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ORIGINAL RESEARCH REPORT

Split-face histological and biochemical evaluation of tightening efficacy using temperature- and impedance-controlled continuous non-invasive radiofrequency energy

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ABSTRACT

Background: Bipolar radiofrequency (RF) is capable of heating dermal collagen fibers and inducing skin tightening by collagen remodeling. **Objective**: To substantiate safety and improvement of skin laxity following skin heating with a novel temperature- and impedance-controlled non-invasive radiofrequency (RF) device by histological and biochemical evaluations. **Methods**: A split-face study was performed on 4 subjects who underwent 8 weekly RF sessions on one side of their face, leaving the other side an untreated control and then underwent facelift procedure. Clinical evaluation by photographs was done prior to the surgical procedure. *Ex vivo* fragments were harvested from both sides and compared. Morphometric analysis of dermal collagen fibers, collagen synthesis, and elastin synthesis evaluations were compared in triplicates. **Results**: Facial skin tightening was apparent in split-face photographs. A significant increase of 7.9% in dermal collagen content, and a significant increase of 34.7% in collagen synthesis were demonstrated in the treated samples. No statistically significant effect on elastin synthesis was detected. **Conclusions**: Skin tightening following treatment with non-invasive RF has proven histologically and biochemically to derive from increase in dermal collagen synthesis and content.

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Radiofrequency; skin tightening; collagen remodeling

Introduction

Non-ablative energy treatments for skin laxity and rhytides have grown rapidly in the last 10 years. Mid-infrared lasers were initially studied for potential efficacy in skin tightening, but the results were modest and, additionally, the devices could often not be used on darker skin types due to risk of discoloration and scarring following absorption of energy by the chromophore melanin (1). Focused ultrasound devices have recently been approved by FDA for skin tightening, but these devices can be associated with significant treatment discomfort requiring analgesia during and after the procedure (2). Furthermore, there have been reports of motor nerve paresthesia following treatments. Radiofrequency (RF) devices have been reported to achieve modest clinical efficacy in skin tightening without many of the limitations of other energy devices, and may therefore represent an ideal treatment option for non-invasive skin tightening (3–5).

Unlike chromophore-dependent lasers, RF devices are bulk heating devices that act through molecular kinetic energy, and are therefore chromophore independent (4). RF devices utilize rapidly oscillating current, which when passed through tissue causes an increase in molecular kinetic energy vibrations; these vibrations lead to friction and ultimately heat production (4). The depth of the heat production is a function of the frequency of the RF energy, with lower frequency devices typically penetrating more deeply. Historically, RF devices utilized monopolar RF that was flowing through the patient's body (6). This may allow for deeper penetration of the monopolar RF energy, but increases the risk of scarring and adverse effects (4). More recently, bipolar and multipolar (multiple and/or sequential bipolar RF systems) have been developed which incorporate both positive and negative electrodes into the handpiece to allow for a safer, more controlled RF exposure, with RF travelling into the soft tissue, about half the distance between the two electrodes. Additionally, in an effort to further reduce the risk of adverse effects including discomfort, thermal burns, and scarring, dynamic moving RF devices which glide across the skin surface have been developed (7–9). These dynamic RF devices are thought to generate a more uniform, consistent thermal experience while reducing the risk of 'hot spots' or excess thermal heating that have been associated with previous static, high peak energy, short pulse duration RF devices (4). In order to best regulate the thermal exposure of the dermis, the ideal system should have built-in thermal and impedance monitoring with real-time feedback within the device to prevent excess heating of the targeted tissue. This

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feedback loop would allow for prolonged thermal exposure, at the desired set temperature, while limiting the risk of excess heating and the associated adverse effects. The RF device deployed in this study possesses all these ideal features.

While many skin-tightening devices report clinical improvement, it is thought that in many cases, the immediate improvement may be related to short-term changes in skin, such as tissue edema or transient collagen contraction. In order to achieve longer lasting results, it is necessary to induce collagen remodeling and neo-collagenesis. The objective of this study was to evaluate the efficacy, safety, and mechanism of action of a novel temperature-controlled, non-invasive, dynamic moving, bipolar radiofrequency device for facial skin tightening. Rather than focusing on temporary clinical outcomes, this study focuses on histologic and structural changes of collagen and elastin following treatment.

Figure 1. Forma™ handpiece comprising of two pairs of electrodes. Each adjacent electrode is similarly charged. The RF flows from one electrode pair to the next.

Materials and methods

The device

Forma™ is a bipolar RF handpiece that works on Fractora™, InMode™ or BodyTite™ platforms (Invasix Ltd./InMode MD Ltd., Israel) among several other optical and RF applicators. The Forma RF travels between each of the two pair of side electrodes for a non-invasive skin treatment. The heat reaches the whole dermis and upper part of hypodermis. Since the RF is continuous and not pulsed, a constant movement of the handpiece over the skin is needed and gel is used to maintain a smooth movement with adequate RF coupling. There are a number of sensors built into the handpiece, including temperature, impedance, and contact, which are used to monitor the activity of the handpiece and skin; the RF is turned off when the impedance falls too fast, contact is lost, or the desired end point temperature is reached. Once the skin temperature falls below the target temperature, a computer feedback loop turns the RF back on and thus the desired thermal end point can be maintained automatically. The user can control the RF energy and the skin surface cut-off temperature. The operator is thus able to acquire and monitor the epidermal temperature and maintain the treatment safely for as long as desired (Figure 1).

Subjects

Four subjects, patients who were undergoing a facelift, were treated with the Forma device on one side of their face on the cheek and retroauricular zone, while the other side of the face was left untreated, serving as control. Subjects were healthy 45–62 years old females. General contraindications included pacemakers, pregnancy and nursing, epilepsy, thyroid dysfunction, diabetes, cardiac arrhythmias, heart disease or uncontrolled hypertension, history of cancer (5 years of remission), liver or kidney disease, medical conditions causing immunosuppression, use of isotretinion (Accutane®) within the previous 6 months, or any other skin disorders or condition such as abnormal wound healing. Diuretics and anticoagulants were also excluded from the study. All participants of the study signed an Informed Consent Form.

Treatment protocol

Treatment zones were one cheek and the one retroauricular area. Ultrasound gel was applied to the treatment zones and the Forma handpiece was constantly moved by circular movements in full contact with the skin.

Patients went through 8 weekly sessions, using RF energy of 50 energy units with a cut-off temperature of 39°C, maintained for 10–15 min over each of the zones. No anesthesia was needed, as the treatment was comfortable, and there were no post-treatment instructions needed, as mild transient erythema and edema subsided within 30–120 min.

Photographs of the patients' face were taken prior the surgical procedure, one week after the last treatment. The patients then underwent facelift and comparative biopsies of 5–6 cm^2 were taken from both treated and untreated control skin.

A series of validated histological and biochemical studies were performed on the biopsied tissues, to analyze the effect of the RF energy on collagen and elastin (10–12), in triplicates for all 4 donors combined; altogether there were 24 samples (12 comparative pairs).

Morphometrical analysis of collagen fibers

Serial sections of 4-μm thickness were obtained and specifically stained for collagen with a picric acid solution containing 0.1% sirius red. Collagen was analyzed by computerized morphometric analysis. For a quantitative analysis of these macromolecules, a computerized image analysis of each section was made. The stained slides were examined by a microscope (Leitz) (magnification ×160) connected with a camera unit (XC-75 CE type) and with a microprocessor (Q520).

The surface of collagen bundles was measured in μm^2. Then, the relative collagen content of the superficial and mid dermis was expressed as a percentage of total surface area.

Collagen synthesis

Skin fragments were enzymatically digested in an acetic acid 0.5 M solution containing pepsin overnight at 4°C. The fibroblastic activity for collagen synthesis was evaluated spectrocolorimetrically (540 nm) to measure the acido-soluble new collagen synthesized after a specific fixation by sirius red staining (Sircoll Collagen Assay, Interchim). The results were expressed in µg collagen per mg skin.

Elastin synthesis

Insoluble elastin was extracted from each skin fragment by oxalic acid to convert into water-soluble α-elastin. The assay was completed using a quantitative dye-binding method with spectrophotometrical measurement at 513 nm. Results were expressed in µg of elastin per mg skin.

Statistical analysis

Mean values and standard deviations were calculated for quantitative variables. The statistical significance of changes recorded concerning these parameters was determined with the Student's paired *t*-test, when $p < 0.05$ is considered statistically significant.

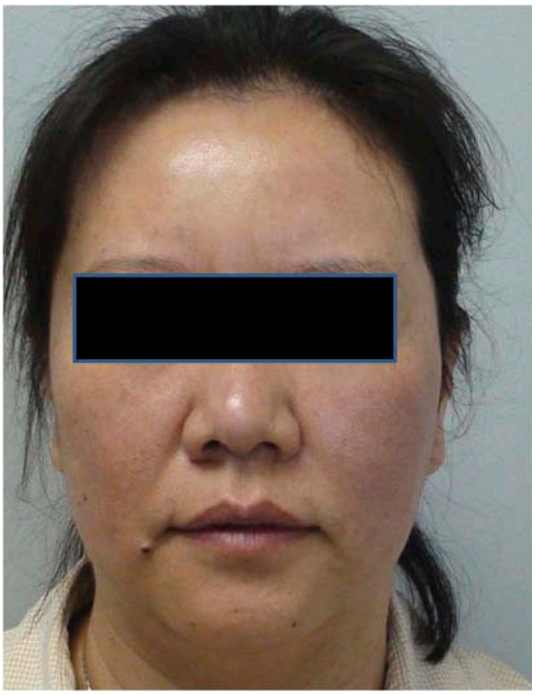

Figure 2. Representative photograph taken one week post 8th split-face treatment by Forma, showing the left treated side versus the right untreated side. Note the treated side has less curved jawline, smoother nasolabial fold, and higher cheekbone.

Results

Photographs

Split-face photos were taken one week after the 8th treatment to evaluate the difference of response to RF by comparing the treated and non-treated sides. A representative photo clearly shows the facial skin tightening that is expressed in jawline, nasolabial folds, and cheekbone following the series of RF treatments (Figure 2).

Histological quantification of dermal collagen by computerized image analysis

The results of histological quantification of dermal collagen are expressed in Table 1 and Figures 3–5. After treatment with the

Table 1. Histological and morphometric analysis of collagen bundles content ($n = 12$).

	Collagen % in dermis
Area	Mean ± SD
Untreated skin	56.67 ± 4.74
Treated skin	61.16 ± 8.97
	$^*p = 0.03$

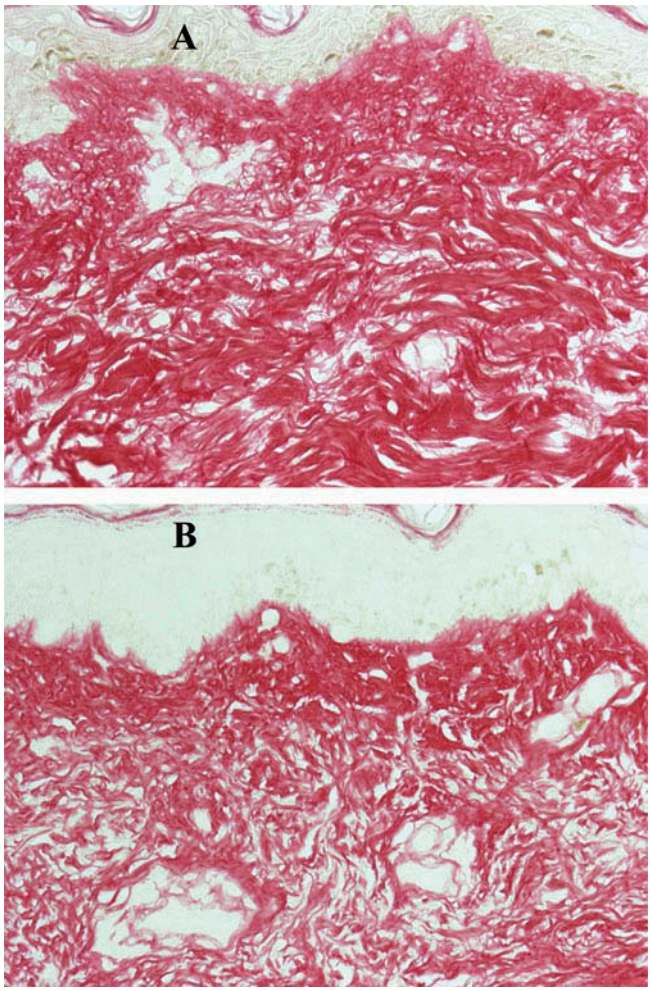

Figure 3. Dermal collagen of subject n°1 from Forma-treated side (A) and from control side (B). Note increase of collagen bundles density in A (Sirius red, ×400).

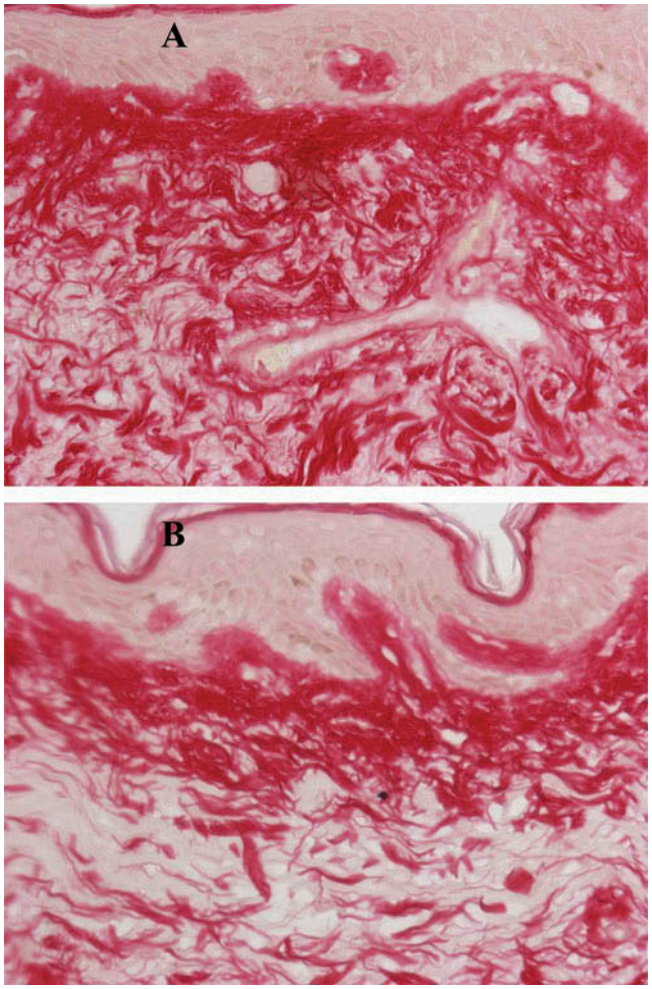

Figure 4. Dermal collagen of subject n°2 from Forma-treated side (A) and from control side (B). Note increase of collagen bundles density in A. (Sirius red, ×400).

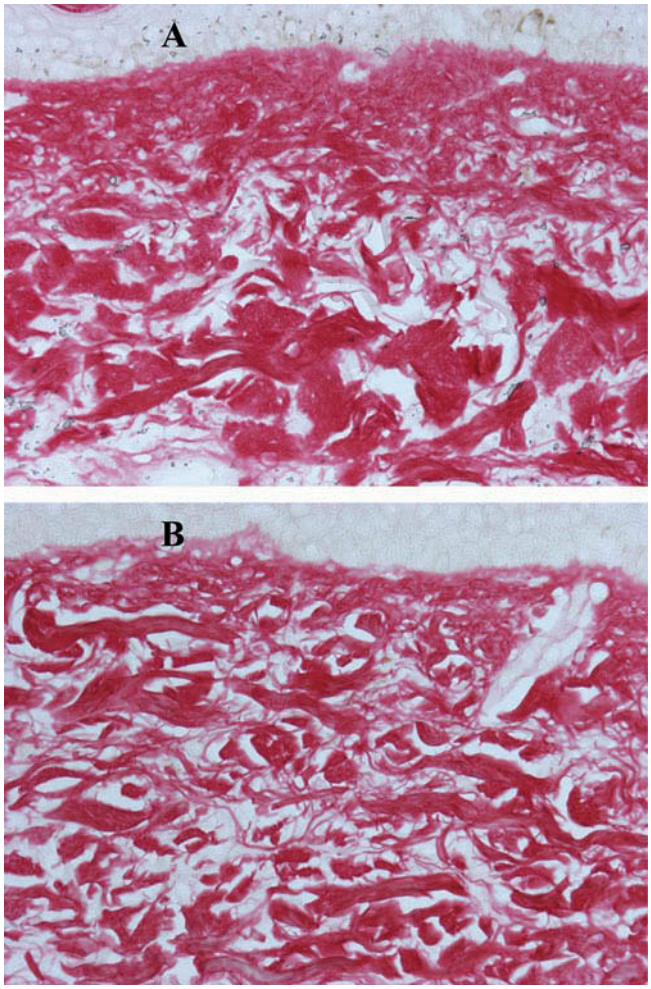

Figure 5. Dermal collagen of subject n°3 from Forma-treated side (A) and from control side (B). Note increase of collagen bundles density in A. (Sirius red, ×400).

Forma on one side, the content of collagen was 61.16% in the superficial and mid dermis versus 56.67% for untreated skin ($p = 0.03$). The difference between treated skin and untreated skin of 7.9% increase in dermal collagen content was statistically significant (paired Student's t-test, $p < 0.05$). These results correlate with Figures 3–5 that show a clear increase of collagen bundles density in the samples taken from the Forma-treated side when compared to the untreated control skin.

Collagen synthesis

The results of collagen synthesis are expressed in Table 2. A significant increase of collagen synthesis was obtained after treatment with Forma with 16.81 µg/mg on the treated side versus 12.48 µg/mg in untreated skin ($p = 0.003$). The difference between treated skin and untreated skin was statistically significant (paired Student's t-test, $p < 0.05$). This stimulation by the non-invasive RF treatment corresponds to an increase in collagen synthesis of 34.7% in the RF-treated skin.

Table 2. Biochemical dosage of collagen ($n = 12$).

	µg collagen/mg of skin biopsy
Area	Mean ± SD
Untreated skin	12.48 ± 5.14
Treated skin	16.81 ± 5.5
	*$p = 0.003$

Table 3. Biochemical dosage of elastin ($n = 12$).

	µg elastin/mg of skin biopsy
Area	Mean ± SD
Untreated skin	2.42 ± 0.69
Treated skin	2.39 ± 0.74

Elastin synthesis

The results of elastin synthesis are expressed in Table 3. Any statistically significant elastin synthesis difference could not be detected, with 2.42 µg/mg on the treated side versus 2.39 µg/mg in untreated skin.

Safety

No complications were recorded.

Discussion

The clinical skin-tightening efficacy of the Forma device in previous clinical studies (13,14) correlates with the histological and biochemical findings in this study. The stimulation of fibroblast metabolism for collagen synthesis and increase of dermal collagen content was demonstrated. Another non-invasive continuous RF device applied to *ex vivo* skin fragments was evaluated by similar technique and showed comparable effects of RF on collagen contents and synthesis rate (15). In the current study, the RF treatment was done *in vivo*, which makes the results even more reliable. The end point of 39°C was 3–4°C lower than the temperature end points of 42–43°C often used clinically and may have actually resulted in less collagen production than can be achieved with higher temperature. The upregulation of collagen synthesis of 34.7% indicates that fibroblasts were actively upregulated in producing collagen. This process takes about 6 weeks to mature and finalize; it is therefore likely that if the biopsies had been taken 4–6 weeks instead of one week after the last treatment, then even greater increases in the collagen content of the dermis (greater than the 7.9% increase) would have been observed. Other studies with suction-coupled RF, treating abdominal skin to end points of 40°C have shown 13.7% more collagen following 6 weekly treatments (16).

No significant effect of RF treatment was detected on elastin tissue in the RF-treated skin. Since elastin content in the dermis is much smaller than collagen content, the result may be due to the method sensitivity, or the small sample size used.

The Forma provides individual adjustment of continuous RF energy to achieve maximum efficacy and safety for each patient. Efficacy is achieved by the possibility to maintain an effective temperature for a long duration; at the same time safety is maintained through constant control and monitoring of a safe pre-determined maximal skin surface temperature. Indeed, no adverse events were recorded clinically, neither shown histologically.

As the Forma treatments were delivered pre-operatively and the upregulation of collagen synthesis was significant, it may be that non-ablative, dynamic RF skin heating may be of benefit preoperatively in cutaneous surgery. The stimulation and upregulation of collagen may shorten healing time and improve scar appearance in facelifts and other skin surgeries, as well as decrease adverse effects such as wound dehiscence and flap failure. Further studies will be necessary to definitively determine these effects.

Forma may also be combined with other modalities, such as radiofrequency fractional ablation and Intense Pulsed Light (IPL) to optimize results according to specific patient needs (15). In the future, a time/temperature–dose–response study would be of use to determine the ideal cut-off skin temperature and exposure times to result in the most collagen production.

Conclusions

The temperature-controlled bipolar RF energy employed by Forma has been shown to be safe and effective in inducing facial skin tightening. The mechanism of skin tightening induced by non-invasive RF has been demonstrated to depend on neo-collagenesis with undetected involvement of elastin.

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