Mechanical Micronization of Lipoaspirates Combined with Fractional CO_2 Laser for the Treatment of Hypertrophic Scars

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Background: Treating hypertrophic scars remains challenging. Stromal vascular fraction (SVF) gel is produced by a purely mechanical process from lipoaspirates, rich in adipose-derived stem cells, and has showed therapeutic potential on scars. However, controversial effects on hypertrophic scars are emerging. This study aimed to assess the therapeutic effects of SVF gel combined with fractional CO_2 laser on hypertrophic scars.

Methods: A rabbit ear hypertrophic scar model was established. SVF gel combined with fractional CO_2 laser was conducted for hypertrophic scars in rabbits. Scar alleviation in rabbits was observed based on the appearance and histology of scars, and the underlying mechanism was investigated by tissue immunologic analyses and quantitative real time polymerase chain reaction. At last, six patients with hypertrophic scar were treated by SVF gel combined with fractional CO_2 laser. Therapeutic effects were assessed using the Vancouver Scar Scale.

Results: Following the treatments, hypertrophic scars became less apparent and softer, the dermis became thinner, and collagen fibers appeared looser and arranged in a more organized pattern. The SVF gel plus fractional CO_2 laser group showed the most obvious improvement. In addition, SVF gel combined with fractional CO_2 laser increased adipogenesis in scar tissue, and adipose tissue regeneration was observed. Hypertrophic scars in patients were alleviated after treatment with SVF gel combined with fractional CO_2 laser.

Conclusions: SVF gel transplantation combined with fractional CO_2 laser showed encouraging therapeutic effects on hypertrophic scars. Although further investigation is necessary, this technique has great potential for clinical application to treat hypertrophic scars. *(Plast. Reconstr. Surg.* 151: 549, 2023.)

Clinical Relevance Statement: This is a new technique for treating hypertrophic scars.

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Hypertrophic scars are accompanied by itching and pain, which cause aesthetically displeasing skin appearances and functional disability, and these complications severely affect quality of life of patients.[1] Many invasive and noninvasive approaches have been used for treating hypertrophic scars[2]; however, to date, the treatment of hypertrophic scars remains challenging, and an optimal treatment program has not yet been established.[3] Growing evidence demonstrates that adipose-derived stem cells (ADSCs) are a promising

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therapeutic strategy for hypertrophic scars.[4,5] Collagenase digestion is required to obtain ADSCs; however, the clinical application of cell products using collagenase is regulated by legislation strictly.[6] Moreover, stem cell therapy derived from in vitro culture is subject to regulatory surveillance. Consequently, the clinical applications of ADSCs are limited. In 2017, Yao et al. described the preparation of a stromal vascular fraction (SVF) gel, which is enriched in ADSCs, obtained by a mechanical processing method, and can be injected with a 27-gauge needle.[7] SVF gel is an autologous product that does not contain any exogenous substances. Clinical studies have shown the successful use of SVF gel for tissue repair and regeneration and antiaging treatment.[7–10] Preclinical study confirmed that SVF gel is effective in treating hypertrophic scars.[11] To confirm the clinical efficacy of SVF gel transplantation for treating hypertrophic scars, we registered a clinical trial treating hypertrophic scars at the Chinese Clinical Trial Registry. However, significant improvements in the pliability and appearance of thick hypertrophic scars after SVF gel transplantation were not found. We speculated that excessive collagen deposition in the hypertrophic scar hindered the migration and survival of ADSCs. Therefore, it is important to provide space for ADSCs to exert their biological effects.

Fractional CO_2 laser is one of the most popular treatment options for hypertrophic scars and results in prolonged positive clinical outcomes.[12] We hypothesized that the dense microporous channels produced by thermal damage caused by the fractional CO_2 laser may provide space for ADSCs to exert their biological effects.

In this study, we investigated the therapeutic effects of SVF gel combined with fractional CO_2 laser (SVF gel plus laser) for hypertrophic scar in a rabbit ear. After treatment with laser, intralesional transplantation of autologous SVF gel was performed. The phenomenon of scar alleviation was observed, and the underlying mechanism was investigated. At last, six patients with hypertrophic scar were treated by SVF gel plus laser, and triamcinolone acetonide combined with fractional CO_2 laser (triamcinolone acetonide plus laser) served as a control.

MATERIALS AND METHODS

Animals and Ethical Approval

Twenty-four female New Zealand white rabbits weighing 2.5 to 3.0 kg were provided by the Animal Experimental Center of the Army Military Medical University (Chongqing, People's Republic of China). All animal experimental procedures were approved by the Committee of Animal Care of the Affiliated Hospital of ZunYi Medical University and conducted in accordance with the guidelines of the National Health and Medical Research Council (China) [KLLY(A)-2019-048].

Rabbit Ear Model of Hypertrophic Scars

The hypertrophic scar model was established according to a previously described procedure.[11] Briefly, the rabbits were anesthetized by an intramuscular injection of sodium pentobarbital (30 mg/kg). A rectangular full-thickness skin defect wound (5.5 cm long and 1.5 cm wide) of each ear was created; the skin and perichondrium were thoroughly removed. Four weeks later, the surface of the healed wounds showed obvious prominence, indicating hypertrophic scar formation. Three rabbits were selected randomly, for a total of six rabbit ear samples; full-thickness skin samples were obtained from the normal skin and scar skin of a rabbit ear by surgery. Skin samples were fixed in 4% paraformaldehyde (Solarbio, Beijing, People's Republic of China), and hematoxylin and eosin (H&E) staining was performed to confirm that the scar model was successfully constructed.

Preparation of the SVF Gel

The SVF gel was prepared as described previously.[11] Briefly, rabbits were anesthetized by an intraperitoneal injection of sodium pentobarbital. Ten milliliters of pure fat was obtained from both inguinal areas and cleared with saline three times. After mincing with surgical scissors, the fat tissue was then mechanically emulsified by transferring between two 10-mL syringes connected by a Luer-lock connector with an internal diameter of 2.4 mm for 1 minute (10 mL/second). The emulsion was centrifuged at 2000 *g* per minute for 3 minutes; the substance below the oil layer was defined as the SVF gel and collected for further use.

In Vivo Animal Experiments and Evaluation

Twenty-four rabbits were used for a total of 48 experimental scar samples. The scar samples were assigned randomly to treatment with SVF gel plus laser ($n = 12$), SVF gel ($n = 12$), laser ($n = 12$), or saline ($n = 12$). The parameters of the fractional CO_2 laser (AcuPulse; Lumenis) under deep mode were as follows: power, 25 mJ; density, 5%. The injection volume of the SVF gel was 0.1 mL/cm^2. General observations of hypertrophic scars were recorded by photographic imaging at different time points. Scar tissues were collected, and H&E staining and Masson trichrome staining were performed according to the manufacturer's instructions at 4 and 12 weeks after treatment.

Immunofluorescence Analysis

For immunofluorescence analysis, the deparaffinized sections were incubated with guinea-pig anti-rabbit perilipin (1:200; Progen, Heidelberg, Germany) and then incubated with secondary antibodies for co-staining: goat anti-pig IgY-488 (1:200; Thermo Fisher Scientific, Cambridge, MA). Nuclei were stained with 4′,6-diamidino-2-phenylindole (1:200; Sigma, St. Louis, MO). Images were acquired using a confocal laser scanning microscope (C1Si; Nikon, Tokyo, Japan).

Western Blotting Assay

For Western blotting, tissue lysates were obtained using radioimmunoprecipitation assay lysis buffer (Solarbio). Thirty micrograms of protein were added to the lane for polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. After blocking, the blotted membranes were incubated with the following primary antibodies at 4°C overnight: PPAR-γ (1:1000; Abcam), C/EBP-α (1:1000; Abcam), and β-actin (1:1000; Abcam) monoclonal antibodies. The blots were then incubated with the corresponding secondary antibodies (1:5000; Abcam) at room temperature for 1 hour. The results were checked using an enhanced chemiluminescence kit (Invitrogen) and analyzed with PC densitometry software (Bio-Rad Laboratories, Hercules, CA).

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed according to the standard procedures. The fold change of each target gene was normalized to that of β-actin mRNA. The primer sequences were as follows:

PPAR-γ:

Forward primer:
5′-CCCTGAGAACCTCATTCCGT-3′.
Reverse primer:
5′-GCAAATGATCCTCCACCCGA-3′.

C/EBP-α:

Forward primer:
5′-ATGAGACTCTTGGTTGGCCG-3′.
Reverse primer:
5′-CCCACCTCACCTCATTGGTC-3′.

β-actin:

Forward primer:
5′-TGTGGCCGAGGACTTTGATT-3′.
Reverse primer:
5′-TACACAAATGCGATGCTGCC-3′.

Patients and Treatment

Fifteen patients with hypertrophic scars in different areas of their bodies were enrolled at the Affiliated Hospital of Zunyi Medical University between October of 2018 and October of 2020. The average age of the patients was 32.67 years (range, 21 to 49 years (Table 1). Inclusion criteria consisted of the following: (1) all patients showed typical hypertrophic scar characteristics and the scars with a duration for at least 3 months; (2) the age of the patients was 18 to 60 years and body mass index was 18.5 to 23.9 kg/m^2; and (3) none of the treated scars exceeded 5% of total body surface area. Exclusion criteria consisted of the following: (1) patients with a history of steroid injection in the past 3 months; (2) patients with underlying blood system disease, immune system disease, hypertension, diabetes, or other metabolic diseases; and (3) patients with mental illness. Six of the patients were treated with SVF gel plus laser. A fractional CO_2 laser (AcuPulse) under the manual mode was performed. The parameters were as follows: power, 1 w; time on, 0.5 msec. After the laser treatment was completed, the SVF gel was transplanted into the scar at a volume of 0.1 mL/cm^2. Nine patients were treated with triamcinolone acetonide plus laser. The laser mode and parameters were consistent with the above. After the laser treatment was completed, the triamcinolone acetonide (40 mg/mL; Zhejiang Xianju Biomedical Co., Ltd., Zhejiang, People's Republic of China) was transplanted into the scar at a dose of 4 mg/cm^2. All the participants provided written informed consent before enrolling in the study. The protocol was approved by the ethics committee of the Affiliated Hospital of ZunYi Medical University (KLL-2019-031).

Efficacy Evaluation before and after Treatment

General observations of hypertrophic scars were recorded by photographic imaging before and after treatment. Scars were also assessed clinically using the Vancouver Scar Scale (VSS)[13] as shown in Table 2, which includes the assessment of vascularity, pliability, pigmentation, and height.

Statistical Analysis

SPSS (Version 17.0; SPSS, Inc., Chicago, IL) statistical software was used for statistical analysis. Differences between the four groups of animal experiments were assessed by one-way analysis of variance test followed by Bonferroni Dunn tests. A paired sample *t* test was used to compare VSS data

Table 1. Summary of Patient Data

Patient	Age (yr)	Sex	External Causes of Scars	Scar Site	Duration of Scar (mo)	History of Scar Treatment	Therapeutic Modalities
1	39	M	Surgery	Front chest	14	Steroid injection, silicone gel	SVF gel plus laser
2	21	M	Acne	Mandibular margin	28	Steroid injection, silicone gel	SVF gel plus laser
3	38	F	Burn	Right hand	5	Silicone gel, elastic compression	SVF gel plus laser
4	23	F	Burn	Mandibular margin and neck	4	Injection of steroids, silicone gel	SVF gel plus laser
5	22	F	Trauma	Shoulders and front chest	28	Steroid injection	SVF gel plus laser
6	36	M	Mosquito bites	Back and front chest	60	Steroid injection, electron beam irradiation	SVF gel plus laser
7	22	F	Surgery	Back	4	Steroid injection, silicone gel	Triamcinolone acetonide plus laser
8	36	M	Trauma	Front chest	6	Steroid injection, silicone gel	Triamcinolone acetonide plus laser
9	21	M	Mosquito bites	Shoulders and front chest	5	Steroid injection, silicone gel	Triamcinolone acetonide plus laser
10	35	M	Mosquito bites	Front chest	36	Triamcinolone injection 6 mo ago	Triamcinolone acetonide plus laser
11	38	F	Surgery	Front chest	4	Steroid injection, silicone gel	Triamcinolone acetonide plus laser
12	27	M	Surgery	Right forearm	23	None	Triamcinolone acetonide plus laser
13	41	F	Surgery	Front chest	8	Steroid injection, silicone gel	Triamcinolone acetonide plus laser
14	42	F	Mosquito bites	Front chest	60	Triamcinolone injection six 6 mo ago	Triamcinolone acetonide plus laser
15	49	M	Mosquito bites	Shoulders and front chest	7	Elastic sleeve	Triamcinolone acetonide plus laser

M, male; F, female.

Table 2. Vancouver Scar Scale Used in Our Study[a]

Vascularity		Pliability		Pigmentation		Height	
Characteristic	Score	Characteristic	Score	Characteristic	Score	Characteristic	Score
Normal	0	Normal	0	Normal	0	Flat	0
Pink	1	Supple	1	Hypopigmentation	1	<2 mm	1
Red	2	Yielding	2	Mixed	2	2–5 mm	2
Purple	3	Firm	3	Hyperpigmentation	3	>5 mm	3
		Ropes	4				
		Contracture	5				

[a]Including assessment of vascularity, pliability, pigmentation, and height.

before and after treatment of patients. A value of $P < 0.05$ was regarded as statistically significant.

RESULTS

Successful Establishment of a Rabbit Ear Model of Hypertrophic Scars

Four weeks after the creation of rabbit ear wounds, reepithelialization was completed, and visible hard scars protruding from the surface of the skin were formed (Fig. 1). In the normal skin of rabbit ears, the dermis was thinner, whereas significantly increased dermal thickness was observed in the scar tissue. Compared to the normal rabbit ear skin tissue, the scar tissue showed dramatically increased dermal thickness. [**See Figure, Supplemental Digital Content 1**, which shows establishment of a rabbit ear model of hypertrophic scars. (*Left*) The dermis (the area above the dotted line) was thinner in the normal skin of rabbit ears. (*Center*) Significantly increased dermal thickness was observed in the scar tissue. *Scale bar* = 200 μm. (*Right*) The dermal thickness was measured and was observed to increase in the scar tissue (**$P < 0.01$), ***http://links.lww.com/PRS/F631***.]

SVF Gel plus Laser Alleviated Hypertrophic Scars

All rabbits survived well, and the ear skin showed no ulceration; the animals were followed up for 12 weeks after treatment. The size of scars gradually decreased, the color became lighter, and the texture became softer after treatment with laser or SVF gel. Scars showed better

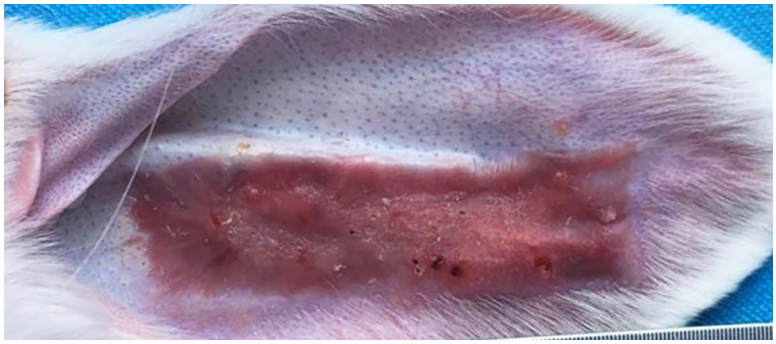

Fig. 1. Establishment of a rabbit ear model of hypertrophic scars. Four weeks later, reepithelialization was completed, and hard scars protruding from the surface of the skin were observed.

improvement in the group treated with SVF gel plus laser than in the other three groups, followed by the SVF gel and laser groups. Surprisingly, scars were almost invisible after 12 weeks in the SVF gel plus laser group. However, a stiff and visibly raised scar persisted for more than 12 weeks in the control group (Fig. 2).

H&E staining and Masson trichrome staining of the scar samples were performed to evaluate the histologic status at 4 and 12 weeks after treatment.

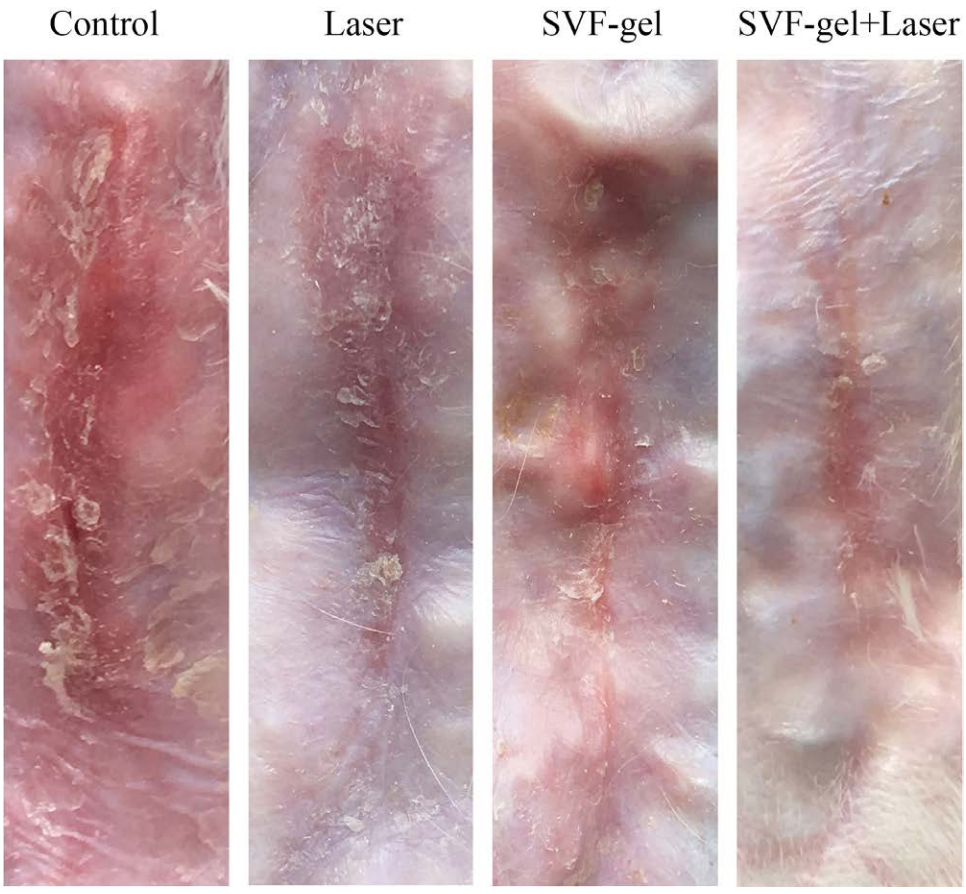

Fig. 2. Gross observation of hypertrophic scar at 12 weeks after treatment. Scars showed better improvement in the group treated with SVF gel plus laser than in the other three groups, followed by that in the SVF gel and fractional CO_2 laser groups. A stiff and visibly raised scar persisted for more than 12 weeks in the control group.

H&E staining showed that reduced inflammatory cell infiltration, thinner dermis, and looser and organized collagen fibers at 4 weeks and 12 weeks after treatment in the SVF gel plus laser group (Fig. 3). [**See Figure, Supplemental Digital Content 2**, which shows histologic detection of scars. (*Above*) H&E staining showed reduced inflammatory cell infiltration and looser and organized collagen fibers at 4 weeks after treatment. (*Center* and *below*) Masson trichrome staining showed that the arrangement of collagen fibers in the SVF gel plus laser group was the most loose and regular, and the density of collagen was the lowest. Subcutaneous adipose tissue was clearly seen in the SVF gel plus laser group and the SVF gel group; however, the control group scarcely showed adipose tissue. *Scale bar* = 50 μm. $^{**}P < 0.01$; $^{*}P < 0.05$, ***http://links.lww.com/PRS/F632***.] Masson trichrome staining showed that the scar tissue was remodeled with renewal and reorganization of collagen fibers in the dermis after treatment. The SVF gel plus laser group showed the most obvious improvement. The quantification of collagen density showed that the control group had the highest collagen density, while collagen density was significantly reduced in the SVF gel plus laser group ($P < 0.05$) (**see Figure, Supplemental Digital Content 2**, ***http://links.lww.com/PRS/F632***).

Interestingly, adipose tissue was clearly observed in scars in the SVF gel plus laser group and the SVF gel group; however, the control group scarcely showed adipose tissue (Fig. 3). Twelve weeks after treatment, the skin of the SVF gel plus laser group showed a normal structure, with a complete epidermis, a thinner dermis layer, and substantial subcutaneous adipose tissue. The H&E and Masson trichrome staining results suggested that the scars treated by SVF gel plus laser achieved a relatively satisfying outcome with remodeling of the extracellular matrix extracellular matrix and subcutaneous adipose tissue regeneration.

SVF Gel plus Laser Increased Adipogenesis in Scars

Immunofluorescence staining of perilipin of scar tissue was performed at 4 and 12 weeks to confirm the regeneration of subcutaneous fat tissue. As shown in Figure 4, at 12 weeks after treatment, very little positive staining was found in the control and laser-alone groups. The SVF gel plus laser group exhibited the strongest positive expression of perilipin, whereas the SVF gel–alone group showed a mild level of positive staining. The results of immunofluorescence staining at 4 weeks after treatment are shown. [**See Figure, Supplemental Digital Content 3**, which shows that there is adipogenesis in the scar. (*Above*) Immunofluorescence staining of perilipin at 4 weeks after treatment. Representative images at 4 weeks and (*center*) quantification results of fluorescence expression at 4 and 12 weeks. The *red arrows* are adipose cells. *Scale bar* = 100 μm. $^{**}P < 0.01$; $^{*}P < 0.05$. (*Below*) Expression of genes such as *C/EPB-α* and *PPAR-γ*associated with adipogenesis was assayed by quantitative real-time polymerase chain reaction. The results showed that the SVF gel plus laser group showed significantly higher expression levels of C/EBP-α and PPAR-γ than the other three groups, followed by the SVF gel–alone and fractional CO2 laser–alone groups. $^{**}P < 0.01$; $^{*}P < 0.05$, ***http://links.lww.com/PRS/F633***.] To determine the effect of SVF gel plus laser on proadipogenesis in scar tissue, we assayed the expression of genes and proteins associated with adipogenesis, such as C/EBP-α and PPAR-γ, by qRT-PCR and Western blot assay. As shown in **Figure, Supplemental Digital Content 3**, C/EBP-α

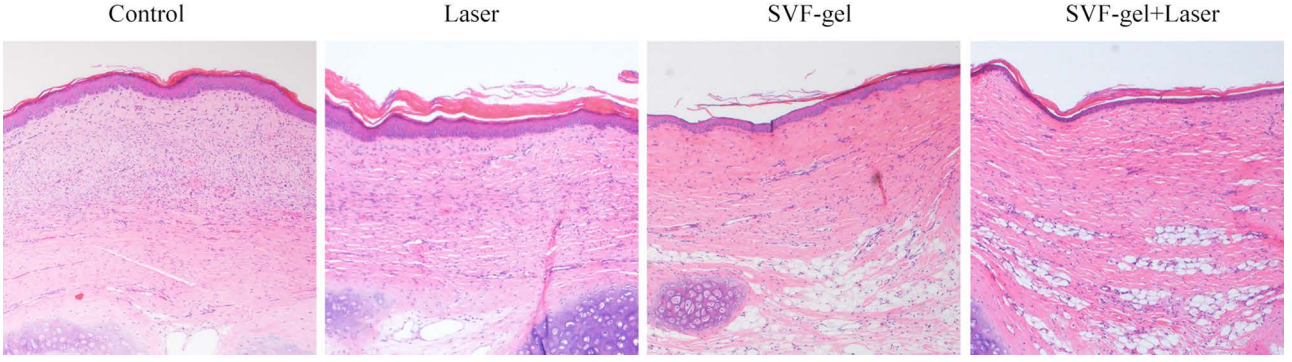

Fig. 3. H&E staining showed that the dermis became thinner and collagen fibers appeared looser and were arranged in a more organized pattern at 12 weeks after treatment. The SVF gel plus laser group showed the most apparent improvement, and the control group showed almost no improvement. *Scale bar* = 200 μm. Interestingly, subcutaneous adipose tissue was clearly seen in the SVF gel plus laser group and the SVF gel group; however, the control group scarcely showed adipose tissue.

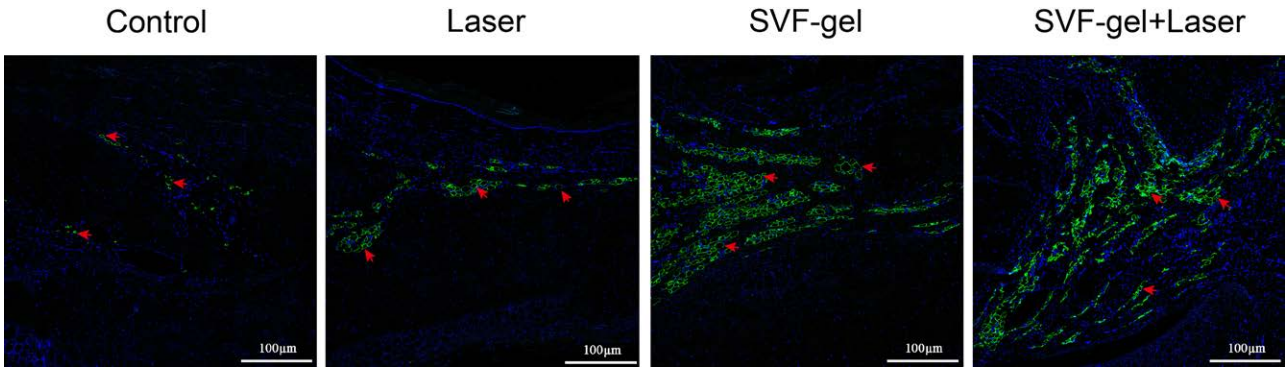

Fig. 4. Immunofluorescence staining of perilipin. The SVF gel plus laser group showed the strongest positive expression of perilipin compared to the other three groups at 12 weeks after treatment. The *red arrows* are adipose cells. *Scale bar* = 100 μm.

and PPAR-γ were expressed at significantly higher levels in the SVF gel plus laser group than in the other three groups as observed in the qRT-PCR assay, followed by the SVF gel–alone and the laser-alone groups. The control group showed the lowest expression level of these proteins as compared to the other groups. The expression of these signature proteins showed the same trend in the Western blot assay (Fig. 5). (**See Figure, Supplemental Digital Content 4**, which shows expression of proteins including C/EBP-α and PPAR-γ, assayed by Western blot assay. The expression of these signature proteins showed the same trend with qRT-PCR in the Western blot assay. $^{**}P < 0.01$; $^{*}P < 0.05$, ***http://links.lww.com/PRS/F634***.)

Hypertrophic Scars in Patients Were Minimized after Treatment with SVF Gel plus Laser

After treatment with SVF gel plus laser, all patients reported that the symptoms of itching and pain at the scar area were significantly relieved. After treatment, the appearance and texture of the scar were improved significantly (Figs. 6 through 8).

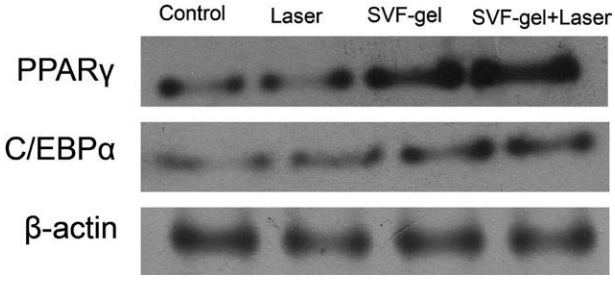

Fig. 5. Expression of proteins such as C/EBP-α and PPAR-γ associated with adipogenesis was assayed by Western blot assay at 12 weeks after treatment. The protein expression of C/EBP-α and PPAR-γ was the highest in the SVF gel plus laser group.

The VSS score was recorded for six patients with SVF gel plus laser before treatment and ranged from 7 to 12 (mean ± SD, 9.67 ± 1.75), whereas after treatment, the VSS score ranged from 3 to 7 (mean ± SD, 4.83 ± 1.47). A significant difference in VSS was observed (Table 3). [**See Figure, Supplemental Digital Content 5**, which shows that hypertrophic scars in patients were alleviated after treatment with SVF gel plus laser and triamcinolone acetonide plus laser, and SVF gel plus laser was better than triamcinolone acetonide plus laser. (*Above, left*) There were significant differences before and after SVF gel plus laser treatment ($^{**}P < 0.01$). (*Above, right*) There were differences before and after triamcinolone acetonide plus laser treatment ($^{*}P < 0.05$). (*Below, left*) The appearance of hypertrophic scars with a duration of 4 months located in front chest; the scar area was increased after triamcinolone acetonide plus laser treatment. (*Below, right*) The difference of VSS before and 6 months after SVF gel plus laser treatment or triamcinolone acetonide plus laser treatment. The difference was statistically significant ($^{**}P < 0.01$). This showed that SVF gel plus laser was better than triamcinolone acetonide plus laser, ***http://links.lww.com/PRS/F635***.]

After treatment with triamcinolone acetonide plus laser, all nine patients reported that the symptoms of itching and pain at the scar area were significantly alleviated. In addition, the texture of the scar became softer and the color became lighter. The VSS score of nine patients before treatment ranged from 7 to 11 (mean ± SD, 9 ± 1.22), whereas after treatment, the VSS score ranged from 5 to 8 (mean ± SD, 6.11 ± 1.26). A significant difference in VSS was observed. However, the area of hypertrophic scar after treatment

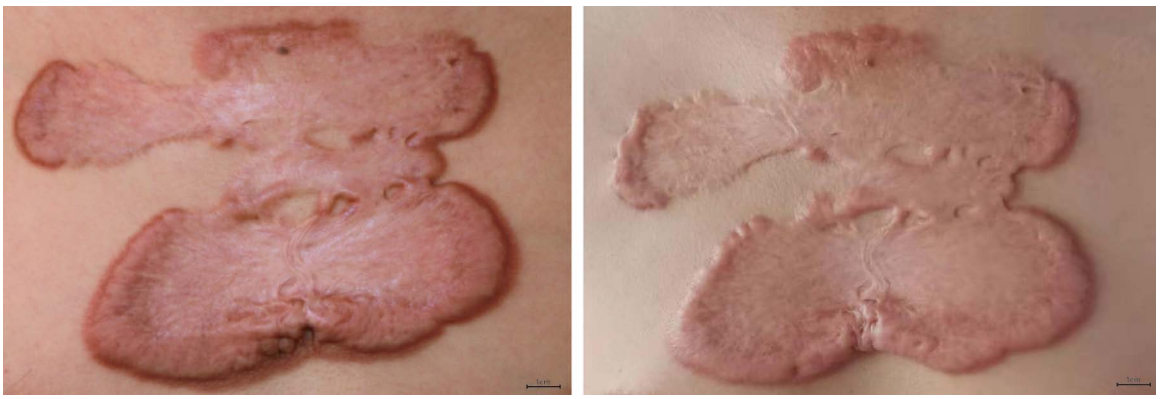

Fig. 6. Representative images of patients' pathologic scars. The appearance of pathologic scars present for 14 months located in the front chest (*left*). Not only were improvements in the color, height, and pliability of the pathologic scars observed, the patient's pruritus symptoms were also well alleviated or even disappeared at 30 months after a combined treatment (*right*).

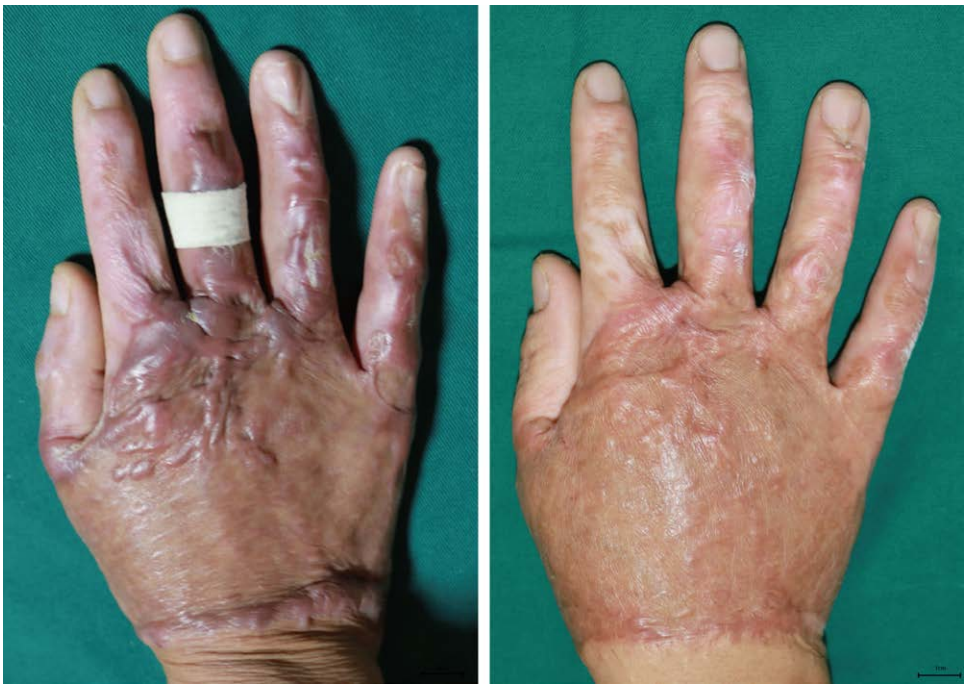

Fig. 7. The appearance of hypertrophic scars with a duration of 5 months located in the right hand (*left*). Not only were improvements in the color, height, and pliability of the pathologic scars observed, the patient's pruritus symptoms were also well alleviated at 32 months after a combined treatment (*right*).

was increased (**see Figure, Supplemental Digital Content 5**, ***http://links.lww.com/PRS/F635***).

The difference of VSS from preoperatively to postoperatively was 4.83 ± 0.75 in the SVF gel plus laser group and 2.89 ± 1.05 in the triamcinolone acetonide plus laser group; the difference was statistically significant (**see Figure, Supplemental Digital Content 5**, ***http://links.lww.com/PRS/F635***, and Table 3). This showed that SVF gel plus laser was better than triamcinolone acetonide plus laser in treating hypertrophic scars.

DISCUSSION

Hypertrophic scars are still a major challenge to treat in modern medicine. In the present study, hypertrophic scar models from the rabbit ear were first treated by SVF gel plus laser. The results

Fig. 8. The appearance of hypertrophic scars with a duration for 4 months located in the mandibular margin and neck (*left*). Improvements in the color, height, and pliability of the pathologic scars were observed at 29 months after a combined treatment (*right*).

Table 3. VSS of 15 Patients with Hypertrophic Scars

	No	Preoperative	Postoperative	Pre–Post	t2	P2
S + L	6	9.67 ± 1.75	4.83 ± 1.47	4.83 ± 0.75	5.175	0.000
T + L	9	9 ± 1.22	6.11 ± 1.26	2.89 ± 1.05	4.914	0.000
t1		0.872	1.795	3.885		
P1		0.399	0.096	0.02		

S + L, SVF gel plus laser group; T + L, triamcinolone acetonide plus laser group; Pre, preoperative; Post, postoperative; t1 or t2, t value of the *t* test of the VSS score before or after the operation or the difference between the two groups; P1 or P2, *P* value of the *t* test of the VSS score before or after the operation or the difference between the two groups.

demonstrated that the appearance of scars was significantly improved, the dermis appeared thinner, and collagen fibers appeared looser and in a more organized pattern. In addition, SVF gel plus laser increased adipogenesis in rabbit ear scars. Not only were the symptoms from patients with hypertrophic scar relieved, but also the appearance was alleviated after treatment with SVF gel plus laser.

Autologous fat grafting has been performed for patients with hypertrophic scars or keloids. Several studies have reported the effectiveness of fat grafting on hypertrophic scars, and adverse effects have been rarely reported.[14,15] The underlying mechanism of fat transplantation involves the delivery of ADSCs from the transferred adipose tissue to the recipient area. The antifibrotic mechanisms of ADSCs are diverse and mostly mediated by paracrine signaling, which activates various antifibrotic molecular pathways.[16–18] Not only the paracrine mechanism but also direct cell-to-cell interaction plays a role in the antifibrotic effect mediated by ADSCs.[19] Co-culture of hypertrophic scar fibroblasts and ADSCs demonstrated that ADSCs not only inhibited the proliferation, migration, and contractility of fibroblasts but also decreased the expression of fibrotic-related or transforming growth factor-β1–induced molecules.[20] However, the concentration of ADSCs in adipose tissue is very low, which may be an important reason for failure to observe significant improvements in the appearance and texture of scars after fat transplantation.[21] In addition, an in-depth study of the survival mechanism after fat transplantation revealed that most of the fat cells die after fat transplantation, and the released oil droplets may aggravate local inflammation, which is also one of the factors that affect its therapeutic effect.[22] Therefore, it is necessary to further optimize the method of ADSC transplantation for hypertrophic scars. The SVF gel was prepared from adipose tissue by mechanical processing, wherein mature fat cells were removed, and the gel contained a high density of ADSCs; thus, this gel could be used as a novel approach for ADSC therapy.[7] Wang et al.[11] reported that an intralesional injection of the SVF gel reduced hypertrophic scarring in a rabbit ear model.

However, in clinical practice, we found that for thicker hypertrophic scars or keloids, the improvement in appearance and symptoms of hypertrophic scars after SVF gel transplantation was very limited. Fractional CO_2 laser is one of the most popular treatment approaches for hypertrophic scars and can be used alone or as an adjuvant in combination with other physical and surgical methods.[23,24] The mechanism of

fractional CO_2 laser treatment of hypertrophic scars involves induction of small three-dimensional zones of thermal damage, referred to as "microscopic thermal zones," which cause collagen remodeling and ultimately lead to improvement in pliability.[25]

In the present study, we used SVF gel plus laser to treat hypertrophic scars. In rabbit ear hypertrophic scar, the results showed that the SVF gel and fractional CO_2 laser exerted a synergistic effect on alleviating hypertrophic scars as evidenced by the smaller size of scars, thinner dermis, and looser and more organized collagen fibers. More adipose tissue regeneration appeared in the scar, and C/EBP-α and PPAR-γ, which are associated with adipogenesis, were highly expressed after treatment with SVF gel plus laser. Adipose tissue regeneration also appeared in the scar after SVF gel alone. We observed an interesting phenomenon, namely, the more the regeneration of adipose tissue, the better the improvement in the appearance of the scar. Previous studies demonstrated that adipocytes are highly active secretory cells that release hundreds of different factors involved in various (pathologic) physiologic processes.[26,27] During skin wound healing, adipocytes are a key component of the intercellular communication that mediate the function of fibroblasts. Mature adipocytes can induce fibroblasts to recruit to the wound and regulate the deposition and remodeling of extracellular matrix.[28] In addition, adipocytes can induce fibroblast reprogramming, and the mechanism is mediated through the direct and indirect activation of PPAR-γ signal and the release of bone morphogenetic protein (BMP)-4 and subsequent stimulation of BMP signal transduction; both of these signaling pathways are known to antagonize transforming growth factor-β and therefore exert an antifibrosis effect.[29] Moreover, PPAR-γ is well established as a prime inducer of adipogenesis.[30] Study also showed that during wound healing, adipocytes regenerate from myofibroblasts, and myofibroblast reprogramming required neogenic hair follicles, which triggered BMP signaling.[31] Areas lacking hair follicle and adipocyte regeneration formed obvious scars, whereas areas with hair and adipocyte regeneration showed scarless healing.[31] When treated with either BMP or when placed with human hair follicles in vitro, human keloid fibroblasts can be reprogrammed into adipocytes.[31] Therefore, we believe that adipogenesis in scars may be one of the mechanisms involved in the treatment of hypertrophic scars.

The present study is the first report of the use of SVF gel plus laser to treat hypertrophic scars. Under the combined intervention of the thermal damage space provided by the fractional CO_2 laser and the lipogenic microenvironment provided by the SVF gel, adipogenesis is better induced in the hypertrophic scar, thereby achieving the purpose of alleviating hypertrophic scars. However, there are still many questions that need to be resolved, such as the source of new adipose cells. Are these cells derived from transplanted SVF cells or transformed from other cells in situ? Further experiments to prove that the adipose tissue has a direct or an indirect impact on the hypertrophic scars are necessary.

Although the present study was limited by a small number of cases, the results demonstrated the benefits of combined treatment with SVF gel and fractional CO_2 laser. These studies are only the preliminary studies of our large-scale clinical studies. A longer follow-up study with additional patients is ongoing, and further investigations to elucidate the underlying molecular mechanisms are being conducted. In addition, glucocorticoids combined with fractional CO_2 laser have been widely used in clinical practice for treating hypertrophic scars, which is convenient. We found that SVF gel plus laser was better than triamcinolone acetonide plus laser in treating hypertrophic scars; it also avoids the complications of triamcinolone acetonide injection (eg, thinning of the skin, dilation of capillaries).

CONCLUSIONS

Our results showed that SVF gel plus fractional CO_2 laser showed encouraging therapeutic effects on hypertrophic scars. Although further investigation is necessary, this technique shows great potential for clinical application to treat hypertrophic scars.

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