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Original Article

Safety and efficacy of three-dimensional vacuum-assisted bipolar plasma skin regeneration for tissue tightening

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Abstract: The aim of the study was to evaluate the safety and efficacy of a new device for body contouring. Sixteen informed and consenting female patients (aged 20-59 years, mean: 32.38 ± 7.26 years) were recruited for this study. The PSR device used here combined bipolar PSR radiofrequency and vacuum technologies. The patients underwent facial or body contouring every 3 weeks for a total of three treatments using a PSR energy of 2.5-3.5 J. Full-thickness skin biopsy specimens from two patients were processed for hematoxylin-eosin, Masson trichrome, and proliferating cell nuclear antigen staining. Samples taken from a mastectomy were assessed *ex vivo* by measuring the markers of movement to visualize adipose contraction. The good results were maintained during a 2- to 3-month follow-up. The histology of the treated tissue biopsies demonstrated normal dermal architecture with healthy collagen and easting fibers in the deep reticular dermis and no evidence of scar tissue or abnormal collagen fibers, as well as active epithermal cellular proliferation. An unsymmetrical fat contraction was qualitatively observed in the *ex vivo* samples. The results of this study showed the efficacy and safety of vacuum-assisted bipolar PSR for three-dimensional tightening. This technology allows for precise soft-tissue modeling with better results than traditional PSR technology, but additional larger studies are needed.

Keywords: Body patterning, lipoprotein lipase, plasma skin regeneration, muscle tonus

Introduction

In 2005, the US Food and Drug Administration approved plasma skin regeneration (PSR) technology for skin rejuvenation and the treatment of wrinkles. Plasma is a state of matter that can be created by adding sufficient energy into a material in the gas state. The application of heat to the skin induces controlled thermal damage, which results in the production of new collagen, a reduction in elastic fibers, and a restructuring of the dermal architecture that can be confirmed histologically [1].

PSR technology can be used at various energy settings. Because of this range of effects, the relative effectiveness and safety are concerns when applying PSR for facial and body contouring. All noninvasive tissue tightening treatments have a limitation, because it is not safe the energy delivered through the skin surface. The energy from PSR devices tends to scatter or be absorbed in the upper layers of the skin, making it difficult to deliver sufficient amounts of energy to the deep dermis without causing damage.

Studies indicate that deeper penetrating energy can be used for treatment of facial rhytides and laxity, body tightening [2]. Energy overlapping and confinement are the physical and biological characteristics of vacuum-assisted bipolar PSR that explain its more effective three-dimensional mechanism of skin tightening [3]. Sufficient energy can be applied from both sides such that the overlapping energies reach and affect the adipose tissue in the core. This should hypothetically allow more precise facial contouring with a smaller chance of adverse thermally related side effects. Moreover, the vacuum-induced mechanical stress on the skin fibroblasts has been reported to lead to collagen secretion, which likely contributes to the clinical efficacy of the technique [4]. In addition,

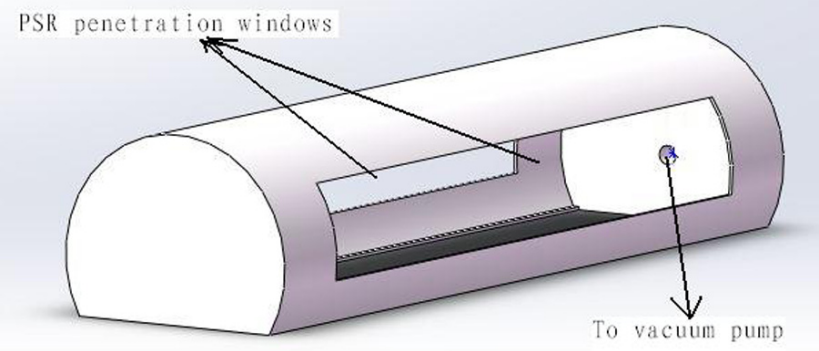

Figure 1. Schematic of the vacuum tube.

Table 1. Subjective patient satisfaction and clinician efficacy grades and the 5-point scale definitions

Grade	Patient satisfaction grades	Grade	Clinician efficacy grades
	Description		Description
5	Highly satisfied	5	Excellent (>85% improvement)
4	Very satisfied	4	Very Good (65%-84%)
3	Satisfied	3	Good (45%-64%)
2	Neither satisfied nor dissatisfied	2	Poor (0%-44%)
1	Dissatisfied	1	Worse (increased girth)

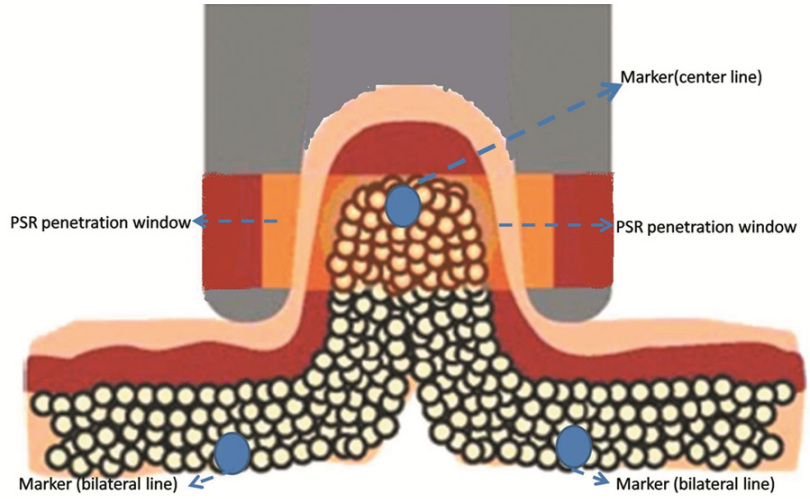

Figure 2. Diagram showing vacuum aspiration of the skin to a predetermined depth between the lateral plates.

increasing blood perfusion at the target area may support the stimulated fibroblast activity [5].

Materials and methods

Vacuum-assisted bipolar PSR device

A traditional PSR device consists of an ultra-high-frequency radiofrequency (RF) generator that excites a tuned resonator and imparts energy to a flow of inert nitrogen gas. The acti vated ionized gas is named plasma and has an optical emission spectrum with peaks in the visible (mainly indigo and violet) and near-infrared ranges. Nitrogen is used as the gaseous source because it purges oxygen from the surface of the skin, thereby minimizing the risk of unpredictable hot spots, charring, and scar formation.

The device used here combines traditional PSR (Rhytec Inc., Waltham, MA, USA) with bipolar irradiation and vacuum technologies. The volume of the vacuum apparatus could be selected from 1 to 5 mL to suctiononly the fold of skin for alignment, thus avoiding off-target tissue structures such as muscle, fascia, and bone. Symmetrical rectangular windows on both side walls of the device allowed the PSR to safely pass into and efficiently deliver energy and heat to the target tissues. By folding the skin, the adipose tissue in the core receives overlapping PSR energy that penetrates from both sides (**Figure 1**).

Clinical protocol

Sixteen female patients (age range: 20-59 years; mean age: 32.38 ± 7.26 years), including nine patients requiring body contouring and seven patients requiring facial contouring, were recruited for this study. Twenty-four sites were treated on the 16 patients: 15 on the body (armpit area: 2, upper arms: 2, abdomen: 5, buttocks: 2, and inner thighs: 4) and nine on

Table 2. Patient details, side effects, and the subjective and objective assessments

Pat. No	Zone	Side effects and grade					Grading scores			
							Patient		Clinician	
							F	L	F	L
1	Armpit area*	p +	e ++	r ++	i +	h +	4/4	4/4	4/4	5/5
2	Abdomen mermaid line*	p -	e +	r ++	i +	h +	3/4	4/5	3/3	5/5
3	Buttocks*	p -	e +	r ++	i +	h +	4/4	4/4	4/5	4/4
4	Inner thighs*	p -	e +	r ++	i +	h ++	5/5	4/4	5/5	4/4
5	Lower cheek*	p +	e ++	r +	i +	h +	4/4	5/4	4/3	5/5
6	Submental area	p -	e +	r ++	i +	h +	4	5	4	5
7	Upper arms*	p -	e +	r ++	i +	h +	4/4	4/5	4/4	4/5
8	Abdomen	p +	e -	r +++	i +	h ++	2	4	3	4
9	Inner thighs*	p -	e +	r ++	i +	h ++	4/5	4/4	4/5	4/4
10	Abdomen	p -	e +	r ++	i +	h ++	2	3	3	3
11	Submental area	p +	e +	r ++	i +	h ++	4	5	4	5
12	Submental area	p -	e -	r +	i -	h -	3	4	4	5
13	Submental area	p -	e +	r ++	i -	h +	3	5	4	5
14	Abdomen	p -	e +	r ++	i -	h +	4	4	3	4
15	Submental area	p +	e -	r +	i +	h +	4	5	5	5
16	Lower cheek*	p -	e +	r +	i -	h +	4/4	4/4	3/4	4/5

F: First assessment, immediately after 1st treatment; L:final assessment; *bilateral; p: pain; e: edema; r: erythema; i: inflammation; h: induration; +++: severe; ++: moderate; +: some; -: none.

the face (lower cheek: 4 and submental area: 5). The study was approved by the Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University, and all subjects gave written informed consent for participation and our use of their clinical images after having been informed of the purpose of the study and possible outcomes.

Patients underwent treatments every 3 weeks for a total of three treatments. The patients arrived 1 h before the procedure and a topical anesthesia was applied without occlusion or other skin preparations. Oral analgesia (one combination tablet of 7.5 mg hydrocodone and 750 mg acetaminophen or one combination tablet of 100 mg propoxyphene and 650 mg acetaminophen) was administered 30-45 min before the procedure. Once the tissue was properly positioned between the two notches on the vacuum tube, the RF generator delivered plasma energy at a distance of 3 mm from the skin through the gap, first to one side and then to the other side. The plasma energy applied from both sides of the folded tissue suctioned inside the tube created a local confined thermal effect. Energies of 2.5-3.5 J per pulse in a single pass were commonly used. The frequency of the pulses varied from 1 to 4 Hz, and the duration of the pulses was between 1 and 6 s. The treatment was ended when significant erythema and warmth radiating from the treated areas was observed. After treatment, the patients were instructed to avoid sun exposure and to apply a bland ointment to the face at least three times daily while the skin was healing.

Clinical analysis

Clinical photographs using a digital camera (EX-Z1050; Casio, Tokyo, Japan) were taken before treatment, 3 days after the first treatment, and 12 weeks after the final treatment. The patients reported their satisfaction with the treatment on a 5-point scale immediately after treatment and at their final assessment session (**Table 1**). The clinical photographs, presented in random order, were assessed for treatment efficacy compared with the baseline by two independent clinicians, who reached a consensus rating following discussion and who also rated the improvement on a 5-point scale. The patient satisfaction index was calculated as the sum of the sites that patients scored as a 4 or 5 expressed as a percentage of the total number of sites. The overall efficacy of treatment was calculated as the sum of the number of sites scored by the clinicians as 4 or 5 expressed as a percentage of the total number of sites.

Histological and immunohistochemical analysis

Full-thickness, 2-mm punch skin biopsy specimens from the inner thigh were obtained before treatment and 40 days after the last treatment from two of the patients. The tissue was stained with hematoxylin-eosin and Masson's trichrome

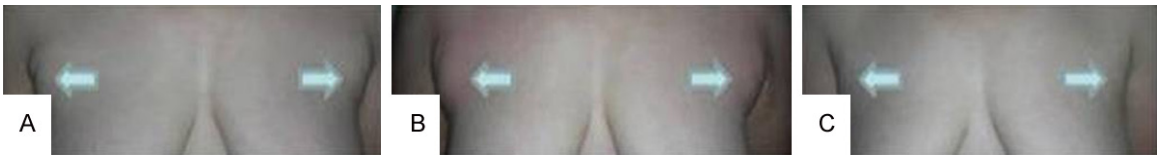

Figure 3. Healing progression in a 44-year-old woman from (A) before treatment, (B) 3 days after the first treatment, and (C) 12 weeks after the last treatment.

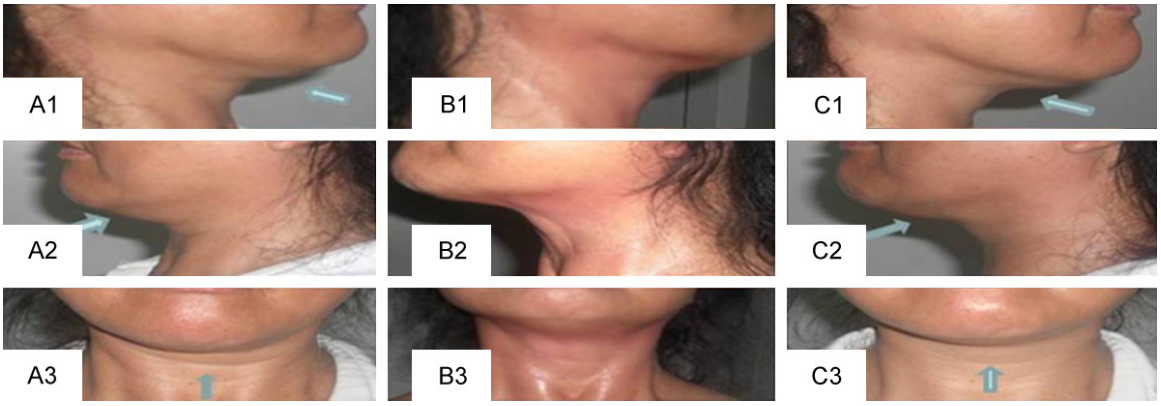

Figure 4. Healing progression in a 55-year-old woman from (A1-3) before treatment, (B1-3) 3 days after the first treatment, and (C1-3) 12 weeks after the last treatment.

to assess the overall tissue response and the presence of collagen in the dermis. The epidermal proliferating cell nuclear antigen (PCNA) content was assessed using an automated immunostainer.

Ex vivo experimental setup

Ex vivo human tissue samples were taken directly after amastectomy and tested within 10 min of excision to minimize any changes in the tissue caused by storage, temperature variation, or change in the extracellular fluid environment, including blood and lymphatic content. For testing, the tissue samples were suctioned into the vacuum tube device. Marks were placed on each side 1 cm from the middle fold line to visualize the tissue displacement. The experimental design setup is shown in **Figure 2**. Each experiment was performed in triplicate.

Results

In vivo clinical vacuum-assisted bipolar PSR

All of the enrolled patients completed the study and the full 12-week follow-up. The procedure was well tolerated with minimal discomfort after the use of the topical anesthesia and adjunctive oral analgesia. No serious unexpected adverse side effects occurred. As with all procedures using thermal energy, the observed side effects included pain, edema, erythema, inflammation, and slight induration. However, these effects were mostly mild and all were transitory, all lasting for less than 7 days (**Table 2**). Erythema and edema are common post-procedure side effects that usually resolve within several days. Pain was decreased through the application of ice after the procedure. There is a chance of slight induration at higher energies, which was treated with a liberal application of bland ointment.

Representative cases

The following three cases, two on the body and one on the submental area, were representatives of the treatment and its effects.

Case 1: A 44-year-old woman (Patient No. 1) with excess armpit fat was shown in Figure 3, before and 3 days after the first body contouring treatment with vacuum-assisted bipolar PSR. The excellent result was clearly visible 12 weeks after the procedure (**Figure 3**).

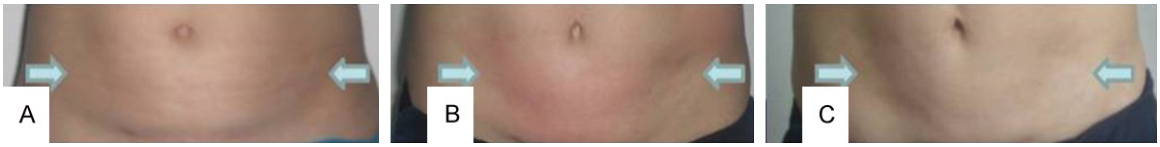

Figure 5. Healing progression in a 34-year-old woman from (A) before treatment, (B) 3 days after the first treatment, and (C) 12 weeks after the last treatment.

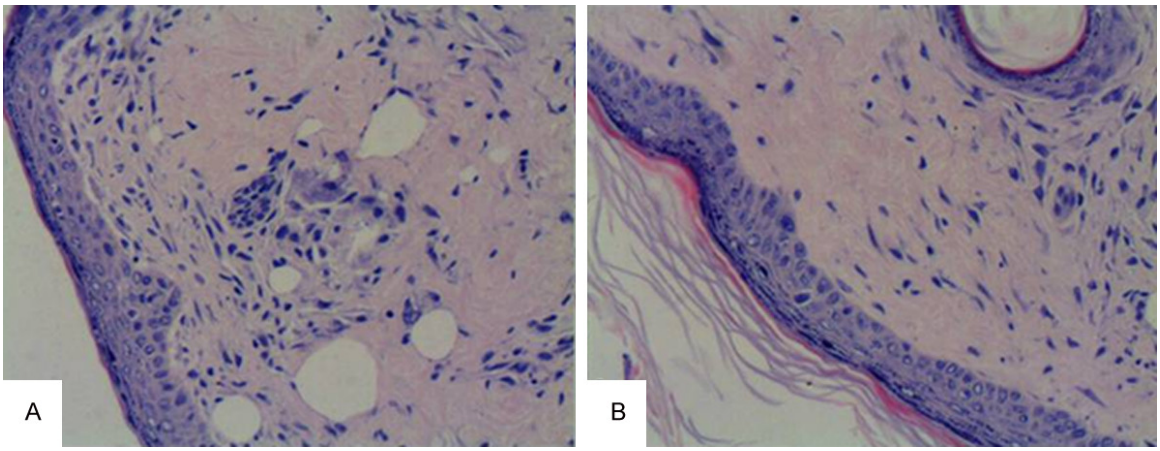

Figure 6. Epidermal and dermal structures shown by hematoxylin-eosin staining (×200) from (A) before treatment and (B) 40 days after the last treatment.

Case 2: The 55-year-old woman (Patient No. 11) required facial contouring on her lower cheeks and submental area. Comparing with the results of the pretreatment condition, 12 weeks after the last treatment (**Figure 4**) showed that the final results were subtle, but very good.

Case 3: A 34-year-old woman (Patient No. 2) wanted abdominal"mermaid line" contouring to pursue a performance career as a belly dancer. The baseline findings and the final results at the 12-week follow-up were shown in **Figure 5**.

Histologic features

The skin biopsies taken from the patients 40 days after the last treatment showed normal dermal architecture in the deep reticular dermis and no evidence of scar tissue or abnormal collagen fibers (**Figure 6**). The tissue specimens were stained with Masson's trichrome to identify the extracellular matrix components, the dermal collagen in particular. No significant changes in the spatial distribution of the collagen bundles were found between the control and untreated areas (**Figure 7**). PCNA was used asa marker of proliferation that reflects DNA repair activity in the tissue. The percentage of PCNA-positive epidermal cells in the treated zone ranged from 10 to 30% compared with 1-5% in the control area (**Figure 8**).

Ex vivo tissue contraction experiments

The amount of adipose tissue contraction is shown in **Figure 9**. The tissue contraction was not symmetrical, with an average displacement of 4 mm on one side and 3 mm on the other side. This asymmetrical behavior may be explained by the non-uniform structure of the connective tissue and asymmetrical geometry of the particular tissue sample. The average marker migration and tissue contraction for the three experiments with adipose tissue was 3.1 ± 0.4 mm.

Discussion

Non-invasive body contouring is a new frontier in aesthetic skin surgery. Initial demonstrations of methods for altering the three-dimensional aspects of the body have led physicians, scientists, and engineers to develop new techniques for changing the topography of the human body, especially as it relates to pockets of fat deposi-

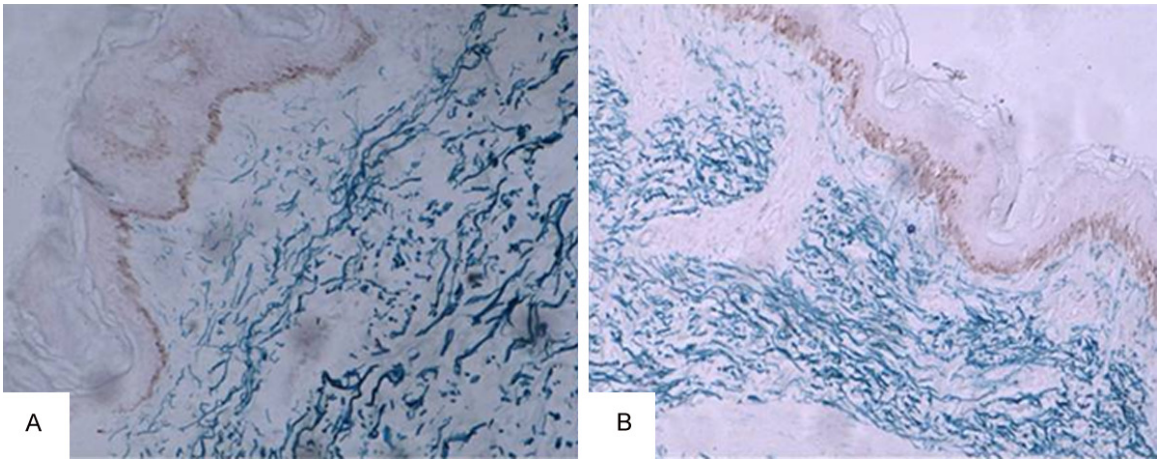

Figure 7. Collagen structures shown by Masson's trichrome staining (×200) from (A) before treatment and (B) 40 days after the last treatment.

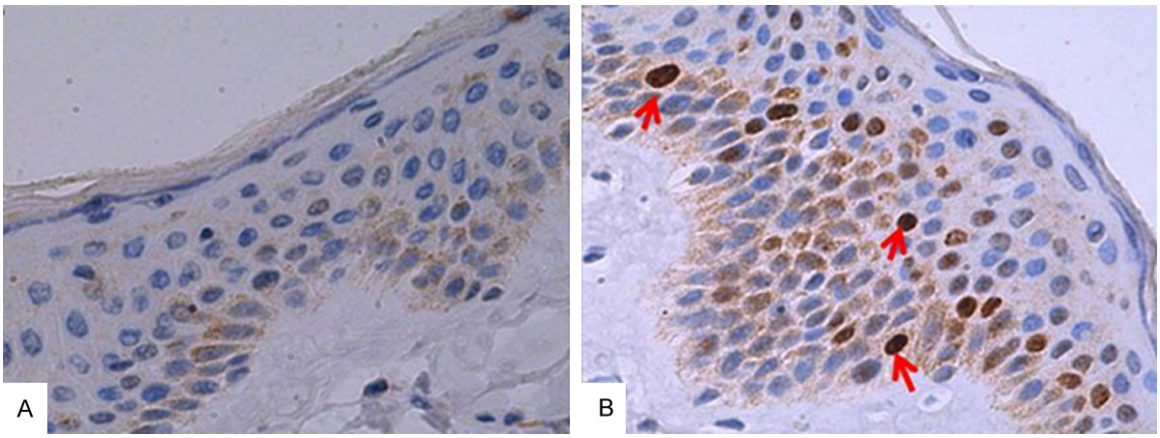

Figure 8. Epidermal proliferating cell nuclear antigen (PCNA) immunostaining (×200) from (A) before treatment and (B) 40 days after the last treatment.

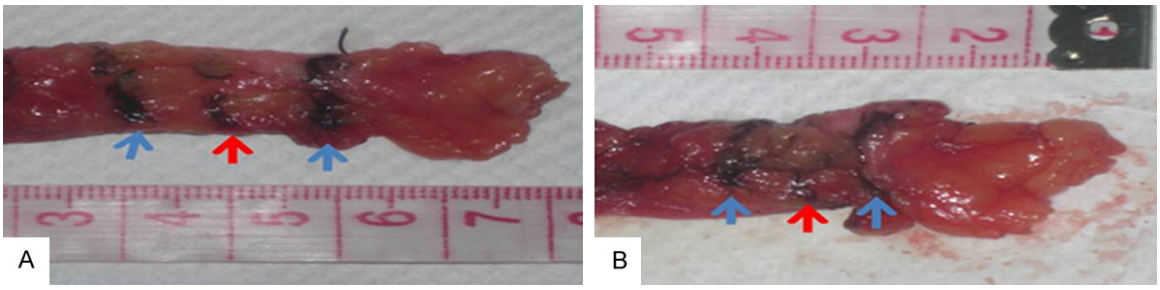

Figure 9. Adipose tissue contraction during PSR energy delivery.

tion, irregular contours or laxity of the skin, and circumferential reduction of the legs, arms, abdomen, and buttocks. A large number of patients who have multiple and diverse secondary body contour deformities after massive weight loss also desire body contouring [6].

PSR is one of the most innovative techniques proposed to induce remodeling of connective tissue. PSR is different than lasers, light sources, and ablative lasers in that it is not chromophore dependent and does not vaporize the tissue. Instead, it leaves a layer of intact desic-

cated epidermis that acts as a natural biologic dressing that promotes wound healing and rapid recovery [7].

Five PSR anti-aging treatment regimens have been reported: PSR 1 (low energy), PSR 2 (low and high energy), PSR 3 (high energy), and PSR 2/3 combinations selected according to the severity of the problem and the recovery time available, and the fifth treatment is the newly US Food and Drug Administration-approved anti-aging procedure for treating non-facial areas of the body. All of these protocols can be used for lines, but higher energy treatments are needed for skin tightening. The main characteristics of the higher energy PSR configuration are the high-power density and the deep power penetration [8]. This energy induces heat-dependent damage that removes the upper epidermis layers, inducing keratinocyte proliferation and differentiation with the consequent de novo synthesis of collagen that acts as a new tissue scaffold [4]. Unfortunately, the high thermal energies required to induce skin remodeling often cause several side effects and unavoidable discomfort for the patients. Thus, we developed a better approach to skin regeneration that combines vacuum with PSR to stimulate the regeneration of connective tissue through distinct pathways.

An *ex vivo* contraction response was observed in adipose tissue containing septal connective tissue and reticular collagen fibers encasing fat cells after vacuum PSR treatment. The immediate contraction of dermal collagen cannot be achieved without burning the skin, which happened when the epidermal temperature exceeded 45°C [9]. This observed fat tissue contraction indicated that PSR energy can be superimposed and the thermal energy is directly deposited into the adipose tissue and subdermal space, thus avoiding heating of the epidermal surfaces.

In this clinical case series, the efficacy of the vacuum-assisted bipolar PSR treatment in reducing the localized or irregular deposits of fat and the smoothing of irregular contours was demonstrated by direct visual and textural evaluations. Some investigators have found more than 90% improvement at 3 months after treatment, comparable to the 39% improvement seen at 6 months after one high-energy single-pass treatment in a study by Potter *et al*. [10]. Because the present study was performed using lower energy treatments, the longer follow-up (6-12 months) may not show further improvement because the diffuse application of intermediate magnitudes of energy over the fat in the core can induce thermally and mechanically mediated contraction of the fat lobules without damaging the adjacent skin [11]. Several participants reported more improvement at the 3-month follow-up than at the earlierswelling transition period. The discrepancy between the investigator and subject evaluations may have resulted from the participants focusing more on improvements in dyspigmentation rather than contour because they were asked to evaluate the overall improvement in rejuvenation.

No hypopigmentation or permanent post-inflammatory hyper pigmentation occurred after vacuum PSR. Patients should be prepared for the risk of some degree of post-inflammatory hyperpigmentation, but this effect has never been reported as permanent. PSR contouring does not physically remove or destroy fat cells. Instead, it reduces localized or irregular deposits of fat, smoothes irregular contours, and rebalances proportions via redistribution. The most commonly treated areas include the abdomen, upper arms, inner thighs, and submental area.

The term tissue contraction may be more accurate for this treatment because the significant area contraction is a result of the strong contribution from the deeper adipose layers. In the present study, we demonstrated that a combination of vacuum with plasma energy provides a potentially effective method to achieve significant treatment outcomes with relatively low plasma energies, and consequently, no side effects.

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Disclosure of conflict of interest

None.

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