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RESEARCH ARTICLE

Stromal vascular fraction isolated from lipo-aspirates using an automated processing system: bench and bed analysis

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Abstract

The heterogeneous stromal vascular fraction (SVF), containing adipose-derived stem/progenitor cells (ASCs), can be easily isolated through enzymatic digestion of aspirated adipose tissue. In clinical settings, however, strict control of technical procedures according to standard operating procedures and validation of cell-processing conditions are required. Therefore, we evaluated the efficiency and reliability of an automated system for SVF isolation from adipose tissue. SVF cells, freshly isolated using the automated procedure, showed comparable number and viability to those from manual isolation. Flow cytometric analysis confirmed an SVF cell composition profile similar to that after manual isolation. In addition, the ASC yield after 1 week in culture was also not significantly different between the two groups. Our clinical study, in which SVF cells isolated with the automated system were transplanted with aspirated fat tissue for soft tissue augmentation/reconstruction in 42 patients, showed satisfactory outcomes with no serious side-effects. Taken together, our results suggested that the automated isolation system is as reliable a method as manual isolation and may also be useful in clinical settings. Automated isolation is expected to enable cell-based clinical trials in small facilities with an aseptic room, without the necessity of a good manufacturing practice-level cell processing area. Copyright © 2012 John Wiley & Sons, Ltd.

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1. Introduction

Subcutaneous adipose tissue is an abundant source of mesenchymal stem/progenitor cells. Through collagenase digestion of adipose tissue, heterogeneous stromal vascular fraction (SVF) can be easily isolated and homogeneous adipose-derived stem/progenitor cells (ASCs) can be obtained after cell sorting or adherent culture of SVF. Findings have shown that ASCs have the capacity to differentiate into various cell lineages (Zuk *et al*., 2002). The utility of these cells has been demonstrated in multiple preclinical animal models and clinical cases (Gimble *et al*., 2010; Casteilla *et al*., 2011). Clinical applications include soft tissue or bone defects, chronic or irradiated ulcers, Crohn's disease, multiple sclerosis, graft-vs-host disease, myocardial infarction and stroke.

The advantages of ASCs include easy access to the source tissue, abundant cell yield per tissue volume and comparable functional potency to bone marrow-derived mesenchymal stem cells (Bajadal *et al.*, 2008). In addition, processing of lipo-aspirates for isolation of SVF is not very difficult in a laboratory and can be done within 90 min. In clinical settings, however, strict control of technical procedures according to standard operating procedures and validation of cell processing conditions are required under current Good Manufacturing Practices (Gimble *et al*., 2011). Thus, an automated system for cell isolation is required in order to avoid human errors and facilitate clinical trials performed under Good Clinical Practice (Hicok *et al*., 2011).

In this study, we evaluated the efficiency and reliability of an automated system for SVF isolation from adipose tissue in both laboratory and clinical studies. We compared SVF cells obtained from the manual and automated

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procedures in nucleated cell yield and viability, ASC yield by culture, and cell composition by flow cytometry. In addition, we have performed a clinical trial utilizing SVF as an additive to fat grafts for soft tissue augmentation/reconstruction [cell-assisted lipotransfer (CAL)] as previously described (Matsumoto *et al*., 2006; Yoshimura *et al*., 2008a, 2008b). The SVF isolated from the automated system was used in the treatment of 42 patients.

2. Materials and methods

2.1. Human adipose tissue samples

Aspirated subcutaneous adipose tissue was obtained from female patients ($n = 6$) undergoing liposuction surgery. Informed consent was obtained from each patient using a protocol approved by the institutional review board.

2.2. Stromal vascular fraction (SVF) isolation with a conventional method

Stromal vascular fraction was manually isolated from the fatty portion of liposuction aspirates, as described previously (Yoshimura *et al*., 2006). Briefly, the aspirated fat tissue was washed with phosphate-buffered saline (PBS) and digested in PBS containing 0.075% collagenase (the same product provided for the automated system below) on a shaker at 37 °C for 30 min. Mature adipocytes and connective tissues were separated from pellets by centrifugation (800 × *g*, 10 min). The pellets were resuspended and filtered through a 100 μm mesh (Millipore, MA, USA) and centrifuged again (800 × *g*, 10 min) to obtain SVF cell pellets. The SVF cell yield was counted using a cell counter (NucleoCounter, Chemometec, Allerod, Denmark), which measures total and/or dead nucleated cells. Counting was performed three times for each sample and the average of triplicated data was shown as sample data.

2.3. SVF isolation with an automated system

The Tissue Genesis Cell Isolation System (Tissue Genesis, Honolulu, HI, USA) was used for automated SVF isolation from aspirated adipose tissue in this study (Figure 1). The automated cell isolation system consists of a machine and a single-use disposable. The sterile disposable unit contains a processing chamber (for tissue digestion, centrifugation and washing), two syringes (for collagenase solution and final product), and other bags and connecting tubes. The whole system basically mimics a manual method and the cell isolation was automatically performed by the programmed sequence set in the machine. In brief, aspirated fat tissue (20–60 ml) was introduced into the chamber and enzymatically digested. The collagenase (Adipase™) used, as well as the whole system and disposables (Adipase™ Loading Kit, Suspension Kit and Front-end Tissue Collection Kit) were provided by the manufacturer. After digestion, the solution was centrifuged and washed in the same chamber. The final cell output was approximately 35 ml SVF cell suspension and the whole process time was consistently 65 min. The nucleated cell number was determined using the cell counter as described above. For clinical application, the SVF solution was centrifuged (700 × *g*, 5 min) to remove the fluid and isolate the SVF cell pellets.

2.4. Cell culture

Freshly isolated SVF cells from the manual and automated methods were plated (30 000 cells/cm^2) on gelatin-coated

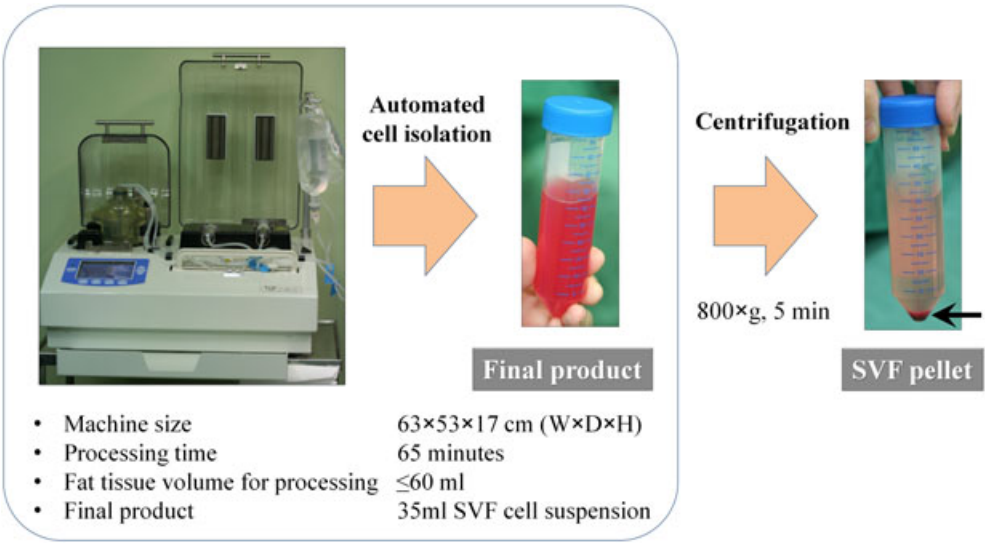

Figure 1. Isolation of stromal vascular fraction (SVF) from adipose tissue using an automated system. An automated instrument (Tissue Genesis Cell Isolation System™) and its disposable kit were used for isolation of SVF cells from human lipo-aspirates. The final product (35 ml SVF cell suspension) was centrifuged (700 × *g*, 5 min) to obtain SVF pellets

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60 mm dishes and cultured at 37 °C in an atmosphere of 5% carbon dioxide (CO_2) in humid air. The culture medium was M-199 containing 10% fetal bovine serum (FBS), 100 IU penicillin, 100 mg/ml streptomycin, 5 mg/ml heparin and 2 μg/ml acidic fibroblast growth factor. After 7 days, cells were detached by trypsinization and nucleated cell number was measured as described above.

2.5. Flow-cytometry analysis

Cell composition of freshly-isolated SVF cells was examined using flow cytometry as described before (Yoshimura *et al*., 2006). The following monoclonal antibodies (MAb) conjugated to fluorochromes were used: anti-CD31–APC, anti-CD34–PE-Cy7 and anti-CD45–FITC (BD Biosciences, San Jose, CA, USA). Multi-colour flow cytometry was performed using an LSR II (BD Biosciences) and cell composition percentages were calculated according to the profile of the surface marker expression.

2.6. Clinical trial

Between August 2009 and May 2011 we performed fat grafting combined with supplementation of SVF cells (CAL) in a total of 42 patients. The SVF was isolated from aspirated adipose tissue (20–60 ml) processed with the automated cell isolation system. Participants provided informed consent using a protocol approved by the institutional review board. The registered number of this clinical trial was UMIN000002454.

CAL was performed as previously described (Yoshimura *et al*., 2008a, 2008b) with minor deviations. Briefly, the fat tissue for cell isolation was first harvested by liposuction under general (for large-volume liposuction) or local (for small-volume liposuction, < 100 ml) anaesthesia. The liposuction site was infiltrated with saline solution containing epinephrine (0.001%). Adipose tissue was suctioned using a special liposuction device with a filter (Lipivage, Genesis Biosystems, Lewisville, TX, USA) and a cannula with a 2.5 mm inner diameter. The aspirated adipose tissue was transferred into the processing chamber of the automated system, which was placed in an aseptic operating room and SVF isolation was performed as described above. During the SVF processing, liposuction was continued to obtain aspirated fat tissue as graft material. The additional lipo-aspirates were centrifuged (700 × *g*, 3 min) and the liquid infranatant was discarded. The supernatant centrifuged fat tissue was mixed with the SVF cells and transplanted to augment the soft tissue in the face or breast. For injection into the face or the breast, a 1 ml syringe with an 18 gauge blunt needle or a 20 ml screw-type syringe (with a threaded plunger) and an 18 gauge, 15 cm long needle was used to obtain diffuse distribution of the graft tissue, respectively. Photographs of each patient were taken before and at each visit after treatment with a high-resolution digital camera (Model D30, Canon, Tokyo, Japan).

2.7. Statistical methods

Results are expressed as mean ± SD. The statistical significance was determined using a *t*-test for all variables. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Nucleated cell yield from lipo-aspirates

To evaluate SVF cell yield, 100 ml aspirated fat tissue (obtained from patients) was divided into two tubes (50 ml each) and processed using either the manual or automated method. The nucleated cell yield was $7.01 \pm 2.43 \times 10^5$ and $7.02 \pm 1.89 \times 10^5$ cells/ml aspirated adipose tissue by the manual and automated method,

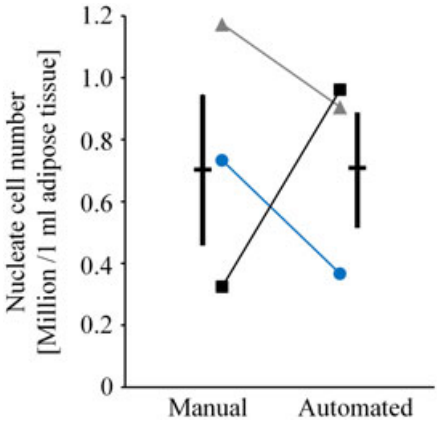

Figure 2. Nucleated cell yield of freshly isolated SVF cells from 1 g aspirated fat tissue. SVF cells isolated with the manual or automated method were measured using a cell counter. Nucleated cell yield/1 g aspirated adipose tissue was calculated. Each line with markers represents individual patient data, and the vertical bars demonstrate the mean ± SD. No significant difference was found between the manual and automated methods ($p = 0.998$)

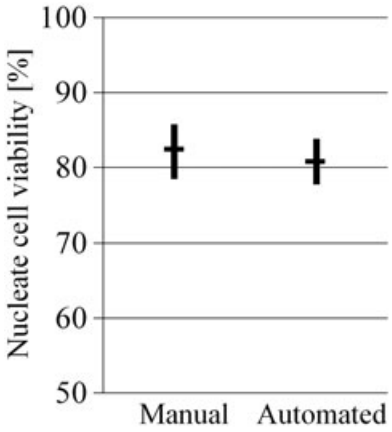

Figure 3. Viability of nucleated SVF cells freshly isolated from lipo-aspirates. Total cell number and dead cell number in SVF cells isolated with the manual and automated methods were separately counted using a cell counter after appropriate staining. No significant difference was found between the two methods ($p = 0.360$)

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respectively ($n = 3$) (Figure 2). There was no statistical difference between methods ($p = 0.998$).

3.2. Viability of freshly isolated nucleated SVF cells

Viability of nucleated SVF cells isolated from five donors was compared between the manual and automated methods. The viability of cells isolated by manual and automated methods was $82.4 \pm 7.7\%$ and $80.7 \pm 7.1\%$, respectively (Figure 3), and there was no statistical significance ($p = 0.360$).

3.3. Cultured ASC yield

Freshly-isolated SVF cells were plated and cultured with 10% FBS in M-199 for 1 week. The nucleated cell count at 1 week was $4.28 \pm 1.03 \times 10^5$ and $3.85\ (\pm 0.38) \times 10^5$/dish for the manual and automated methods, respectively (Figure 4). There was no statistical difference between the two methods ($p = 0.704$).

3.4. Cell composition of SVF

Cell composition of freshly-isolated SVF was analysed by flow cytometry. Expression of CD45, CD31 and CD34 was surveyed to classify the SVF cells into four cell populations; (a) $CD45^+$ haematopoietic cells (leukocytes); (b) $CD31^+/CD34^+/CD45^-$ vascular endothelial cells; (c) $CD31^-/CD34^+/CD45^-$ adipose-derived stromal/stem cells (ASCs); and (d) $CD31^-/CD34^-/CD45^-$ other cells. The other cells ($CD31^-/CD34^-/CD45^-$) are considered to contain vascular pericytes, fibroblasts, etc. Flow-cytometric analysis indicated that cell compositions of SVF obtained through the manual and automated method were comparable to each other (Figure 5).

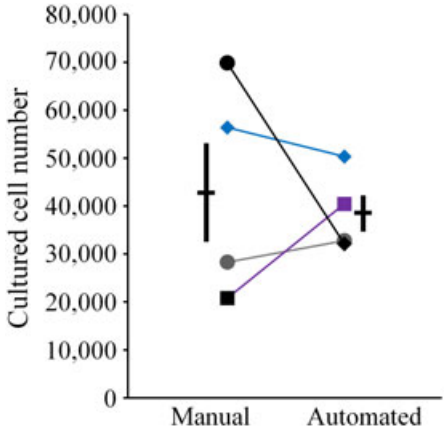

Figure 4. Nucleated cell count of SVF culture for 1 week. SVF cells isolated with the manual or automated method were cultured on a 60 mm gelatin-coated dish (seeded at 2.0×10^5 cells/dish) for 7 days. Most of the cultured cells at 7 days are assumed to be adipose-derived stromal/stem cells (ASCs). Each line with markers represents individual patient data and the vertical bars demonstrate the mean ± SD. There was no significant difference between the manual and automated methods ($p = 0.704$)

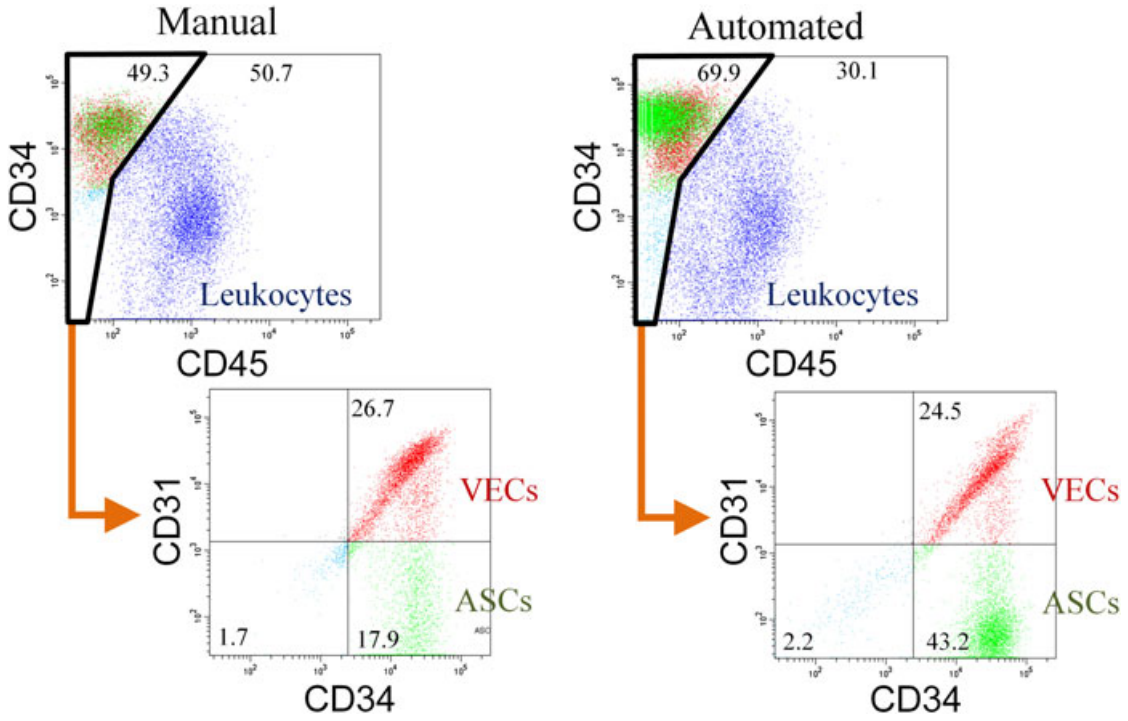

Figure 5. Multi-colour flow cytometric analysis of freshly isolated SVF cells. Cell compositions of SVF are $CD45^+$ cells (leukocytes), $CD31^+$ cells [vascular endothelial cells (VECs)], $CD31^-/CD34^+$ cells [adipose-derived stem/progenitor cells (ASCs)] and $CD31^-/CD34^-$ cells (other cells). The figures showed representative data of SVFs isolated from the manual and automated methods. There was no significant difference in cell composition between the two methods

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3.5. Clinical results

CAL using SVF isolated with the automated method was performed in the breasts of 27 patients and the faces of 15 patients; the patient data are summarized in Table 1. All patients were Japanese and were followed up for 3–18 months; they were aged 20–66 and body mass index (BMI) was in the range 16.8–27.5 kg/m^2. The volume of transplanted fat tissue was 16.8 ± 3.1 ml for facial rejuvenation ($n = 13$) and 29.4 ± 9.4 for facial reconstruction ($n = 2$). For the breasts, 217 ± 60 ml were injected per side for cosmetic augmentation ($n = 24$), while 225 ± 48 ml was injected for reconstruction after mastectomy ($n = 3$). SVF was isolated from 20–60 ml (53.6 ± 2.6 ml, $n = 42$) of aspirated adipose tissue and nucleated cell number of SVF was $7.0 \pm 3.5 \times 10^5$, with cell viability of 84.3 ± 7.6%.

Although neither volumetric measurement of tissue augmentation nor comparative evaluation between the manual and automated method were performed, all the patients showed clinical improvement without any serious side-effects or complications. The treated tissue generally showed minimal changes in the stabilization phase (3–12 months after surgery), suggesting that the healing and remodelling of the grafted tissue (between surgery and 3 months) were satisfying (Yoshimura *et al.*, 2011). The overall results suggested that the SVF cells isolated with the automated method can be as safely used as those obtained from the manual isolation. Representative cases are shown in Figures 6 and 7.

4. Discussion

In this study, we tested an automated cell isolation system to evaluate its efficiency, reliability, feasibility and safety, because such automated systems potentially stand to benefit ASC-based therapeutic use in the future by preventing technical human errors, inconsistency, training efforts for technicians, variability of technician skills, management and validation of aseptic facilities, etc. The results of this comparison between the manual and automated methods showed no significant difference in freshly-isolated SVF cell yield, SVF cell viability, cultured ASC number and SVF composition.

Adipose tissue contains various types of cells including mature adipocytes, ASCs, vascular endothelial cells, resident macrophages and lymphocytes, pericytes, smooth muscle cells, fibroblasts and others. After collagenase digestion of adipose tissue, nucleated cells other than mature adipocytes can be extracted as SVF cells. Based on our previous numerical measurement of each cell type, it was roughly estimated

Table 1. Summary of patients in the clinical trial

Patient numbers	Gender (male/female)		42 (4/38)
	Face	Rejuvenation	13
		Reconstruction	2
	Breast	Augmentation	24
		Reconstruction	3
Age in years (range)			43.2 ± 13.0 (20–66)
BMI in kg/m^2 (range)			20.0 ± 2.4 (16.8–27.5)
SVF	Nucleated cell number (/ml)		$7.0 \pm 3.5 \times 10^5$
	Viability (%)		84.3 ± 7.6
	Nucleated viable cell number (/mL)		$6.0 \pm 3.2 \times 10^5$
Injection volume (ml)	Face	Rejuvenation	16.8 ± 3.1
		Reconstruction	29.4 ± 9.4
	Breast	Augmentation	217 ± 60
		Reconstruction	225 ± 48

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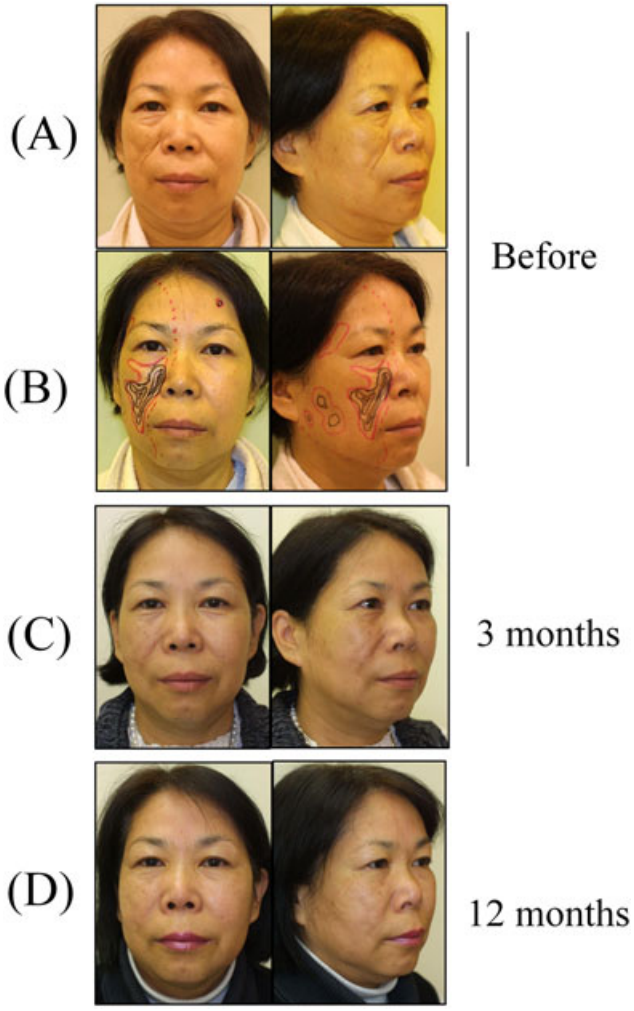

Figure 6. Clinical case 1. A 57 year-old female with facial lipodystrophy induced by erythaematosus profundus underwent cell-assisted lipotransfer to reconstruct the soft tissue defect on the right cheek. SVF cells were isolated from 25 ml aspirated fat tissue, which was processed using the automated method; 23 ml centrifuged fat tissue was injected with the SVF cells. Frontal and oblique views (A, B) before surgery at 3 months (C) and at 12 months (D); (B) shows drawings of the preoperative defect

that 1 g intact adipose tissue contains approximately 4–5 million cells, including 1 million adipocytes, 1 million ASCs, 1 million vascular endothelial cells and 1 million other cells (Suga *et al*., 2008; Eto *et al*., 2009). Previous reports showed that freshly-isolated SVF nucleated cell yield from 1 g (or ml) lipo-aspirated tissue was variable among samples and has been recorded as containing $4.04 \pm 2.06 \times 10^4$–$1.31 \pm 0.50 \times 10^6$ cells, probably due to different protocols in harvesting fat tissue and isolating of SVF (Aust *et al*., 2004; Boquest *et al*., 2005; Caspar-Bauguil *et al*., 2005; Yoshimura *et al*., 2006; Mitchell *et al*., 2006; Lin *et al*., 2008). The standard SVF yield appears to be 300 000–1 000 000 cells/gram. The viable SVF number extracted from the automated system are within this range, suggesting that the isolation procedure was successfully performed. However, based on our estimation of cell number in the adipose tissue (Eto *et al*., 2009), it is suggested that only 20–30% of SVF cells (potentially 3–4 million cells in 1 g intact adipose tissue) were isolated as viable cells by both methods as well as previously reported methods. It has been difficult to extract most of SVF cells as viable cells, although many efforts have been made to improve and standardize the isolation method for many years. Thus, the efficiency of SVF cell isolation may be an important matter of future improvement.

We cultured the SVF cells for 1 week and confirmed that comparable numbers of viable ASCs were contained in the SVF from both the manual and automated methods. SVF compositions are known to be variable among samples (Yoshimura *et al*., 2006), probably due to inconsistency in haemorrhage volume and tissue-degrading factors such as surgical techniques, storage/ transportation time and temperature (Matsumoto *et al*., 2007). We have examined the cell population profile by measuring the main four populations within the SVF: haematopoietic cells, ASCs, endothelial cells and other cells, without detecting notable differences between the two methods utilized in this study. This proportion is different from SVF processed from excised fat tissue, which contains more ASCs and fewer haematopoietic cells compared to aspirated fat tissue (Eto *et al*., 2009).

By culturing SVF cells for 1 week, we evaluated viable and functional ASC number included in the SVF and found no significant difference between the manual and automated methods. Although SVF contains different types of cells, most of the cells which survive more than 5 days in DMEM medium were ASCs, as we reported previously (Yoshimura *et al*., 2006).

Processing time of the manual and automated methods was also comparable (80 and 75 min, respectively), although the maximum tissue volume to process was limited (to 60 ml) in the current version of the automated system. Processing time in the automated system is consistently 65 min. However an additional 10 min was added to overall processing time to account for the necessity to centrifuge ($700 \times g$, 5 min) the 35 ml final SVF cell suspension product from the automated system to obtain an SVF pellet.

The manual isolation in a clinical setting requires skilled technicians and sterile condition such as in an aseptic cell processing room. Due to its relatively compact size ($63 \times 53 \times 17$ cm) and weight (53.6 kg), the automated system can be put on a wheeled wagon or cart and be easily moved into an operating room. In the clinical trial, viable SVF cell yield per adipose volume was relatively consistent and comparable to the laboratory data. The preliminary clinical survey showed that clinical outcomes were generally satisfactory without leaving any serious side-effects such as oil cyst formation, suggesting that SVF cells are not harmful and may be similarly functional compared to the previous studies with the manual isolation (Yoshimura *et al*., 2008a, 2008b). Because our study does not have control groups without SVF supplementation or with supplementation of SVF isolated by a manual method, further comparable prospective randomized controlled clinical studies with longer follow-up are necessary to make a final conclusion on the functionality of the SVF cells.

In conclusion, our results suggested that cells freshly isolated with the automated system are comparable to those isolated manually and may also be reliably used in a

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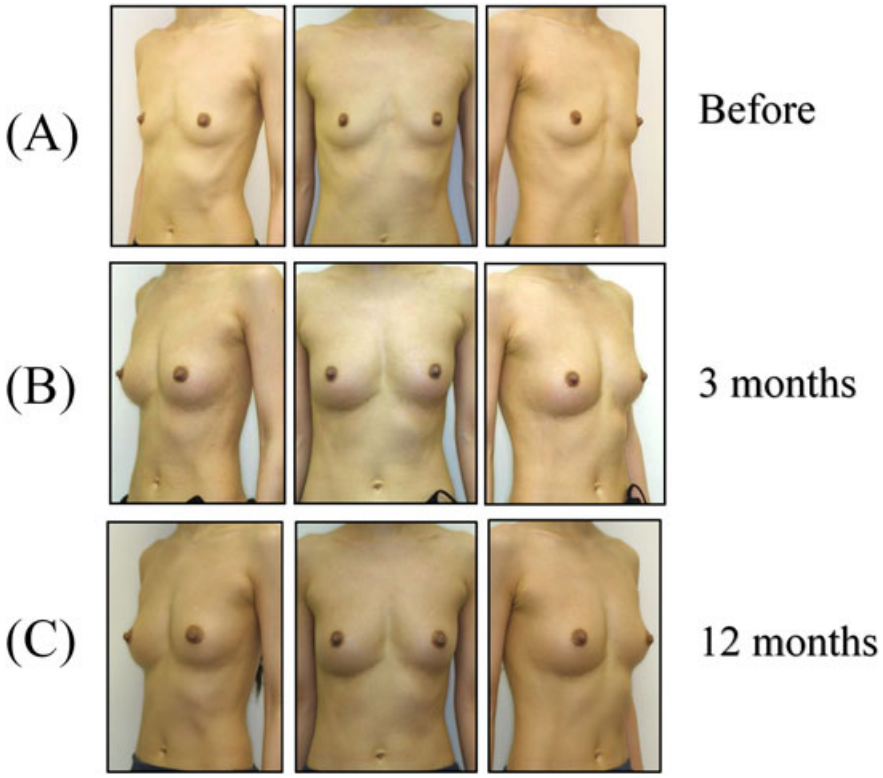

Figure 7. Clinical case 2. A 41 year-old female underwent cell-assisted lipotransfer for breast augmentation. SVF cells were isolated from 60 ml aspirated fat tissue, which was processed using the automated method. Centrifuged fat tissue (209 ml on the left and 231 ml on the right) was injected with the SVF cells. Frontal and oblique views (A) before surgery, (B) at 3 months and (C) at 12 months

clinical setting. Automated isolation can prepare a therapeutic dose of SVF cells and enables cell-based clinical trials in small facilities with an aseptic room, without the need for a good manufacturing practice-level cell processing area.

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References

Aust L, Devlin B, Foster SJ, *et al*. 2004; Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy* **6**: 7–14.

Bajada S, Mazakova I, Richardson JB, *et al*. 2008; Updates on stem cells and their applications in regenerative medicine. *J Tissue Eng Regen Med* **2**: 169–183.

Boquest AC, Shahdadfar A, Fronsdal K, *et al*. 2005; Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after *in vitro* cell culture. *Mol Biol Cell* **16**: 1131–1141.

Caspar-Bauguil S, Cousin B, Galinier A, *et al*. 2005; Adipose tissues as an ancestral immune organ: site-specific change in obesity. *FEBS Lett* **579**: 3487–3492.

Casteilla L, Planat-Benard V, Laharrague P, *et al*. 2011; Adipose-derived stromal cells: their identity and uses in clinical trials, an update. *World J Stem Cells* **3**: 25–33.

Eto H, Suga H, Matsumoto D, *et al*. 2009; Characterization of adipose tissue structure and cellular components: differences between aspirated adipose tissue and excised adipose tissue. *Plast Reconstr Surg* **124**: 1087–1097.

Gimble JM, Bunnell BA, Guilak F, *et al*. 2011; Adipose-derived stromal vascular fraction cells and stem cells: let's not get lost in translation. *Stem Cells* **29**(5): 749–754.

Gimble JM, Guilak F, Bunnell BA. 2010; Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. *Stem Cell Res Ther* **1**: 19.

Hicok KC, Hedrick MH. 2011; Automated isolation and processing of adipose-derived stem and regenerative cells. *Methods Mol Biol* **702**: 87–105.

Lin K, Matsubara Y, Masuda Y, *et al*. 2008; Characterization of adipose tissue-derived cells isolated with the Celution system. *Cytotherapy* **10**: 417–426.

Matsumoto D, Sato K, Gonda K, *et al*. 2006; Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. *Tissue Eng* **12**: 3375–3382.

Matsumoto D, Shigeura T, Sato K, *et al*. 2007; Influences of preservation at various temperatures on liposuction aspirates. *Plast Reconstr Surg* **120**: 1510–1517.

Mitchell JB, McIntosh K, Zvonic S *et al*. 2006; Immunophenotype of human adipose-derived cells: temporal changes in stromal associated and stem cell-associated markers. *Stem Cells* **24**: 376–385.

Suga H, Matsumoto D, Inoue K, *et al*. 2008; Numerical measurement of viable and non-viable adipocytes and other cellular components in aspirated fat tissue. *Plast Reconstr Surg* **122**: 103–114.

Yoshimura K, Eto H, Kato H, *et al*. 2011; Stem cell manipulation for soft tissue repair/reconstruction. *Regen Med* **6**(6, suppl): 29–37.

Yoshimura K, Sato K, Aoi N, *et al*. 2008a; Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesth Plast Surg* **32**: 48–55.

Yoshimura K, Sato K, Aoi N, *et al*. 2008b; Cell-assisted lipotransfer for facial lipoatrophy: efficacy of clinical use of adipose-derived stem cells. *Dermatol Surg* **34**: 1178–1185.

Yoshimura K, Shigeura T, Matsumoto D, *et al*. 2006; Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol* **208**: 64–76.

Zuk PA, Zhu M, Ashjian P *et al*. 2002; Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* **13**: 4279–4295.

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