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TWO-TREATMENT PROTOCOL FOR SKIN LAXITY USING 90-WATT DYNAMIC MONOPOLAR RADIOFREQUENCY DEVICE WITH REAL-TIME IMPEDANCE MONITORING

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Two-Treatment Protocol for Skin Laxity Using 90-Watt Dynamic Monopolar Radiofrequency Device With Real-Time Impedance Monitoring

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ABSTRACT

Multiple devices are currently on the market that employ radiofrequency to non-invasively treat skin laxity and wrinkle reduction. The study device was a unique monopolar radiofrequency device FDA cleared for the treatment of wrinkles and rhytids. The delivery system allows constant monitoring of the real-time local skin impedance changes, which allows radiofrequency energy to be more uniformly dosed over an entire treatment area.

Objective: The objective was to validate effectiveness of a modified treatment protocol for a unique monopolar radiofrequency device, which has been engineered with greater power and self-monitoring circuitry.

Methods: Twenty-four female subjects received bilateral monopolar radiofrequency treatments to the mid and lower face from the sub malar region to the submentum. Subjects completed 1 and 3 month follow ups with digital imaging. Skin biopsies (on 4 subjects) and ultrasound measurements (on 12 subjects) were completed.

Results: Assessments demonstrated a reduction in skin laxity of 35%, a reduction in fine lines/wrinkles of 42%, and a reduction in the appearance of global photodamage of 33%. Expert photograding demonstrated 92% of subjects showing at least a mild improvement in skin laxity at three months post treatment. 50MHz ultrasound measurements in 12 subjects showed an increase of 19% in skin density. Histology showed a marked increase in dermal collagen and elastin fibers in two subjects who demonstrated a clinically noticeable reduction in skin laxity and minimal changes in two subjects who demonstrated minimal clinical improvements. There were no significant adverse events reported.

Conclusion: This modified radiofrequency device and treatment protocol was well tolerated and produced improvements in the appearance of skin laxity and overall anti-aging effects in the majority of subjects. Objective measurements including ultrasound and histology help explain the clinical outcome.

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INTRODUCTION

The appearance of the face and neck is profoundly affected in the aging process. There is decreased tissue elasticity coupled with rearrangement of facial volume that is compounded by the effects of gravity.[1] As the appearance of the face and neck is a primary concern of many people, ways in which to tighten the skin are increasingly in demand. One such method to address this concern is radiofrequency treatment, which produces an electrical current that uses the resistance within the various skin layers to convert the delivered energy into thermal energy.[2] Radiofrequency creates oscillating electrical current, causing vibration and collisions between charged molecules, thus resulting in the production of heat as described by Belenky, et al.[3] This radiofrequency heating occurs regardless of skin chromophores or skin type and is not dependent upon selective photothermolysis but rather heating of water. Thus, hydration of tissues in the radiofrequency treatment area is important. There are many different types of radiofrequency devices using multiple types of radiofrequency energy, temperature ranges, and target depths.[4,5] Radiofrequency heat has different biological and clinical effects, depending upon the method of delivery and depth of heating. In the dermis, which is comprised of collagen, elastin, and ground substances, radiofrequency mediated thermal stimulation of this matrix results in an immediate and temporary change in the helical structure of the collagen.[6] It is also believed that radiofrequency thermal stimulation results in a micro-inflammatory stimulation of fibroblasts, which produces new collagen (neocollagenesis) and new elastin (neoelastogenesis), as well as other proteins to enhance dermal structure.[7,8]

To allow a constant and consistent radiofrequency energy delivery, there is a need for the power to be maximized and normalized for skin impedance. Some first generation radiofrequency skin tightening devices offer skin impedance measurements, but these measurements are not real-time measurements of the target area. Some radiofrequency devices use impedance of one sampled area to "average" impedance at the beginning of treatment. This initial one-time impedance measurement is used for the duration of the treatment and does not allow for changing

power relative to impedance from one area to the next, thus yielding an uneven thermal energy distribution. Without active real-time impedance measurement, some areas of the skin could be treated too intensely (risk of burning, higher in bony areas) while elsewhere the therapeutically optimal temperatures may not be attained.

The monopolar radiofrequency device used for the study is a monopolar radiofrequency device (Exilis Elite, BTL Industries Inc) that is FDA cleared for the "primary treatment of dermatologic and general surgical procedures for non-invasive treatment of wrinkles and rhytids". The delivery system allows constant monitoring of the real-time local skin impedance changes during radiofrequency skin treatment. This impedance compensation system controls, or tunes, the current supply while the circuitry automatically compensates for impedance changes. Energy flow is controlled and the microprocessor automatically keeps the power output equivalent even in areas of higher/lower impedance allowing the operators to use high power settings without compromising safety. The system design enables the energy be consistently dosed over the entire treatment area.

"Early equipment designs would continue to put energy through the system even when the contact point was too small, generating a burn or blister in the treated skin. The double grounded electrode ensures that once sufficient contact is lost, no energy is delivered."

These features are highlighted in this system through the following modifications to the original device. First the device has a double grounded electrode that is monitored several times per second and assists in stopping energy flow when sufficient contact is not made with the tissue to be treated. This helps eliminate the previous generation, electro cautery-based issue of arcing when sufficient contact is not made with the tissue. Early equipment designs would continue to put energy through the system even when the contact point was too small, generating a burn or blister in the treated skin. The double grounded electrode ensures that once sufficient contact is lost, no energy is delivered. Next, the hardware/software interface following these measurements allows for even distribution of heat through variable impedance regions as shown on infrared imaging (all treated regions appear to be a uniform temperature despite any impedance differences). These modifications are part of the "Impedance Intelligence" system, which is an automatic self-adjusting system delivering the energy amount based on measured capacitive, inductive, and resistive parameters of the skin-applicator borderline. It consists of impedance measurement circuit connected to the energy delivery system, changing the parameters of the delivered energy according to the impedance measurement.

Previous histologic porcine skin studies demonstrated safety of the study device. One study involved 4 serial treatments at one-month intervals. Photomicrographs taken from special stained sections were analyzed using stereological analysis, which is a computer-based image processing and analysis technique for quantitative analysis of skin structures histological sections. Mean percentage of collagen volume was calculated with results showing that while average baseline collagen volume was 9%, the post four treatments average volume was 23%, an increase in collagen volume of 154%. Histology with temperature measurements also demonstrated that the reported temperature range (42-43C°) can generate this type of tissue response (Data on file, BTL Industries Inc, Framingham, MA).

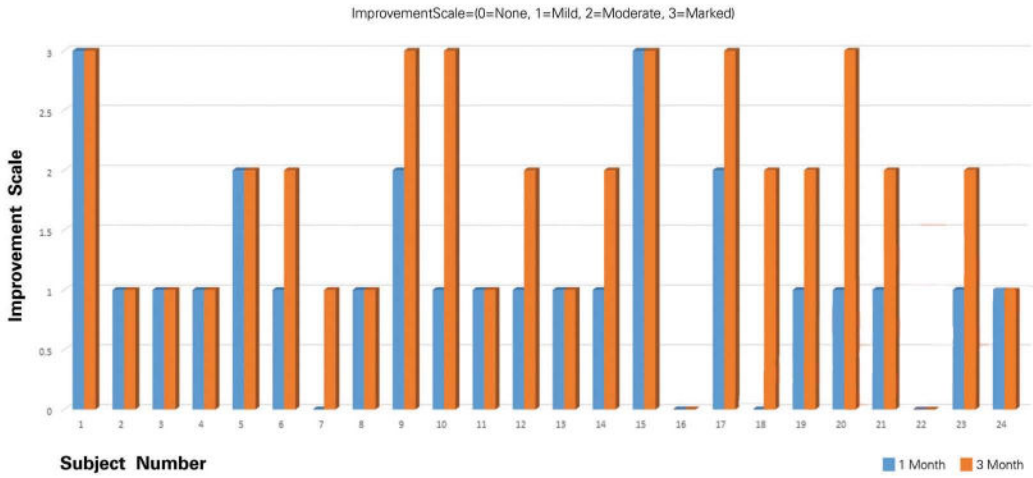
MATERIALS AND METHODS

Study Design

Our study enrolled 26 female subjects with 24 completed at two investigative sites both under IRB approval. Subjects were between 25 and 65 years of age (average age = 57) who exhibited mild to moderate laxity of the submentum, mid, and lower face. Subjects received two (2) treatments 10-14 days apart (+/- 4 days) using the monopolar radiofrequency system (Exilis Elite, BTL Aesthetics). The starting settings were 90 Watts and with the device set to emit a continuous wave of energy (100% duty factor for radiofrequency transmission). The power setting (Watts) was titrated based on subjects' verbal response for heat tolerance. The treatment was administered as follows: 1) Treated area from sub malar region to mandible for 6 minutes; 2) Treated submentum from lateral aspect of area to midline for 4 minutes; 3) Returned to sub malar region and treated for 3 minutes. Treated mandible region for 3 minutes; 4) Returned to submentum and treated for 4 minutes; 5) Repeated steps 1-4 on the contralateral side of face. Temperature of the skin was monitored using an external infrared radiometer and maintained at 42-43 C°. Typical total treatment time averaged 40-45 minutes, and no topical anesthetic or oral pain medications were used.

The subjects were consented, had a medical history taken, and had assessments for Skin Laxity/Sagging, Erythema, Edema, Fine Lines/Wrinkles, and Global Improvement prior to the first treatment, 1 month, and 3 months post final treatment. Digital images were also taken using the VECTRA- M3, VISIA CA and Intellistudio (Canfield Scientific, Passaic NJ). Subjects underwent assessment using a 0-3 rating scale (0=Normal, 1=Slight, 2=Moderate, and 3=Severe) at all time points. Ultrasound imaging using the TPM DUB SkinScanner (Taberna Pro Medicum, Germany) on the left lower cheek (within the treatment area) was

FIGURE 1. Improvement in skin laxity.



performed in a series of 3 measurements using a 50MHz scanner head (shallower depth of penetration but higher resolution images) This was repeated in the same location at the 1 and 3 month follow up visits. Finally, 4 subjects were selected to have pre and 3 month post final treatment 2 mm skin biopsies from the submentum for histological examination. Biopsies were taken a minimum of 1 cm apart to minimize wound healing artifact.

Imaging Methods

Standardized images of the face were captured using 3 different imaging units: the Canfield Intellistudio, VISIA-CA (high resolution/megapixel cameras with a stereotactic head positioning device) and VECTRA-M3. Photographs were taken prior to any treatment (clean and dry face only). No makeup was worn during the photographs, including foundation, blush, eye shadow, lipstick and mascara.

Three ultrasound images (per visit, per scanner frequency) for 12 subjects at a single site were analyzed using the device software for Skin Analysis. Data for each measurement included skin thickness and density. These values were averaged to give individual subject skin thickness and densities for each time point. The subject skin density and thickness measurements for the selected 12 subject subset, were then combined to determine the average effect of the treatment on skin density and thickness for each scanner.

The biopsies were placed in 10% buffered formalin, paraffin embedded and cut into 5 micron sections. Masson Trichrome stain for visualization of collagen and Verhoeff stain for visualization of elastin fibers were performed on a section for each biopsy subject. The prepared slides were viewed on a Leica-DM IRB inverted microscope fitted with a Nikon Camera system (DS-Fi2 Visible light Camera) and the NIS Imaging Suite was used to form a composite image of the entire section, which was then visually assessed for increased staining of the selected proteins/fibers.

RESULTS

Clinical Observations

On the 0-3 point assessment scale, 35% reduction in skin laxity/sagging ($P < 0.0001$), 35% reduction in fine lines/wrinkles ($P < 0.0053$), and 33% reduction in the severity of global photodamage ($P < 0.009$) were recorded. No edema was observed. An incidental finding was a statistically significant reduction in background erythema ($P = 0.0109$) in some subjects.

Photo assessment by blinded expert graders revealed 79% of subjects had mild improvement in each of three categories measured (fine lines/wrinkles, skin laxity, and overall skin texture). For skin laxity, 92% of subjects showed a minimum of mild improvement at the 3-month time point (Figure 1). Average improvement from one month to three months achieved statistical significance (as determined by a two-tailed paired t test) of 38% improvement in fine lines/wrinkles ($P = 0.0011$), 64% improvement in skin laxity ($P = 0.0003$), and 50% improvement in overall skin texture ($P = 0.0011$).

Ultrasound

Ultrasound images from the 50MHz detector revealed an average of 19% increase in skin density at 3 months (Figure 2) and a statistically significant linear increase in skin density over the course of the study ($P < 0.01$; $R^2 = 0.2645$, $P = 0.0014$, respectively). This was calculated using a two-tailed One-Way ANOVA with post-hoc Dunnett's Multiple Comparisons test and test for a linear trend.

Histological Imaging

Histologic examination of the four biopsies showed significant increase in dermal collagen and elastin fibers throughout the

FIGURE 2. Average of all subjects density readings at all time points from 50MHz Ultrasound unit in the lower cheek. An increase of 19% in skin density was observed.

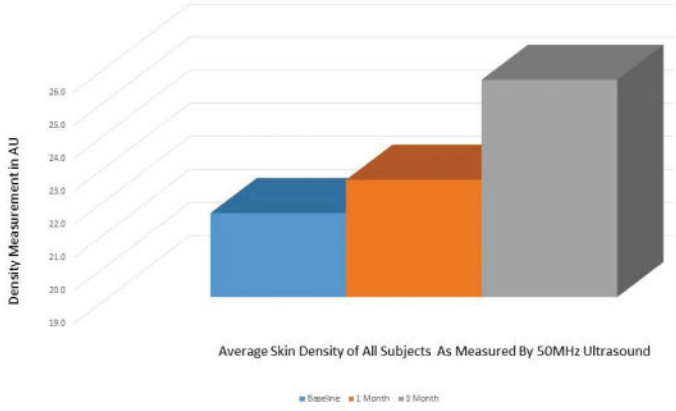
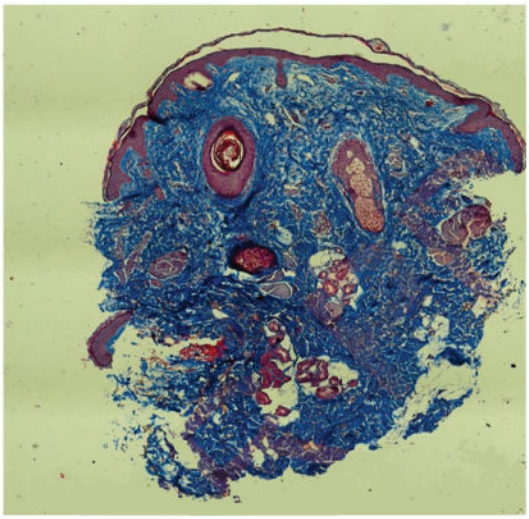


FIGURE 3a. Masson staining in one subject demonstrates increased collagen (blue) at 3 months post treatment.

Pre



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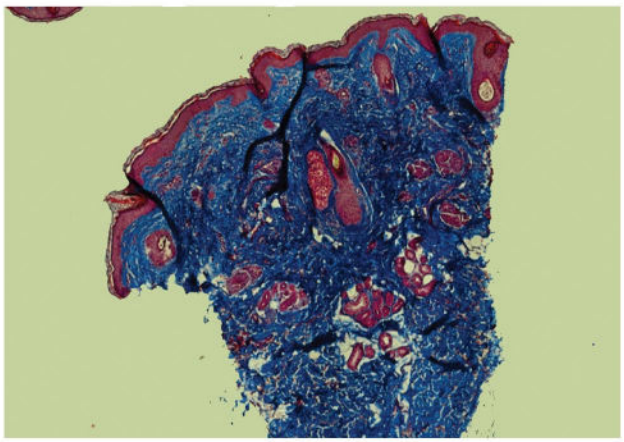
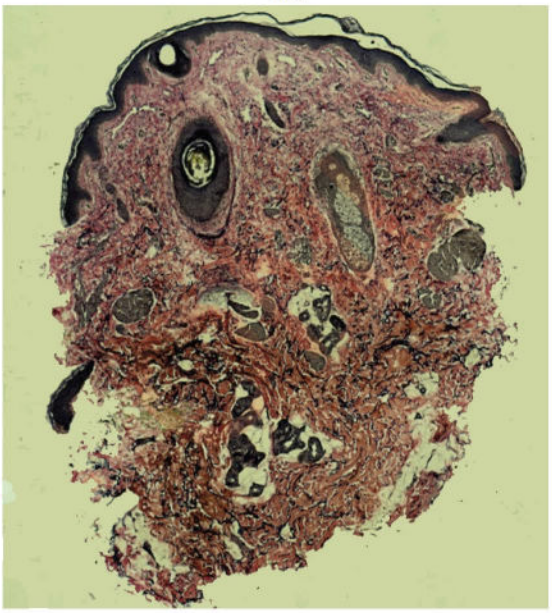


FIGURE 3b. Verhoeff staining in one subject demonstrates increased elastin fibers (black), with more uniform pattern and distribution at 3 months.

Pre



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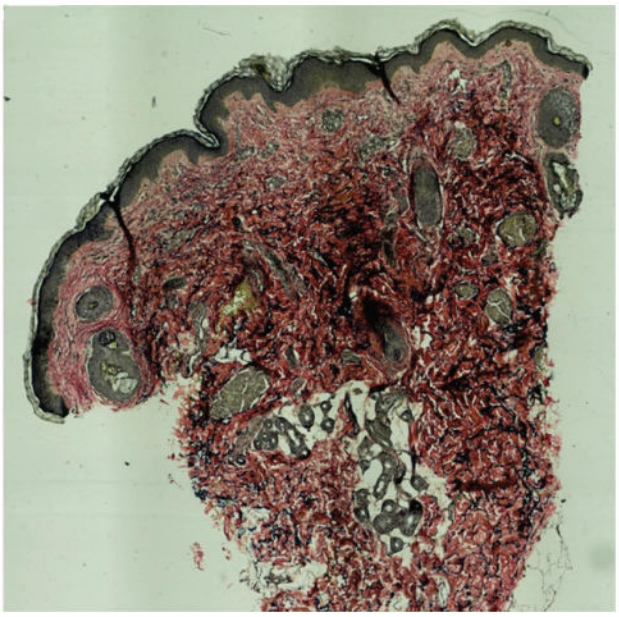
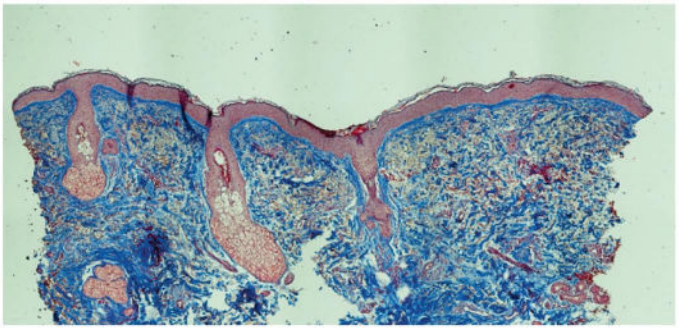


dermis in two of the four subjects. Two subject's full thickness images are presented below (Figures 3a, b, and 4a, b). Clinical images of these same subjects demonstrated significant clinical improvement, consistent with the histological findings (Figures 5 and 6). There were minimal changes in the biopsies and clinical grading from the other two subjects. The histology results correlate well with the respective subjects' clinical response (marked improvement for the two with visible collagen and elastin deposition and minimal improvement for

FIGURE 4a. Masson staining in one subject demonstrates increased collagen (blue) at 3 months post treatment.

Pre



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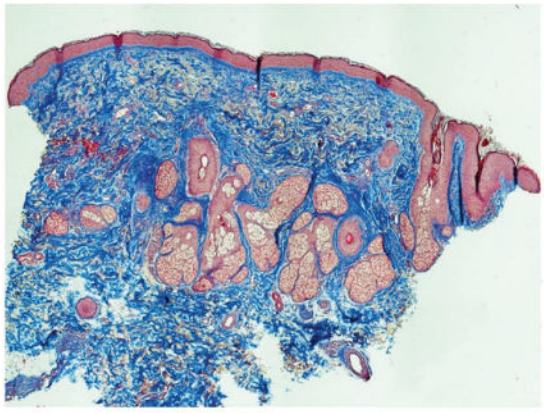

FIGURE 4b. Verhoeff staining in one subject demonstrates increased elastin fibers (black), with more uniform pattern and distribution at 3 months.

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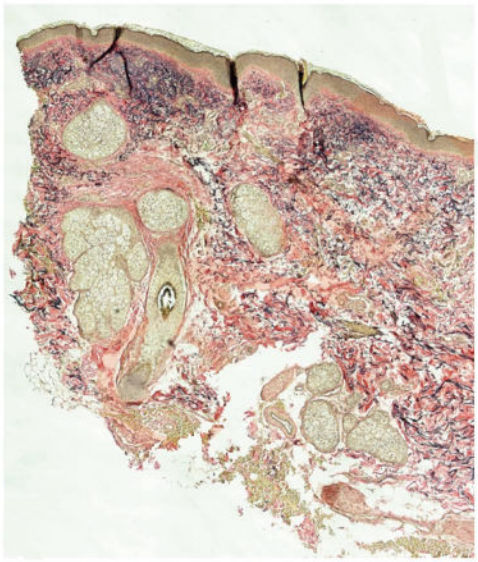

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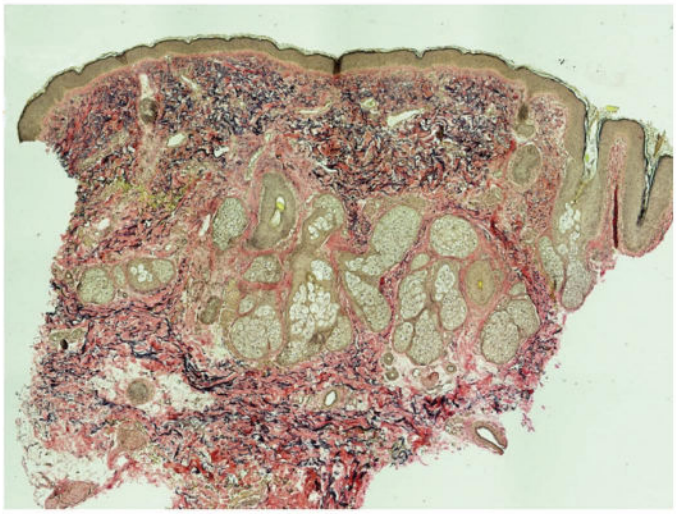


the others). Tightening around the jawline, under the chin and neck are clearly demonstrated (Figure 7).

CONCLUSION

This study demonstrates that a novel monopolar radiofrequency device with increased fluence and continuous impedance monitoring used with a new two treatment protocol achieves measurable clinical benefits. The data supports previous statements that mention by maintaining a lower skin temperature for longer treatment times measurable benefits can be achieved. Thus, it is possible to achieve clinical results without pain and downtime. The study data indicates an increase in overall skin density, collagen, and elastin deposition/organization, and some improvement in fine lines/wrinkles as well as overall skin texture in the majority of subjects. There was also an unanticipated decrease in background erythema in many subjects, the mechanism of which is unknown.

The data analysis also revealed other items that warrant further study and discussion. The first is the variation in the results from subject to subject. Each subject was treated using the same standardized protocol such that time on tissue was consistent for every subject. We propose that the cause of variance in the results may be due to the wide age range of the subjects as well as individual heat tolerance. While time on tissue was standardized, the titration of energy was based on tissue response as well as subjects' reported sensation of heat. The subject variation could be addressed in future studies through a tighter selection process as the current subjects were selected from a wide range of potential candidates as this study was designed to mimic broad clinical usage and not to selectively treat subjects that would respond well to the treatment. It is also worth noting that some subjects experienced early results (1 month) but did not improve as expected at 3 months; the reason for this is currently unknown. Future studies that examine the longevity of the results are also warranted. It would be of benefit to follow the subjects

FIGURE 5. Clinical photo of subject at baseline and 3 months following 2 radiofrequency treatments.

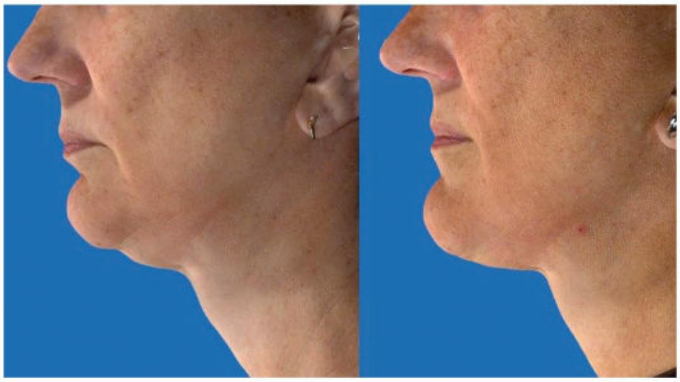


FIGURE 6. Clinical photo of subject at baseline and 3 months following 2 radiofrequency treatments.

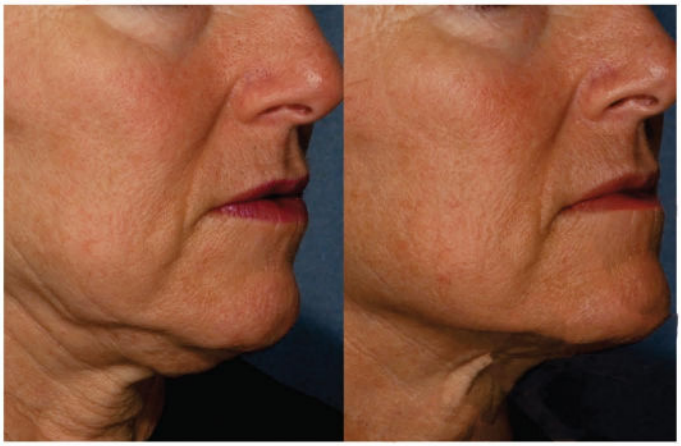
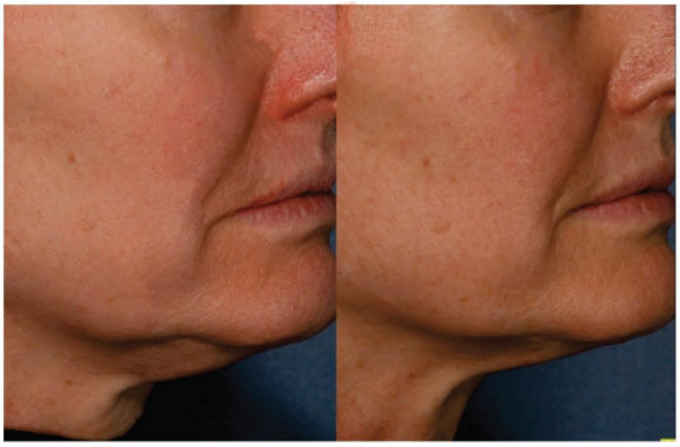


FIGURE 7. Clinical photo of subject at baseline and 3 months following 2 radiofrequency treatments.



for 6 months or longer to determine if repeated treatments are needed as commonly associated with similar types of treatments, or if this device has an additional advantage of increased improvement duration.

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DISCLOSURES

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ORIGINAL CONTRIBUTION

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Evaluation of the safety and efficacy of a monopolar nonablative radiofrequency device for the improvement of vulvo-vaginal laxity and urinary incontinence

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Summary

Background and objective: Vaginal childbirth, natural process of aging, congenital factors, and surgical interventions are considered the main causes of vulvo-vaginal laxity driven by changes in collagen and elastin fibers. This causes a loss of strength and flexibility within the vaginal wall. As a result, women may experience lack of sensation and stress urinary incontinence (SUI)—the condition of involuntary loss of urine associated with activities that cause an increase in intra-abdominal pressure (eg, sneezing, coughing, and lifting). Both vaginal laxity and urinary incontinence significantly affect patients' quality of life (QoL).

The aim of this study was to evaluate efficacy and safety of a noninvasive radiofrequency device when used to treat SUI and vulvo-vaginal laxity through its heating effect which stimulates collagen and elastin fibers.

Methods: Twenty-seven women (average age 44.78±10.04 years) with indications of mild/moderate SUI as well as vulvo-vaginal laxity were treated with a monopolar radiofrequency device. The treatment course consisted of three once-a-week sessions. Each session included intravaginal treatment followed by treatment of labia majora and the perineum.

Improvement in the SUI condition was evaluated by applying the International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF). Data were collected at the baseline, after the last treatment and at 1-month follow-up visit.

Vaginal laxity was assessed by subjective vulvo-vaginal laxity questionnaire (VVLQ). Data were collected before the 1st treatment and during the 1-month follow-up visit.

Patient's satisfaction was recorded using a satisfaction questionnaire. Data were collected after the last treatment and at the 1-month follow-up visit. Any adverse events related to the treatments were monitored.

Results: On a scale of 0 to 5, the average frequency of urine leak improved from "2-3 times a *week*" (2.15±1.03 points prior to treatment) to "*once a week*" (1.00±0.78 points post-treatment), and on to "*never*" (0.44±0.51 points at the 1-month follow-up visit). Sixteen subjects (59.3%) reported decrease in the amount of

leakage, with 15 women (55.6%) becoming completely leak-free at the 1-month follow-up. At the 1-month follow-up visit, 24 subjects (88.9%) expressed their condition's interference with everyday life decreased and 17 patients (62.9%) said the condition did not interfere with their everyday life at all as a result of the treatment. All results are statistically significant ($P<.05$). No adverse events were recorded.

All subjects reported improvement in vaginal laxity, from average perception of "*very loose*" (2.19±1.08 points prior to treatment) to "*moderately tight*" (5.74±0.76 points at the 1-month follow-up visit).

During the follow-up visit, 89% of the patients "*agreed*" or "*strongly agreed*" that their *SUI condition* improved, and 93% of the patients "*agreed*" or "*strongly agreed*" that their gratification during intercourse improved. None of the subjects reported dissatisfaction.

Conclusion: The study confirmed the monopolar radiofrequency method as an effective and safe treatment of SUI and vulvo-vaginal laxity. The treatments were well tolerated by all subjects with no adverse effects.

KEYWORDS

extra-vaginal, intravaginal, labia majora, noninvasive tightening, perineum, radiofrequency, sexual gratification, SUI, urinary incontinence, vaginal laxity, vulvar laxity

1 | INTRODUCTION

Stress urinary incontinence (SUI) is a condition of involuntary urine leakage from the urethra considered to be a hygiene and/or social problem.[1] Statistical data show that the most affected part of the population are women, with approximately 35% of all women worldwide affected.

Urinary incontinence (UI) is frequently linked to vulvo-vaginal laxity, which encompasses laxity of both the vaginal introitus and labia majora. This condition is most commonly linked to sexual dissatisfaction due to limited friction, feeling of looseness, and orgasmic dysfunction; all leading to lower sexual gratification during intercourse. Both of these conditions lead to a decreased quality of life (QoL) including social isolation, decreased self-confidence, and lower sexual gratification during intercourse.[2,3]

The major risk factors for the development of SUI and vulvo-vaginal laxity include childbirth, advancing age, hysterectomy, recurrent urinary tract infections, smoking, medications such as diuretics, sedative-hypnotics and alpha blockers, the presence of comorbid diseases, and excessive weight.[2,4–6] The conventional methods for treating this condition include medications, pelvic floor muscularity strengthening (exercising and/or electro stimulation), surgical procedures, and lifestyle changes (such as quitting smoking or losing weight).[7–9]

Radiofrequency (RF) is one of the more innovative approaches to treating SUI and vulvo-vaginal laxity. It has gained significant popularity in recent years due to its noninvasiveness, absence of adverse events, and fast results. The mechanism of action is based on elevating the temperature of the treated tissue to initiate biological changes. RF-generated heat stimulates the tissue matrix of collagen, elastin, and ground substances and results in immediate change in the helical structure of the collagen. Additionally, neocollagenesis and neoelastogenesis are triggered due to micro-inflammatory stimulation of fibroblasts.[10] It is also believed that the production of sex steroid precursor dehydroepiandrosterone (DHEA) is activated. DHEA supports estrogen production in the vulvo-vaginal cells which plays a big role in rejuvenating and stimulating the vaginal tissue and collagen.

The aim of this study was to investigate the efficacy and safety of a monopolar radiofrequency device for transvaginal treatment of SUI and vulvo-vaginal laxity.

2 | MATERIALS AND METHODS

2.1 | Participants

Twenty-seven women aged between 28 and 66 (mean age 44.78±10.04 years) participated in this nonrandomized, prospective, multicentric study. Only subjects who experienced mild-to-moderate stress urinary incontinence (minimum level 1 in the frequency of leakage based on ICIQ-UI SF form, ie, experiencing leakage at least once a week) and vaginal laxity (maximum level 5 of vulvo-vaginal laxity based on VVLQ questionnaire, ie, defined as no more than "slightly tight") were enrolled. Prior to the study, 19 subjects (70.4%) evaluated their vulvovaginal tightness as "moderately loose" or "very loose," 18 subjects (66.7%) reported they leak urine at least two or three times a week. Twenty-six subjects (96.3%) had a history of at least one prior delivery. The exclusion criteria included the following: abnormal cell cytology; positive urine culture; bleeding in the vulvo-vaginal area; pregnancy or breastfeeding; metal implants; unwillingness or incapability to complete the entire study protocol; any other contraindication listed by the device manufacturer. All patients were

consented. The study was approved by an independent ethics committee.

2.2 | Therapy provision

The therapy course consisted of three once-a-week (± 2 days) treatment sessions with monopolar radiofrequency device (Exilis Ultra 360, BTL Industries Inc., Boston, MA). Each treatment session consisted of an intravaginal and subsequent extra-vaginal treatment. For intravaginal treatment, the starting power was set to 30 points and 80% duty factor. The intravaginal tip was applied to the mucosal surface of the vaginal introitus behind the hymenal ring, was moved deeper inside the vaginal canal to a depth of approximately 10 cm over the course of 2.5 seconds, and then was moved back to the vaginal introitus over the course of the next 2.5 seconds. This repetitive movement continued for 5 minutes. The energy was adjusted based on patient's feedback. For extra-vaginal treatment, the initial power was set to 90 points and 100% duty factor. The extra-vaginal tip was applied to the labia majora using slow circular motions for 3 minutes on each side; the energy was adjusted based on patient's feedback. Then the extra-vaginal tip was applied to perineum using slow circular motions for 3 minutes; the energy was adjusted based on patient's feedback.

2.3 | Outcome measures and statistic evaluation

The SUI condition was assessed by applying the standardized International Consultation on Incontinence Questionnaire - Urinary Incontinence Short Form (ICIQ-UI SF).[11] Data were collected before the first, after the third (last) treatment and during the 1-month follow-up visit. Average improvement was calculated.

Vaginal laxity was assessed by nonstandardized subjective vulvo-vaginal laxity questionnaire (VVLQ) using 7-point Likert scale (BTL Industries Inc.). Data were collected before the first treatment and during the 1-month follow-up visit. Average improvement was calculated.

All outcome data were tested for statistical significance by means of *t* test, where levels of $P<.05$ were deemed statistically meaningful.

Patients' satisfaction with the treatment results was evaluated using a 6-point Likert scale satisfaction questionnaire. The questionnaire consisted of the following statements: (1) "My UI has been improved" and (2) "My sexual gratification has been improved", with the following possible answers: strongly disagree (1); disagree (2); slightly disagree (3); slightly agree (4); agree (5), strongly agree (6). Data were collected after the third (last) treatment and during the 1-month follow-up visit.

3 | RESULTS

All 27 patients completed the study. All treatment sessions were conducted in accordance with the treatment protocol. No adverse events or side effects were observed.

3.1 | Urinary Incontinence

The outcome data and the results from ICIQ-SF and VVLQ are presented in Table 1.

The average frequency of urine leak improved from "*2-3 times a week*" (2.15±1.03 points prior to treatment) to "*once a week*" (1.00±0.78 points post-treatment), and on to "*never*" (0.44±0.51 points at the 1-month follow-up visit). Twenty-six subjects (96.3%) reported improvement of at least one level, with 15 subjects (55.6%) showing improvement of two or more levels when comparing the baseline to the follow-up visit.

Sixteen of the enrolled subjects (59.3%) also reported decrease in the amount of leakage, with 15 women (55.6%) becoming completely leak-free at 1-month follow-up.

At 1-month follow-up, 24 subjects (88.9%) expressed their condition's interference with everyday life decreased, with 12 individuals (44.4%) reporting improvement of three or more levels on a 0-10 scale. Seventeen patients (62.9%) said the condition does not interfere with their everyday life anymore.

All measured results were proven statistically significant ($P<.05$).

3.2 | Vaginal laxity

On a scale of 1-7, the average vulvo-vaginal laxity improved from "*very loose*" (2.19±1.08 points prior to treatment) to "*moderately tight*" (5.74±0.76 points at the 1-month follow-up visit). Twenty-seven subjects (100%) reported improvement of at least two levels, with 23 subjects (85.2%) showing improvement of three or more

TABLE 1 Changes in Stress urinary incontinence (SUI) and vulvo-vaginal laxity

Questionnaire	Score range	Pretreatment	Post-treatment	*P* value	1 mo post-treatment	*P* value	Improvement (Pre to Post)	Improvement (Pre to 1 mo post)	*P* value
ICIQ-UI SF									
Frequency	(0-5)	2.15±1.03	1.00±0.78	<.001	0.44±0.51	<.001	1.15±0.53	1.70±0.87	<.001
Volume	(0-5)	1.04±0.19	0.70±0.47	<.05	0.44±0.51	<.001	0.33±0.48	0.59±0.50	<.05
Interference	(0-5)	3.41±2.34	1.26±1.32	<.001	0.59±0.93	<.001	2.15±2.01	2.81±2.20	<.05
VVLQ									
Tightness	(1-7)	2.19±1.08	n/a	n/a	5.74±0.76	<.001	n/a	3.56±0.97	n/a

Data are mean±SD.

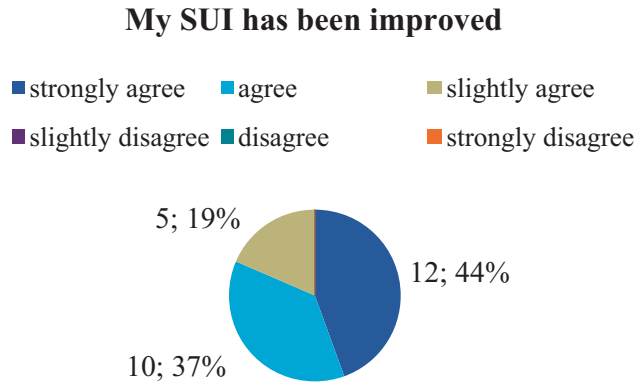

FIGURE 1 Stress urinary incontinence (SUI) improvement (Post-treatment)

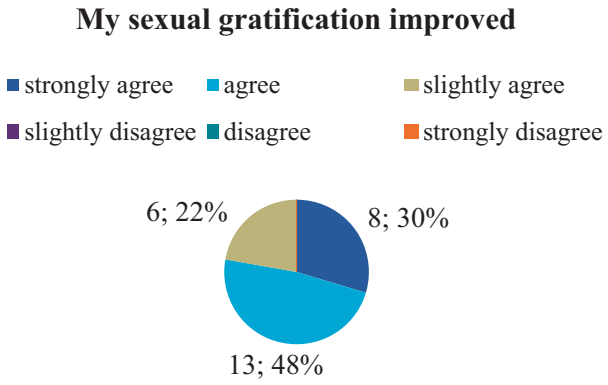

FIGURE 2 Stress urinary incontinence (SUI) improvement (1-month follow-up visit)

levels when comparing the baseline to the follow-up visit. 1 month after the last treatment, all (100%) subjects evaluated their vulvo-vaginal sensation to be slightly, moderately or very tight.

3.3 | Patient satisfaction

The data from the satisfaction questionnaire are presented in Figures 1-4.

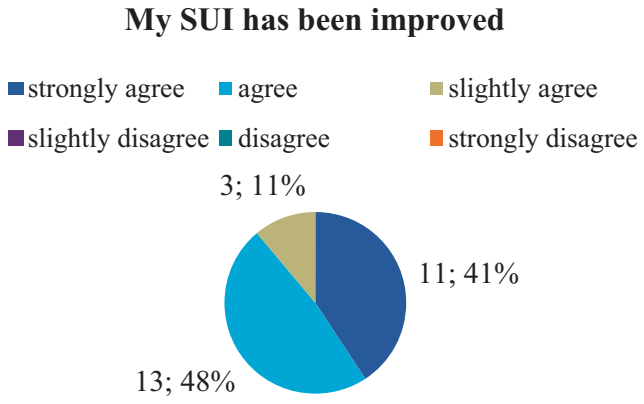

FIGURE 3 Sexual gratification improvement (Post-treatment)

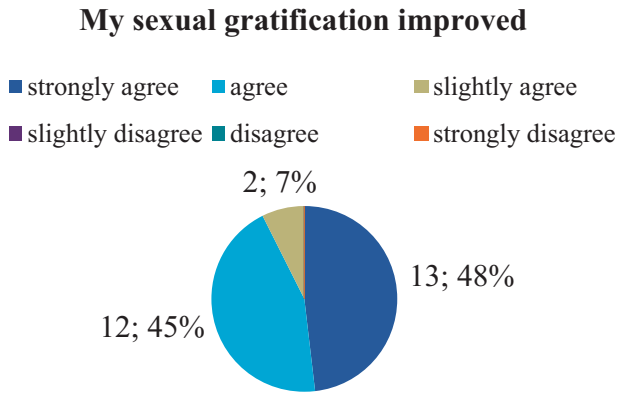

FIGURE 4 Sexual gratification improvement (1-month follow-up visit)

Eighty-one percentage of the patients "*agree*" or "*strongly agree*" that their *SUI condition* improved post-treatment compared to the baseline, and the share increased to 89% during the follow-up visit. The remaining 19% and 11%, respectively "*slightly agreed.*" None of the subjects reported dissatisfaction (score 0-3).

Seventy-eight percentage of the patients "*agree*" or "*strongly agree*" that their gratification during intercourse improved post-treatment compared to the baseline, and the share increased to 93% during the follow-up visit. The remaining 22% and 7%, respectively, "*slightly agreed.*" None of the subjects reported dissatisfaction (score 0-3).

4 | CONCLUSION

The primary goals of the study have been met as the monopolar radiofrequency treatments demonstrated good results both in terms of efficacy, and safety in all evaluated areas. The results show zero nonresponding subjects when treating vulvo-vaginal laxity and 3.7% of nonresponders when evaluating improvement in SUI in terms of frequency of leakage. Most subjects also reported decrease in the amount of leakage and improvement with the interference in their everyday life. In addition to the originally designed areas of improvement which were monitored, subjective perception of better lubrication during intercourse as a result of the treatments was reported by the majority of the patients.

Improvement in treated conditions was reported immediately after the last treatment session and was even more significant after the 1-month follow-up visit. Improvement of results with time is driven by the collagen remodeling process which takes up to 90 days to fully complete. It should be investigated by future studies with longer follow-ups to understand how the results develop over time.

Patients reported high satisfaction rate when evaluating improvement in SUI conditions and in sexual gratification. The treatments were well tolerated by all subjects; no adverse events were observed. This study demonstrates efficacy and safety of a monopolar radiofrequency for SUI and vulvo-vaginal laxity treatments. Every patient is likely to recognize the improvement at different points in time depending on their individual physiological processes. This

study captures significant improvement in the treated conditions at the 1-month follow-up visit. Although further controlled study is needed to confirm the data and evaluate the long-term effects in the endovaginal treatment.

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Short Time Efficacy and Safety of Focused Monopolar Radiofrequency Device for Labial Laxity Improvement—Noninvasive Labia Tissue Tightening. A Prospective Cohort Study

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Background and Objective: To evaluate safety and efficacy of focused monopolar radio frequency (RF) device for non-invasive labia tissue tightening and improvement of labial laxity.
Methods: This prospective cohort study participants were 17 female subjects aged between 27 and 56 years with lax skin at the labia area. All subjects received four consecutive treatments at 7-day intervals with RF device (Exilis Protege IntimaR, BTL Industries Inc., Boston, MA). The primary efficacy outcome measure was defined as one or more point improvement on 1–4 scale for vulva appearance determined by three blinded evaluators. Digital photographs were taken at the baseline and 1 month after the last treatment. Sexual gratification was assessed with Female Sexual Functioning Index (FSFI) and patient discomfort by Visual Analogue Scale (VAS).
Results: An average 2.9 (of maximum 4) points improvement rate in vulvar appearance was observed ($P < 0.01$). Mean of the total FSFI score enhanced from initial 75–87% ($P < 0.001$). Resultant 4.7 (18%) points increase was achieved. Ninety four percent of subjects reported mild to none discomfort during the treatment. No adverse events during the study course were reported.
Conclusion: The present study demonstrates the positive effect of focused monopolar RF device for non-invasive labia tissue tightening. The treatment is effective and safe with high patient satisfaction. Lasers Surg. Med.

Key words: radio-frequency; vulvar laxity

INTRODUCTION

Radiofrequency is often used in dermatology to treat skin laxity, thyrides, acne vulgaris, scaring, and cellulite [1].

Childbirth vaginal trauma and process of ageing are fundamental in developing sexual dysfunction related to vaginal and vulvar relaxation. Unpleasant aesthetic appearance of vulva is additional factor that deepens negative psychological response, embarrassment, anxiety, and lack of confidence [2]. Vaginal laxity remains usually underreported, although the majority of women patients consider this condition as bothersome with significant impact to their relationship. The visual aspect and functionality of introitus is marked most often as being responsible for sexual disorder and reduced quality of life (QoL) [3]. Although woman often will not talk about vaginal laxity with her physician for several reasons, there is a rising concern about the problem of unpleasant vulvar outlook. According to the American Society for Aesthetic Plastic Surgery annual report [4] there were 7,535 vaginal rejuvenation procedures performed in the U.S. in 2014, an increase of 48.6% from the 5,070 performed in 2013. Most of patients (55.1%) were in mid-generative phase of life (19–34 years of age). Following the recent recommendations 'cosmetic vaginal/vulvar surgery' include labiaplasty, labia minora reduction, excess or redundant clitoral prepuce reduction, labia majora reduction or augmentation, labia majora divergence repair, perineal skin reduction and mons pubis reduction [5].

However, there is a risk of serious adverse effects resulting from surgery procedures. Complications such as bleeding, infection, iatrogenic asymmetry, poor wound healing and overcorrection may require medical intervention [6]. Additional financial costs and extended recovery time have induced a growing trend in non-invasive cosmetic procedures; increase of 521% since 1997, now account for 84% of total cosmetic procedures and 42% of $12 billion in total expenditures [7].

The firmness and solidity of connective tissue strongly correlate with collagen structure and quantity as collagenesis

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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not only diminishes in the course of the aging process, but is also significantly affected by the destruction of collagen fibrils due to childbirth trauma [8]. RF has been employed widely in many protocols for skin laxity treatment [9]. By increase in temperature, RF waves break up intermolecular cross-links and stabilize collagen triple-helix structure, thus resulting in the shrinkage ad thickening of collagen fibers. Additionally, RF induced micro-inflammatory environment will stimulate fibroblasts to produce new collagen and elastin fibers via the natural wound healing response of the skin, starting averagely 1 month after the treatment [10].

The aim of this study was to assess the effects of monopolar RF delivered to the determined areas of vulva for the treatment of its laxity including independent visual evaluation and changes in sexual function/level of sexual gratification.

MATERIALS AND METHODS

Study participants were 19 female subjects aged between 27 and 56 years with lax and sagging skin at the vulvar region. We gathered information on medical history, demographics, health and reproductive factors at baseline screening visit.

Inclusion criteria for entering the study were: age 21–60 years; history of vaginal delivery; evidence of vulvar skin laxity; negative pregnancy test; normal cell cytology; a vaginal canal, introitus, and vestibule free of injuries and bleeding.

The exclusion criteria were: pregnancy and/or breast-feeding or planning to become pregnant during the study period; an active sexually-transmitted disease; acute bacterial or viral infection; implantable pacemaker or cardio converter/automatic defibrillator; malignant tumor; impaired immune system; active collagen diseases; blood disorders; anticoagulant therapy; dermatological condition requiring systemic or topical therapy in the treatment area; Isotretinoin in the past 12 months; metal implants; varicose veins; any other medical condition that, in the investigator's opinion, would interfere with the subject's participation in the study.

The study was approved by the Ethics committee of the University of Rijeka, School of Medicine in Rijeka, Croatia, and written informed consent was obtained from all of the participants prior to enrollment in the study.

The procedure was performed with the device that delivers 3.25 MHz focused monopolar RF energy by a non-invasive contact electrode with power range from 1 to 90 W, therapy circle time of 30 seconds and encompassing a treatment area of 3.2 cm^2. (Exilis Protégé Intima®, BTL Industries Inc., Boston, MA). A disposable adhesive return pad was used for the grounding. Starting parameters were the initial power of 90 W with continuous energy emission (100% duty factor). Generous amount of the ultrasound gel was applied to the treated area for close contact between the handpiece and the skin. During the treatment surface temperature of treated skin was between 40–43°C.

Each patient received four consecutive treatments at 1-week intervals for labia tissue tightening. The follow-up visit was scheduled 1 month after the final procedure. Treatment area was divided into five zones: labium major right; labium minor right; labium major left; labium minor left; and perineum/vestibular area. Following the protocol, listed areas were treated in previous order exactly, precisely focusing on one zone—avoiding extension to the surrounding regions (e.g., groin or thigh area). Probe was covered with single use hygienic membrane. Treatment was performed with smooth slow elliptical moves covering whole treated zone. Generous amount of the ultrasound gel was used to allow perfect contact between the probe tip and the skin. Each zone was treated for 4:30 minutes with total time of procedure around 24 minutes. No anesthetics were used before or during the session.

Digital photographs of external genitalia were obtained at baseline, before and after each of four sessions and 1 month after the last procedure (2 months from baseline). We used Sony Exmor R IMX145 8-megapixel camera with wide aperture f/2.4, HD (1080 p) at 30 frame/s, IR filter and standardized LED flash. Position of the fixed camera was at the distance of one meter from the patient in litothomy position. Photographs were taken by the same investigator.

The randomized images taken at the baseline and 1 month after the last procedure were evaluated by three blinded observers (two marketing experts and a physiotherapist). They were not previously trained and were not introduced in the treatment method in order to avoid any possible bias. Their evaluations were assessed, as primary outcome, on a 4-point scale of vulvar appearance (0 = no change, 1 = mild change, 2 = moderate change, 3 = excellent change) (Appendix A). Follow-up scores were compared with baseline for statistical significance.

As a secondary outcome the results of Female Sexual Functioning Index (FSFI) were analyzed at the baseline and 1 month post final treatment [11]. The FSFI is a 19-item questionnaire that has been developed as a multidimensional self-report instrument to assess the key dimensions of sexual function in women. It provides scores on six domains of sexual function: desire, arousal, lubrication, orgasm, satisfaction, and pain. The domain "pain" describes the intensity of pain during the intercourse. The higher the number is the pain is less intense. Maximal individual domain score is 6 and maximal total FSFI score is 36 points. A score $\leq$26.55 is classified as female sexual disorder [12].

Patient discomfort during the treatment was assessed with Visual Analogue Scale (VAS), 10-level rating scale (1 = no pain, 10 = worst possible pain) [13].

All statistical analyses were performed using Excel Analysis ToolPack. A statistically significant difference was set at $P < 0.05$.

RESULTS

The present study included 19 women. Two participants dropped off for reasons not connected to the study. Total of 17 participated. Their age was between 27 and 56 years, with mean age of 44.6 ± 8.6 years.

Clinical photographs assessed by three blinded evaluators showed overall improvement in vulvar appearance.

Excellent improvement was recorded in 5 (28%), moderate 8 (45%), mild 3 (20%), and no change in 1 (8%) participants (Appendix B).

Average improvement rate in vaginal appearance was 2.9 based on a four point evaluation scale ($P < 0.01$), and 92% of assessments were at least 2 or higher.

A total of 34 (17 before treatment, 17 at follow-up visit) questionnaires were completed. In all the FSFI domains statistically significant improvement was obvious ($P < 0.01$) except in "pain" domain, where improvement was also recorded, but was not statistically significant ($P = 0.061$), that is, pain during the intercourse was not the issue at the basal assessment (Table 1, Fig. 1). Seven examinees' (41%) total FSFI score before treatment was below the cut-off level (26.55) for female sexual disorder (FSD group). The average total FSFI score increased from initial 26.5 ± 4.89 to 31.2 ± 3.68 or from 75 to 87% that is a 4.7 (95%CI 3.3–6.1) point score enhancement, or relative improvement of 18% in the average. Based on a Student's t-test for paired samples the overall FSFI score improvement was statistically significant ($P < 0.001$) (Table 1, Fig. 2). Only 2 (12%) participants have not meet the clinically relevant change (FSFI total score improvement ≤ 2). Comparison was also made between FSD group and the group of patients with baseline FSFI scores in normal range. After the intervention FSD group whose average baseline FSFI score was 22.5 ± 5.09 shifted to 29.1 ± 4.83 with 6.6 (95%CI 4.1–9.1; $P = 0.001$) average score improvement or relative enhancement of 29%. On the other hand, group of patients with baseline FSFI score in normal range changed from 29.3 ± 2.0 to 32.7 ± 1.66, in the average 3.4 (95%CI 1.9–4.8; $P = 0.001$) absolute, or 12% relative improvement. Improvement of FSFI score was statistically significantly more expressed in FSD group than in the group with normal baseline values ($P = 0.011$).

No adverse events were observed during the study. Slight side effects like erythema and edema of treated tissue restituted in a few hours. The therapy was very well tolerated; based on ten point visual analog scale 94 % reported mild to no pain during the treatment. Only one subject reported moderate pain (4 of 10).

DISCUSSION

Our study showed that monopolar radiofrequency improved sexual functioning measured by FSFI. No intravaginal treatment was performed. As we reported previously, vaginal distension syndrome may be efficiently treated with Er: YAG laser [14].

Recent studies report RF use for vaginal laxity suggesting subjective improvement in self-reported vaginal tightness, sexual function and decreased sexual distress with 6–12 month effectiveness [15–19]. Millheiser L.S. et al. reported on RF treatment of vaginal introitus laxity after vaginal delivery ($N = 24$) [15]. Using the same tool, FSFI questionnaire, they found the comparable improvement of score in FSD group after 1 month follow up as we did (24.1 ± 2.4–29.9 ± 2.9 and 22.5 ± 5.09–29.1 ± 4.83; respectively) and in total sample as well (27.4 ± 3.6–31.1 and 27.2 ± 4.7–31.2 ± 3.6; respectively). In FSD group the average change in Millheiser study was 5.8 (24%) compared to 6.6 (29%) in our study. In total sample Millheiser et al. found 3.7 (14%) versus 4.7 (18%) in our research. Using Vaginal Laxity and Sexual Satisfaction Questionnaire primarily designed for their study, they concluded that responses to the questionnaires suggested subjective improvement in self-reported vaginal tightness.

Short time follow-up was set to assess the effects at the visit 2 months from the baseline. Originally, we planned second follow-up at sixth month from the baseline, but we finally decided to focus only to the short-term outcomes to prevent large lost-to-follow-up due to the obstacles with recruitment. Our decision was based on the well-known thermal energy effect that results not only with immediate break of collagen cross-links but also future process of neocollagenesis starting averagely 1 month after RF treatment [10]. Moreover, recent studies that use radiofrequency techniques for skin tightening reported the effect duration up to six [16,17] to twelve [18,19] months.

Sekiguchi et al. [15] reported long-term effectiveness of a single nonsurgical procedure with monopolar radiofrequency for the treatment of vaginal introitus laxity. They observed clinically relevant change in FSFI total score in 63% of subjects, while our results showed that 87% of participants have improved their sexual function. The difference may have occurred because we have applicate RF monopolar energy on skin parts of vulva, not to "the mucosal surface of vaginal introitus behind the hymenal ring" only [16], so the treated area was wider. Vaginal birth trauma affects not only perineal tissue but also vulva

TABLE 1. FSFI: Total and Domain Scores (Mean ± SD; Percentage Score)

	Before treatment	At 1 month follow-up	Absolute difference	Relative difference (%)	P
Desire	3.5 ± 0.93 (58%)	4.3 ± 0.74 (72%)	0.81	23	0.005
Arousal	4.3 ± 0.95 (72%)	5.2 ± 0.68 (87%)	0.94	22	<0.001
Lubrication	4.9 ± 1.08 (82%)	5.5 ± 0.64 (92%)	0.62	13	0.002
Orgasm	4.8 ± 1.24 (81%)	5.5 ± 0.83 (91%)	0.65	14	0.002
Satisfaction	4.7 ± 1.33 (78%)	5.4 ± 1.0 (89%)	0.66	14	0.006
Pain	4.9 ± 1.15 (82%)	5.3 ± 0.98 (88%)	0.37	8	0.061
FSFI total	26.5 ± 4.89 (75%)	31.2 ± 3.68 (87%)	4.7	18	<0.001

$P = t$-test for paired samples statistical significance of the difference.

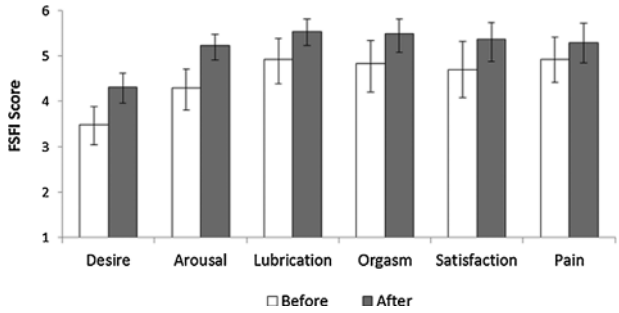

Fig. 1. FSFI domain scores before (white bars) and 1 month after the last procedure (dark bars); errorlines represent means' 95% confidence intervals.

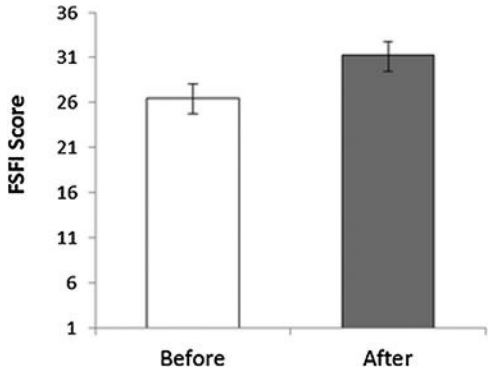

Fig. 2. Total FSFI score before (white bar) and 1 month after the last procedure (dark bar); error linesrepresent means' 95% confidence intervals.

in toto. That is, distension of connective tissue and skin will often result in certain level of vulvar laxity including not only introitus and perineal region but both pair of labia as well. Consequence is unpleasant presentation of vulva with open introitus. In our study we have employed the protocol that covers treatment of entire vulva in order to cover all the sub-regions affected through the process of vaginal birth. Also, they have found statistically borderline difference in the desire domain of FSFI at the 1-month follow up ($P = 0.043$) versus relevant difference in our sample ($P = 0.005$). Although our participants did not evaluate the clinical images before and after the intervention, these images were presented to them. That could possibly influence their sense of overall improvement in sexual gratification.

Our results may indicate that the model of wider area treatment of vulvar tissue, not introitus only that was discussed by Alisond RM [20] as well, is more effective as tightening of labia majora, labia minora and perineum/vestibulum contributes to the positive perception of aesthetic improvement.

Protocol, treatment schedule and number of treatments suggested by the various investigators is still not standardized as some authors propose even eight sessions of RF treatment for vulvar laxity [21]. The importance of uniform approach is needed for clinical recognition of the method.

Short follow up period is clear limitation of our study. Non-existence of control sham group is another shortcoming as interpretation of results may be biased by possible placebo effect.

CONCLUSION

Present study demonstrates the positive short time effect of focused monopolar RF device for non-invasive labia tissue tightening. Safety parameters of this radiofrequency system allow high patient and operator compliance. During the entire procedure the device informs the operator about the therapeutic method, the type of the therapy applied, the set power, and other necessary data. Energy emission is constant, keeping excellent control of impedance allowing optimal power delivery with maximal safety.

We believe that this monopolar RF device is a good minimally invasive alternative to treat vulvar laxity as it is safe, painless and effective. Also, there is a need for extension of follow up period and better design with a proper control sham group included. Future randomized controlled studies in this therapeutic field are needed as well.

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APPENDIX A

Please identify the improvement of the treated area according to following samples of improvement pictures.

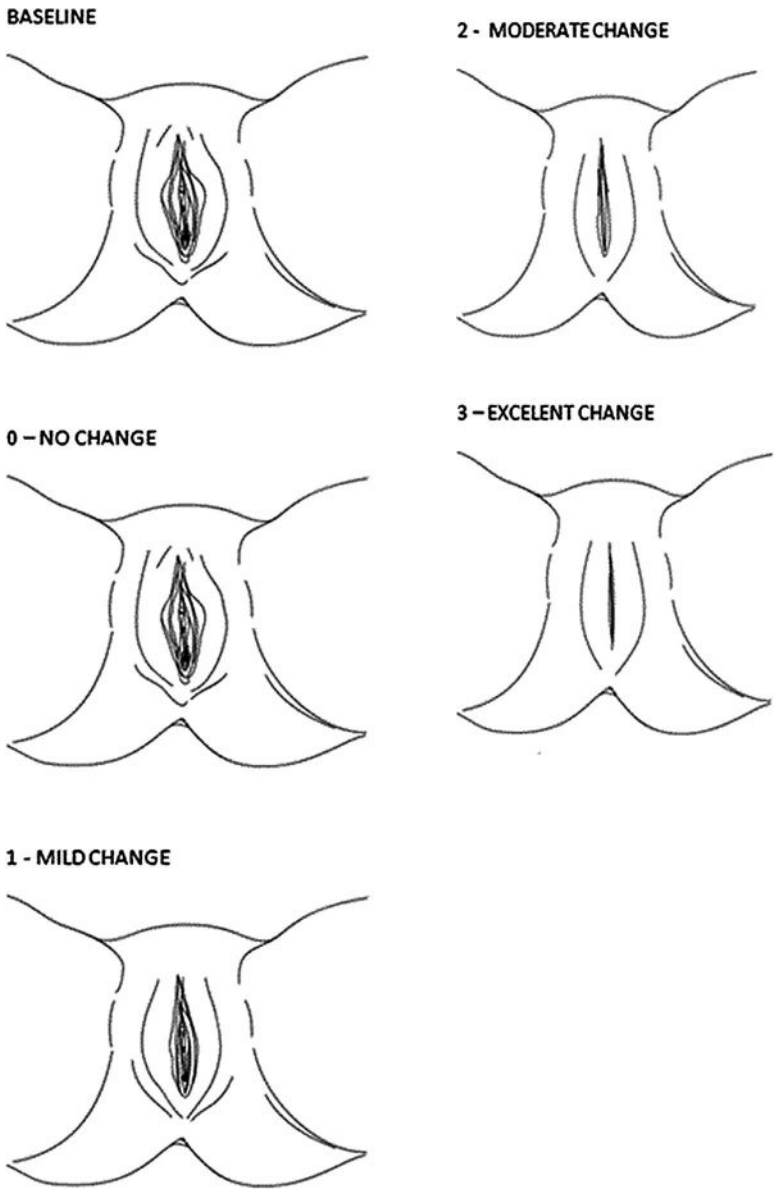

Fig. A1. Evaluation scoring map.

APPENDIX B

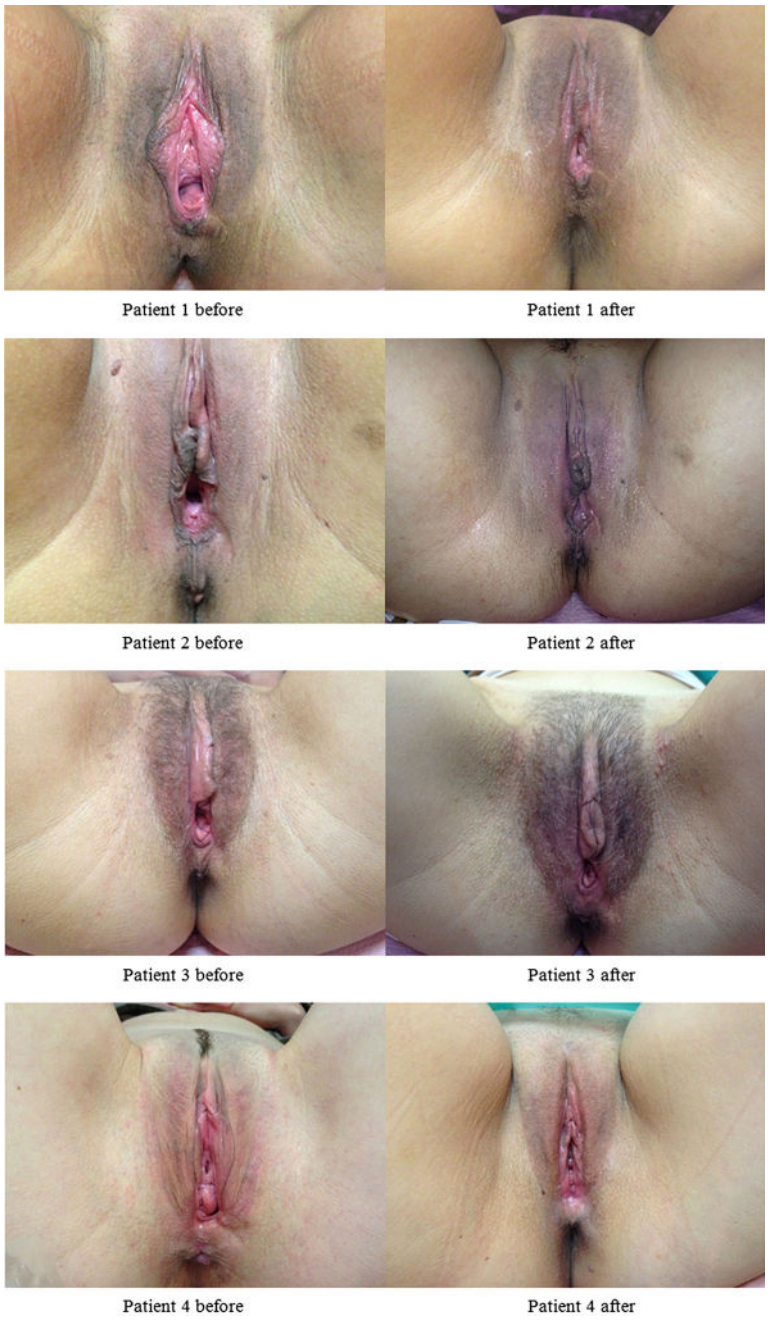

Fig. A2. Before and after photographs (samples).

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Noninvasive Radio Frequency for Skin Tightening and Body Contouring

Robert A. Weiss, MD, FAAD, FACPh

The medical use of radio frequency (RF) is based on an oscillating electrical current forcing collisions between charged molecules and ions, which are then transformed into heat. RF heating occurs irrespective of chromophore or skin type and is not dependent on selective photothermolysis. RF can be delivered using monopolar, bipolar, and unipolar devices, and each method has theoretical limits of depth penetration. A variant of bipolar delivery is fractional RF delivery. In monopolar configurations, RF will penetrate deeply and return via a grounding electrode. Multiple devices are available and are detailed later in the text. RF thermal stimulation is believed to result in a microinflammatory process that promotes new collagen. By manipulating skin cooling, RF can also be used for heating and reduction of fat. Currently, the most common uses of RF-based devices are to noninvasively manage and treat skin tightening of lax skin (including sagging jowls, abdomen, thighs, and arms), as well as wrinkle reduction, cellulite improvement, and body contouring.
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KEYWORDS radio frequency, radio waves therapeutic use, rejuvenation, skin aging

Consumer demand for noninvasive methods for skin tightening, wrinkle reduction, body contouring, and cellulite reduction is continuously growing. Patients want to look better and younger, in part due to the fear of being replaced by younger colleagues in a competitive job market. As less time for recovery means less time lost from work, patients are seeking noninvasive cost-effective procedures requiring minimal downtime to diminish skin laxity and smooth irregular body contours.

Sagging jowls are the manifestation of loss in elasticity with the resulting skin drooping, and many patients request noninvasive methods to contract or tighten the skin. These noninvasive methods include lasers, both nonablative fractional and macro spot size, focused ultrasound, and radio frequency (RF). Use of RF is typically reserved for deeper skin heating without causing ablation of the epidermis and dermis. RF devices are within the frequency range of 3 kHz to 24 GHz, which comprise the so-called ISM-RF bands, which are reserved for industrial, scientific, and medical (ISM) uses. We are most familiar with ISM bands for Wi-Fi 2.5- to 5-GHz radio bands used in the industrial sector, and this information can help explain to patients why RF is a commonplace modality. RF can be used not only to induce contraction of skin but also, in certain iterations, for reduction of fat.

By manipulating skin cooling, RF can be used for heating and reduction of fat. As the accessibility of food calories increases in Western civilization, so do the methods of reducing the effects of fat accumulation. Multiple noninvasive modalities to induce adipocyte apoptosis in pockets of fat have recently become obtainable. These modalities primarily aim at targeting the properties of fat that differentiate skin from muscle, thus resulting in the selective removal or dissolution of fat, otherwise known as lipolysis. Currently available noninvasive fat removal methods use heating, cooling, laser, RF, and ultrasound sources to more selectively target adipocytes.

The medical use of RF is based on an oscillating electrical current that forces collisions between charged molecules and ions, which are then transformed into heat. Water is the main target for this process. As a result, RF heating occurs irrespective of chromophore or skin type and is not dependent on selective photothermolysis but rather heating of water. RF-generated tissue heating has different biological and clinical effects, depending on the depth of tissue targeted, the frequency used, and the specific cool-

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ing of the dermis and epidermis. The depth of penetration of RF energy is inversely proportional to the frequency. Consequently, lower frequencies of RF are able to penetrate more deeply. RF technology also has the ability to noninvasively and selectively heat large volumes of subcutaneous adipose tissue. By selecting the appropriate electric field, one can obtain greater heating of fat or water.

RF can be delivered using the monopolar, bipolar, and unipolar devices described later in the text. The dermis is composed of collagen, elastin, and ground substances. RF-mediated thermal stimulation of this matrix results in an immediate, although temporary, change in the helical structure of collagen.[1] It is also believed that RF thermal stimulation results in a microinflammatory stimulation of fibroblasts, which produces new collagen (neocollagenesis) and new elastin (neoelastogenesis), as well as other substances to enhance dermal structure.[2] RF thermal stimulation of adipose tissue is believed to result in a thermal-mediated stimulation of adipocyte metabolism and augmented activity of lipase-mediated enzymatic degradation of triglycerides into free fatty acids and glycerol. Induction of apoptosis of fat cells is another proposed mechanism.

Methods of RF Delivery

Monopolar

The ISM bands were first established at the International Telecommunications Conference of the International Telecommunication Union held in Atlantic City in 1947. Initial use of RF for medicine included the pinpoint coagulation of blood vessels during surgery. This was the first use of monopolar RF, requiring the patient to have a grounding plate in contact with the skin. RF-induced heat ablation has been applied to other fields of dermatology, including soft-tissue (basal cell carcinoma) ablation, endovenous ablation of the saphenous system varicosities, and the treatment of vascular abnormalities. Currently, the most common uses of RF-based devices are to noninvasively manage and treat skin tightening of lax skin, wrinkle reduction, cellulite improvement, and body-contouring enhancement by influencing adipocytes. There are many devices on the market that have wide-ranging methods of RF delivery (Table 1). RF devices may be monopolar, meaning the patient is grounded and the RF is delivered through the skin, into the body, and ultimately to the grounding electrode. Typically, RF travels through structures with the highest water content with the greatest resistance of fat.

These monopolar devices may be delivered in a stamped mode in which a short cycle of 1-2 seconds is delivered while the handpiece is held in place (Thermage Solta Medical, Hayward, CA). Alternatively, monopolar RF may be delivered by either continuous movement or dynamically, where RF is delivered in a continuous pulse with constant rotation of the handpiece (Exilis, BTL, Prague, Czech Republic). In the static stamped method, a single pulse is delivered; the handpiece is then moved to an adjacent marked area and fired again. This is performed for hundreds of pulses until a premarked area is treated. Each pulse is measured for temperature while spray cooling is applied so the skin temperature does not exceed 45°C. With dynamic monopolar RF, the handpiece is continuously moved, and specific areas of laxity can be targeted in a relatively short time to reach a final temperature. The surface temperature measurements are continuously monitored, and the measurement tool is often built into the handpiece. The dynamic devices are quicker and require more technique and skill; the stamped devices are more tedious and take longer, but are easier to perform.

Bipolar

When using the bipolar method of RF delivery, the RF travels to and from the positive and negative poles, which are usually built into the handpiece. With a specific distance between the electrodes, the depth of penetration and heating is predetermined by the spacing of the electrodes and typically confined to within 1-4 mm of the skin surface. It is commonly stated that the depth of penetration is half the distance between the electrodes, but there is little evidence to support this assertion. Multiple variations of the bipolar RF concept include:

1. Fractional or fractionated RF constructed of mini-bipolar electrodes (eMatrix,e2, Syneron/Candela)
2. Bipolar-insulated needle electrodes mechanically inserted into the dermis (ePrime, Syneron/Candela)
3. Bipolar RF combined with other modalities, including diode laser or IPL (Polaris, Syneron/Candela)
4. Multiple bipolar electrodes at different distances apart firing sequentially to achieve different depths (Endymed PRO, Endymed)

Unipolar

Another form of delivery is unipolar, in which there is one electrode, no grounding pad, and a large field of RF emitted in an omnidirectional field around a single electrode. This is analogous to a radio tower broadcasting signals in all directions. Some devices new to the market are now labeled to be tripolar or multipolar but are variations of the 3 basic delivery methods (ie, monopolar, bipolar, and unipolar). Other energy sources (eg, laser or IPL) can be combined with RF. Large arrays of technologies use RF to smooth and tighten skin and reduce fat. Each of these devices have unique names and marketing associated with them, but there are individual advantages and disadvantages (Table 1). The most common uses of RF-based devices are to noninvasively manage and treat skin tightening of lax skin, including sagging jowls, abdomen, thighs, and arms, as well as wrinkle reduction, cellulite improvement, and body contouring.

RF Devices

Thermage or ThermaCool

The first device approved for RF skin contraction was the Thermage (Solta Medical, Hayward, CA) monopolar RF device, which was cleared by the US Food and Drug Administration in 2002 for sale in the United States for general sur-

Table 1 RF Devices for Skin Tightening (Courtesy of BTL, Prague, CR)

Product	Manufacturer	Frequency	Output Energy	Delivery System	Features
Monopolar Devices					
Exilis	BTL	3.4 MHz	Up to 120 W/90 W	Contact cooling	Monopolar energy flow control, safety system, built-in thermometer, no risk of overheating
CPT Comfortable Pulse Technology	Thermage Solta	6.78 MHz	400 W		New handpiece with TENS and vibrations to improve patient comfort. Pain nerval interceptors get confused and busy (vibrations, cooling, heating)
Cutera	TruSculpt	1 MHz		4″ handpiece	Handpiece that reads out once optimal temperature is reached of 43-45°C
Ellman	Pelleve	4 MHz alternating	Levels	4 small handpieces: 7.5, 10, 15, 20 mm	Several handpieces for smaller areas. Can use unit as an electrocautery unit also
Bipolar Devices					
Accent Elite	Alma	40.68 MHz	Up to 200 W	1 handpiece bipolar	
VelaShape II	Syneron	NA	IR - up to 35 W RF up to 50 W	Handpiece w bipolar RF, IR, suction	
eMatrix	Syneron	NA	25 J/cm^3	NO	
Apollo—TriPollar	Pollogen	1 MHz	50 W	3 handpieces Large, med, small	
Reaction	Viora	0.8, 1.7, 2.45 MHz	Body 50 W Face 20 W	SVC suction Vaccuum, cooling 4 modes - 0.8, 1.7, 2.45 + multichannel	
V-touch	Viora	?	?	SVC suction Vaccuum, cooling 3 handpc—0.8, 1.7, 2.45	
EndyMed PRO 3 Deep 3 Poles	EndyMed Medical	1 MHz	65 W	4 handpieces body contour, body tight, facial tightening, fractional	3 deep RF, skin tightening HP, body contouring HP, facial tightening HP, fractional skin resurfacing HP
Venus Concept - 8 Circular Poles	Venus Freeze	RF: 1 MHz, Mag: 15 Hz	RF: up to 150-W Mag flux: 15 Gauss	Large handpiece 8 poles 5 mm apart, dual mode = bipolar + magnetic field	Multipolar RF and magnetic pulse
TiteFx	Invasix	1 MHz	60 W	Bi–RF + vaccuum	Bipolar w suction real-time epidermal temperature monitor

gical use. In 2004, clearance for periocular wrinkles was obtained. The initial indication that was promoted was treatment of the forehead for eyebrow elevation. Soon after, dermatologists were testing the device for treatment of sagging jowls and skin tightening in other body areas, such as the abdomen and thighs. The US Food and Drug Administration cleared Thermage for body contouring in 2006. Larger handpieces to cover larger areas were also introduced (Fig. 1).

There are 3 components to the ThermaCool device: (1) the RF generator, (2) the handheld tip with a thin membrane, and (3) a cryogen unit. To deliver cooling, other units use Peltier cooling. Sensors in the thin membrane tip measure temperature and tissue contact. The membrane electrode is designed to disperse energy uniformly across the skin surface in a process termed capacitive coupling, which creates a zone of increased temperature at depths of 3-6 mm.[3] The depth of heating depends on the size and geometry of the treatment tip.[4] Theoretically, the device heats the dermis from 65 to 75°C, the temperature at which collagen denatures, whereas cooling allows epidermal temperatures between 35 and 45°C. Zelickson and colleagues evaluated the effects of RF (ThermaCool) on 2 samples of human abdominal skin treated with energy ranging from 95 to 181 J.[1] The treatment effect was evaluated using light and electron microscopy of punch biopsies taken immediately and up to 8 weeks after treatment. Immediately after treatment, a mild perivascular and perifollicular infiltrate was observed. At 0, 3, and 8 weeks after treatment, electron microscopy revealed collagen fibrils with greater diameter (shortening of collagen fibers) compared with collagen fibers evaluated pretreatment, up to 5 mm deep in the skin.[5]

The most recent model, the ThermaCool NXT, has incorporated additional features with several tips and handpieces. These include tips for the body and eye and a handpiece for cellulite. A new Comfort Plus Technology tip is also available, which incorporates massage with RF energy delivery, increasing the speed of the procedure yet making it more comfortable by blocking pain using vibration.

Some studies have analyzed the use of RF devices on subcutaneous fat and circumferential reduction in the size of

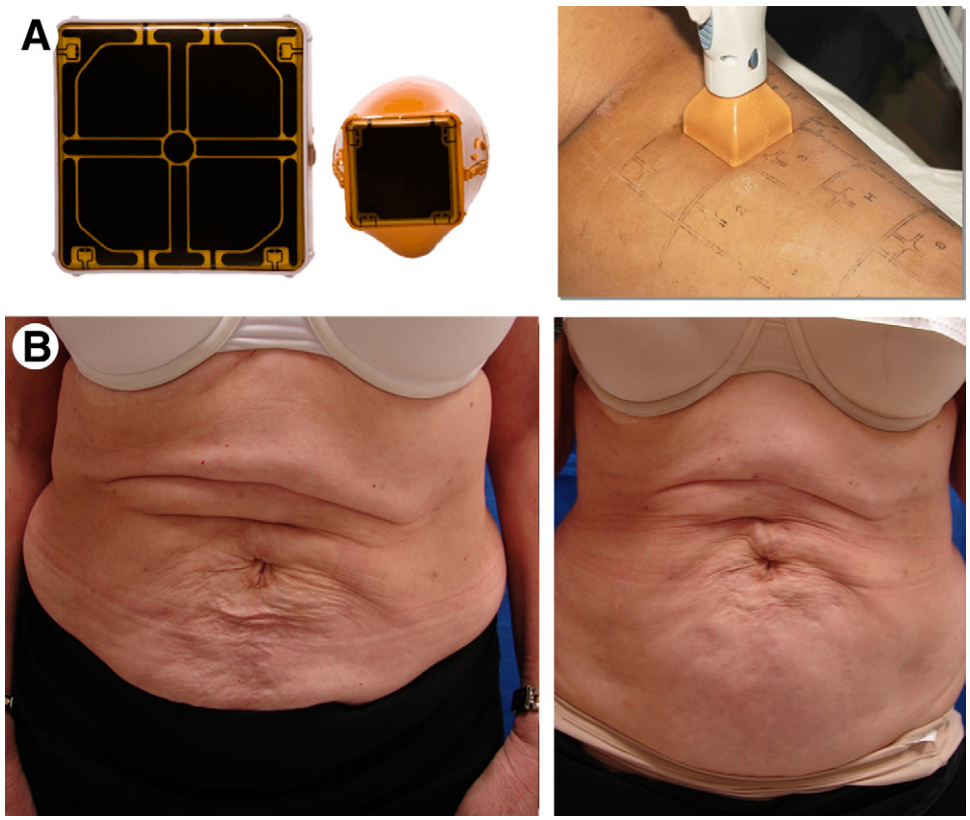

Figure 1 (A) Large (3 cm) vs small (1 cm) handpieces for ThermaCool monopolar device (Solta, Hayward, CA). Using the larger 3-cm handpiece, it is possible to treat larger areas like the abdomen. (B) Clinical result of skin tightening on the abdomen using ThermaCool device. Seen at 2 weeks after one treatment.

treated areas. A summary of studies is shown in Table 2. An interesting study used the ThermalCool TC (Solta Medical, Inc, Hayward, CA) device with the Thermage Multiplex Tip to evaluate its effect on abdominal skin laxity and waist circumference.[13] Twelve subjects were treated in this study, and results demonstrated an average decrease in waist circumference of 1.4 cm at the 1-month follow-up visit. Another study using a different monopolar RF device was able to demonstrate that adipocyte cell death occurred from thermal injury and was evident starting at 9 days after treatment.[14] Foamy histiocytic and granulomatous infiltrates were observed after cell death around the adipose tissue, but no increase in circulating lipid levels was seen.

Jacobson et al[9] treated 24 patients with laxity of the neck, nasolabial folds, marionette lines, and jawline using the ThermaCool system. Each patient received 1-3 monthly treatments that consisted of 2 passes on the forehead, 3 on the cheek, and 1 on the neck using 106-144 J. Seventeen of the

Table 2 Studies Evaluating the Effects of Monopolar RF

Authors	Type of Study	Results
Fitzpatrick et al[6]	Multicenter nonrandomized blinded clinical trial	Periorbital wrinkles improvement and brow elevation; 50% patient satisfaction
Bassichis et al[3]	Nonrandomized nonblinded	Objective improvement in brow elevation, but majority of patients do not appreciate improvement
Nahm et al[7]	Nonrandomized nonblinded split face	One side of brow treated in 10 patients, objective elevation on treated site in all 10
El-Domyati et al[8]	Histologic study	Six patients with statistically significant increase in collagen I and III, and newly synthesized collagen, while elastin was decreased
Jacobson et al[9]	Nonrandomized nonblinded	Subjective improvement in lower face skin tightening
Alster et al[10]	Nonrandomized nonblinded	Subjective improvement for cheek laxity and submental laxity
Weiss et al[11]	Retrospective chart review	Largest patient experience in >600 patients, few adverse events, all short-term, 85% patient satisfaction
Zelickson et al[1]	Electron microscopy	Abdominal skin collagen fibril contraction with tissue contraction and thermally mediated wounding, new collagen production seen

Adapted with permission from Lolis et al.[12]

24 patients showed improvement by 1 month after treatment, and results continued to improve 3 months after treatment. Transient burning pain was described by most of the patients. Patients who underwent multiple treatments and passes had greater results. Alster and Tanzi reported similar findings with the ThermaCool system, with improvement in moderate cheek laxity and nasolabial folds in 30 patients treated with monopolar RF.[10]

Weiss et al[11] published a retrospective chart review to establish the rate and seriousness of side effects, as well as patient satisfaction. More than 600 patients were treated using the ThermaCool device for mild laxity. Patients were treated with multiple passes with fluences of 74-130 J/cm^2 using a 1-, 1.5-, or 3-cm^2 tip. The most common side effects were erythema and edema lasting <24 hours. Transient erythema resolved within 5-20 minutes, with <5% reporting erythema lasting up to 72 hours. The most significant side effects occurred with the original 1-cm^2 tip and included 1 case of superficial crusting that resolved in 1 week, 1 case of a slight depression on the cheek that lasted for 3.5 months, 3 cases of subcutaneous erythematous papules, and 3 cases of neck tenderness lasting 1-4 weeks. The overall rate of unexpected adverse side effects with the first-generation device was 2.7%, but with subsequent generations and using the multiple-pass lower-energy treatment algorithm, no adverse effects have been seen. Patient satisfaction was high at 90%.

Monopolar RF has also been used to treat active cystic acne to inhibit sebaceous activity and promote dermal contouring. A study including 22 patients with moderate to severe active cystic acne reported improvement using stamped monopolar RF.[15] Patients were treated in 1-3 sessions using 65-103 J/cm^2. A 75% reduction in active acne lesion count was seen in 92% of patients, and a 25%-50% reduction occurred in 9% of patients. Often a decrease in active lesions was accompanied by improvement of underlying scarring. These results have not been duplicated in other studies.

Exilis Device

The Exilis (BTL, Prague, Czech Republic) is a novel RF dynamic monopolar device that combines focused monopolar RF delivery with a number of built-in safety features, including Peltier cooling (Fig. 2). The Exilis system delivers the energy through 2 different hand applicators, one designed for the face and one designed for the body. The goal of treatment is to raise the surface temperature to 40-42°C for 4-5 minutes for each region treated. When this temperature is reached, the patient feels a comfortably warm sensation. The handpiece is in continuous motion so the areas of skin with the most laxity can be specifically targeted. This has been termed dynamic monopolar RF. Additionally, Peltier cooling can be adjusted up or down to allow targeting of skin or subcutaneous tissue. For example, to drive heating more deeply, the skin is cooled and protected, allowing heat to reach into the subcutaneous fat. Alternatively, to get maximum effect on skin laxity, cooling is turned off and heating of the skin occurs quickly, with minimal effect on subcutaneous fat.

For the body applicator, the skin temperature is monitored and continuously displayed by an on-board infrared temperature sensor. When the device senses spikes in RF delivery, these spikes are automatically reduced. Constant monitoring of energy flow through tissue (impedance) detects tip contact with skin. The device is equipped with an energy flow control system, which automatically shuts off the device when the tip contact and/or energy flow is disrupted and virtually eliminates the risks of burns. The energy flow control allows use of high power (watts), which then leads to faster treatment times while ensuring the greatest level of safety and comfort.

This device also warns when RF is not being delivered. Experiments have shown that the increased temperature effect is seen as much as 2 cm below the skin with surface cooling (Fig. 3). The primary advantage of this system is the ability to target skin laxity or contour deformities. A precise depth of penetration combined with the focused thermal effect due to advanced controlled cooling allows total body and full face applications. A cohort of 30 patients who were treated with the Exilis device on the jowls and neck for rhytid and laxity, as well as submental fat pad reduction, were followed for 6 months (Abstract accepted for presentation at the American Society for Laser Medicine & Surgery's 33rd ASLMS Annual Conference; April 3-7, 2013; Boston, Massachusetts). The age range was 31-66 years. Additionally, 14 of the facial treatment patients were also treated for "jiggly" fat pads or loose skin on the arms between the shoulder and elbow. Circumference was measured mid-arm. The treatment target was fat pad and circumferential reduction and/or tightened skin. Patients were weighed and photographed before and after the study and were instructed to continue with their current lifestyle and not to change their nutrition, caloric intake, or physical activity routines.

The treatment protocol is 10 minutes for a 20 × 25-cm area, maintaining surface temperatures of 40-42°C, for 4 treatments, with each treatment spaced at 7-10 days. Skin temperatures at the end of a treatment cycle were typically 40°C, which rapidly dropped at the conclusion of the treatment. The patients were treated lying down comfortably, with the treatment area exposed. A water-based gel (face) or mineral oil (body) was applied to the treatment area before the onset of treatment. Baseline temperature before treatment was typically 32°C.

The energy and treatment times were adjusted according to the area being treated. For the face, typically 30 W with 100% duty cycle was used. For the body, 50-80 W with 100% duty cycle was used. The RF applicator was applied to the skin and maintained contact with the skin through each 30-second treatment cycle. Circular motions or to-and-fro motions were used to keep the tip moving over the treatment area. The key is not to allow the RF applicator to stop moving but to focus on areas of greatest concern. According to patient feedback, the energy was adjusted up or down, as tolerated, to achieve a sustained surface temperature of 40-42°C with a rapid slope up from baseline.

In a recent study, 20 subjects had 4 circumferential treatment sessions with the Exilis device for the upper arm (Abstract accepted for presentation at the American Society for

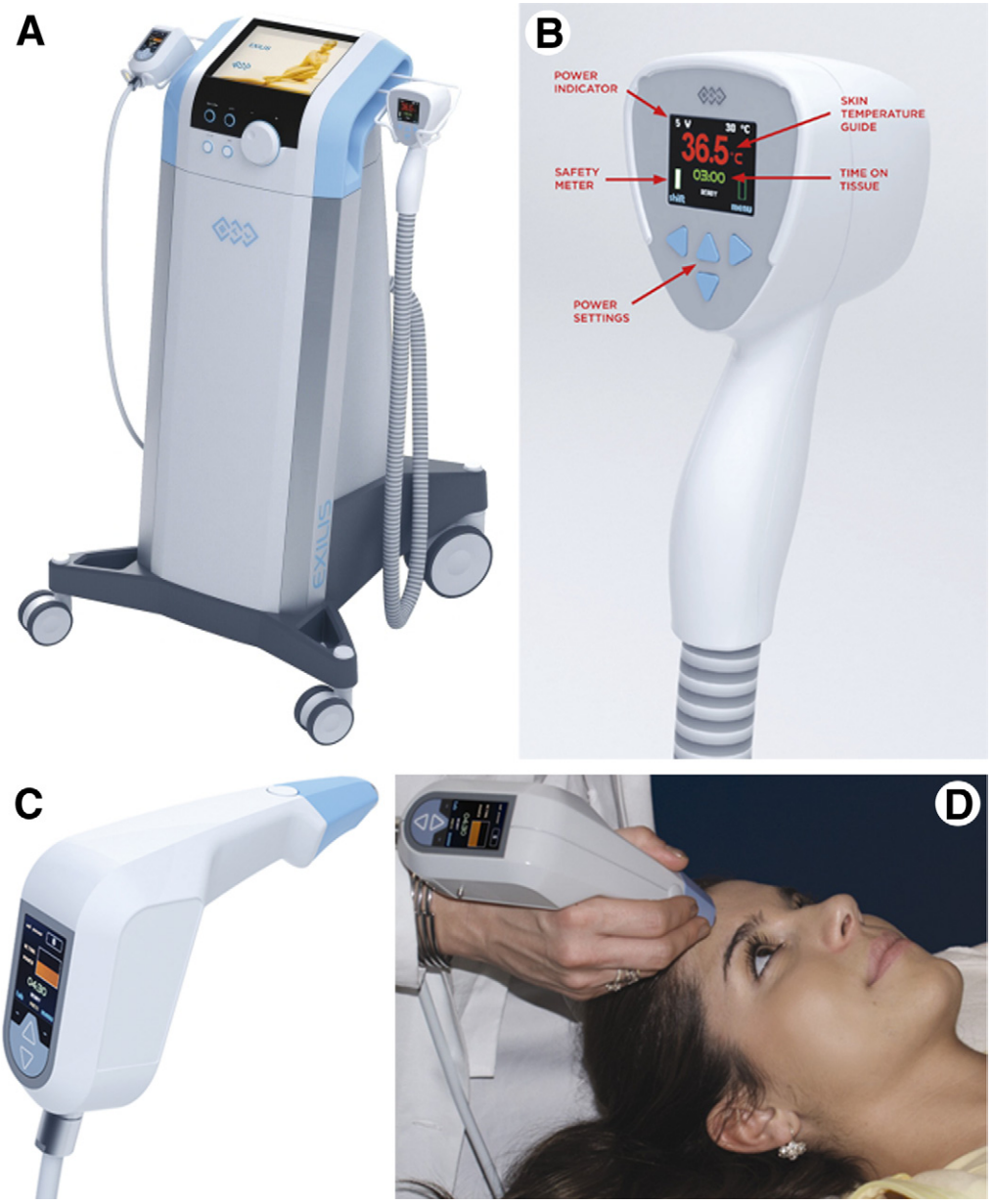

Figure 2 Device design for Exilis system (BTL, Prague, Czech Republic). (A) Unit with 2 handpieces. (B) Body treatment handpiece with temperature monitoring. (C) Facial treatment handpiece design. (D) Treatment with facial handpiece.

Laser Medicine & Surgery's 33rd ASLMS Annual Conference; April 3-7, 2013; Boston, Massachusetts). Treatment outcome was not measured by images or circumference but by ultrasound thickness of the fat layer. Measurements were taken at precise reproducible points on the arm. Authors reported average posterior fat reduction for the arm of 0.5 cm vs 0.02 for the untreated control arm. This was a statistically significant measurement of fat reduction by ultrasound fat layer thickness. Examples of clinical results for monopolar RF devices are shown in Fig. 4.

Accent Unipolar Device

The Accent (Alma Lasers, Inc, Fort Lauderdale, FL) RF system is designed for continuous skin contact using 2 handpieces: the unipolar to deliver RF energy to the subcutaneous adipose tissue for volumetric heating, and the bipolar to deliver RF energy to the dermis for nonvolumetric heating. It uses both unipolar and bipolar RF and delivers different depths of RF current to the skin, theoretically bipolar for more superficial heating and unipolar for deeper dermal heating. Several clinical trials describe its use in reducing the appearance of cellulite and its effects on tissue tightening.[16-18] In a randomized, blinded, split-design study, 10 individuals (aged 32-57 years) with a clinically observable excess of subcutaneous fat and cellulite (minimum grade 2 out of 4) on the thighs received up to 6 unilateral treatments at 2-week intervals with unipolar RF. All participants responded to a mean of 4.22 treatments, with a range of 3-6 treatments. Blinded evaluations of photographs using the cellulite grading scale demonstrated an 11.25% mean improvement. The treatment was painless, and side effects included minimal to moder-

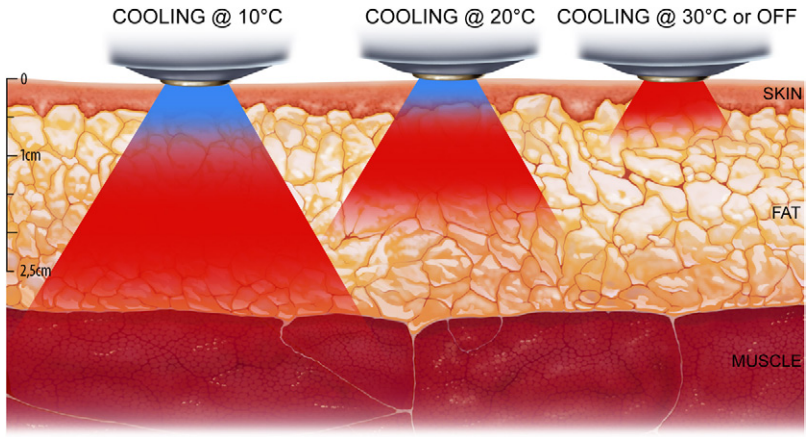

Figure 3 Monopolar RF depth can be controlled by cooling, depths of >2 cm can be achieved (Courtesy: BTL, Prague, Czech Republic).

ate erythema, which resolved within 1-3 hours. No crusting, scarring, or dyspigmentation was observed. However, clinically visible and quantified improvement did not achieve statistical significance.

Combination RF and Light (ELOS)

The most widely used combination RF systems are those that use IPL, a diode laser, or infrared light. One system (Aurora SR, Syneron Medical, Ltd, Yokneam, Israel) uses IPL as its optical energy source, with wavelengths between 400 and 980, 580 and 980, and 680 and 980 for different targets or chromophores. RF energies up to 25 J/cm^3 can be generated with dermal penetration of 4 mm.[19] Another system (Polaris WR, Syneron Medical, Ltd) is a combined 900-nm diode laser with RF energy. Optical and RF energies are delivered simultaneously through the bipolar electrode tip. Optical energy fluences range from 10 to 50 J/cm^2 and RF energies from 10 to 100 J/cm^3.[12] Another ELOS device (VelaSmooth, Syneron Medical, Ltd) uses a combination of infrared light (700-2000 nm), RF energy, and suction with mechanical massage for the treatment of cellulite.[20] All devices have been reported to lead to moderate improvement.

Bipolar RF Plus Vacuum

The Aluma RF plus vacuum device (Aluma System Lumenis, Inc, Santa Clara, CA) is composed of an RF generator, a handpiece, and a tip with 2 parallel electrodes. When the handpiece with the tip is placed perpendicular to the surface of the skin, the system produces a vacuum, which suctions a small area of skin.[21] The skin becomes a U-shaped area with

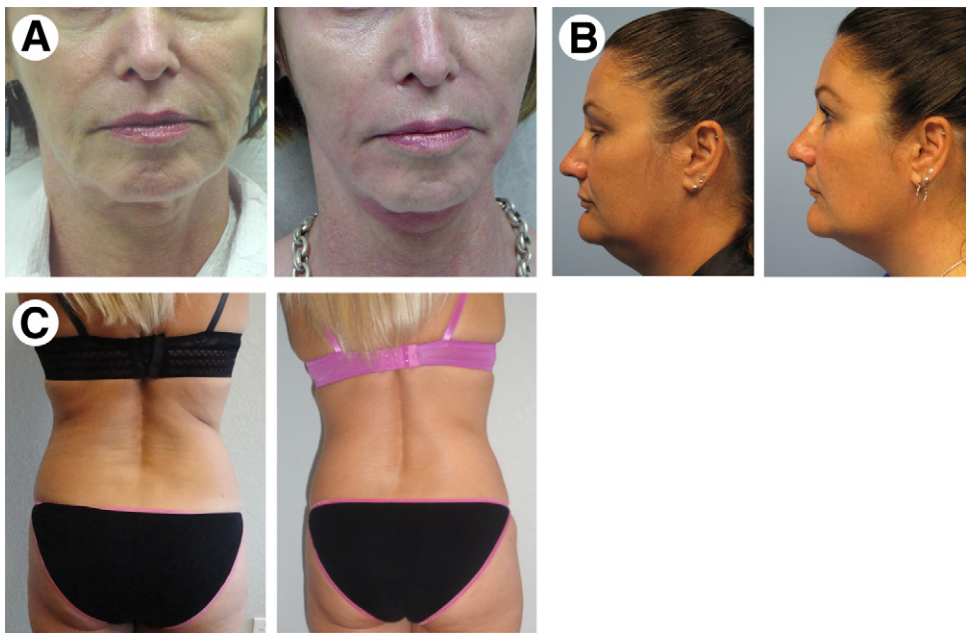

Figure 4 Clinical results with monopolar RF. (A) Before and immediately after third treatment for sagging. (B) Submental before and after 4 treatments. (C) Flanks (muffin top) before and after 3 treatments. Treatments using Exilis BTL monopolar device.

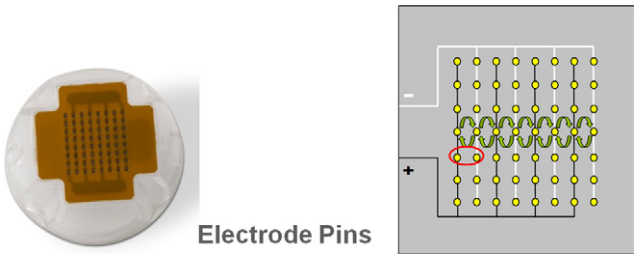

Figure 5 Fractional RF tip showing flow of current from microelectrodes. Flow is from positive to negative (Syneron/Candela).

epidermis on both sides and dermis and connective tissue in the middle. The design is to allow the energy emitted to reach the middle and deep dermis. When 46 patients with 8 facial treatments, every 1-2 weeks, were evaluated, statistically significant improvement in facial wrinkles was observed.[22] A low incidence of adverse events such as burning and crusting was reported. Another study reported clinical improvement in 30 patients treated with 6-8 cycles of the vacuum plus RF system. Patients were treated for multiple clinical conditions, including periocular and glabellar wrinkles, striae distensae, and acne scars. By histology, there was less collagen atrophy and greater interstitial edema of treated skin compared with untreated skin, which showed atrophic dermal collagen with elastotic changes.[23]

Fractional RF

Fractional RF is another form of bipolar RF delivery with mini-electrodes. The concept is that RF is omnidirectional so that dots of RF spread out from the point of contact in comparison with laser, in which the energy is attenuated in a sharp fashion in interaction with tissue (Fig. 5). Fractional RF has been used mainly for skin rejuvenation. Less than 1-mm thermal injuries are formed in a patterned fractional array directly to the reticular dermis. The area directly in contact with and below the array of microneedles or electrodes is selectively heated, whereas the areas between the targeted areas are left intact. A prospective multicenter study was conducted on 35 subjects who received 3 treatments on their entire face with a fractional device (eMatrix RF, Syneron/Candela Medical, Ltd).[24] Clinical improvement was assessed 4 weeks after the last treatment using photographic analysis. Eighty-three percent of patients showed improvement in skin brightness, 87% in skin tightness, and 90% in smoothness and wrinkling. Subjects undergoing facial treatment had minimal pain, no permanent side effects, and no significant downtime. Investigators' assessment for improvement in skin texture correlated with subjects' evaluation and was >40% for approximately 50% of subjects. Eighty percent of the subjects were satisfied with the results. Higher energy levels and lower coverage rates produced better esthetic results along with less pain.

Safety

A recent advance has been a novel kind of RF energy delivery system that allows constant monitoring of the real-time local skin impedance changes during RF skin treatment (BTL Elite, BTL Aesthestics, Prague, Czech Republic). This Impedance Compensation system controls the energy supply while the circuitry automatically compensates for impedance changes. Energy flow is controlled, and the computer automatically keeps the heating power on the optimal level even in areas of higher/lower impedance, allowing the operators' use of high power settings without compromising safety. The system enables the energy to be evenly and precisely dosed over the whole treatment area, allowing for maximum clinical efficacy. This is important because impedance varies greatly in areas of the face where the bone is close to the skin surface. Without monitoring of impedance, this may lead to overheating and blistering. Arcing and serious skin damage may occur when the contact between the applicator and the tissue is not ideal or the applicator is lifted, but with the new circuitry, energy is immediately cut off when sudden impedance changes are detected.

Conclusions

RF is commonly used for tissue heating and tightening. Competitive technologies include vacuum massage, infrared laser technologies, high-frequency focused ultrasound, cavitation frequency ultrasound, RF energy, and various hybrid energy devices combining some or all of the above. Monopolar RF excites molecules (2-3 million times per second) to create desirable heating effects on collagen and subcutaneous tissues. Many devices use a combination of heat and cooling to noninvasively deliver RF energy to specific depths in tissue, which produces a predictable response, notably collagen remodeling, to achieve desired cosmetic results for wrinkle reduction, tissue tightening, and body contouring. Monopolar RF plays an important role in our practice for treatment of sagging jowls and mild body contouring. It is a safe technology, which is continually being made safer.

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THERAPEUTIC HOTLINE

Efficacy of monopolar radiofrequency on skin collagen remodeling: a veterinary study

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ABSTRACT: The aesthetic market offers various radiofrequency treatments for the reduction of wrinkles and rhytids. Even though this not an uncommon aesthetic therapy, there is considerable lack of clinical evidence on the various energy delivery systems available (unipolar, bipolar, tripolar, multipolar, etc.). The purpose of this study was to demonstrate the efficacy of a monopolar radiofrequency device (Exilis Elite, BTL Industries Inc., Boston, MA, USA) on the skin collagen in an animal model. The study treatment was done on the abdominal area of the potbellied Vietnamese mini pigs in the Veterinary Research Institute facility. All pigs were treated once per week for 4 weeks. The treatment area was sized 20 × 10cm. The surface temperature was kept in the therapeutic interval from 39°C to 43°C and the therapy lasted for 10 minutes after reaching the therapeutic temperature. Biopsy samples were taken before the therapy and at the 3-month follow-up. The histology samples were stained and magnified (×400) before computer processing. The collagen volume was calculated using the stereological analysis and the data were statistically processed (using the nonparametric two-sample *t*-test). The collagen content tissue increased from average of 9.0% before the therapy up to 25.9% after the 3-month follow-up period. The statistical comparison of 54 samples taken before and after the treatment acknowledged the significant difference ($p = 0.018$). The stereological analysis proved large-scale improvement of collagen in the treated area. We have observed that the monopolar radiofrequency therapy significantly increases collagen remodeling.

KEYWORDS: collagen, radiofrequency, tightening

Introduction

The global market for aesthetic treatments offers various techniques for wrinkle reduction. Some of them are based on cosmetics (vanishing creams) or on manual therapy (massage), whereas others represent device-based therapies. Many established therapies typically reduce wrinkles by inducing collagen neogenesis. It is well known that an internal temperature above 42°C stimulates fibroblasts to produce more collagen; some treatments involve tissue heating to the

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clinical endpoint using different types of energy such as light, ultrasound, or radiofrequency (RF) (1).

This study shows how monopolar RF device can effect collagen distribution in the treated area on Vietnamese mini pigs after 10-minute exposure to the temperature in the range of 39°C to 43°C.

Materials and methods

The study protocol was approved by the Institutional Animal Care and Use Committee and the Ethics Committee for Animal Protection of the Ministry of Agriculture. The laboratory providing procedure operates in accordance with the Good Laboratory Practices standards. The procedures employed minimized or avoided causing pain, discomfort or distress of the animals. The animal care was in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and with the Law on the Protection of Animals Against Cruelty. The number of pigs (3) used for this study was the minimum required to meet the scientific and regulatory guidelines for this type of study.

Meyer et al. (2) showed that Vietnamese pig's skin is very similar to human skin and a good model in the meaning of function or disease. All pigs enrolled in this study were fed by cereal diet for swine (25 g/kg). All pigs were in good health conditions before and during the study duration. The room temperature was kept at 20°C and all animals were monitored continuously by the camera (2).

Treatment procedure

The therapy was administrated once per week for the period of 4 weeks. The ventrolateral part of the left flank (20 × 10 cm) was submitted to the treatment. The untreated right flank served as a control. Before the set of the treatments and after the 3-month follow-up period biopsy samples from each animal were taken from the skin (nine samples per each animal before and after treatment on the treated side as well as 3 on the untreated side before and after). The disposal circular blade (Kruse Buster biopsy punch 6mm) was rotated down through the epidermis and dermis, and into the subcutaneous fat, yielding 8 to 10 mm cylindrical core of tissue sample, and carefully removed. The tissue punch biopsy samples were taken from the treated rectangles and untreated opposite side as control samples. Cylindrical-shaped samples of the tissue were carefully removed and cut off to avoid crush artifact and damage to the fragile tissue, divided into smaller portions for different media (formol, RLT, and Lenly), and stored appropriately. The elliptical-shaped wound was made by stretching the skin perpendicular to the lines of least skin tension before incision, allowing easier closure by a single suture. For stereological analysis, the samples were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, containing 7% sucrose (2,3). The animals were treated for 10 minutes after reaching the therapeutic temperature range (39°C to 43°C). The monopolar RF handpiece with embedded loopback-based energy delivery system was used for the treatment. The average power setting was 85W with the duty factor set at 100%. To reach the homogenous clinical outcome, it was necessary that the treated area be evenly heated by the operator's movements. The surface temperature was measured using both an external infrared thermometer and an external thermal imager.

Collagen analysis

To preserve the tissue, tissue specimens were submitted to the fixation by 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). The specimens were processed (by dehydration), cleared and infiltrated with the paraffin wax, embedded in a cube, and finally sectioned by a microtome and placed on a microscope slide (27 histology samples before, 27 after the follow-up). Photomicrographs taken from the specially stained sections were analyzed using a simple and reliable software stereological method (Excilis, BTL, Prague, Czech Republic SOFO, SK). The stereological analysis (a computer-based image processing and analysis technique) was used on the skin structures in color histological sections for quantitative analysis.

The Goldner's trichrome method was performed for stereological analysis to quantify connective tissue and fat. Microphotographs were captured at the magnification of ×400. Collagen content was counted by the stereological processing software in the top of the cellular epidermis and at the dermal-epidermal junction. Consequently, the volume of the cellular layer of the epidermis, epidermal thickness and the ratio of the dermal-epidermal junction surface area to the in-plane surface area were calculated (see FIG. 1). The collagen in the tissue specimen was calculated using the pixel by pixel analysis. All images were acquired

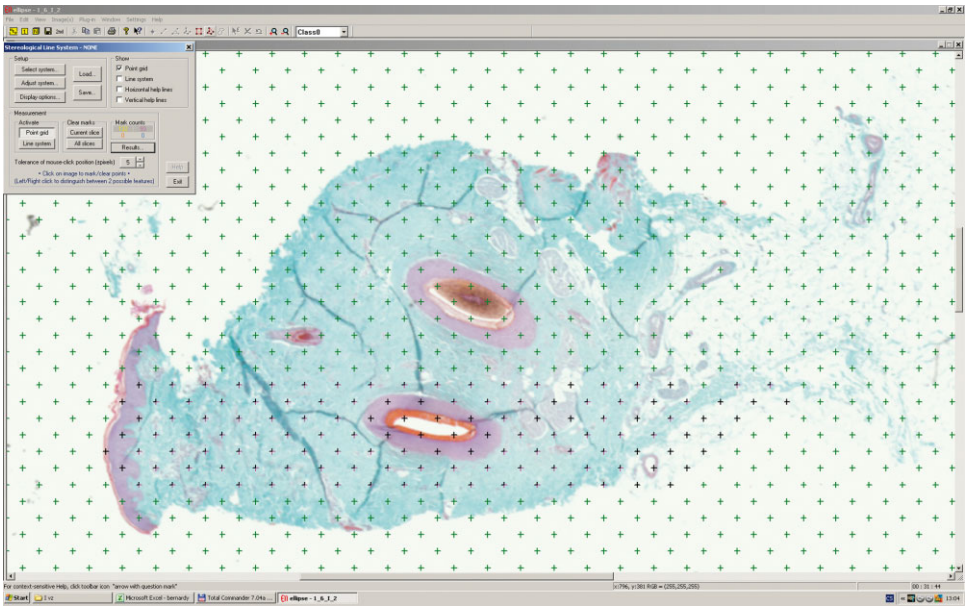

FIG. 1. Stereological analysis.

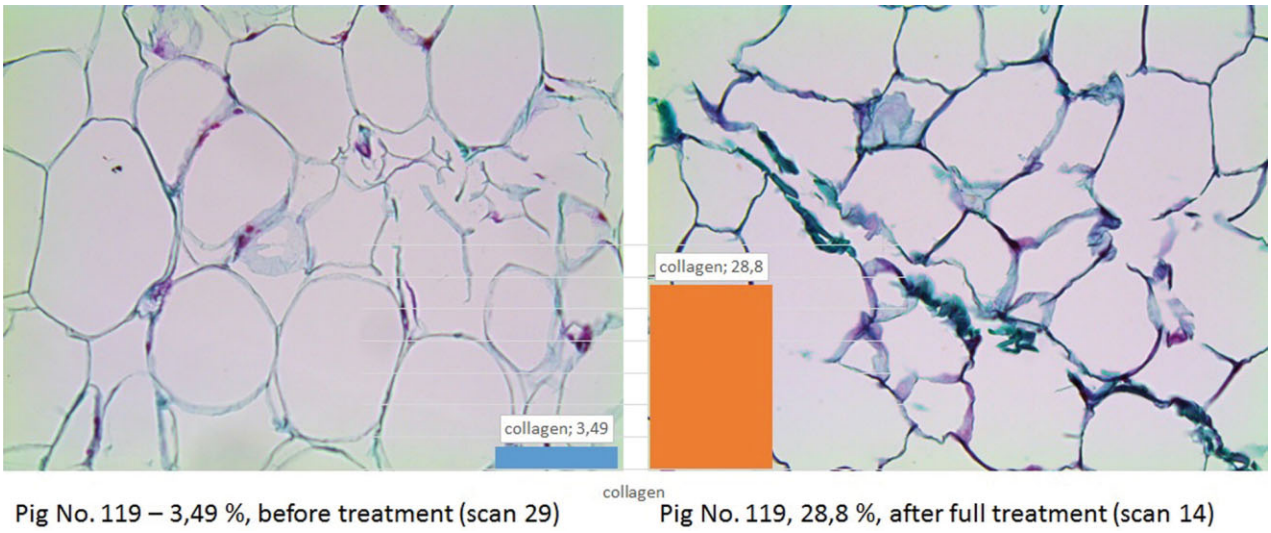

FIG. 2. Results of stereological analysis.

under the same magnification. Mean percentage of collagen was calculated, together with cellular densities and collagen densities in the papillary and reticular layers of the dermis (4,5).

Results

The stereological analysis counts collagen percentage in an observed specimen. Prior to the analysis, the collagen is selectively marked using the Goldner's trichrome method so that the computer can analyse pixel per pixel collagen density. FIG. 2 shows the collagen marked by green and the total percentage of the counted collagen in the sample.

The results are summarized in Table 1, showing average values, minimum and maximum values, and the standard deviation before the therapy and after the follow-up. Statistical calculation (nonparametric two-sample t-test) was applied on the data set and the $p = 0.018$ was calculated.

Discussion

Numerous medical devices are described as skin tightening device, among them ablative and

Table 1. Collagen in treated and untreated skin – changes after treatment

Variable collagen – treated skin	Samples *n*	Average (%)	Minimum (%)	Maximum (%)	Standard deviation
Before	27	9.0332	3.4965	16.7832	3.2522
After 3-month follow-up	27	25.8990	10.2564	51.5790	10.5163
Variable collagen – untreated skin	**Samples *n***	**Average (%)**	**Minimum (%)**	**Maximum (%)**	
Before	9	9.0332	3.1536	14.8390	
After 3-month follow-up	9	9.1597	2.9664	15.0923	

nonablative lasers, high intensity focused ultrasound, and RF from unipolar to multipolar. They are all used to heat the skin. By heating to 40–45° Celsius, heat initiates a repair mechanism laying down new collagen. New collagen production is induced resulting in tightening of the skin. Higher temperatures, in the 65° range, will denature collagen and cause contraction. In many devices, there is little evidence that the expected results really can be achieved. It is difficult to heat the dermis to a temperature that is effective and still maintain safe temperatures for the skin surface. If a critical temperature is not reached, no stimulation of collagen results. So, there is a desire to prove the efficacy of a new technology, as it was done for the device used in this study. Our findings are supported by similar results in other studies. One study quantitatively examined the effects of monopolar RF treatment on in vivo rabbit dermal collagen fibrils and the dermal response in six RF groups that underwent two passes of RF treatments (10 and 20 W). After monopolar RF treatment, the rabbit skin clearly showed changes in the collagen network structure, whereas normal group showed tangled nanostructures. Monopolar RF treatment leads to underlying collagen contracture and promotes new collagen formation. A multi-pass treatment of low-energy RF led to the highest contraction of collagen fibrils at the nanostructural level, compared with a single pass of high-energy RF (6). Another study histologically demonstrated that type I and III collagen increased significantly in the dermis after mRF treatment. The amount of stem cells did not affect the increase in collagens (7).

Conclusion

The aim of this study was to prove efficacy of the monopolar RF device on the collagen remodeling of a Vietnamese pig's skin. The value of collagen structures in the samples elevated from the average of 9.0% before the therapy to 25.9% after the 3-month follow-up period compared with no change in samples of untreated areas. The statistical significance test was calculated as $p = 0.018$ (predetermined significance level was set to $p \leq 0.05$), meaning that the efficacy of the treatment to the collagen improvement and remodeling is considered as significant.

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A Focused Monopolar Radiofrequency Causes Apoptosis: A Porcine Model

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ABSTRACT

Background: The purpose of this study was to demonstrate the effect of monopolar, focused radiofrequency (RF) with embedded cooling on subcutaneous skin structures. Specifically, the study was to prove that the monopolar RF with cooling can selectively heat fat, causing disintegration of adipocytes and programmed cell death (apoptosis) of the subcutaneous fat cells.

Methods: A non-invasive monopolar RF device with cooling (Exilis Elite, BTL Industries, Framingham, MA) was used to reduce abdominal fat in a porcine model. The study was done on 3 Vietnamese pigs in a certified veterinary facility. The treatment was delivered to an area the size of 20 x 10cm. The treatment duration was 11 minutes, 30 seconds. Biopsy samples were taken before the first treatment, 1 hour post each treatment, as well as 8 and 20 hours post each treatment. Programmed cell death (apoptosis) was monitored using the TUNEL method. The temperature was measured on the skin surface by an infrared thermal imager and built-in IR thermometer, and by an internal probe inserted into various depths of the subcutaneous layer. The internal probe placement was monitored by diagnostic ultrasound examination.

Results: The temperature in the treated adipose tissue was higher compared to the skin surface temperature. The average temperature gradient observed was 3.1°C. Due to the temperature gradient the skin surface remained intact, while subcutaneous layers showed significant changes. The TUNEL method proved large-scale apoptosis of fat cells after each treatment. The apoptotic index increased from 7% before the first treatment to an average of 53.4%, 39.6%, 40.2%, and 44.7% respectively for each treatment. In the three-month follow up the apoptotic index dropped back to 11.7%. Histology, blood biochemistry and hematology samples showed mild to no signs of inflammation in the treated area.

Conclusion: The study has shown that use of monopolar, focused radiofrequency can induce substantial apoptotic process in a porcine model. The data suggests that the monopolar, focused radiofrequency device can be used for reduction of fat and body shaping.

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INTRODUCTION

The demand for safe and effective devices for non-invasive body shaping and reduction of fat has steadily risen over the last decade. Many modalities have been developed to target adipocytes, including ultrasound, radiofrequency, and various cooling and light based devices.[1,2,5]

In this study, we evaluated the ability of a monopolar focused radiofrequency device to induce apoptosis in the subcutaneous fat. The device delivers uniform heating at controlled depths to the subcutaneous tissue, due to its adjustable built-in cooling system. The clinical efficacy was intended to safely and efficiently deliver maximum power and speed of high frequency radio waves using an active cascade of hardware and software safety elements.

METHODS

This study was carried out in a veterinary and a laboratory certified to Good Laboratory Practices (GLP) standards. Animal care was in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and with the law on the Protection of Animals Against Cruelty. The protocol of the study was approved by the Institutional Animal Care and Use Committee (IACUC) and the Committee for Animal Protection of the Ministry of Agriculture of the Czech Republic. Procedures used conformed to accepted practices and to minimize or avoid causing pain, distress, or discomfort to the animals. In those circumstances in which study procedures were likely to cause more than momentary or slight pain or distress, the animals received appropriate analgesics or anesthetics. During anesthesia the life functions and pain perception of treated animals were monitored to assure full insensibility during painful treatment and correct recovery. The number of animals selected for use in this study was considered to be the minimum (OECD Principles) number necessary to meet scientific and regulatory guidelines for this type of study.

Study Design Justification: Swine is a suitable animal model due to the similarity between human and swine dermal and subcutaneous structures. Additional anatomical similarities with humans include renal morphology, eye structure, skin, and tooth development. The pig is also one of few animals that will voluntarily eat to obesity.[7,8] The 3 study animals were housed individually and were continuously monitored by cameras. The room temperature was maintained at 20°C. Cleaning of the stall and surrounding area was performed on a daily basis. Food feeders were sanitized twice a week. During the acclimation and study period, animals were fed with complete cereal diet for swine (CDP), in the amount of 25g per kilogram of the body weight of the CDP provided per animal per day. The quality of the water was monitored during the whole study period. The acclimation period was 13 days. No prophylactic or therapeutic treatment was needed during the acclimation or study periods. Only animals in good health were used for the study.[6]

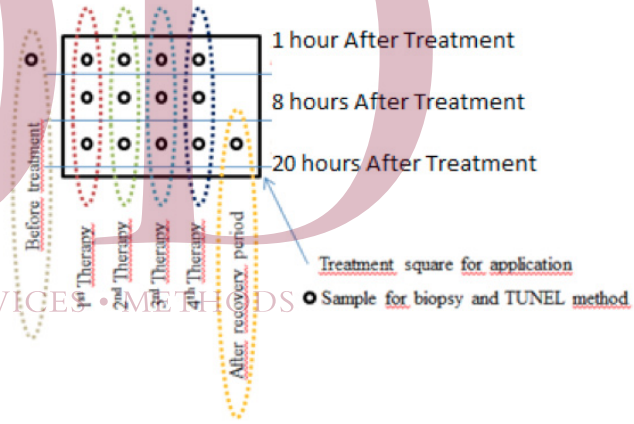
"Induction of the death of adipocytes through apoptosis is emerging as a promising strategy for the prevention and treatment of excess fat due to the destruction of adipocytes via this mechanism, resulting in reduced body fat."

Treatment Procedure: The area (20 x 10cm) of the skin was selected on the experimental animal in the abdominal region and labeled with a pen-marker (See Figure 1).

The treatment was administered 4 times with a week interval between treatments. The initial output setting was 95Watts with applicator tip cooling set at 10°C (See figure 10). The desired skin temperature was 42.5°C, minimum exposure temperature level was 39°C, and maximum exposure temperature level was 43°C. The surface temperature was measured continuously and output power was adjusted according to the temperature measurement. The internal tissue temperature was periodically measured by thermal probe during the exposure. Anesthesia was administered during each treatment, and during biopsy. Blood samples were taken before the first treatment, after each treatment, and after the three-month follow-up period. Tissue samples for the TUNEL method were taken from the treated area before the first treatment, after each treatment (3 samples after each treatment – 1 hour after therapy, 8 hours after therapy, and 20 hours after therapy), and after the three-month follow-up period. (For the location and summary of samples see Figure 2: Biopsy location in the treatment area). Autopsy and histological samples of skin, liver, kidney, and lungs were taken at the end of the recovery period.

FIGURE 1. Application area.

FIGURE 2. Biopsy location in the treatment area.



Clinical Observations

All swine were observed for clinical signs, morbidity, or mortality once a day during acclimation and during the treatment period. Onset, duration, and severity of any signs were recorded. The investigation included: changes of skin, eyes, and mucous membranes, respiratory, circulatory, and autonomic and central nervous system, somatomotor activity, and behavior pattern, changes in gait, posture, and response to handling, and the presence of clonic or tonic movements and stereotypes.

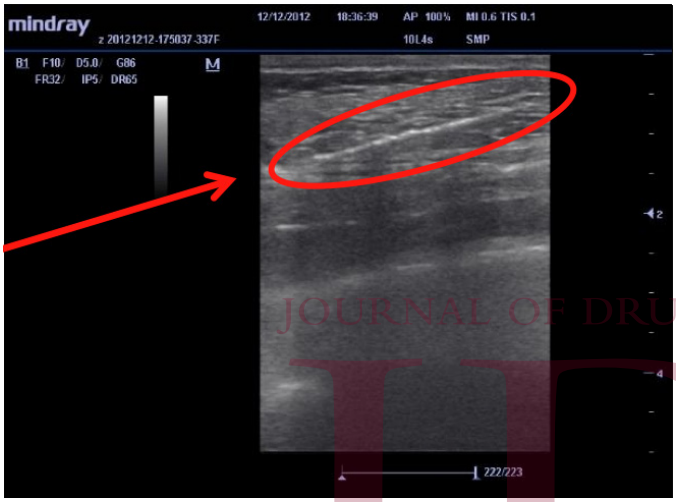
Clinical Procedures

The temperature of superficial structures was monitored by an infrared thermo imager during each treatment. The type of thermo imager used was the FLUKE Ti32. (FLUKE Corp., Everett, WA) The temperature of the cutaneous and subcutaneous tissue layers was measured by the TC-08 8-channel T-probe needle thermometer manufactured by Pico Technology Limited, UK, and placed under the control of USG Mindray M5Vet. Output power and other settings were recorded for each treatment.

Preservation of Samples

The full-blood samples were evaluated immediately, centrifuged sera was deep frozen, and punch biopsies and autopsy

FIGURE 3. Thermal probe inserted within the subcutaneous tissue.



samples of skin, liver, kidney, and lung were preserved in formalin and prepared for paraffin-embedded tissue sections for further research. For stereological analysis the samples were fixed in 3% glutaraldehyd in 0.1M cacodylate buffer, pH 7.2, containing 7% sucrose.

Histological Examination

The tissue specimens were submitted to the fixation by 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline) for tissue preservation, processing (dehydration), clearing and infiltrating the tissue with paraffin wax, embedding the specimen in a cube and finally sectioning by a microtome to be placed on a microscope slide. The specimens were further stained by haematoxylin eosin.

Cell Death Monitoring

For apoptosis (programmed cell death), formaldehyde-fixed and paraffin-embedded tissue sections were analyzed by in situ TdT-mediated dUTPX nick-end labeling (TUNEL) staining, ie, visualizing the DNA fragmentation by TUNEL kit (Apoptosis Detection Kit), S7100, Scintilla. The results were evaluated and calculated in percentage of stained cells.

RESULTS

Apoptotic Index

Apoptotic index describes the percentage of the stained cells in the specimen, which were marked as apoptotic cells. The described TUNEL method indicates apoptotic cells through color change of nucleus in the histology sample. The cells with a brown nucleus indicate apoptosis while cells with a blue nucleus indicate viable cells (See Figure 4).

Before the treatment, the average apoptotic index average was 7.0%. The apoptotic index reached average levels of 53.4%, 39.6%, 40.2%, and 44.7%, respectively, in four consecutive treatments, from tissue samples taken at 1, 8, and 20 hours after the therapy. At the three-month follow-up the average dropped to 11.7%. The average, minimum and maximum apoptotic index two weeks before therapy, during each therapy and at the three month follow up is shown in Table 1 and Figure 5.

FIGURE 4. Adipocyte (A) apoptotic nucleus is stained brown (B), other nuclei are blue (C) (400x).

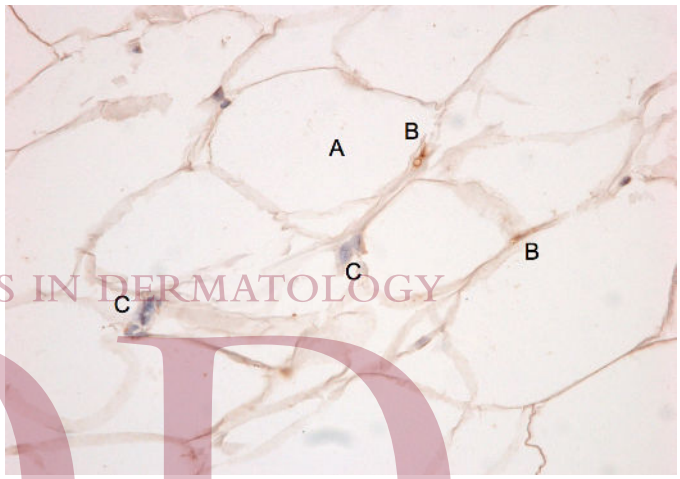


FIGURE 5. Graph of average apoptotic index.

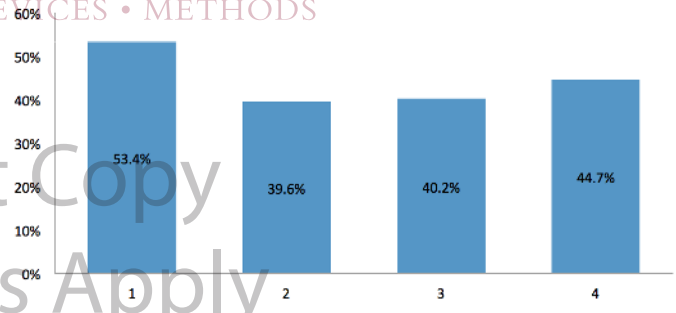


TABLE 1.

Apoptotic Index Results (%) Two Weeks Before Therapy, During Each Therapy, and at the Three-Month Follow-up

Treatment	Measurements (n)	Average Apoptosis (%)	Minimum	Maximum	St. Dev
2 weeks before	3	7.00	4.00	10.00	3.00
1	9	53.44	34.00	64.00	9.72
2	9	39.56	27.00	50.00	6.46
3	9	40.22	17.00	57.00	14.32
4	9	44.67	32.00	60.00	11.32
follow up	3	11.67	5.00	18.00	6.51

The apoptotic index values in percentages from individual animals are shown in Table 2 and Figure 6.

FIGURE 6. Graph of apoptotic index values (%) in individual animals.

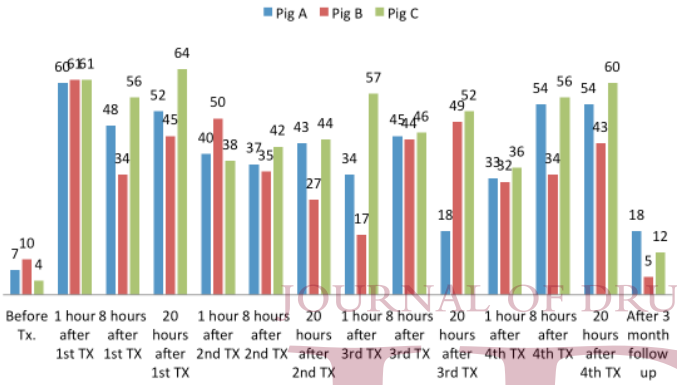


TABLE 2.

Apoptotic Index Values (%) in Individual Animals

TUNEL Test							
Time after Treatment (h)	Animal	2 Weeks Before	1	2	3	4	Follow up
1	A	7	60	40	34	33	18
1	B	10	61	50	17	32	5
1	C	4	61	38	57	36	12
8	A		48	37	45	54	
8	B		34	35	44	34	
8	C		56	42	46	56	
20	A		52	43	18	54	
20	B		45	27	49	43	
20	C		64	44	52	60	

The course of an apoptotic index of each animal after each treatment did not show significant differences, ie, there are not great rises and falls in average values of separate treatments. The comparison before (before any treatment) and after (just after the last treatment, up to 20 hours after) mean showed statistically significant results at nearly all treatments at $P \leq 0.05$ and very close to $P \leq 0.01$ in some cases.

Histology

The fragmentation of fibrous tissue within the cutaneous stroma and subcutaneous structures was visible in the histology specimens. The following images show intact adipocyte cell walls pre- treatment and decomposed or disrupted cell walls post-treatment four.

Thermal Profile Measurement

The temperature of the subcutaneous layers was measured using the 8-channel T-probe needle thermometer inserted to a maximum depth of 3cm within the tissue. Location of the probe was

FIGURE 7. Histology samples.

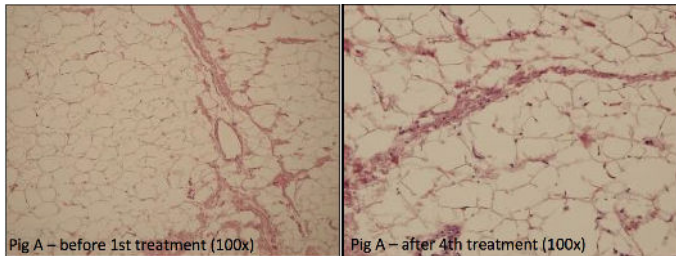


FIGURE 8. Thermal probe position verification using ultrasound measurement.

verified using diagnostic ultrasound. The surface temperature and deep tissue temperature measurement results are recorded in Figure 9: Graph of results of skin and fat temperature in time.

The measurement verified that the adipose tissue was heated more than skin during the therapy. The thermal gradient of adipose tissue and skin surface increased in time until saturation. The average thermal gradient (from 2:30 till 11:30 min) was 3.1°C. Maximum skin temperature was 43.0°C and maximum fat temperature was 45.6°C.

FIGURE 9. Graph of results of skin and fat temperature in time.

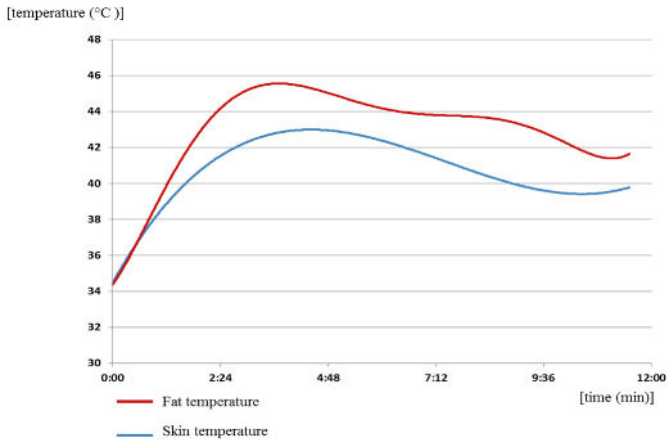
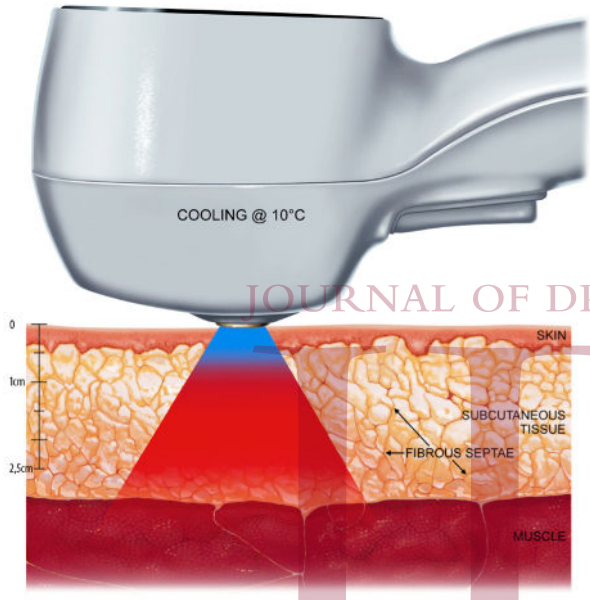


FIGURE 10. Cooling with corresponding depth of heat penetration.



Histology Results

Samples of skin from the treated area, liver, kidney and lung from all three swine for histopathological examinations were taken. No abnormalities were observed in the inner organs during pathology examination. Histology, blood biochemistry and hematology samples showed mild to no signs of inflammation in the treated area.

DISCUSSION

Apoptosis is the main mechanism for regulating cell death in many tissues. Induction of the death of adipocytes through apoptosis is emerging as a promising strategy for the prevention and treatment of excess fat due to the destruction of adipocytes via this mechanism, resulting in reduced body fat.[9]

The aim of this study was to demonstrate the effect of a monopolar radiofrequency device with embedded cooling on the cutaneous and subcutaneous structures in a porcine model. Multiple treatments of the skin and subcutaneous tissue demonstrated apoptosis in the adipose tissue. The apoptotic index increased from an average of 7% prior to start of the treatment to an average of 53.4%, 39.6%, 40.2%, and 44.7% after each of the four treatments. The thermal gradient (difference in temperature between the skin and the adipose tissue) was on average 3.1 degree Celsius and confirmed that the temperature was higher in subcutaneous tissue compared to the skin surface. The laboratory, histological or pathological analyses did not indicate any safety risks or side effects

In conclusion, the results of this study support the hypothesis that a focused monopolar radiofrequency treatment can induce apoptosis in adipose tissue via heat activation. Based on the findings of this preclinical animal study, human clinical studies looking at the improvement in body shaping as well as the potential for an effective approach for the prevention of excess body fat, is warranted.

DISCLOSURES

The authors have not disclosed any conflict of interest.

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