha-Station, (3) Cytori Celution 800/CRS System, and (4) Medi-Khan's Lipokit with MaxStem. Identical lipoaspirate samples from five separate volunteer donors were used to evaluate system process time, viable cell yield, composition, residual enzyme, and operating costs.

**Results:** The mean processing time ranged from 88 to 115 minutes. The highest mean number of viable nucleated cells was obtained using the Celution System ($2.41 \times 10^5$ cells/g) followed by the Multi Station ($1.07 \times 10^5$ cells/g). Lipokit and Cha-Station systems yielded nearly a log fewer nucleated cells ($0.35 \times 10^5$ cells/g and $0.05 \times 10^5$ cells/g, respectively). The Celution System also yielded significantly more endothelial cells, $CD34^+/CD31^-$ cells, and adipose-derived stem cells (colony-forming unit–fibroblast). Residual enzyme levels observed with the Multi Station, Cha-Station, and Lipokit, respectively, averaged 5.1-, 13.0-, and 57-fold higher than that observed with the Celution System.

**Conclusions:** Although all systems generated measurable amounts of stromal vascular fraction, significant variability exists in the number, identity, and safety profiles of recovered viable cells. Side-by-side clinical trials will be required to establish the relevance of these differences. (*Plast. Reconstr. Surg.* 132: 932e, 2013.)

Autologous fat grafting is an accepted treatment for a variety of clinical indications, including soft-tissue augmentation, improvement of irradiated and traumatized tissue fields, and many cosmetic applications. Improvement in the results and reliability of fat grafting by augmentation of the graft with autogenous additional vascular-associated progenitor cells found within the stromal vascular component of adipose tissue is an emerging technique and the subject of numerous preclinical and clinical investigations.[1–8] The accumulation of data documenting safety and efficacy of a cellular approach to fat grafting has driven clinical demand for systems that allow separation of stromal vascular fraction cells from lipoaspirate in the operating room. As a result, several manual, semiautomated, and automated stromal vascular fraction isolation systems are now commercially available to generate stromal vascular fraction–enhanced fat grafts in the operating room at the point of care. It is difficult for the

*From the University of Southern California Keck School of Medicine; the John Wayne Cancer Center; and Cedars-Sinai Medical Center.*
*Received for publication March 16, 2013; accepted June 4, 2013.*

DOI: 10.1097/PRS.0b013e3182a80652

**Disclosure:** *A CHA Biotech Cha-Station machine and a Cytori Celution device were loaned for this study at no charge by the respective manufacturers. Some reagents and disposable supplies were also provided at no cost.*

clinician to assess the performance of the available separation methods because of the paucity of independent comparative performance data available. Furthermore, the data that have been reported in the literature to date regarding stromal vascular fraction isolation methodologies are not directly comparable because of different metrics and endpoint assays used to characterize them.[8–11] Clinically important parameters necessary to compare systems include process time, volume capacity, yield, viability, surface marker identity, safety profile of the cells, and capital and operating costs. The purpose of this article is to report a prospective, blinded comparison of the performance of four commercially available adipose tissue stromal vascular fraction cell separation systems operated in a controlled clinical outpatient surgery environment using identical fresh lipoaspirate.

## PATIENTS AND METHODS

### Tissue Collection

The study received approval from the Cedar-Sinai Medical Center Institutional Review Board, and all adipose tissue donors provided informed consent before the procedure. Lipoaspirate was obtained using tumescent liposuction under a protocol standardized for tumescent solution volume per area, harvest cannula, and vacuum pressure. The lipoaspirate was harvested from the abdomen of five healthy female donors undergoing a tumescent liposuction procedure using 25- to 28-mmHg vacuum with a 2.5-mm blunt tip cannula. Following collection, excess blood and lipoaspirate fluid was removed by decantation; 600 to 800 cc of aspirated tissue was available for processing from each donor. The lipoaspirate samples were not further purified before processing using the four systems.

### Lipoaspirate Processing

Collected lipoaspirate from each patient was mixed gently to homogeneity and then aliquoted aseptically into four fractions. The four commercially available systems tested in this comparison were the Multi Station, an open, manual processing system including shaker/heater and high-capacity centrifuge incorporated under a biosafety hood with high-efficiency particulate air filtration and ultraviolet light (PNC International, Gyeonggi-do, Republic of Korea) (Fig. 1, *above*, *left*); the Cha-Station, a closed semiautomated processing system (CHA Biotech, Kangnamgu, Republic of Korea) (Fig. 1, *above*, *right*); the Celution 800/CRS System, a closed automated processing system (Cytori Therapeutics, Inc., San Diego, Calif.) (Fig. 1, *below*, *left*); and Lipokit with MaxStem, a closed, manual processing system (Medi-Khan, West Hollywood, Calif.) (Fig. 1, *below*, *right*). All systems were physically located at the point of care in the surgical facility and were operated according to manufacturers' instructions for use. Tissue processing to obtain stromal vascular fraction was performed concurrently in all four systems. The volume processed in each system was selected from the midrange of the processing volumes specified by the manufacturer. Total processing time was recorded. Samples collected from each device were assigned a random identification number to blind endpoint assay technicians to the identity of the source processing system used to obtain the stromal vascular fraction sample.

### Stromal Vascular Fraction Analysis

Total viable nucleated cell recovery and percentage cell viability were determined using a NucleoCounter (ChemoMetec, Allerød Denmark). Viable cell numbers were normalized to the gram mass of tissue processed. Cellular identity of stromal vascular fraction was determined by flow cytometric analysis for expression of the cell surface markers CD31, CD34, and CD45. The frequency of adipose stem cells was estimated using the colony-forming unit–fibroblast clonogenic assay.[9] Residual collagenase activity in the stromal vascular fraction output from each processing system was determined using a commercially available florescent ligand cleavage assay (EnzChek Gelatinase Assay; Life Technologies, Carlsbad, Calif.).

### Colony-Forming Unit–Fibroblast Clonogenic Assay

Cells recovered from the different devices were diluted to two concentrations (1000 cells per six-well plate well and 5000 cells per six-well plate well) in standard adipose stromal cell growth media (Dulbecco's Modified Eagle Medium:F12 with 10% fetal bovine serum and 1% penicillin/streptomycin antimycotic). Colonies were grown for 10 to 14 days, depending on the growth rate of the cells. In culture plates where colonies were rapidly approaching each other, the assay was stopped so that we could avoid being unable to count the colonies as a result of overgrowth. At the end of the assay, the culture plates were rinsed with phosphate-buffered saline once and then fixed with neutral buffered formalin for 15 minutes. Cell colonies were counted using phase contrast microscopy. All six wells of each plate were counted, but the wells generating the highest and lowest colony numbers were discarded, and

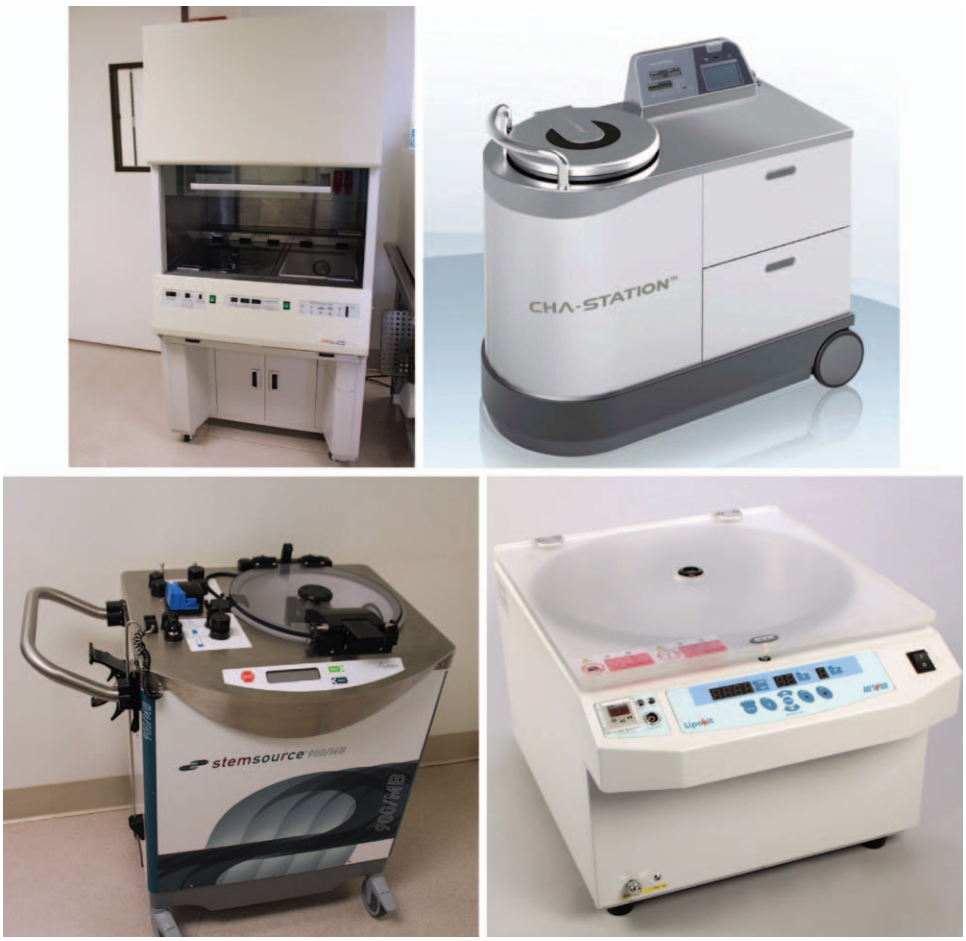


**Fig. 1.** Four different systems. (*Above*, *left*) PNC's Multi Station; (*above*, *right*) CHA Biotech Cha-Station; (*below*, *left*) Cytori Celution800/CRS System; and (*below*, *right*) Medi-Khan's Lipokit with MaxStem.

the average and standard deviation of the four remaining wells were calculated to generate the final frequency percentage value.

### Statistical Analysis

The mean nucleated cell yield and viability, colony-forming unit–fibroblast (percentage), residual protease activity, and cell population components were compared across processing systems using a mixed-effects linear model with processing system as a fixed effect and patient as a random effect. A covariance structure with unequal variances across the processing systems was assumed, unless there was evidence of homogeneous variances. Histograms of the residuals and quantile-quantile plots were examined for violations of the normality assumption. The Tukey honestly significant difference method was used to correct for the multiple comparisons across processing systems. The Tukey comparison of means test was used to compare differences. Statistical significance was defined as $p < 0.05$. Error bars in the figures represent standard deviations.

## RESULTS

### Stromal Vascular Fraction Characterization

The mean number of viable nucleated cells recovered from each gram of processed tissue is shown for each system in Figure 2. On average, the Celution System yielded over two-fold as many viable nucleated cells as the Multi Station system and 7- and 36-fold more than the Lipokit and Cha-Station, respectively ($p < 0.05$ for all comparisons). Nucleated cell viability was greatest with the Celution System (93 ± 2 percent; mean ± SD), followed by Cha-Station (87 ± 12 percent) and Lipokit (72 ± 15 percent), and lowest in samples generated with the Multi Station (57 ± 21 percent).

Comparison of the cell types in the population generated by the different devices also demonstrated significant differences. The composition

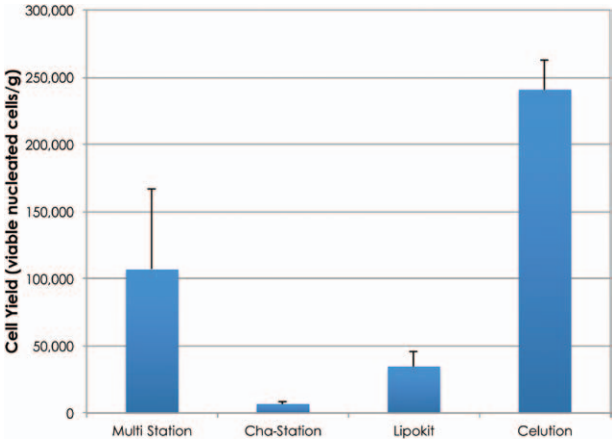


**Fig. 2.** Nucleated cell yield. Mean number for the five study subjects of viable nucleated cells recovered per gram of tissue processed. The Celution System had the greatest cell yield; $p = 0.049$ (for comparison with Multi Station), $p < 0.001$ (for Cha-Station), and $p = 0.004$ (for LipoKit) (Tukey-adjusted $p$ values).

of cells obtained by means of the Lipokit, Multi Station, and Cha-Station were similar in relative frequency of the major stromal vascular fraction cell populations. However, the number of viable cells obtained using the Cha-Station was too few for flow cytometry to be performed in three of the five experimental runs it completed. By contrast, the Celution System yielded a cell population containing a higher percentage of endothelial cells ($CD34^+/CD31^+$, $p = 0.003$ for Celution versus the other three systems) (Fig. 3, *above*, *left*) and a trend toward a significantly higher percentage of $CD34^+/CD31^-/CD45^-$ cells (a population that includes progenitor cells and other stromal and vascular cell types) (Fig. 3, *above*, *right*) ($p = 0.056$ for Celution versus the other three systems). The significantly higher content of progenitor cells in samples processed using the Celution System was confirmed using the biological and functional colony-forming

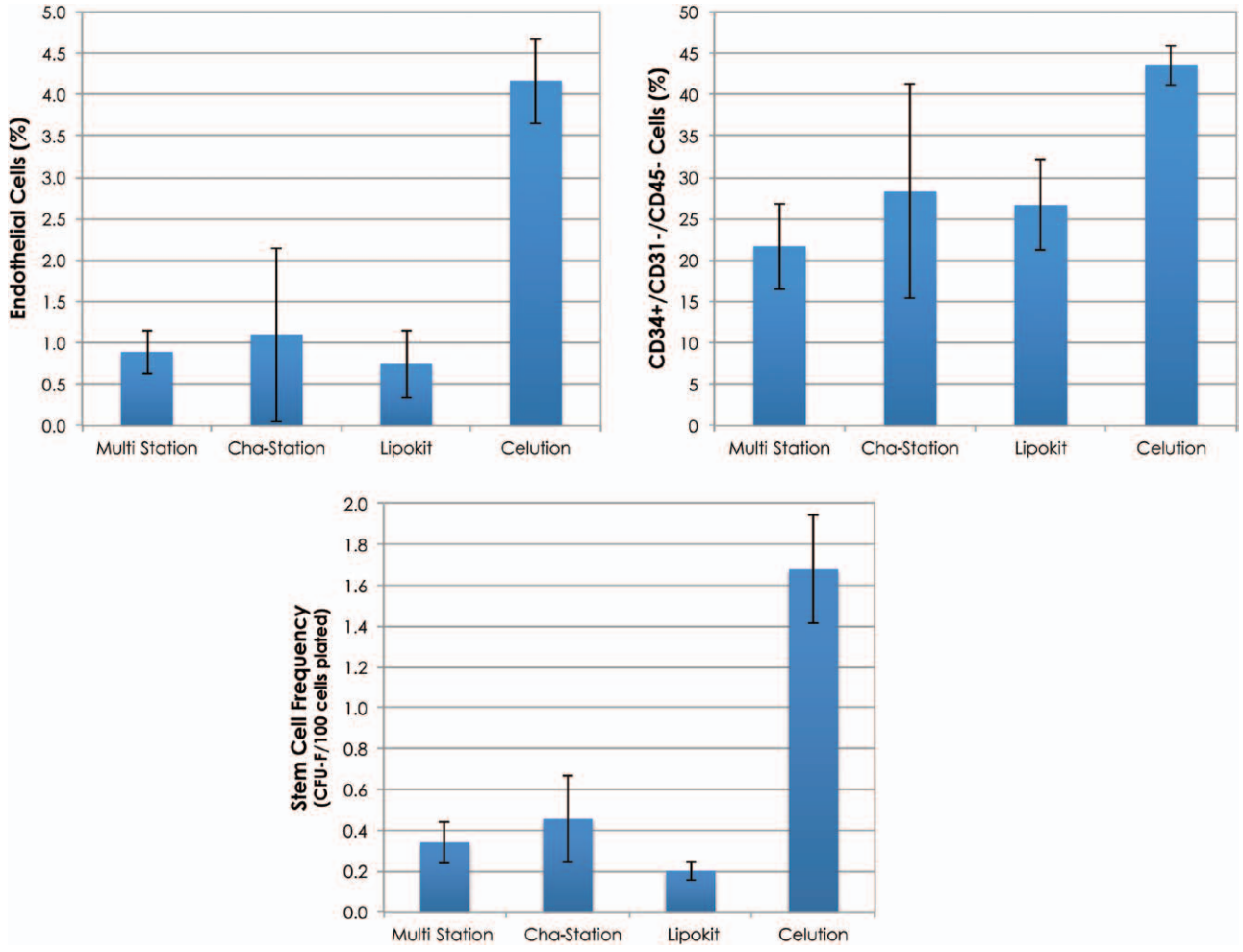


**Fig. 3.** Compositions of cell subpopulations generated by the four devices. (*Above*, *left*) Percentage of endothelial cells (defined as $CD34^+/CD31^+$ cells). (*Above*, *right*) Frequency of $CD34+/CD31^-/CD45^-$ cells. (*Below*) Frequency of stem cells defined as the number of colonies (colony-forming unit–fibroblasts) per 100 viable nucleated cells plated. *CFU-F*, colony-forming unit–fibroblasts.

unit–fibroblast assay (Fig. 3, *below*) ($p = 0.002$ for Celution versus the other three systems).

As a result of the higher frequency of endothelial cells, $CD34^+/CD31^-/CD45^-$ cells, and colony-forming unit–fibroblasts combined with the higher total nucleated cell yield, the Celution System exhibited a significantly greater yield of all three key cell types (Fig. 4) ($p < 0.005$ for Celution versus the other three systems). For example, the output of the Celution System yielded, on average, 6.6-fold more colony-forming unit–fibroblasts per gram of tissue than the Multi Station, 63.6-fold more than with Lipokit, and 99.3-fold more than the same tissue processed with the Cha-Station (Fig. 4, *below*).

### Residual Collagenase Activity

All four systems use a proteolytic enzyme solution composed primarily of type I and type II collagenases. The concentration of enzyme remaining in the cell suspension at the end of processing is a relevant parameter in establishing safety benchmarks for cellular therapies. The amount of residual collagenase activity in the final cell output of these systems is shown in Figure 5. Stromal vascular fraction generated with the Celution System, Cha-Station, and Multi Station contained statistically significantly less collagenase activity than the stromal vascular fraction from the Lipokit system ($p < 0.0001$) and was consistently lower than the other two systems, although the difference did not reach significance because of the very high variability in residual enzyme levels of stromal vascular fraction from the Multi Station and Cha-Station systems. When data were normalized within each run to eliminate possible donor effects, the output of the Multi Station contained an average of 5.1-fold greater enzyme activity than the output of the Celution System; the Cha-Station contained an average of 13.0-fold more enzyme activity than the Celution System; and the Lipokit contained an average of 57-fold more enzyme activity than the Celution System.

### Processing Economics

System processing parameters are listed in Table 1. The Cha-Station and Celution System had the shortest operating cycles (approximately 90 minutes), and the Multi Station and Lipokit with MaxStem were the slowest (approximately

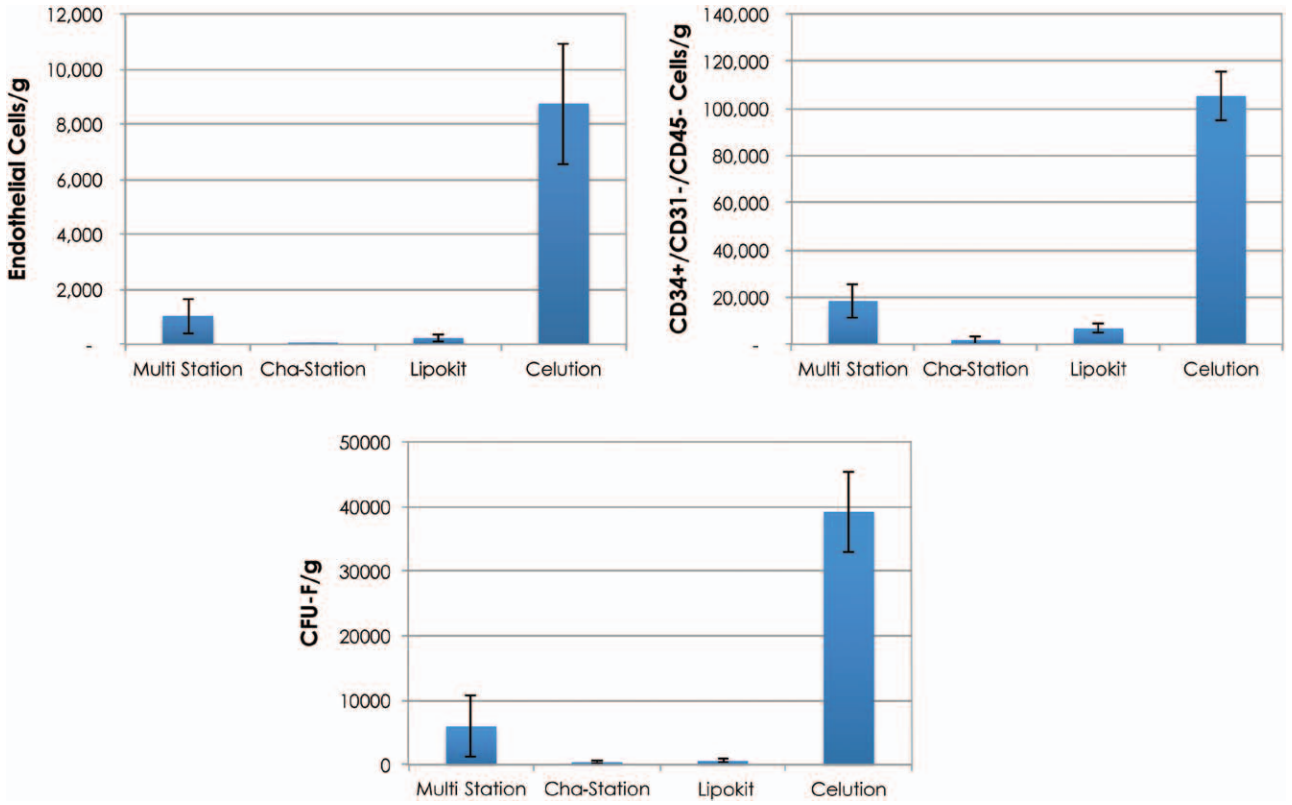


**Fig. 4.** Yield of key cell populations. (*Above*, *left*) Yield of endothelial cells ($CD34^+/CD31^+$ cells) per gram of tissue processed. (*Above*, *right*) Yield of $CD34^+/CD31^-/CD45^-$ cells) per gram of tissue processed. (*Below*) Yield of colony-forming unit–fibroblasts per gram of tissue processed. *CFU-F*, colony-forming unit–fibroblasts.

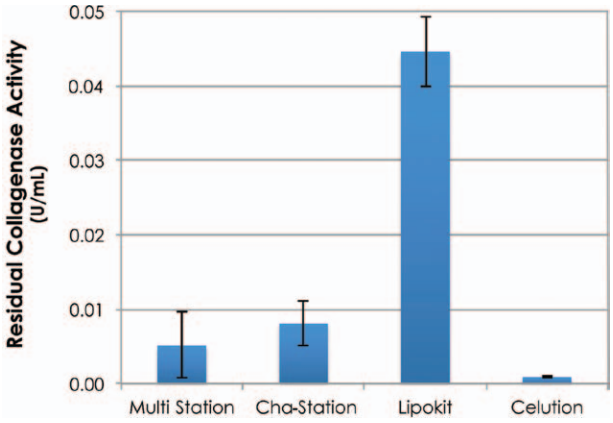


**Fig. 5.** Comparison of residual protease activity. The Lipokit yielded the highest residual protease activity, with a Tukey-adjusted value of $p < 0.0001$ for all comparisons with the other devices.

110 minutes). The Cha-Station was not included in the fifth run because of a mechanical failure that prevented processing from being completed. No other runs of the four systems were excluded from the results. Single-use reagents and supply cost was highest using the Celution System ($1950 per process). The manual-based Multi Station system was least expensive ($460 per process). When costs are normalized to stem cell (colony-forming unit–fibroblast) content (a likely surrogate indicator of therapeutic efficacy), the Celution System yielded the highest number of adipose stromal cell progenitors per supply and reagent dollar spent, whereas the LipoKit system yielded the lowest number of adipose stromal cells. When the maximal processing capacity per unit time was factored in, the Celution yielded twice the number of adipose stromal cells per dollar as the Multi Station and 10 times the number of these cells per dollar compared with the LipoKit.

## DISCUSSION

Establishment of standards for point-of-care cell stromal vascular fraction isolation is essential for evaluation of the growing body of clinical studies using stromal vascular fraction–supplemented fat grafting. The major variables include volume of lipoaspirate; residual enzyme levels; and nucleated cell yield, viability, and composition. Cost and total processing time are also important practical considerations. A similar prior study (Aronowitz and Watson, 2012 presented at the International Federation for Adipose Therapeutics and Science 2012 meeting) compared three separation platforms but did not determine cell identify or confirm counts with the colony-forming unit assay.

This prospective, blinded study evaluated these factors across four commercially available platforms in a real-time operating room setting. Each of the four systems produced measurable viable nucleated cells in a time frame consistent with clinical use of autogenous cells at the point of care in the operating room (<120 minutes). However, they achieved this using different engineering designs and degrees of automation. The Multi Station is basically a compact biology laboratory. It requires a technician to perform virtually all steps in the process manually. In contrast, the Celution System is fully automated and requires only manual placement of the disposable component. The systems also differ in the time allowed for the collagenase to digest, the neutralization process, and the time and intensity of centrifugation.

For the clinician to evaluate different means of preparing stromal vascular fraction cells, it is critical to know how the quality and quantity of cells obtained from different systems compare. Despite the apparent similarities in the processing approach, when operated in an identical clinical setting with the same lipoaspirate, the systems differed consistently and significantly with respect to the measured parameters. For example, the Celution and Multi Station systems produced the highest yield of nucleated cells (Fig. 2), although the Multi Station showed considerable variation in the cell number recovered and the lowest cell viability of all systems. The high degree of variation

**Table 1. Summary of Cell-Processing Parameters***

| Device | Operating Time (min) | Tissue Processed (ml) | Processing Volume Capacity (ml) | Disposable Cost | Operation Type | Collagenase Units/50 g Tissue | ASC Progenitors/$ Spent |
|---|---|---|---|---|---|---|---|
| Celution | 90 ± 16 | 100–180 | 100–360 | $1950 | Automated | 7 mg Celase (Cytori) | 250 |
| Multi Station | 115 ± 13 | 100–150 | 25–400 | $460 | Manual | 35 Wunsch units | 50 |
| Lipokit with MaxStem | 111 ± 18 | 60–100 | 25–100 | $530 | Semiautomated | 35 Wunsch units | 5.6 |
| Cha-Station | 88 ± 23 | 80–180 | 25–180 | $710 | Semiautomated | 35 Wunsch units | 0.8 |

ASC, adipose stromal cell.
*The calculated costs include all single-use disposable and enzyme costs per cycle irrespective of volume processed but exclude labor and capital equipment cost, which are variable between institutions and countries in which the systems are operated.

in cell yield is likely related to operator technique because the Multi Station is a fully manual system. Cell yields were notably lower from the Cha-Station and Lipokit with MaxStem, although the relative viability of the cells surpassed the Multi Station results.

It is also reasonable to assume that the types of cells present within the cell population will affect efficacy; for example, if the stromal vascular fraction cells are heavily diluted by blood leukocytes, the same total nucleated cell dose will contain substantially fewer true stromal vascular fraction cells. Flow cytometric and clonogenic assay analysis of stromal vascular fraction subpopulations was attempted for all samples. Unfortunately, the number of nucleated cells obtained using the Cha-Station was too few to perform flow cytometry in three of the five experimental runs it completed. Analysis of samples obtained from the other systems revealed that the cellular composition of the Lipokit, Multi Station, and MaxStem systems contained significantly lower levels of endothelial cells, $CD34^{+}/CD31^{-}$ cells, and stem cells (colony-forming unit–fibroblasts) than the Celution System (Fig. 3). In terms of putting these systems into practice, the key factor is not so much the relative frequency of these cells but the yield or absolute number obtained. The yield of three key stromal vascular fraction cell populations, including stem cells, was considerably greater in tissue samples processed using the Celution System (Fig. 4). The mean colony-forming unit–fibroblast yield obtained with the Celution System was 20.7-fold greater than that obtained with Multi Station, 145-fold greater than Cha-Station, and 36.0-fold greater than Lipokit with MaxStem. It should be noted that although the colony-forming unit fibroblast assay is correlative, it is not the criterion standard assay for identifying stem cells. Differentiation assays, which are beyond the scope of this study, might further characterize the stem cell potential of the cell products.

Residual protease levels also showed considerable variation between systems, with levels observed using the Celution System trending toward lower than with use of the other three systems (Fig. 5). When data are normalized within each run to eliminate possible donor effects, the output of the Multi Station contained an average of 5.1-fold greater enzyme activity than the output of the Celution System; the Cha-Station contained an average of 13.0-fold more enzyme activity than the Celution System, and the Lipokit contained an average of 57-fold more enzyme activity than the Celution System.

The clinical significance of residual collagenase activity in humans is not determined, but it should be noted that intracerebral collagenase injection is widely used to induce hemorrhagic stroke in animal models,[12] and Santyl (Smith & Nephew, London, United Kingdom), a collagenase-based ointment, is used for active débridement of wounds.[13]

Control of variability of these parameters must also be considered. Importantly, variability was considerably smaller with the Celution System than with any of the other systems, as evidenced by the relative size of the error bars in the figures and other data shown in this article. For example, in the data for stem cell yield per gram of tissue processed, the size of the standard deviation of the data is 180 percent of the mean for Multi Station, 96 percent for Cha-Station, 76 percent for Lipokit, and only 36 percent for the Celution System.

It is also important to evaluate the cost of different approaches. The Multi Station had the lowest disposable cost per run but exhibited the greatest degree of variability in cell recovery. The Celution System had the highest disposable cost but, as noted above, outperformed the other systems in all key output quality parameters. Of course, the cost of disposables and reagents does not represent the full cost of processing. This study did not account for important cost factors such as labor, amortized capital investment in equipment, cost of operating room time, or fixed overhead.

## CONCLUSIONS

In conclusion, this study demonstrates the feasibility of stromal vascular fraction isolation at the point of care using a variety of commercially available systems. It is significant that viable nucleated cell count correlated well with the colony-forming unit count and calculated nucleated cell count, as this indicates the reliability of these tests in comparing isolation methods in a clinical setting. The Celution System demonstrated the highest and most reproducible cell output. Furthermore, the residual enzyme activity, an important consideration as safety standards are established for this emerging technology, was lowest with the Celution System.

***Joel A. Aronowitz, M.D.***
University of Southern California Keck
School of Medicine and
Cedars-Sinai Medical Center
8635 West 3rd Street, Suite 1090W
Los Angeles, Calif. 90048
dra@aronowitzmd.com

## ACKNOWLEDGMENTS

*Technical support in training for operation of each of the processing systems was provided by representatives of the respective manufacturers. The authors also acknowledge the editorial assistance of Kevin Hicok in preparation of the article.*

## REFERENCES

1. Zhu M, Zhou Z, Chen Y, et al. Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention. *Ann Plast Surg.* 2010;64:222–228.
2. Matsumoto D, Sato K, Gonda K, et al. Cell-assisted lipotransfer: Supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. *Tissue Eng.* 2006;12:3375–3382.
3. Yamamoto T, Gotoh M, Kato M, et al. Periurethral injection of autologous adipose-derived regenerative cells for the treatment of male stress urinary incontinence: Report of three initial cases. *Int J Urol.* 2012;19:652–659.
4. Akita S, Yoshimoto H, Akino K, et al. Early experiences with stem cells in treating chronic wounds. *Clin Plast Surg.* 2012;39:281–292.
5. Sterodimas A, de Faria J, Nicaretta B, Papadopoulos O, Papalambros E, Illouz YG. Cell-assisted lipotransfer. *Aesthet Surg J.* 2010;30:78–81.
6. Koh KS, Oh TS, Kim H, et al. Clinical application of human adipose tissue-derived mesenchymal stem cells in progressive hemifacial atrophy (Parry-Romberg disease) with microfat grafting techniques using 3-dimensional computed tomography and 3-dimensional camera. *Ann Plast Surg.* 2012;69:331–337.
7. Calabrese C, Orzalesi L, Casella D, Cataliotti L. Breast reconstruction after nipple/areola-sparing mastectomy using cell-enhanced fat grafting. *Ecancermedicalscience* 2009;3:116.
8. Doi K, Tanaka S, Iida H, et al. Stromal vascular fraction isolated from lipo-aspirates using an automated processing system: Bench and bed analysis. *J Tissue Eng Regen Med.* E-published ahead of print March 22, 2013.
9. Hicok KC, Hedrick MH. Automated isolation and processing of adipose-derived stem and regenerative cells. *Methods Mol Biol.* 2011;702:87–105.
10. Wu CH, Lee FK, Suresh Kumar S, et al. The isolation and differentiation of human adipose-derived stem cells using membrane filtration. *Biomaterials* 2012;33:8228–8239.
11. Lin CS, Xin ZC, Deng CH, Ning H, Lin G, Lue TF. Defining adipose tissue-derived stem cells in tissue and in culture. *Histol Histopathol.* 2010;25:807–815.
12. Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. *Stroke* 1990;21:801–807.
13. Mosher BA, Cuddigan J, Thomas DR, Boudreau DM. Outcomes of 4 methods of debridement using a decision analysis methodology. *Adv Wound Care* 1999;12:81–88.