ORIGINAL ARTICLE

Prospective Multicenter Clinical Trial of a Minimally Invasive Temperature-Controlled Bipolar Fractional Radiofrequency System for Rhytid and Laxity Treatment

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BACKGROUND A minimally invasive fractional bipolar radiofrequency (FRF) was developed.

OBJECTIVE To evaluate safety and efficacy of FRF in reducing face and neck rhytides and laxity.

MATERIALS AND METHODS This prospective, open-label, multicenter clinical trial enrolled 100 subjects with mild to severe facial and neck rhytides and laxity at seven centers in a per-protocol analysis. One single-pass FRF treatment was administered through five 32 g-needle electrode pairs at a preselected real-time fixed temperature of 62 to 78°C, energy duration for 3 to 5 seconds, and impedance restrictions of 200 to 3,000 Ohms, ensuring intradermal delivery. Five blinded dermatologists and plastic surgeons graded randomized standardized baseline and follow-up photographs of 53 and 42 subjects at 3- and 6-month follow-up intervals, respectively, using the Fitzpatrick wrinkle and Alexiades-Armenakas laxity scales. Subject assessments and adverse events were recorded in 100 subjects.

RESULTS Blinded evaluations revealed correct pre- and post-treatment identification in 100% of scored cases, mean improvement of 25.6% on the Fitzpatrick Wrinkle Scale and 24.1% on the Alexiades-Armenakas laxity scale at 6 months, and 100% response rate for rhytides and 95% for laxity. Subgroup analysis revealed maximal rhytid reduction in the mean target temperature of 66.7, energy duration of 4.2 seconds, and volume of denatured collagen of mm^3 denatured collagen group. Adverse events included transient erythema, edema, and ecchymoses, resolving within 1 to 5 days, and two incidents of temporary pinpoint depressions. More than 90% of subjects were satisfied or very satisfied.

CONCLUSION Real-time temperature-controlled FRF is a highly reproducible, safe, effective nonsurgical treatment of face and neck rhytides and laxity and provides important insights into neocollagenesis, neoelastogenesis, and clinical outcomes.

The authors have indicated no significant interest with commercial supporters.

The demand for minimally invasive procedures to reverse the signs of aging has inspired the development of modalities with greater efficacy than preceding generations. Until now, the criterion standard for treating skin laxity on the face and neck was the surgical facelift, but it has the disadvantages of significant perioperative risks, prolonged wound healing, and complications such

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as scarring and poor aesthetic outcome. In contrast, noninvasive laser resurfacing technologies have yielded only modest improvements in skin laxity and required multiple treatment sessions.[1-3]

To improve the efficacy and predictability of nonsurgical treatments, a minimally invasive bipolar radiofrequency (RF) system was developed with real-time tissue temperature feedback that delivers fractional RF (FRF) thermal pulses to the reticular dermis.[4,5] This system safely created thermal injury zones in a fractional manner within the reticular dermis without damage to the dermal–epidermal junction, epidermis, or subcutis.[4] Adnexal structures such as sweat and sebaceous glands, as well as hair follicles, were spared during FRF treatment. In a subsequent study, it was shown that reticular dermal volume, cellularity, and hyaluronic acid and elastin content had increased significantly 10 weeks after FRF treatment.[6] The collagen chaperone HSP47 triggered the dermal remodeling process in part, which led to complete replacement of fractional thermal injury zones with new dermal tissue by 10 weeks after treatment.[6,7] Definitive evidence of neocollagenesis and neoelastogenesis suggested that the replacement tissue closely resembled normal dermis and addressed dermal volume and elasticity.

Although skin laxity reduction had been difficult to achieve without surgical procedures, it was recently demonstrated in a randomized, blinded, controlled trial that FRF resulted in 37% the skin laxity reduction to the face and neck relative to the surgical facelift.[5] The histologic evidence of neoelastogenesis was clinically correlated with less skin laxity and greater elasticity.[5,8] A decrease in the Young modulus of 5 to 12% and in snap back time of 10 to 16%, features characteristic of younger skin, were observed 3 months after treatment.[8] The aim of the present clinical trial was to prospectively assess the safety and efficacy of the bipolar FRF system for treatment of facial and neck rhytides and laxity using standard, well-accepted grading scales in a large, multicenter clinical trial with blinded evaluations.

Materials and Methods

Subject Selection

The Western institutional review board approved the study protocol. One hundred consecutive subjects were enrolled at seven centers across the United States. Eleven board-certified dermatologists and plastic surgeons performed treatments. The inclusion criteria were healthy individuals aged 19 and older, presence of mild to severe facial rhytides or laxity, and ability to attend scheduled follow-up visits. The exclusion criteria were history of implanted device; silicone, fat, collagen, or synthetic material in the treatment area; cosmetic surgery in the treatment area in the last 3 years; RF in the last year; lasers or intense pulsed light in the last 6 months; isotretinoin use in the last year; history of active smoking in the past 10 years; bleeding disorder; collagen vascular disease; anaphylaxis; active, chronic, or recurrent infection; compromised immune system (e.g., diabetes); lidocaine hypersensitivity; hypertrophic scarring; keloid formation; women currently pregnant or breastfeeding; and systemic anticoagulants, thrombolytics, chemotherapy, corticosteroids, or anabolic steroids (standard inhaled or nasal corticosteroids were acceptable).

FRF Treatment

Treatments were conducted using a minimally invasive bipolar FRF system (ePrime; Syneron, Yokneam Illit, Israel). ePrime delivers bipolar FRF pulses using five microneedle electrode pairs deployed into the dermis at an angle of 20 to 25° with the exposed portion extending from 1 to 2 mm beneath the skin surface. The proximal half of each 6-mm microneedle is electrically insulated to restrict the applied energy to the reticular dermis. During treatment, epidermal temperature is maintained at 10°C using a cooling device to protect the papillary dermis and epidermis. Temperature sensors embedded within the microneedles and a

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real-time temperature feedback algorithm were used to reach and maintain the target temperature across a wide range of clinically relevant dermal impedances.[4,7]

After informed oral and written consent, standardized preoperative digital photographs were taken. The skin was cleansed and anesthetized as previously described.[4–8] Local anesthetic and regional blocks were previously shown to be effective at eliminating discomfort without affecting the volume of thermal injuries.[4,7] The treatment area, marked using a sterile marking pen, extended from the nasolabial and melolabial folds medially to the preauricular region laterally to the periocular zones superiorly to the inferior margins of the submental and submandibular regions inferiorly (Figure 1). All subjects received one single-pass treatment. After each pulse, the microneedle array and cooling device were withdrawn, repositioned, and activated on the next insertion; this was repeated until the target zone was fully treated. The distance between two adjacent treatments was 4 to 8 mm for mild to moderate and 3 to 4 mm for severe laxity or rhytides. The number of insertions averaged 164 ± 52 (range 50–300).

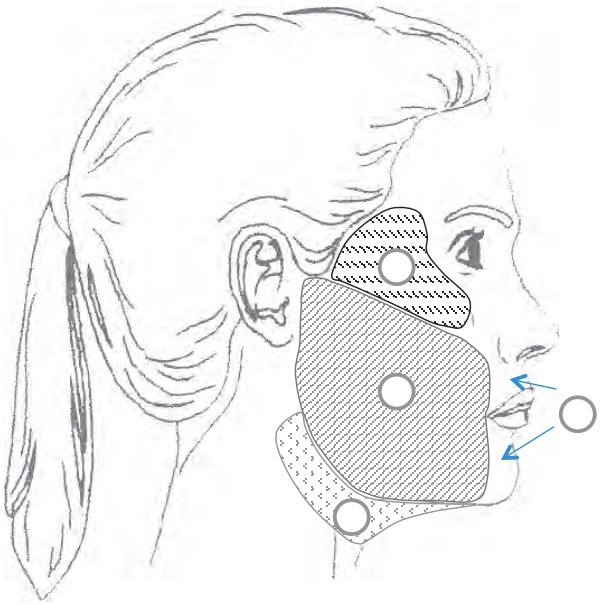

Figure 1. Treatment zone distribution. All subjects received fractional bipolar radiofrequency applications in the lower lateral facial side (zone 1). The periorbital region (zone 2) was treated in 31% of subjects, the perioral and mental region (zone 3) in 9%, and the submental and submandibular region (zone 4) in 25%.

Assessment of Safety and Efficacy

Subjects were followed up 1 day and 1 and 3 months after treatment; 6-month follow-up was conducted at a subset of clinical sites. Digital photographs were taken and subjects assessed for complications. Random sets (baseline, follow-up) of en face photographs per scored subject were used for blinded grading by two board-certified dermatologists and one board-certified facial plastic surgeon using the Fitzpatrick wrinkle scale[9] and Alexiades-Armenakas laxity scale.[1,10] The frequency with which evaluators could identify the before-and-after photos was also tabulated. At 3-month follow-up, subjects rated improvement of rhytides, laxity, global appearance (no response, worse, none, mild, moderate, significant, dramatic), and overall satisfaction (no response, very dissatisfied, dissatisfied, neutral, satisfied, very satisfied). At 6-month follow-up, subjects rated the 3-month interval improvement (no response, worse, none, some, considerable), global appearance, and overall satisfaction using the same scales.

Statistical Analysis

A per-protocol analysis was used, calling for discontinuation of further blinded physician grading upon receipt of 510 k clearance for wrinkle treatment. Safety data and self-assessment surveys were collected on all 100 subjects who attended the scheduled and voluntary follow-up visits. Means and standard deviations (SD) were calculated for 3- and 6-month results using each cohort's matched baseline cohort. Data were considered significant at $p < .05$. The 99% confidence interval (CI) for the grading results was between 1.1 and 1.9 grade improvements for rhytides and between 0.5 and 0.7 for laxity at 3 months. Cohen's d-statistic was calculated to ensure that the study was sufficiently populated.

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Results

Demographic Characteristics and Treatment Parameters

The study methodology was selected in accordance with strict Food and Drug Administration (FDA) guidance. During the study period, data were analyzed and presented at required FDA meetings to evaluate the device's safety and efficacy. When grading was completed on 53 and 42 female subjects for the mandatory 3-month and voluntary 6-month post-treatment follow-ups, respectively, the FDA found the data to be statistically significant and adequate, and further blinded physician evaluation was discontinued. Using a per-protocol analysis approach, safety and efficacy data presented below for the 3- and 6-month post-treatment cohorts relied on matched baseline cohorts.

Mean age was 54.5 ± 7.9 (range 38–75) for the 3-month cohort and 55.3 ± 6.9 (range 44–70) for the 6-month cohort. All subjects enrolled were Fitzpatrick skin type I to IV, with the majority being II or III. No statistically significant variation in mean body weight was observed between baseline (3-month cohort, 137 ± 21 lb; 6-month cohort, 137 ± 23 lb) and 3 (136 ± 22 lb) or 6 months (133 ± 18 lb) after treatment. Mean lesion target temperature was 67.7 ± 4.6°C for the 3-month cohort and 67.4 ± 4.6°C, for the 6-month cohort (Table 1). Mean duration was 4.5 ± 0.9 seconds and 4.4 ± 0.9 seconds, respectively, and mean insertion number was 158 ± 49 (range 52–257) and 156 ± 52 (range 52–257), respectively.

Blinded Physician Evaluator Outcomes

In the pre- and post-treatment identification analysis, evaluators were asked to identify the pre vs. post photo for each subject in randomized, not paired photographs. They made a selection in 86% of the cases presented, and abstained in making a selection in 14%. Blinded physician evaluators correctly selected the baseline and post-treatment photographs in 100% of cases in which a selection was made. Typical results obtained for zone 1 treatment with low settings (e.g., 62–64°C, 3–4 seconds) are presented in Figure 2.

For the 3-month cohort, mean baseline rhytid score was 6.1 ± 1.7 (Table 2). For this cohort, 96% of subjects showed improvement in rhytides, with a mean reduction of 1.5 grades (Table 2), corresponding to 24.0% mean improvement after a single treatment. Six months after treatment, 100% of subjects showed improvement in rhytides, with a mean reduction of 1.6 grades, corresponding to 25.6% mean improvement after a single treatment (Table 2).

The mean baseline laxity score for the 3-month cohort was 2.8 ± 0.6, with 94% of this cohort's subjects showing improvement in skin laxity (Table 2). The mean reduction in laxity score was 0.6 grades, which corresponded to 22.3% mean improvement after a single treatment. For the 6-month cohort, 95% of subjects showed improvement in skin laxity, with a mean reduction of 0.7 grades, corresponding to 24.1% mean improvement after a single treatment. Confidence

TABLE 1. Summary of Treatment Parameters

	3-Month Cohort	*6- Month Cohort*
Treatment Parameter	*Mean ± SD (Range)*	
Target temperature, °C	67.7 ± 4.6 (62–78)	67.4 ± 4.6 (62–78)
Treatment duration seconds	4.5 ± 0.9 (3–5)	4.4 ± 0.9 (3–5)
Number of insertions*	158 ± 49 (52–257)	156 ± 52 (52–257)
Thermal injury zones	790 ± 245 (260–1,285)	780 ± 260 (260–1,285)

*Number of five microneedle pairs. Each needle pair results in one fractional thermal injury zone.

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Figure 2. Rhytid and laxity improvement 3 months after fractional bipolar radiofrequency treatment. Before (A, C, E) and after (B, D, F) treatment to zone 1 using 196 insertions at 62°C for 3 seconds, 189 insertions at 64°C for 5 seconds, or 226 insertions at 64°C for 3 seconds with corresponding volumes of thermal injury of 0.75, 1.5, and 1 mm^3, respectively.[3]

TABLE 2. Rhytid and Laxity Grading Results

	Baseline	*3 Months After Treatment*	*6 Months After Treatment*
Rhytides			
Score, mean ± SD	6.1 ± 1.7		
Grade improvement, mean (%)		1.5 (24.0)	1.6 (25.6)
99.99% confidence interval		1.1–1.9	1.1–2.0
Subjects showing improvement, %		96	100
Laxity			
Score, mean ± SD	2.8 ± 0.6		
Grade improvement, mean (%)		0.6 (22.3)	0.7 (24.1)
99.99% confidence interval		(0.5–0.7)	(0.5–0.8)
Subjects showing improvement, %		94	95

SD, standard deviation.

interval testing showed significance at 99.99% and the Cohen d-stat test indicated that our study was adequately powered for all sets of grades at both time points (Table 2).

Subgroup analysis revealed that greater (30%) improvement (2.1-grade reduction) was observed when the baseline rhytid score was 7 (Figure 3A). Subjects with a baseline rhytid score of 3 to 7

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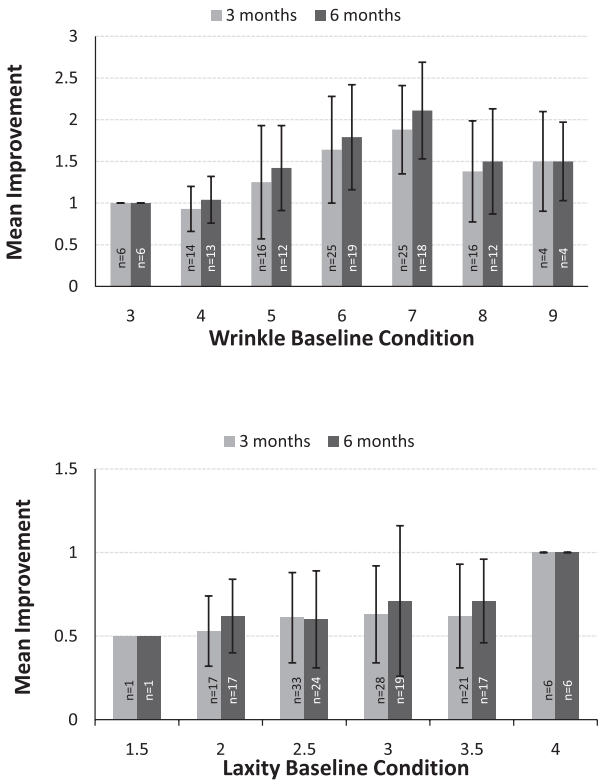

Figure 3. Subgroup analysis of rhytid and laxity scores 3 and 6 months after FRF treatment. Means ± SD (ranges) are shown.

achieved mean improvement of 26 to 30% at 6 months after treatment (Figure 3A). Mean improvement decreased to 19% and 17% for subjects with baseline scores of 8 and 9, respectively. For laxity, maximum improvement (33%) was observed at 6-month follow-up when baseline scores were less severe (1.5, Figure 3B). Maximal grade improvement (1.0) was observed in subjects with the most-severe baseline scores (4, Figure 3B).

Subgroup analysis of subjects with greater (>20%) improvement in rhytid scores was conducted with regard to treatment parameters employed (Table 3). Subjects with less (<20%) improvement were treated at a mean target temperature of 69.5°, for a treatment duration of 4.9 seconds and a calculated volume of fully denatured collagen of 3.02 mm^3. In contrast, those with greater (>20%) improvement were treated at a mean target temperature of 66.7°, duration of 4.2 seconds, and calculated volume of fully denatured collagen of 2.00 mm^3 (Table 4). *p*-values were .08 for temperature, .03 for duration, and .05 for volume, indicating that higher temperature, longer treatment duration and greater volume of fully denatured collagen were correlated with less improvement in rhytides. Differences in the total number of needle insertions ($p = .88$), baseline rhytid conditions ($p = .42$), and age ($p = .77$) between the two subgroup were not statistically significant.

Subject Self-Assessment and Satisfaction Outcomes

Table 4 shows that most subjects reported moderate to significant improvement in rhytides (79%), laxity (77%), and global appearance (69%) 3 months after treatment. Dramatic improvement was reported in an additional 2 to 6% of subjects across all indicators. Three months after treatment, 51% of subjects reported being very satisfied, with an additional 36% being satisfied (Table 5). The percentage of subjects who were satisfied or very satisfied increased from 87% 3 months after treatment to 91% 6 months after treatment. By 6 months after treatment, 86% of subjects reported continued improvement in their overall appearance, and 14% reported no additional improvement. No subjects reported an interval worsening of results. In addition, 90% of subjects reported an overall

TABLE 3. Statistical Analysis: Console Settings and Corresponding Volumes

Improvement from Baseline	*Target Temperature, °C*	*Duration, Seconds*	*Volume, mm^3*
≤20%, mean ± SD	69.5 ± 4.5	4.9 ± 0.3	3.02 ± 1.36
>20%, mean ± SD	66.7 ± 4.5	4.2 ± 1.0	2.00 ± 1.49
p-value	.08	.03	.05

SD, standard deviation.

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TABLE 4. Subject Self-Assessment Scores 3 Months After Treatment (n = 53)

Self-Assessment Scale	*Rhytides*	*Laxity*	*Global*
	n *(%)*		
No response	1 (2)	1 (2)	2 (4)
Worse	0 (0)	0 (0)	0 (0)
None	1 (2)	1 (2)	2 (4)
Mild	8 (15)	7 (13)	9 (17)
Moderate	27 (51)	17 (32)	15 (28)
Significant	15 (28)	24 (45)	22 (41)
Dramatic	1 (2)	3 (6)	3 (6)

TABLE 5. Subject Satisfaction Scores

Satisfaction Score	*3 Months After Treatment,* n = *53*	*6 Months After Treatment,* n = *42*
	n *(%)*	
Very dissatisfied	0 (0)	0 (0)
Dissatisfied	0 (0)	0 (0)
Neutral	7 (13)	4 (9)
Satisfied	19 (36)	1 (43)
Very satisfied	27 (51)	20 (48)

impression of benefits of moderate or better, with 59% falling in the significant or dramatic category.

Adverse Events

All subjects returned to normal activities within 24 hours. Minimal to moderate erythema was observed in 100% of subjects and resolved in 2 to 12 hours (Figure 4). Sixty-five percent reported generalized edema, which resolved within 1 to 5 days. Focal, palpable, but not visible edema at insertion sites was present in 100% of subjects and resolved in 2 to 4 weeks; subjects who applied cold compresses immediately after the procedure had no to minimal edema. Sixty-five percent of subjects reported mild postoperative discomfort that resolved within 24 hours and rarely required acetaminophen. When topical anesthetic was used alone, focal isolated ecchymosis was observed in 92% of subjects, could be covered with makeup, and resolved within a maximum of 7 to 10 days; infiltration with lidocaine and dilute epinephrine greatly reduced its incidence and severity. When present, 52% of subjects rated bruising as mild, 27% as moderate, and 21% as severe. Two subjects experienced a total of seven erosions that healed leaving punctuate depressions measuring 1 mm in diameter and 0.5 mm deep (Figure 5). Nineteen subjects received additional treatment periorbitally without any adverse reactions or complications. All subjects were advised to avoid makeup for 24 hours, reported resuming normal activities after 24 hours, and were able to mask focal ecchymosis with makeup during the postoperative period.

Figure 4. Clinical findings immediately after fractional bipolar radiofrequency treatment.

Figure 5. Superficial pinpoint punctate depressions secondary to incomplete electrode insertion. Two instances of this complication were observed in the 100 patient study and resolved completely during follow-up.

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Discussion

Recently, successful generation of fractional thermal injuries directly within the reticular dermis using a minimally invasive temperature-controlled bipolar RF investigational device was reported.[4] Based on this pilot study, we further refined the treatment parameters and examined the safety and efficacy of the FRF system on facial and neck rhytides and laxity in a prospective multicenter clinical trial presented here.[11] Our study demonstrates that a single FRF treatment results in consistent significant improvements in rhytides and laxity as assessed by randomized, blinded evaluation using quantitative grading. The 100% and 95% response rates for rhytides and laxity (Table 2), respectively, 6 months after treatment indicates that a single FRF treatment generates reproducible efficacy in all patients. An undemanding recovery profile with resumption of normal activities at 24 hours, no wound care, 1 to 5 days of down-time, and a promising safety profile with only two instances of punctate atrophy that resolved during the follow-up period accompanied this consistent efficacy for a nonsurgical modality (Figure 5).

Our clinical data indicate that substantial improvement is achieved by 3 months after treatment, with continued improvement to at least 6 months (Table 1). This clinical improvement is consistent with the timeline for increases in collagen, elastin, and hyaluronic acid and the corresponding volumization, elasticity, and skin laxity improvements previously reported after FRF treatment.[4,6–8,12] A unique series of molecular events are induced that differ from those with ablative laser resurfacing and underlie these changes.[6] FRF does not cause significant collagen and elastin degradation, as evidenced by relatively mild increases in metalloproteinase (MMP) 1 and 3 expression—important catabolic enzymes responsible for collagen degradation.[6,13] Rather, it causes grater expression of the anabolic (remodeling) enzymes MMP-13 and MMP-9 at 4 weeks after treatment,[13,14] suggesting that the continued clinical improvement observed here through at least 6-month follow-up is, at least in part, due to sustained MMP-13 activity because this enzyme plays an important role in long-term dermal remodeling. Thus, the current clinical data combined with our previous molecular findings support the hypothesis that FRF treatment promotes a primarily anabolic wound healing response throughout the inflammatory and proliferative phases and unique elevations in collagen, elastin, and hyaluronic acid that correlate clinically with volumization and rhytid and laxity reduction over 6 months.

The treatment parameters tested here, including temperature, treatment duration, and volume of denatured collagen, provide important insights into optimizing clinical outcome. To achieve dermal volume of injuries in the range of a few cubic millimeters, a few seconds of RF application is needed to allow the passive thermal conduction to increase the lesion size after target temperature is reached. As shown in Table 3, higher temperatures (mean 69.5°C), longer treatment duration (mean 4.9 seconds), and larger volume of fully denatured collagen (mean 3 mm^3) per insertion correlated with less (<20%) improvement in rhytides. In contrast, lower temperatures (mean 66.7°C), shorter treatment duration (4.2 seconds), and smaller volume of fully denatured collagen (mean 2 mm^3) correlated with greater (>20%) improvement in rhytides that was statistically significant. Based on the results, the current recommended setting is 67°C and 4 seconds, with insertion spacing of 3 to 4 mm between adjacent applications. The percentage of volumetric coverage for the recommended setting is 7.4%, assuming 4-mm spacing between adjacent insertions and 2-mm dermis thickness (Table 6). The current findings that lower temperatures for a shorter time and less volume of fully denatured collagen is more effective at reducing rhytides is highly instructive to the laser resurfacing field.

These findings support the authors' hypothesis that partially denatured collagen is more effective at inducing neocollagenesis and consequently rhytid

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TABLE 6. Volume Coverage Percentage for Different Settings

Settings	*Volume Coverage, %**
62°C, 3 seconds (low end of clinical study)	2.6
67°C, 4 seconds (preferred setting)	7.4
78°C, 5 seconds (high end of clinical study)	19.1

*Assuming 4-mm spacing and 2-mm dermal thickness.

reduction than fully denatured collagen. Two potential explanations for such a theory are presented here. The first relates to the heat-induced changes in collagen that signal induction of neocollagenesis. Heat-mediated partially denatured collagen appears to be more effective at inducing wound healing than fully denatured collagen. Partial heat denaturation of type I collagen results in dissociation of its triple helical structure, leading to exposure of internal arginine–glycine–aspartate (RGD) cell adhesion sequences that are inaccessible in native type I collagen conformation.[15,16] RGD-dependent integrin interactions play a significant role in regulating MMP function, releasing extracellular matrix fragments and exposing cryptic epitopes that affect fibroblast phenotype and wound healing.[20,21] Partially denatured collagen helps wounds heal 38% faster than normal skin equivalents in vitro.[22] Partially denatured collagen induces wound healing through the binding of $\alpha v\beta 3$ integrin binding to RGD sequences that are exposed upon denaturation and unwinding of the triple helix. Mitogen-activated protein kinase and subsequent intracellular pathways correlate these events to matrix remodeling and stem cell differentiation.[20] As shown previously,[6] heat-denatured type I collagen does not induce collagenase expression, in contrast to native-type I collagen, which also binds keratinocytes during wound healing through integrins $\alpha 1\beta 1$, $\alpha v\delta$, and $\alpha 2\beta 1$.[21] Partially heat-denatured collagen is thus more effective at inducing a dermal wound healing response, which is distinct from the processes that exposure of native type I collagen required for wound re-epithelialization induces, as is the case after standard ablative resurfacing. Thus, FRF-mediated heat injury resulting in partially denatured type I collagen may induce, through exposure of intact RGB sequences, greater dermal wound healing and consequently greater rhytid-reduction than fully denatured collagen, as our data support.

Another potential explanation for the current findings that larger volumes of fully denatured collagen (3 mm^3) are less effective at rhytid reduction than smaller volumes (2 mm^3) may be the role of hypoxia central to the thermal lesions. Hypoxia centrally to relatively larger thermally induced fully denatured lesions of dermal tissue may impair the wound healing response, further explaining the current findings. Oxygen is a prerequisite for successful wound healing because of the increased demand for reparative processes such as cell proliferation, bacterial defense, angiogenesis, and collagen synthesis. Many experimental and clinical observations have shown wound healing to be impaired with hypoxia.[22] Prolyl 4-hydroxylases (P4Hs) have central roles in the synthesis of collagens and the regulation of oxygen homeostasis. All P4Hs require Fe_2+, 2-oxoglutarate, oxygen, and ascorbate.[23] Thus, if a thermally induced dermal lesion exceeds a certain threshold of size, such that hypoxia occurs centrally to the lesion, this would be counterproductive to the induction of wound healing and correlate with less clinical efficacy of rhytid reduction.

Real-time temperature feedback built into the bipolar needle RF system was selected to optimize the reproducibility of thermal injuries in the deep dermis and may explain the 100% response rate in rhytid reduction across the study at 3- and 6-month follow-up. The low sensitivity of the thermal injury volumes and dermal tissue temperature to clinically relevant skin impedances reported earlier[7] allows for infiltration of electrically conductive anesthetic in the dermal space to manage pain and bruising yet to create consistent thermal injuries without being limited by pain tolerance or other factors.

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Perioperative discomfort was fully controlled through a combination of topical anesthetic (lidocaine/tetracaine) or nerve block, followed by local infiltration of 0.25% lidocaine with 1:400,000 epinephrine in the deep dermis. The real-time temperature feedback system unique to this device allows one to precisely reach and maintain precise dermal temperature, such as 67°C to optimize collagenesis and elastogenesis, and to minimize hypoxia as discussed above.

The precise intradermal real-time temperature control may also explain the minimal side effects in the 100-patient trial. Subjects experienced transient erythema lasting approximately 2 days; focal, localized bruising resolving within 7 to 10 days; and visible swelling resolving within 1 to 5 days. The microneedle punctures cause erythema directly, whereas bruising and swelling are related to the anesthetic technique and are minimized through the addition of dilute epinephrine and postoperative cold compresses. The most significant safety concern associated with this procedure is the avoidance of superficial injuries. The proximal halves of the microneedles inserted into the skin are insulated to protect the epidermal and superficial dermis from heating. It is mandatory to visually confirm proper needle insertion in skin before applying RF energy, because superficial insertion was accountable for the two incidences of punctate atrophy, which resolved.

The patient surveys revealed notable laxity improvements and global improvements in skin quality. Subject self-assessment scores at 3-month follow-up indicated greater improvement for laxity than rhytides, with 51% reporting significant to dramatic improvement in laxity, versus 30% for rhytides (Table 4). A serendipitous finding was that 47% of subjects reported significant to dramatic improvement in global appearance, suggesting that, although the thermal injury was localized to the reticular dermis, the wound healing process spread to involve the overlying dermis and epidermis. This spread may occur through passive diffusion gradients of important signaling molecules involved in thermal-induced wound healing. This is consistent with our prior findings of collagen chaperone HSP47 expression extending beyond the peri-injury zone by day 28 and through 10 weeks after treatment.[6] In addition, it has previously been postulated that the presence of healthy zones of spared tissue during fractional treatments amplifies signal transduction, allowing cross-talk between the dermis and epidermis during wound healing.[6,24] Finally, the epidermal and papillary dermal injury induced by the microneedle insertion process may in part explain the global skin quality results, because similar textural changes have been observed after microneedling.[14,24]

Conclusion

The results of this multicenter clinical trial suggest that minimally invasive RF with real-time temperature feedback treatment may provide predictably consistent clinical results and become a valuable nonsurgical option for the treatment of facial skin laxity and rhytides. The real-time feedback data presented here have refined our understanding of the ideal parameters required for induction of neocollagenesis and neoelastogenesis and optimal clinical outcomes.

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References

1. Alexiades-Armenakas M. Rhytides. Laxity and photoaging treated with a combination of radiofrequency, diode laser, and pulsed light and assessed with a comprehensive grading scale. J Drugs Dermatol 2006;5:609–16.
2. Alexiades-Armenakas M. Assessment of the mobile delivery of infrared light (1100–1800 nm) for the treatment of facial and neck skin laxity. J Drugs Dermatol 2009;8:221–6.
3. Alexiades-Armenakas M, Dover JS, Arndt KA. Unipolar versus bipolar radiofrequency treatment of rhytides and laxity using a mobile painless delivery method. Lasers Surg Med 2008;40:446–53.
4. Hantash BM, Ubeid AA, Chang H, Kafi R, et al. Bipolar fractional radiofrequency treatment induces neoelastogenesis and neocollagenesis. Lasers Surg Med 2009;41:1–9.
5. Alexiades M, Rosenberg D, Renton B, Dover J, et al. Blinded randomized, quantitative grading comparison of minimally Invasive, fractional radiofrequency and surgical face-lift to treat skin laxity. Arch Dermatol 2010;146(4):396–405.
6. Hantash BM, Renton B, Berkowitz RL, Stridde BC, et al. Pilot clinical study of a novel minimally invasive bipolar microneedle radiofrequency device. Lasers Surg Med 2009;41:87–95.
7. Berube D, Renton B, Hantash BM. A predictive model of minimally invasive bipolar fractional radiofrequency skin treatment. Lasers Surg Med 2009;41:473–8.
8. Willey A, Kilmer S, Newman J, Renton B, et al. Elastometry and clinical results after bipolar radiofrequency treatment of skin. Dermatol Surg 2010;36:877–84.
9. Fitzpatrick RE, Goldman MP, Satur NM, Tope WD, et al. Pulsed carbon dioxide laser resurfacing of photo-aged facial skin. Arch Dermatol 1996;132:395–402.
10. Alexiades-Armenakas M. A quantitative and comprehensive grading scale for rhytides, laxity, and photoaging. J Drugs Dermatol 2006;5:808–9.
11. Alexiades-Armenakas M, Willey A, Kilmer S, et al. Prospective open-label multicenter clinical trial evaluating the efficacy of fractional radiofrequency for the treatment of facial rhytids and laxity. Amer Soc Las Surg Med 2010;?????:????.
12. Alexiades-Armenakas MR, Dover JS, Arndt KA. The spectrum of laser skin resurfacing: nonablative, fractional, and ablative laser resurfacing. J Am Acad Dermatol 2008;58:719–37.
13. Orringer JS, Kang S, Johnson TM, et al. Connective tissue remodeling induced by carbon dioxide laser resurfacing of photodamaged human skin. Arch Dermatol 2004;140:1326–32.
14. Park KY, Kim HK, Kim SE, Kim BJ, et al. Treatment of striae distensae using needling therapy: a pilot study. Dermatol Surg 2012 38:1823–8.
15. Har-el R, Tanzer ML. Extracellular matrix. 3: evolution of the extracellular matrix in invertebrates. FASEB J 1993;7:1115–23.
16. Yamamoto M, Yamato M, Aoyagi M, Yamamoto K, et al. Identification of integrins involved in cell adhesion to native and denatured type I collagens and the phenotypic transition of rabbit arterial smooth muscle cells. Exp Cell Res 1995;219:249–56.
17. Heino J. The collagen receptor integrins have distinct ligand recognition and signaling functions. Matrix Biol 2000;19: 319–23.
18. Mauney JR, Kirker-Head C, Abrahamson L, Gronowicz G, et al. Matrix-mediated retention of in vitro osteogenic differentiation potential and in vivo bone-forming capacity by human adult bone marrow-derived mesenchymal stem cells during ex vivo expansion. J Biomed Mater Res 2006;79:464–75.
19. Egles C, Shamis Y, Mauney JR, Volloch V, et al. Denatured collagen modulates the phenotype of normal and wounded human skin equivalents. J Invest Dermatol 2008;128(7):1830–7.
20. Abraham LC, Dice JF, Lee K, Kaplan DL, et al. Phagocytosis and remodeling of collagen matrices. Exp Cell Res 2007;313 (5):1045–55.
21. Sudbeck BD, Park WC, Welgus HG, Pentland AP, et al. Collagen-stimulated induction of keratinocyte collagenase is mediated via tyrosine kinase and protein kinase C activities. J Biol Chem 1994;269(47):30022–9.
22. Schreml S, Szeimies RM, Prantl L, Karrer S, et al. Oxygen in acute and chronic wound healing. Br J Dermatol 2010;163(2): 257–68.
23. Myllyharju J. Prolyl 4-hydroxylases, key enzymes in the synthesis of collagens and regulation of the response to hypoxia, and their roles as treatment targets. Ann Med 2008;40(6): 402–17.
24. Hantash BM, Mahmood MB. Fractional photothermolysis: a novel aesthetic laser surgery modality. Dermatol Surg 2007;33:525–34.

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