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

Kyung Suk Koh, MD, PhD, Tae Suk Oh, MD,* Hoon Kim, MD,* In Wook Chung, MD,* Kang Woo Lee, MD,* Hyo Bo Lee, MD,* Eun Jung Park,* Jae Seob Jung,* Il Seob Shin, PhD,† Jeong Chan Ra, PhD,† and Jong Woo Choi, MD, PhD, MMM**

Background: Parry-Romberg disease is a rare condition that results in progressive hemifacial atrophy, involving the skin, dermis, subcutaneous fat, muscle, and, finally, cartilage and bone. Patients have been treated with dermofat or fat grafts or by microvascular free flap transfer. We hypothesized that adipose-derived stem cells (ASCs) may improve the results of microfat grafting through enhancing angiogenesis. We evaluated the utility of ASC in microfat grafting of patients with Parry-Romberg disease by measuring the change in the hemifacial volumes after injection of ASCs with microfat grafts or microfat grafts alone.

Methods: In April 2008, this investigation was approved by the Korean Food and Drug Administration and the institutional review board of the Asan Medical Center (Seoul, Korea) that monitor investigator-initiated trials. Between May 2008 and January 2009, 10 volunteers with Parry-Romberg disease (5 men and 5 women; mean age, 28 y) were recruited; 5 received ASC and microfat grafts and 5 received microfat grafts only. The mean follow-up period was 15 months. Adipose-derived stem cells were obtained from abdominal fat by liposuction and were cultured for 2 weeks. On day 14, patients were injected with fat grafts alone or plus (in the test group) 1×10^7 ASCs. Patients were evaluated postoperatively using a 3-dimensional camera and 3-dimensional CT scans, and grafted fat volumes were objectively calculated.

Results: Successful outcomes were evident in all 5 patients receiving microfat grafts and ASCs, and the survival of grafted fat was better than in patients receiving microfat grafts alone. Before surgery, the mean difference between ipsilateral and contralateral hemiface volume in patients receiving microfat grafts and ASCs was 21.71 mL decreasing to 4.47 mL after surgery. Overall resorption in this ASC group was 20.59%. The mean preoperative difference in hemiface volume in those receiving microfat grafts alone was 8.32 mL decreasing to 3.89 mL after surgery. Overall resorption in this group was 46.81%. The preoperative and postoperative volume differences between the groups was statistically significant ($P = 0.002$; random-effects model [SAS 9.1]).

Conclusions: Adipose-derived stem cells enhance the survival of fat grafted into the face. A microfat graft with simultaneous ASC injection may be used to treat Parry-Romberg disease without the need for microvascular free flap transfer.

Received December 11, 2011, and accepted for publication, after revision, May 29, 2012.

From the *Department of Plastic and Reconstructive Surgery, Ulsan University, College of Medicine, Seoul Asan Medical Center; and †RNL Bio Seoul, Korea.

This article has been presented at the 2009 Meeting of the International Federation for Adipose Therapeutics and Science and at the 20th Congress of the European Association for Cranio-Maxillo-Facial Surgery (2010), Bruges, Belgium.

Conflicts of interest and sources of funding: none declared.

Reprints: Jong Woo Choi, MD, PhD, MMM, Department of Plastic and Reconstructive Surgery University of Ulsan, College of Medicine Asan Medical Center, 388-1 PungNap-2Dong, SongPa-Gu, Seoul, 138-736, Korea. E-mail: pschoi@amc.seoul.kr.

ISSN: 0148-7043/12/6903-0331
DOI: 10.1097/SAP.0b013e31826239f0

Key Words: adipose tissue, Romberg disease, hemifacial atrophy, clinical application, mesenchymal stem cell, ASC

(*Ann Plast Surg* 2012;69: 331–337)

Cell-based therapy, a field of tissue engineering that has rapidly expanded in the time since the technique was introduced in the 1980s, has gathered further impetus from recent research that has extended our understanding of the basic biology of stem cells and tested clinical applications thereof.[1–3] Use of adult stem cells is relatively free of ethical concerns, and such cells are almost as multipotent as are embryonic stem cells. Of the 2 prominent tissue sources of adult stem cells, adipose tissue–derived mesenchymal stem cells (ASCs) are easier to harvest in larger quantities than are bone marrow stem cells.[4,5] The potential of ASCs to differentiate into mesenchymal tissue, including bone, cartilage, muscle, and fat, has been verified in vitro. In clinical settings, adult stem cell therapy using ASCs may be used synergistically with microfat graft techniques to manage a variety of soft tissue deficiencies. Although the exact mechanism is unknown, ASCs contribute to vasculogenesis around the tissue.[5,6]

Parry-Romberg disease is a rare condition that results in progressive hemifacial atrophy, involving the skin, dermis, subcutaneous fat, muscle, and, finally, cartilage and bones. The etiology of the disease has not been determined.[7,8] Because simple microfat grafts have a potential limits especially when a large amount of fat is used, patients with this disease are generally treated with microvascular free flap transfer. Although free tissue transfer can be effective for restoring the atrophic soft tissues, this technique also has a limitation such as sagging deformity in the long term.[9,10] On the contrary, microfat grafts minimize the postoperative drooping even in the long term. Thus, a microfat graft technique has been introduced for progressive hemifacial atrophy but is limited by unpredictable absorption of grafted fat when a large amount of fat is used.[11]

This investigation was designed to overcome these limits. We hypothesized that ASCs, which contribute to angiogenesis, may improve the survival of the microfat graft. We therefore evaluated the potency of ASCs in microfat grafting and explored whether a combination of a microfat graft and ASC therapy could be used to treat patients with Parry-Romberg disease. We measured fat absorption and facial volume change after injection of ASCs and microfat grafts, or microfat grafts alone, into atrophied hemifaces using the 3-dimensional (3-D) camera and 3-D CT.

MATERIALS AND METHODS

In April 2008, this investigation was approved by the Korean Food and Drug Administration (KFDA) and the institutional review board of the Asan Medical Center (Seoul, Korea) that monitors investigator-initiated trials. Ten volunteers with Parry-Romberg

disease (5 men and 5 women; mean age, 28 y) without risk factors including smoking, diabetes, hypertension, vascular disease, infectious disease, and other diseases were recruited between May 2008 and January 2009. Of these volunteers, 5 (2 men and 3 women) were treated with ASC and microfat grafts and 5 (3 men and 2 women) were treated with microfat grafts alone. They were randomly allocated to the each group. Group 1 (control group): patients with Parry-Romberg disease treated with microfat grafts alone (n = 5). Group 2 (experimental group): patients with Parry-Romberg disease treated with microfat grafts and simultaneous human ASC injection (n = 5).

On the day of surgery, abdominal fat was harvested and injected into the hemiatrophic side of the face using the microfat grafting technique of Coleman based on the difference in volumes between each face measured in advance (Fig. 1). Fat was harvested from the abdominal tissue using the Coleman fat grafting technique. Briefly, approximately 100 g of lower abdominal fat tissue was harvested and placed in a 50-mL conical tube, which was covered with wet saline gauze before further processing. Adipose-derived stem cells were obtained from the abdominal fat by liposuction and cultured to passage 0 to 3 until each culture yielded 1×10^7 cells; the cells were confirmed as mesenchymal, thus not hematopoietic, stem cells, by FACS (Fig. 2).

Human ASCs were extracted from adipose tissue and isolated using standard protocols from the International Society for Cellular Therapy[12]: (1) Adipose tissue was chopped as fine as possible with aseptic scissors and transferred to a fresh tube. (2) Collagenase I was added, and incubation continued for 3 hours with stirring (100 rpm) at 37°C in a 5% (vol/vol) CO_2 incubator. (3) The mixture was transferred to a conical tube, and after washing the spinner flask with 5 mL of phosphate-buffered saline and combining this suspension with the originally transferred contents, the tube was centrifuged at 1500 rpm for 5 minutes. (4) The supernatant was removed, and the pelleted material was resuspended in Dulbecco modified essential medium containing 10% (vol/vol) fetal bovine serum and filtered through a sieve to remove debris. (5) The solution was again centrifuged at 1500 rpm for 5 minutes, resuspended in culture medium, seeded into a culture flask, and cultured overnight at 37°C under 5% (vol/vol) CO_2. (6) The culture was examined the next day, and cell adherence was confirmed under a microscope. The medium was removed, and the cells were washed with 5 mL of phosphate-buffered saline and suspended at 1×10^6 cells/mL to form seeding scaffolds. To confirm that cells isolated in this manner were indeed mesenchymal stem cells, adherent cells were grown in vitro at 37°C under 5% (vol/vol) CO_2, with medium replacement every 2 days. The cells were confirmed as mesenchymal stem cells based on the criteria of the International Society for Cellular Therapy, in that they grew as attached cells in culture flasks; were positive for CD105, CD73, and CD90; negative for CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-DR; and were capable of differentiating into osteoblasts, adipocytes, and chondroblasts. The cells were subcultured in 3 stages (Fig. 2).

On day 14, patients were injected with secondary fat grafts and test patients simultaneously received 1×10^7 ASCs. At this time, 30% overcorrections of measured differences in the hemifacial volumes were done with stored fat.

The analysis include the gross appearance, patient satisfaction score (visual analog scale 0–5), and measurement of hemifacial volume using a 3-D camera and 3-D CT scan. For the confirmation of the accuracy of the measurement with 3-D camera, interrater and intrarater errors were calculated using the volume data that were obtained by 10 medical personnel. In addition, these values, on average, were used for this analysis.

Patients were postoperatively evaluated using a 3-D camera (Vectra; Canfield Scientific, Inc) and 3-D CT scans, and grafted fat volumes and absorption levels were objectively calculated (Fig. 1).

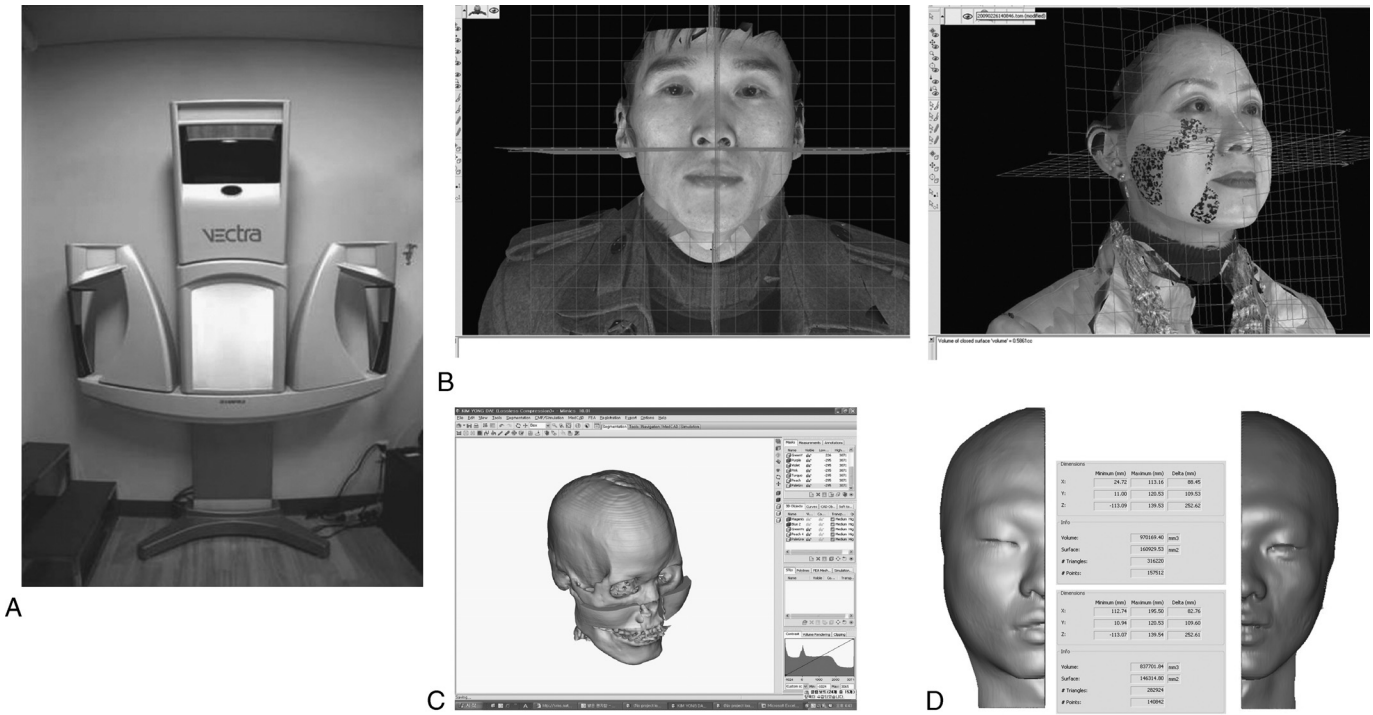

FIGURE 1. A, 3-D camera (Vectra; Canfield) used in this investigation. B, 3-D images on the software program using the 3-D camera including initial axis collaboration and volume measurement with 3-D camera. C, 3-D CT data image with volume rendering technique. D, The comparison of the volumes in each hemiface with 3-D CT. This revealed us the difference of hemifacial volumes on each face.

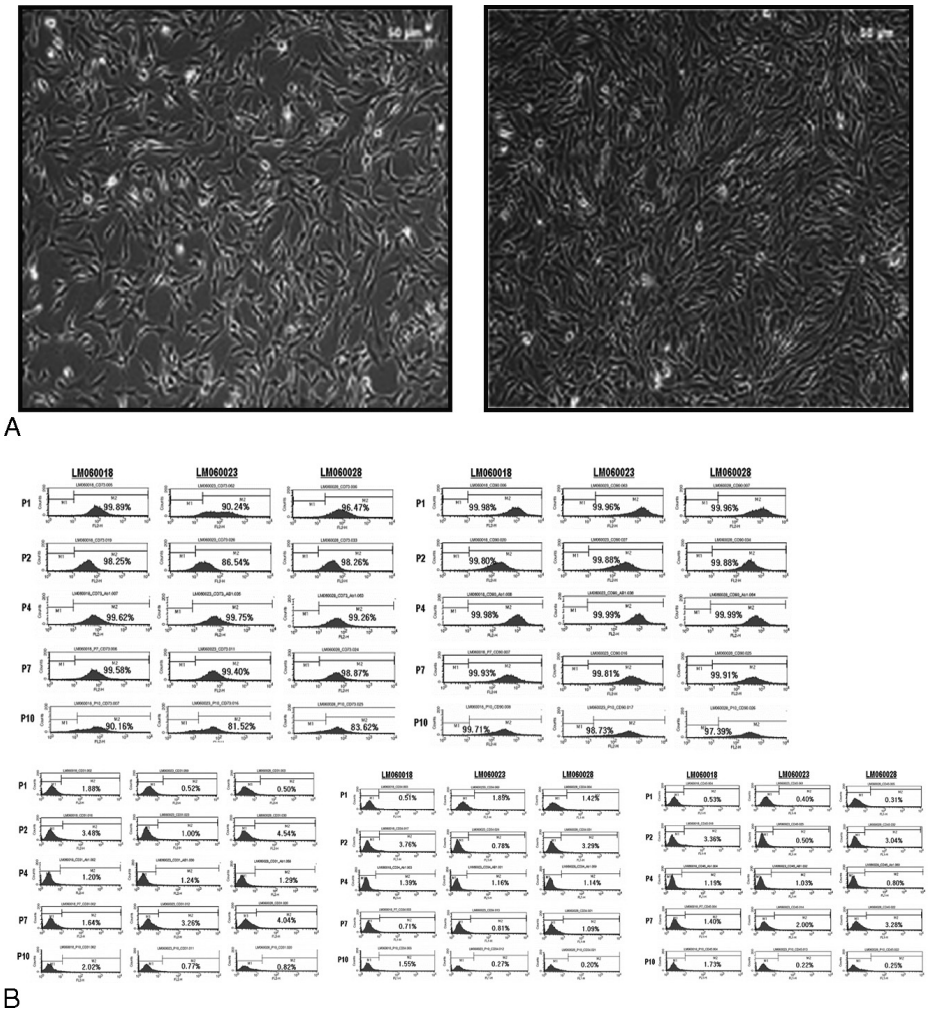

FIGURE 2. A, Spindle-shaped mesenchymal stem cell 3 days after incubation. B, CD typing to confirm the characteristics of the mesenchymal stem cells.

For the reference, the contralateral normal hemifacial volume was used. Thus, the difference between the affected and nonaffected hemifacial volume was measured for the evaluation of the hemifacial volume change after microfat injection. At 2 months after the secondary fat injection, 3-D evaluation was started. For statistical analysis of the volume data from 3-D camera and 3-D CT scan, the random-effects model and mixed-effects model were used, respectively.

Preoperative and postoperative blood tests included complete blood cell count, smooth muscle antibody, and urinalysis. The patients were followed up monthly for at least 12 months to detect any adverse effects of cell therapy. The mean follow-up period was 15 months.

RESULTS

Two-dimensional photometry and patient satisfaction analysis were performed. The soft tissue contour of the hemiface was evaluated at 1, 6, 12, and 18 months after implantation. Patients who received ASCs and microfat grafts showed more retention of soft tissue augmentation and restored acceptable facial symmetry. In contrast, more fat absorption was observed in patients who received microfat grafts only. Moreover, mean patient satisfaction (visual analog scale, 1–5) was higher in the experimental group (4.5) than in the control group (3.1).

Before using the 3-D camera, intrarater and interrater errors by users of the 3-D camera was measured to minimize errors in the estimation of hemifacial volume using the camera. Ten medical personnel measured the volume differences. 3-D photogrammetric analysis revealed that the interrater and intrarater reliabilities were 0.801 and 0.828, respectively, indicating that the instrument was very reliable (Table 1).

In advance to the actual application of microfat graft with ASC, differences in hemifacial volumes were measured using the 3-D camera. Before surgery, measurements from the 3-D camera indicated that the mean volume difference between the 2 hemifaces was

TABLE 1. Intracorrelation and Intercorrelation Coefficient Showing Interobserver and Intraobserver Variability

		95% Confidence Interval	
	ICC*	Lower Limit	Upper Limit
Interrater	0.801	0.756	0.847
Intrarater	0.828	0.790	0.866

*intraclass correlation coefficient

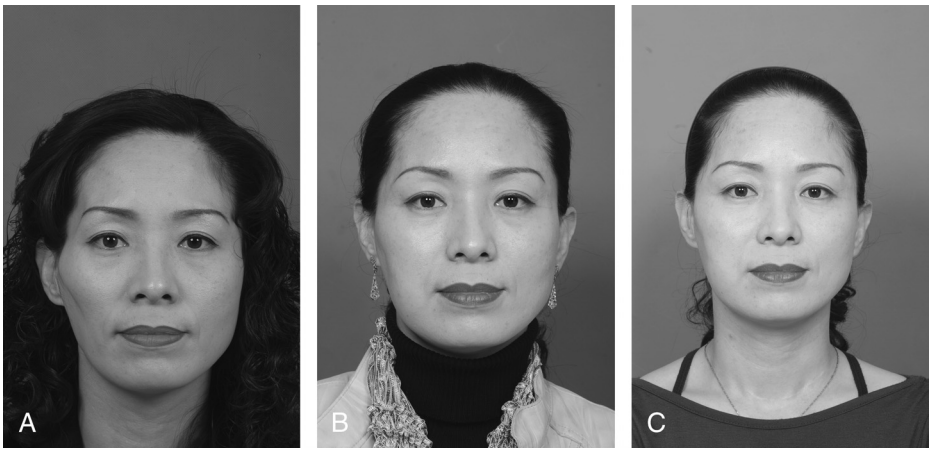

FIGURE 3. A, Preoperative view of Parry-Romberg disease (experimental group). B, Six months after the ASC and microfat grafting. C, Twelve months after the treatment.

21.71 mL in the experimental group. In the control group, the mean preoperative volume difference was 8.32 mL.

At day 0, on average, 22.5 mL of fat was injected in patients in the experimental group. On the contrary, on average, 9.3 mL of fat was injected in patients in the control group. At day 14, 7.4 mL of fat, on average, was injected in patients in the experimental group for the 30% overcorrection. On the contrary, on average, 3.2 mL of fat was secondarily injected in patients in the control group for the 30% overcorrection.

The amount of decrease in postoperative hemifacial volume change was smaller in the experimental group than that in the control group (Figs. 3–6). Volume differences in individual patients are shown in Table 2. Measurements from the 3-D camera indicated that, before surgery, the mean volume difference between the 2 hemifaces was 21.71 mL in the experimental group, decreasing to 4.47 mL after surgery (Table 2). Overall resorption in this group was 20.59% (Figs. 3–5 and 7B). In the control group, the mean preoperative volume difference was 8.32 mL, decreasing to 3.9 mL after surgery. Overall resorption in this group was 46.81% (Figs. 6 and 7A). The preoperative and postoperative volume differences between the groups were statistically significant ($P = 0.002$; random-effects model, SAS 9.1; Table 3 and Fig. 7C).

Three-dimensional CT analysis with volume rendering (Figs. 1C, D) was done to confirm the results obtained using the 3-D camera. We obtained CT scans both before and 6 months after surgery. After CT scanning, volume rendering techniques were used to analyze hemifacial volumes. The 3-D CT analysis showed similar results with those using the 3-D camera. Mean fat uptake was 2.51-fold higher ($P = 0.046$; mixed-effects model) in the experimental group than in the control group.

DISCUSSION

We focused on the clinical utility of ASCs in patients with progressive hemifacial atrophy. This is the first clinical application approved by the KFDA for using cultured ASCs in plastic surgery in South Korea. We chose patients with Parry-Romberg disease because the condition is relatively rare, and it is not possible to completely correct the facial atrophy by conventional methods, even including the use of microvascular free flaps.[10–12] Moreover, the fact that the atrophy is hemifacial allowed us to obtain control values for estimating facial volume. Using data from the contralaterally preserved healthy hemiface, we could evaluate volume changes on the side with disease.

Although several reports have described the properties of ASCs both in vitro and in vivo, few workers have explored real clinical

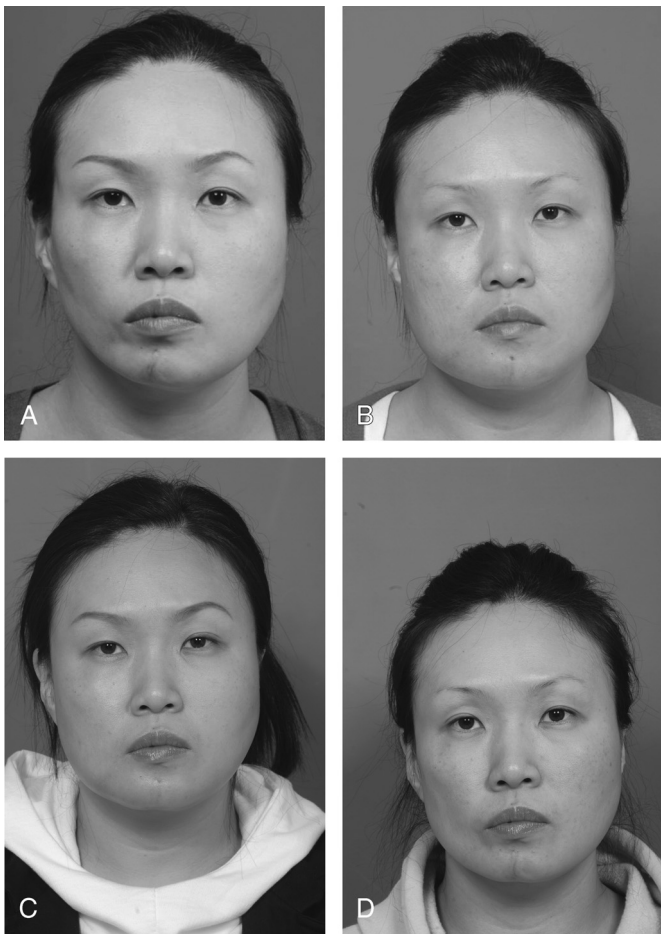

FIGURE 4. A, Preoperative view of Parry-Romberg disease (experimental group). B, Six months after the ASC and microfat grafting. C, Twelve months after the treatment. D, Eighteen months after the treatment. After losing 15 kg with diet, an unanticipated marked regression of the injected fat was observed.

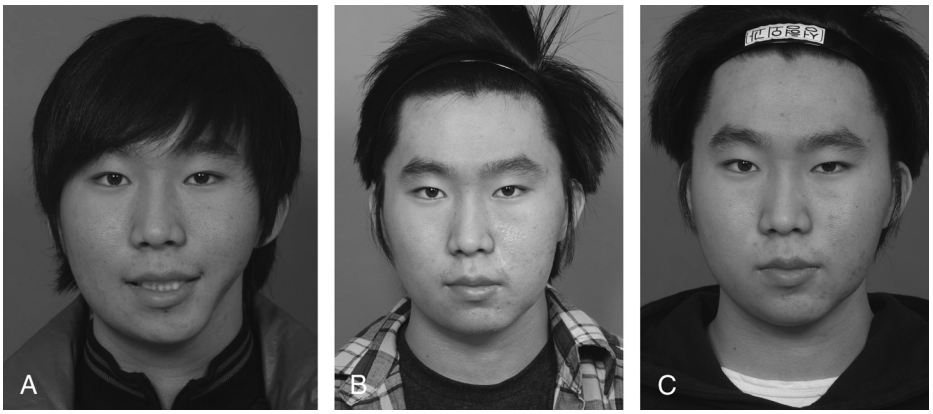

FIGURE 5. A, Preoperative view of Parry-Romberg disease (experimental group). B, Six months after the ASC and microfat grafting. C, Twelve months after the treatment.

applications. Stem cells have paracrine effects and an immune surveillance capacity, but roles for ASCs in clinical situations have not been clearly determined. Previous work on tissue engineering and gene therapy has indicated that a considerable gap exists between experimental potential and actual clinical application.[13–17] For example, the in vitro manipulation of stem cells necessary to induce differentiation along a desired lineage may result in unpredictable adverse effects when the cells are used clinically. The increased interest in adult stem cells contrasts sharply with our poor understanding of specific clinical applications and metabolic activities of such cells. Functional assays of stem cells are usually performed in vitro. Despite efforts to mimic site-specific conditions, in vitro assays expose cells to environments that differ considerably from those of the original in vivo locations. Because such artificial conditions may introduce experimental artifacts, it is important to study the clinical efficacy of stem cells further.

Although various techniques have been used to measure facial volume, serial measurement tools are impossible. More recently, 3-D CT with volume rendering has been introduced, enabling measurement of distance, area, and volume. However, this technique cannot be used for serial measurements because of the potential radiation hazards and high cost.[18–20] In contrast, the 3-D camera is free from such limitations, enabling serial measurement of facial volume. To validate efficacy of the camera and to estimate measurement bias, we statistically evaluated intrarater and interrater errors and found that measurements of hemifacial volume differences were reliable (Table 1). We therefore used the 3-D camera for serial evaluation of facial volume. To confirm our results, we compared the results of 3-D photogrammetry with those of 3-D CT using a volume rendering technique. Any minor differences were attributable to head position. Because CT scans were obtained with subjects in the supine position, facial soft tissue tended to droop slightly and scans were mildly affected by the head posture. The camera can determine the correct midline axis, whereas CT scans cannot. However, such factors resulted in only small differences between results of the 2 test modalities, showing that, overall, CT supported the validity of 3-D photography.

To date, patients with Parry-Romberg disease have been treated with simple fat grafts, dermofat grafts, bony augmentation, or microvascular free flaps. Although free flaps might be the method of choice, the technique has limitations, including a relatively long operation time, a risk of facial nerve injury, and the possibility of long-term flap drooping. In contrast, recent advances in microfat grafting techniques allow enhanced correction of soft tissue deficiencies. Such techniques also have limitations when used to treat patients with moderate-to-severe Romberg disease because absorption of grafted fat is rather extensive, especially when a large amount of fat is used. To overcome this limitation, we performed microfat grafting simultaneously with injection of cultured augmented ASCs. We hypothesized that 1×10^7 cultured ASCs should enhance survival of injected

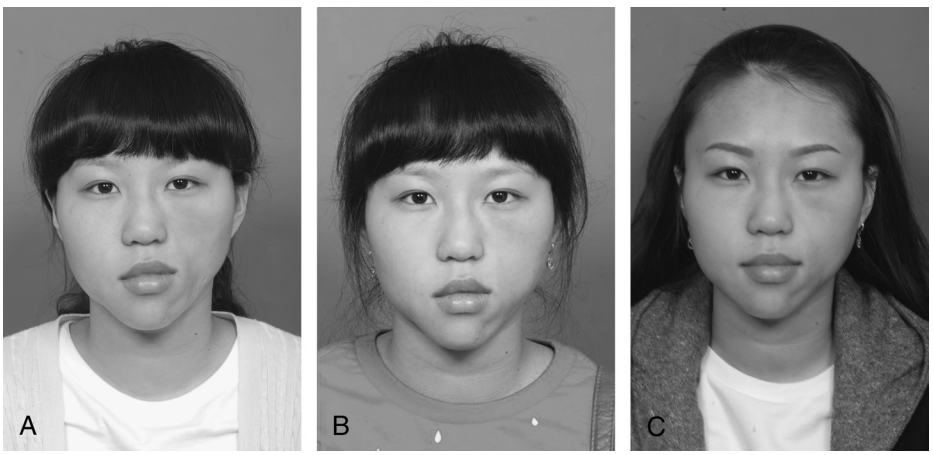

FIGURE 6. A, Preoperative view of Parry-Romberg disease (control group). B, Six months after the ASC and microfat grafting. C, Twelve months after the treatment.

TABLE 2. Differences (mL) in Both Sides of the Face (Abnormal and Normal Sides) in the Experimental and Control Groups

		Amount of Fat Injection, mL		Mean Difference, mL	
	Patient No.	Day 0	Day 14	Before Surgery	After Surgery
Experimental group	1	17.5	4.5	−16.199	1.078
	2	19	11	−16.942	−1.646
	3	31	9.5	−33.395	−7.496
	4	24.5	5	−23.694	−3.594
	5	20.5	7	−18.306	−6.201
	Mean	22.5	7.4	−21.707	−4.472
Control group	6	7.5	2	−6.262	−3.68
	7	8	3.5	−7.194	−3.397
	8	10.5	3	−9.78	−4.944
	9	7	3	−6.185	−2.836
	10	13.5	4.5	−12.169	−4.64
	Mean	9.3	3.2	−8.318	−3.899

microfat lobules via a paracrine effect or another mechanism. We found that injection of ASCs together with microfat grafting decreased fat absorption, from 46.81% to 20.59%.

The amount of injected fat could not be directly correlated with hemifacial volume because of soft tissue resistance during the actual application of fat to the hemiface. Correction of hemifacial atrophy to a desired volume requires a greater amount of fat, but we sought to minimize the number of procedures. We therefore injected the correct volume of fat, as determined preoperatively using photography, at the first surgery associated with fat harvest. Two weeks later, we injected cultured ASCs and performed additional fat grafting using stored fat. We did get 30% overcorrection done to obtain satisfactory results. Because we could calculate the hemifacial volumes after the swelling period, this overcorrection did not give us any bias at all. After the swelling subsided, we measured hemifacial volumes using the 3-D camera and CT. Thus, our results refer to the upper threshold of correction using ASCs plus fat in patients with Romberg disease. In addition, this study only evaluated the hemifacial volume based on the 3-D camera and 3-D CT because the KFDA did not allow the tissue biopsy after the procedures. Further evaluation including the histologic confirmation would be needed later.

In contrast to the satisfactory outcomes observed in patients with moderate-to-severe disease who received ASCs plus microfat grafting, such outcomes were not achieved in patients who received conventional microfat grafting alone. Three of the 5 patients in the control group requested more than 5 microfat grafts after the cessation of the investigation. Despite these efforts, patient satisfaction was relatively low. Moreover, because the control group included the patients less affected by Romberg disease, their expectation seemed much higher compared with those of the experimental group. That would be the other reason for the different satisfactory responses between groups.

TABLE 3. Statistical Analysis of Preoperative and Postoperative Changes in the Hemifacial Volume Using Random-Effects Model

	LSMEANS	SE	*P*
Experimental group	17.4357	1.9608	<0.001
Control group	4.4121	1.9608	0.055
	Estimate	**SE**	***P***
Experimental − control	13.023	2.7729	0.002

Estimate = change in facial volume in the experimental group − change in facial volume in the control group.
Changed values = postoperative − preoperative.
Analysis: random-effects model.
Program: SAS 9.1.
LSMEANS indicates least squares means (mean of change).

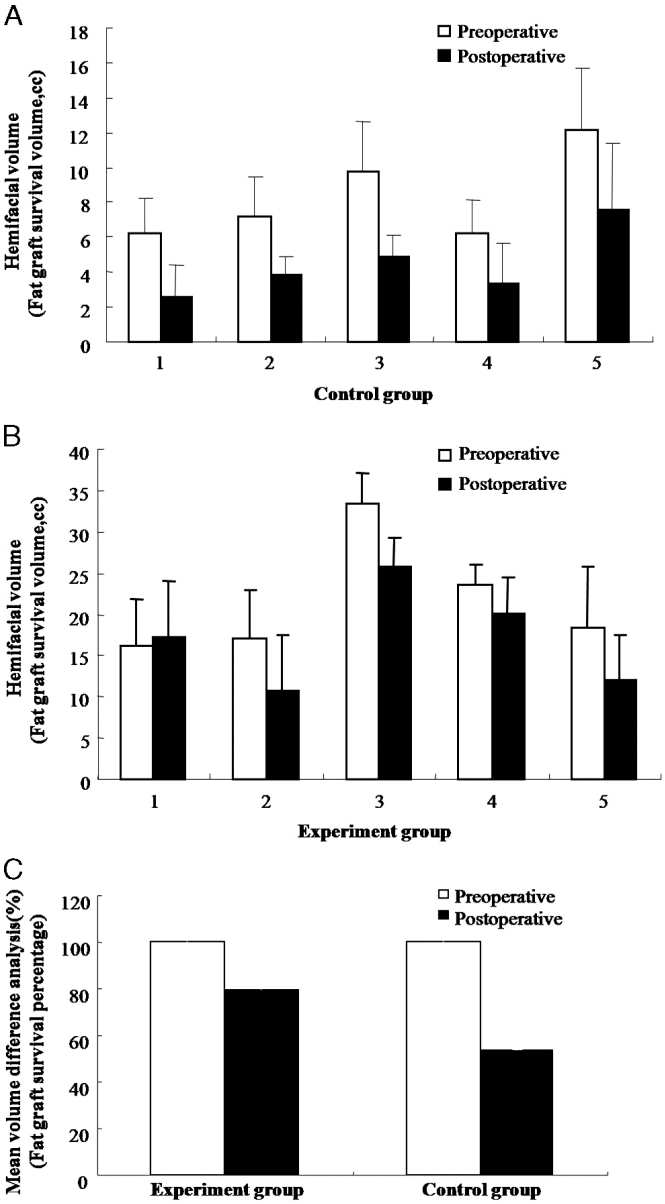

FIGURE 7. A, Preoperative and postoperative changes of the volume differences between the affected and unaffected sides of the face in the control group. B, Preoperative and postoperative changes of the volume differences between the affected and unaffected sides of the face in the experimental group. C, Comparison of the control and experimental groups.

Many investigators are concerned about the potential complications of stem cell therapy.[21,22] We therefore measured laboratory parameters monthly and performed CT scans after 6 months. These tests revealed no complications or hazards associated with ASC use in patients with Romberg disease for up to 2 years. Interestingly,

patient 2 showed a satisfactory outcome after 18 months. Later, this patient, who was obese, intentionally lost about 15 kg over 6 months. Although her face atrophied somewhat on both sides, the lesional hemiface lost a greater volume than did the contralateral hemiface—a difference that may have been attributable to a variation in fat location (Fig. 4D). Also, the abdominal fat injected on the lesional side may have been more affected by dieting because, during dieting, abdominal fat is lost before fat at other locations is metabolized.

Although the KFDA did not permit us to biopsy tissue samples for histologic examination, our results showed that ASC therapy plus microfat grafting enhanced fat survival in a relevant clinical application. Despite the controversial nature of stem cell therapy, this technique can be an alternative for the treatment of Parry-Romberg disease. Investigation with a larger number of patients is being scheduled as the next step.

CONCLUSIONS

Our results suggest that ASCs enhance the survival of fat grafted into the face. Moreover, it may be possible to treat patients with Parry-Romberg disease using a combination of microfat grafting and simultaneous ASC injection, without the need for microvascular free flap transfer. Our results also indicate that ASCs may have other clinical applications in patients requiring fat grafting.

ACKNOWLEDGMENT

Informed consent was received for publication of the figures in this article.

REFERENCES

1. Ulicna M, Danisovic L, Vojtassak J. Does cell therapy and tissue engineering represent a promising treatment of diabetic foot ulcers? *Bratisl Lek Listy.* 2010;111:138–143.
2. Cichutek K, Granzer U. The framework is established: progress in gene therapy, cell therapy and tissue engineering medicine [in German]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*. 2010;53:1–3.
3. Yamada Y, Nakamura S, Ito K, et al. A feasibility of useful cell-based therapy by bone regeneration with deciduous tooth stem cells, dental pulp stem cells, or bone-marrow–derived mesenchymal stem cells for clinical study using tissue engineering technology. *Tissue Eng Part A*. 2010;16:1891–1900.
4. Bochev I, Elmadjian G, Kyurkchiev D, et al. Mesenchymal stem cells from human bone marrow or adipose tissue differently modulate mitogen-stimulated B-cell immunoglobulin production in vitro. *Cell Biol Int*. 2008;32:384–393.
5. Zhu Y, Liu T, Song K, et al. Adipose-derived stem cell: a better stem cell than BMSC. *Cell Biochem Funct*. 2008;26:664–675.
6. Zuk PA. The adipose-derived stem cell: looking back and looking ahead. *Mol Biol Cell*. 2010;21:1783–1787.
7. Guerrerosantos J, Guerrerosantos F, Orozco J. Classification and treatment of facial tissue atrophy in Parry-Romberg disease. *Aesthetic Plast Surg*. 2007;31:424–434.
8. Miller MT, Sloane H, Goldberg MF, et al. Progressive hemifacial atrophy (Parry-Romberg disease). *J Pediatr Ophthalmol Strabismus*. 1987;24:27–36.
9. Asai S, Kamei Y, Nishibori K, et al. Reconstruction of Romberg disease defects by omental flap. *Ann Plast Surg*. 2006;57:154–158.
10. Hintringer T, Furnschlief E, Bauer M. Reconstruction of facial contour in hemifacial atrophy (Romberg disease) using a free revascularized scapula-fat-fascia flap [in German]. *Wien Med Wochenschr*. 1991;141:118–120.
11. Avelar RL, Goelzer JG, Azambuja FG, et al. Use of autologous fat graft for correction of facial asymmetry stemming from Parry-Romberg syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109:e20–5.
12. Gratama J, Kvalheim G, Orfao A. Standardization of cell analysis methods in clinical cellular therapy programs: a challenge for ISCT. *Cytotherapy.* 2006;8:528–529.
13. Arnalich-Montiel F, Pastor S, Blazquez-Martinez A, et al. Adipose-derived stem cells are a source for cell therapy of the corneal stroma. *Stem Cells*. 2008;26:570–579.
14. Crop MJ, Baan CC, Korevaar SS, et al. Human adipose tissue–derived mesenchymal stem cells induce explosive T-cell proliferation. *Stem Cells Dev.* 2010;29.
15. De Girolamo L, Sartori MF, Arrigoni E, et al. Human adipose-derived stem cells as future tools in tissue regeneration: osteogenic differentiation and cell-scaffold interaction. *Int J Artif Organs*. 2008;31:467–479.
16. Heydarkhan-Hagvall S, Schenke-Layland K, Yang JQ, et al. Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. *Cells Tissues Organs*. 2008;187:263–274.
17. Knippenberg M, Helder MN, Doulabi BZ, et al. Adipose tissue–derived mesenchymal stem cells acquire bone cell-like responsiveness to fluid shear stress on osteogenic stimulation. *Tissue Eng*. 2005;11:1780–1788.
18. Goldman MP, Skover GR, Payonk GS. Three-dimensional imaging techniques in the assessment of facial volume augmentation. *J Drugs Dermatol*. 2009;8:1113–1119.
19. Hong HJ, Hong JW, Koh SH, et al. A three-dimensional analysis of the relationship among lower facial width, bony width, and masseter muscle volume in subjects with prominent mandible angles. *J Craniofac Surg*. 2009;20:1114–1119.
20. Nelson LA, Michael SD. The application of volume deformation to three-dimensional facial reconstruction: a comparison with previous techniques. *Forensic Sci Int*. 1998;94:167–181.
21. Gil L, Styczynski J, Komarnicki M. Infectious complication in 314 patients after high-dose therapy and autologous hematopoietic stem cell transplantation: risk factors analysis and outcome. *Infection*. 2007;35:421–427.
22. Hayes-Lattin BM, Curtin PT, Fleming WH, et al. Toxic megacolon: a life-threatening complication of high-dose therapy and autologous stem cell transplantation among patients with AL amyloidosis. *Bone Marrow Transplant*. 2002;30:279–285.