Dermatologic Therapy, Vol. ••, 2015, ••–••
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DERMATOLOGIC THERAPY
ISSN 1396-0296

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

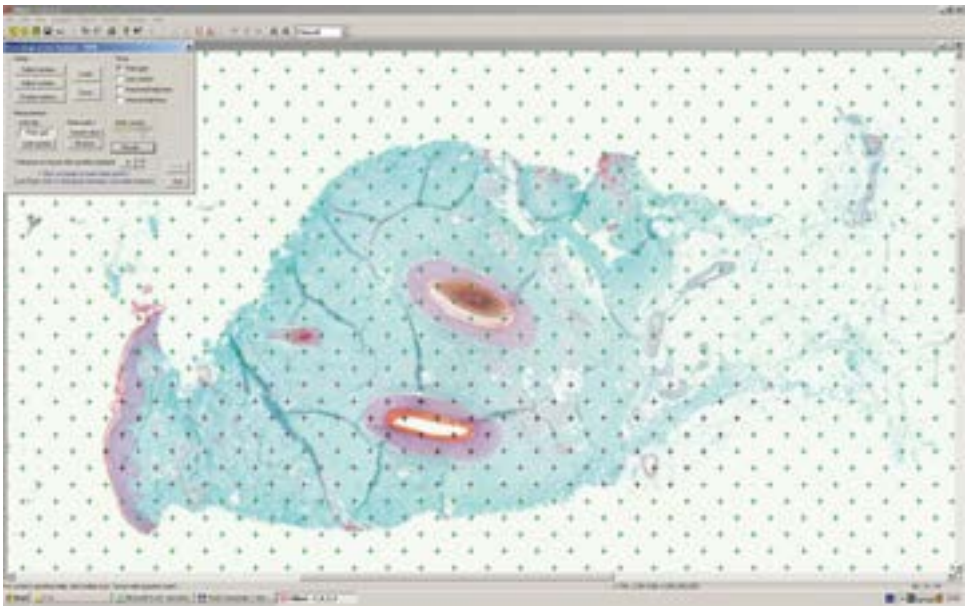

FIG. 1. Stereological analysis.

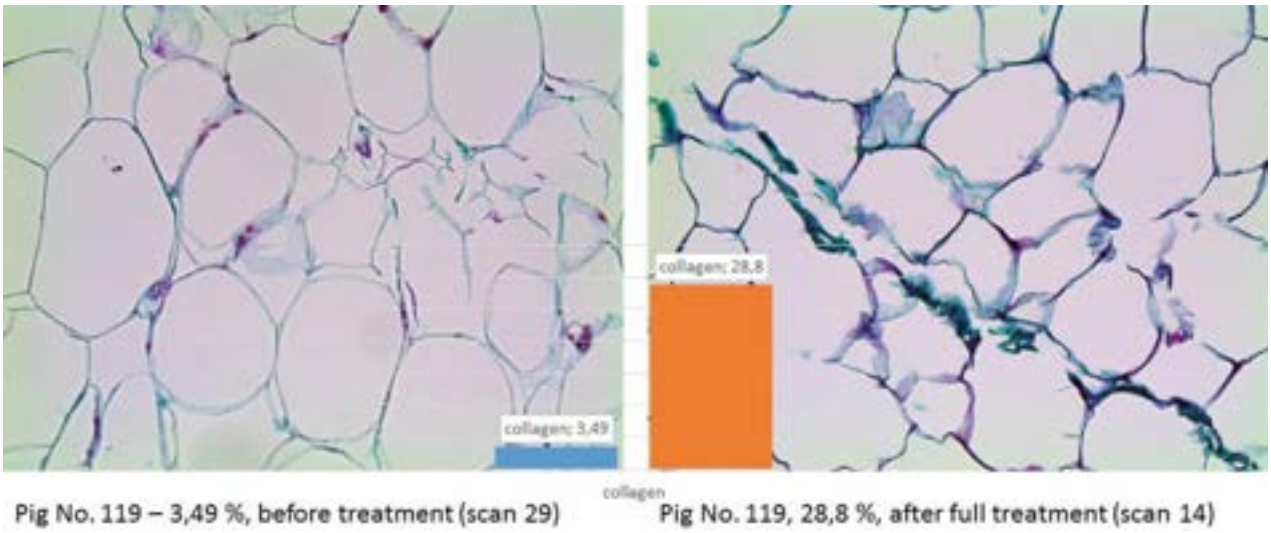

FIG. 2. Results of stereological analysis.

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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Received: 15 January 2019 | Revised: 19 August 2019 | Accepted: 4 September 2019

DOI: 10.1111/jocd.13159

ORIGINAL CONTRIBUTION

JCD Journal of Cosmetic Dermatology WILEY

Safety and mechanism of action of noninvasive radiofrequency treatment for vaginal laxity: Histological study in the swine vaginal model

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Abstract

Background: Structural changes in collagen and elastin fiber density have been previously evaluated by qualitative histological studies; however, quantitative evaluations are lacking.

Aim: To evaluate quantitative changes in collagen and elastin fibers in the vaginal wall in a porcine model after volumetric radiofrequency heating with an intravaginal applicator.

Methods: An animal model was used (domestic pig, multipara: 5.67 ± 0.94 deliveries, 3 years of age). Three pigs under general anesthesia were treated (8-minute, vaginal canal area) once per week for the course of three weeks. There were 2 follow-up evaluations at one and four weeks. Histology specimens were obtained via punch biopsy under ultrasound control. Ultrasound video measurements of the vaginal wall thickness were also obtained. Tissue samples were stained by H&E as well as stains for collagen and elastin fibers.

Results: Elastin ($P < .001$) and collagen ($P < .01$) fiber density increased after every treatment. The measured increase in fibers was highest at the one-week follow-up. Elastin accounted on average for 51.46 ± 16.86% of the tissue examined (increase of 36.8% points), while collagen accounted on average for 44.83 ± 18.92% (increase of 17.1% points). The number of synthetically active cells was increased by 16%. While vaginal wall thickness did show an increase of 1.66 mm (32%), this tendency was not statistically significant ($P > .05$).

Conclusion: Results suggest that volumetric heating of vaginal tissue produced quantitative improvement in the connective tissue organization in a porcine study. Neocollagenesis and neoelastogenesis were observed with an increased number of synthetically active cells.

KEYWORDS

histology, intravaginal, neocollagenesis, neoelastogenesis, radiofrequency volumetric heating

1 | INTRODUCTION

Biomechanical properties of the vaginal wall are defined by elastin and collagen fibers produced by fibroblasts or fibrocytes, the cells which are able to synthesize the cellular matrix.[1] Over a lifetime, a woman's vaginal wall tissue will lose elasticity and stretch as a result of decreases in both collagen and elastin fibers. Factors that contribute to this loss of elasticity and stretch include vaginal childbirth, hormonal changes caused by menopause, or age-related alternations of connective tissue.[1-3] Additionally, thinning of vaginal epithelium, changes in vaginal microflora, and pH are also observed.[4,5]

These alternations in connective tissue organization may also lead to health and psychological issues associated with the urinary-reproductive system. Women may experience dissatisfaction during sexual activity and incontinence issues.[6] These issues have generated interest from patients to search for options to reduce symptoms and restore vaginal integrity.

Historically, the options available for patients were surgical, topical, or involved systemic medications.[3] Dissatisfaction due to expense, postoperative pain, or temporary results from topical treatments has contributed to the development of new therapeutic options. Currently, there are numerous radiofrequency and laser energy-based noninvasive devices targeting vaginal mucosa for rejuvenation.[3,4]

Nonablative radiofrequency (RF) devices produce heat in the range of 40-45°C to stimulate fibroblasts within the treated tissue. Activation of fibroblasts by RF energy induces synthesis of new collagen and elastin fibers (neocollagenesis and neoelastinogenesis[3,7]) as well as cellular matrix, and they result in increased vaginal integrity. One advantage of nonablative RF treatment compared to fractional laser treatments for vaginal rejuvenation is no downtime is required.

Laser devices (CO_2 and Er:YAG) induce changes in vaginal tissue through inflammation and wound healing resulting from vaporization of extracellular water in treated tissue. Utilizing fractional beam technology, laser devices are able to create microzones of tissue injury, separated by intervening areas of untreated tissue, which hastens its recovery.[2,4]

While the effects of RF energy on collagen fiber distribution in skin have been described and quantified by previous research,[7,8] there is no available scientific literature, detailing quantitative changes in both collagen and elastin following intravaginal RF treatments. Existing histology studies have evaluated only qualitative changes in collagen density, or distribution and activation of fibroblast cells.[9] A quantitative assessment might provide insight into the physiological response of vaginal tissue to RF-induced heating.

The objective of this veterinary study was to describe quantitatively the changes in collagen and elastin fibers organization in vaginal tissue after RF volumetric heating delivered as series of consecutive treatments. To comprehensively monitor this dynamic process, the vaginal tissue will be examined after the each appointment. The domestic pig multipara vaginal model was used due to its similarity to human loose vaginal tissue and has advantages over sheep because of regional anatomic differences in collagen content.[10,11]

2 | MATERIALS AND METHODS

The veterinary study protocol was approved by the Institutional Animal Care and Use Committee, and the Ethics Committee for Animal Protection of the Ministry of Agriculture of the Czech Republic. The study was carried out in an animal house, and results were processed in laboratory (Veterinary Research Institute, Brno, Czech Republic), which operates in accordance with Good Laboratory Practices standards. All procedures were performed under standard general anesthesia and appropriately monitored by licensed veterinary physicians.

Radiofrequency treatments were administered once per week for 3 weeks, with 1-week and 1-month follow-up evaluations. Radiofrequency energy was applied using the Exilis Ultra 360 intravaginal applicator (BTL Industries Inc) which delivers 360° radial energy distribution. Treatments were performed on the proximal-mid vaginal canal area while the operator was manually and slowly moving the applicator back and forth to ensure that treated tissue will be heated uniformly. Treatment time of each session was 8 minutes. Therapy parameters were set according to the manufacturer's recommendation (energy setting at 60 units with duty factor set at 80%). The delivery of real power depends on the impedance of treated tissue. Treatment device has an impedance compensation system which controls, or tunes, the current supply and enables the consistent delivery over the entire area.

Vaginal wall biopsies (approximately from 3 to 7 cm from the vaginal introitus) were obtained for histological evaluation after each treatment and at each follow-up session using punch biopsy modified for intravaginal mucosal biopsy (4 × 8 mm Eppendorf vaginal forceps for cervical biopsy in humans). To ensure vaginal tissue is intact during the delivery of RF energy, no sampling preceded treatment. The biopsies were taken in order to minimize tissue damage while including epithelium, lamina propria, and partially also the muscular layer. All biopsy specimens were taken under sonography control, at least 1 cm apart, while the consecutive biopsies were always taken

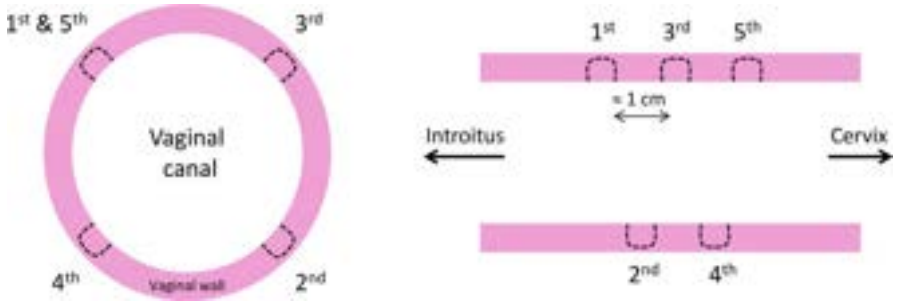

FIGURE 1 Illustrative description of sampling. There were five biopsies in each pig (after 1st, 2nd, and 3rd treatment and both follow-up sessions) spaced at least by 1 cm, taken under sonography control from the area distant up to 7 cm from introitus. The consecutive biopsies were taken from the opposite side of healthy tissue, cross-sectional view on the left side and horizontal section on the right

TABLE 1 The average amount of collagen and elastin fibers and the number of synthetically active cells (mean ± SD) after each treatment (Tx) and follow-ups (FU)

Measurement	1st Tx	2nd Tx	3rd Tx	1-wk FU	1-mo FU
Collagen fibers (%)	27.70 ± 6.03	36.02 ± 7.82	42.69 ± 15.80	44.83 ± 18.92	38.57 ± 10.55
Elastin fibers (%)	14.62 ± 8.97	37.82 ± 15.37	41.86 ± 22.97	51.46 ± 16.86	51.07 ± 15.14
Number of cells (–)	1504.60 ± 775.48	1624.18 ± 1069.14	1782.17 ± 480.97	554.00 ± 104.60	696.40 ± 262.96

from the opposite side of intact and healthy tissue to avoid the risk of inappropriate sampling as well as the impact on histological properties (Figure 1). One biopsy specimen was obtained from each pig at each sampling point.

Specimens were preserved in 10% buffered formalin solution, embedded with paraffin wax, and finally sectioned to 5-µm-thick slices by using microtome. For further examination, the slices were stained for detection of collagen (trichrome), elastin (orcein), and nuclei of fibroblasts and fibrocytes (eosin). Fifteen unique slices from one biopsy were obtained for evaluation of each quantity per sampling (N = 45 measurements in total pear each time point). Slices were distributed under random numbers to the two skilled evaluators. Each slice was computationally processed by NIS-Elements version 4.5 software and evaluated according to the selected regions of interest (ROI) with respect to lamina propria. Size of ROI ranged from 0.5 to 0.7 mm^2. The percentage of collagen and elastin fibers was determined, as well as the overall number of synthetically active cells (nuclei of fibroblast and fibrocytes).

A domestic pig (N = 3, in average 5.67 ± 0.94 deliveries) multipara vaginal model which should represent the atrophic changes in vaginal tissue (decreased amount of collagen and elastin, thinner epithelium, etc) was used. All 3 pigs were in good health conditions, individually housed, environmentally monitored, and fed by complete cereal diet for swine.

The primary data points of the study were to identify quantitatively changes in collagen and elastin fibers produced in vaginal tissue, as a response to treatment. The secondary outcome was to assess ultrasound video measurement of the pig's vaginal wall to document changes in epithelial thickness. These observations were performed on each swine and administered in the same time frame as treatments, immediately after each therapy, and at both follow-ups using GE LOGIQ e machine (intravaginal gynecology transducer, 4-10 MHz). Safety of the therapy was documented by observation of possible adverse events such as swelling and different composition of vaginal wall thickness. Additionally, an external examination has been performed (eg, redness of external genitalia, frequent urination, and hunched back posture).

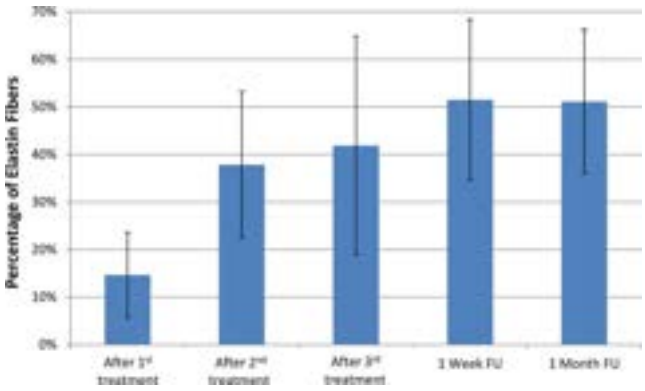

FIGURE 2 Elastin content (mean ± SD) measured after the therapies and at the follow-ups. The highest amount of elastin fibers was observed at the 1-wk follow-up

The variance between individual measurements was tested using the repeated-measures ANOVA. Post hoc analysis was performed by the Tukey-Kramer method to identify the differences between measurements separately. Statistical significance of vaginal wall thickness measurements was verified by the nonparametric Friedman test. The analysis was performed with significance level α set at 5% for all the used statistic tests.

3 | RESULTS

After the first measurement, we observed 27.70 ± 6.03% of collagen and 14.62 ± 8.97% of elastin fibers in average in the specimens, while there were 1504.60 ± 775.48 fibroblast and fibrocytes nuclei counted. The summarized results obtained during the course of study from all animals are shown in Table 1. Visualization of these results is shown in Figures 2, 4, and 6.

The average percentage of elastin fibers showed increase after each treatment session (Figure 2). The highest amount of elastin content in the specimens was observed at the first follow-up visit, when elastin on average accounted for 51.5% of the studied area. At both follow-up visits, the elastin content remained at a stable maximal value of 51%. In comparison with the first measurement, there was an overall 36.8% point increase in the elastin fiber amount at the last follow-up visit. The changes were statistically significant, with $P < .001$. An illustration of increased amount of elastin fibers is shown in Figure 3.

Similar results were obtained by analysis of collagen content, with $P < .01$ ($P = .0067$). After each treatment session, the average percentage of collagen fibers increased compared to the previous measurement (see Figure 4). The highest amount of collagen content was measured after the first follow-up. It was nearly 45% of the studied area. In comparison with the first measurement, this represents increase of 17.1% points. At the 1-month follow-up, a slight decrease in collagen fiber amount was observed, with the average dropping to 38.5%. Illustration of increased amount of collagen fibers is shown in Figure 5.

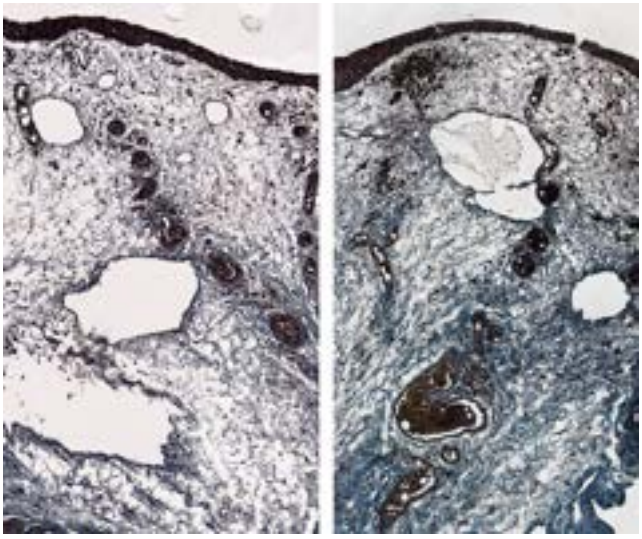

FIGURE 3 Comparison of elastin amount after the 1st treatment (left) and 1-mo follow-up (right), orcein staining. There was observed 37% point increase in elastin content (from 18% to 55%). Average increase at the 1-mo follow-up was 36.5% points

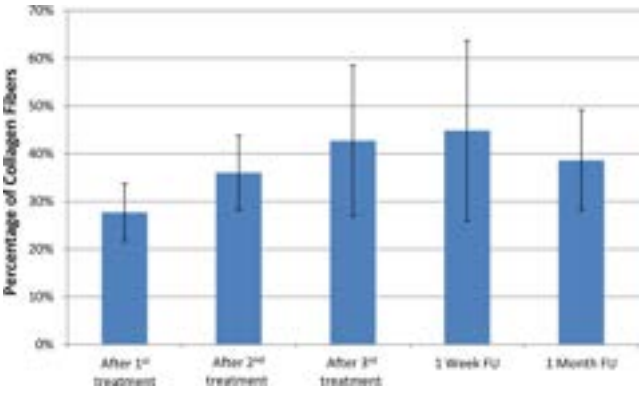

FIGURE 4 Collagen content (mean ± SD) as measured after the therapies and at the follow-ups. The highest amount of collagen fibers was observed at the 1-wk follow-up

Histological assessment of the synthetically active cells showed a statistical significant change in their average number between tissue stimulation and follow-up ($P < .001$). The highest number of cells was presented in the specimens taken immediately after the last treatment. There was an average 278 (increase of 16%) newly observed nuclei between the first (1504 nuclei) and third (1782 nuclei) measurement. At the follow-up visits when the tissue was not stimulated, we observed nearly three times fewer nuclei (average change of 288% compared to the 3rd measurement). The number of observed nuclei was constantly increasing during the course of the treatment (Figure 6).

Figure 7 shows a visible increase in the vaginal wall thickness, measured by ultrasound. These data were obtained by measurement of pig number 2. The results show a maximal increase in the vaginal wall thickness by 1.66 mm (32%) one week after the 3rd therapy. At the 1-month follow-up, the improvement slightly decreased to 1.28 mm (24%). However, in general the results of ultrasound

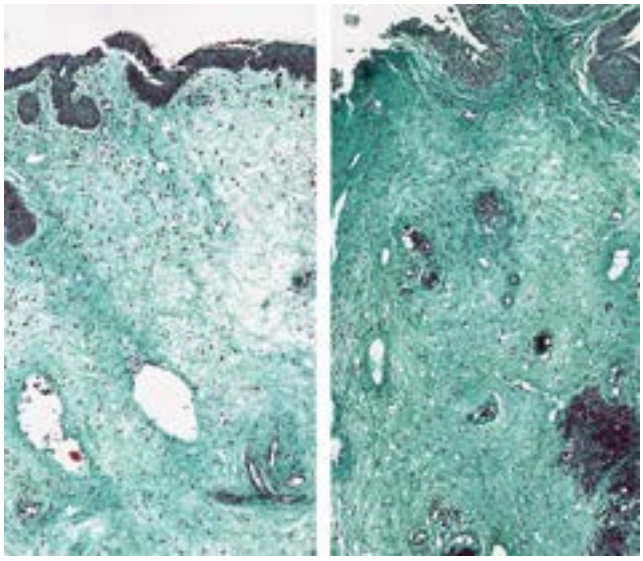

FIGURE 5 Comparison of collagen amount after the 1st treatment (left) and 1-mo follow-up (right), trichrome staining. There was observed 17% point increase in collagen content (from 26% to 43%). Average increase at the 1-mo follow-up was 10.9% points

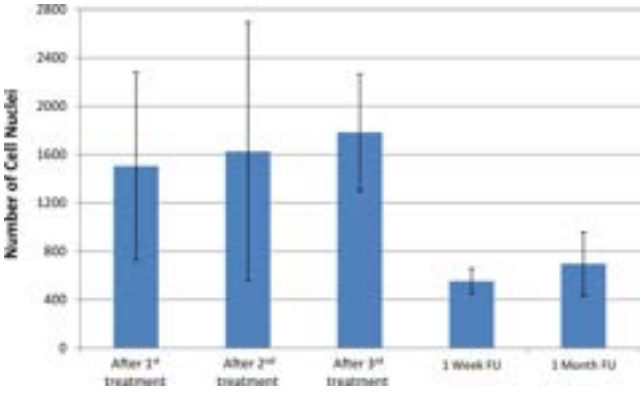

FIGURE 6 Number of cell nuclei (mean ± SD) measured after the therapies and at the follow-ups. The highest number of cells was observed after the 3rd treatment

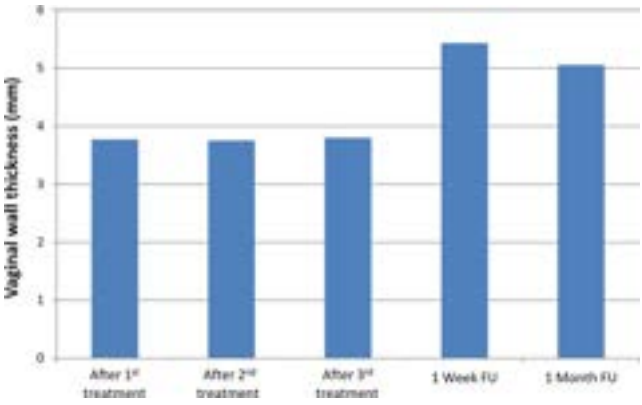

FIGURE 7 Vaginal wall thickness measurement. It was measured 32% increase at the 1-week follow-up in pig No. 2 and 24% increase at the 1-mo follow-up, respectively

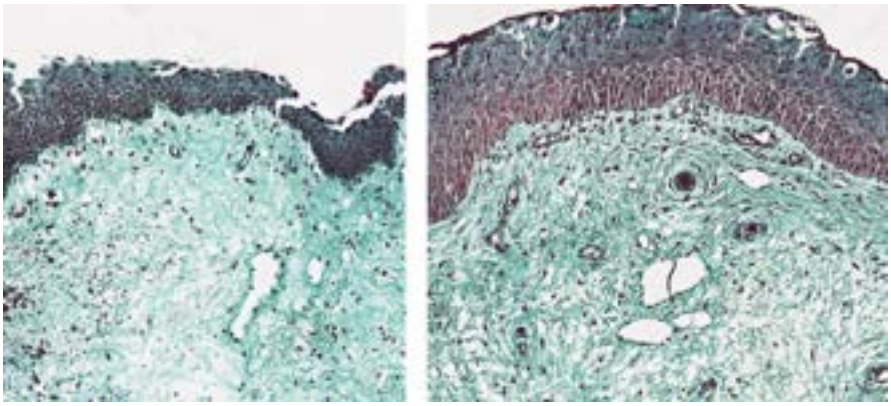

FIGURE 8 Comparison of vaginal wall thickness after the 1st treatment (left) and 1-month follow-up (right), trichrome staining. Epithelium is visibly thicker after 3 treatments. Lamina propria shows a denser arrangement of connective tissue

measurement of vaginal wall thickness did not show statistical significance ($P > .05$). Visible increase in epithelium in studied specimens is shown in Figure 8.

No adverse events were observed during or post-treatment with any of the treatment subjects.

4 | DISCUSSION

This study provided quantitative documentation of an increase in collagen and elastin content produced in the vaginal tissue of a domestic pig. Results of this veterinary study are thought to be comparable to the physiological response of human vaginal tissue, due to the biological similarities in vaginal tissue composition.[10]

We documented that RF intravaginal treatment demonstrated a statistically significant increase in collagen and elastin density and increased number of fibroblast and fibrocyte nuclei in domestic pigs' vaginal tissue. The RF treatments resulted in increased amounts of both collagen and elastin. As can be seen in Figure 3 and Figure 5, at follow-up the lamina propria appears to be more compact with a denser arrangement of fibers. Similarly, the improvement in mucosal architecture with denser lamina propria was also observed in the human patients who received Er:YAG treatment.[6]

Directly after each RF treatment, an increased amount of synthetically active cells in the upper layers of the vaginal wall was documented. The results imply that activation and recruitment of the synthetically active cells are possibly linked to the application of RF-induced heating. This might be explained by the local accumulation of fibrocytes, the circulating spindle-shaped cells which contribute to the tissue remodeling due to their rapid recruitment.[12] In addition, after the adequate stimulation, fibrocytes revert to the fibroblast state, characterized by enhanced synthetic activities. As a result, the neocollagenesis and neoelastogenesis are triggered due to the micro-inflammatory stimulation of synthetically active cells.[12,13] The largest increase was observed after the last treatment (see Figure 6) as there were an average 1782 cells in examined samples. This cellular increase is likely responsible for the early increment of the collagen and elastin fibers. These new fibers are essential for enhanced vaginal wall tightness and improved strength.[14] At follow-up evaluations, there was no stimulation of vaginal tissue, and fewer cells were present. Although fewer cells were observed, the results showed that basis for collagen and elastin fiber formation had been already laid during the course of treatment sessions. At the 1-month follow-up, the amount of elastin content was maintained, while collagen amount showed slight decrease yet still substantially elevated levels.

de Landsheere et al[1] suggested that the density of elastin fibers is primarily responsible for the vaginal wall stiffness. This relation between the density of fibers and rigidity of the tissue was most evident when evaluating lamina propria layer compared to other layers of vaginal wall. Results obtained by the histological examination revealed an average 36.8% point increase in elastin fibers when compared to the first measurement. Due to similarities in both porcine and human vaginal wall anatomies, one can infer that human vaginal tissue should demonstrate increased vaginal wall stiffness due to synthesis of elastin fibers (besides neocollagenesis).

Some of the achieved results visualized in Figures 2, 4, and 6 showed a certain amount of variability, which is expressed by the standard deviation. This is most probably caused by preparation of samples to computational examination and complicated procedure workflow. Prior the ROI selection, the samples must be fixed in formaldehyde, embedded in paraffin, cut into slices, and stained. The software-based evaluation depends also on the operator-dependent selection of ROI.

Data set obtained from the ultrasound measurements of vaginal wall thickness was not large enough to document the statistical significance despite an increased measurement. There was observed visibly thicker and more cellular vaginal epithelium as it is shown in Figure 8. At the 1-month follow-up, the well-developed larger epithelial cells with greater amount of cytoplasm content are noticeable in comparison with the smaller and rounder cells with less cytoplasm observed after the 1st treatment. The maximal measured increase in vaginal wall thickness was 32% 1 week after the last therapy and 24% at the last follow-up (see Figure 7). No measurements of vaginal wall thickness were obtained from biopsies to correlate these findings as specimens did not include the muscular layer of the vaginal wall in full, the adventia layer was not assessed as well.

Responses of vaginal tissue to RF or laser treatments have been documented by previous animal and human histological studies, but only qualitative changes in its composition were evaluated.[2,6,9,15-19] Therefore, it is difficult to compare the efficacy of individual treatments. Furthermore, according to Vos et al[9] it is gentler toward the tissue to use a nonablative source of energy rather than high-energy, ablative devices. Tissue is stimulated gradually without interruption of the epithelial surface in contrast to reparative healing of the ablated tissue.

Findings of previously published studies have confirmed that treatments by energy-based devices (RF and lasers) lead to activation of fibroblasts, production of new extracellular fibers, and overall changes in density. According to patient self-evaluation from human histological studies, the achieved remodeling of the vaginal tissue results in tissue tightening, significant improvement of the vaginal wall relaxation, and an increased comfort during sexual intercourse.[6]

The major limitation of this study is absence of baseline evaluation. Prior to the experiment setup, sampling was broadly discussed by the operating team. While the vaginal tissue is rich on the capillary network, we decide to not obtain samples before the therapy to prevent any possible influence of the results and by blood effusion. Therefore, herein published results document only the post-treatment changes in vaginal tissue including its composition and viability. Besides the baseline evaluation, the future studies might also include longer follow-up observation to examine the durability of the increased elastin and collagen amount in porcine vaginal wall.

5 | CONCLUSION

This study quantitatively documented the ability of volumetric radiofrequency heating by the Exilis Ultra 360 intravaginal applicator to remodel vaginal tissue in a domestic pig model. Neocollagenesis and neoelastinogenesis were observed along with increased number of fibroblasts and fibrocytes as a response to treatment. Vaginal wall thickening, which was documented by the ultrasound measurement, was not statistically significant. Further studies focused on measuring vaginal wall thickness following RF therapy are required to verify this upward tendency.

CONFLICT OF INTEREST

The authors have no commercial interest in BTL and received no compensation for this study. David E. Kent and Jan Bernardy have no relevant conflicts to declare.

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How to cite this article: Kent DE, Bernardy J. Safety and mechanism of action of noninvasive radiofrequency treatment for vaginal laxity: Histological study in the swine vaginal model. *J Cosmet Dermatol*. 2019;00:1–6. https://doi.org/10.1111/jocd.13159

Accepted: 23 September 2017

DOI: 10.1111/jocd.12448

ORIGINAL CONTRIBUTION

WILEY JCD Journal of Cosmetic Dermatology

Treating multiple body parts for skin laxity and fat deposits using a novel focused radiofrequency device with an ultrasound component: Safety and efficacy study

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Summary

Background and objectives: Growing demand for noninvasive skin tightening and reduction in fat results in an increasing pressure for devices with good clinical efficacy, consistency of results, and high patient comfort. The objective was to validate clinical efficacy and versatility of a novel device, which combines radiofrequency (RF) and ultrasound for treating skin laxity and fat deposits.

Methods: We treated 34 subjects with facial skin laxity and/or abundant body or arm fat deposits. Subjects were divided based on their indications. Ten subjects received treatments to the face, 7 subjects to arms, 8 subjects to thighs, and 9 subjects on abdomen. All patients received 4 treatments on a weekly basis. Photographs of patients were assessed by blinded evaluators to recognize the baseline images from the 3-month follow-up images. Patient comfort and satisfaction were evaluated using a 5-point Likert scale questionnaire. Any adverse events were recorded.

Results: Patient images were correctly recognized in >90% of cases in all study groups. Patient questionnaires showed overall satisfaction with the therapy course and results. On a scale of 1 to 5, the patients agreed (4.1) that they are satisfied with the results that the treatment is comfortable (4.1) and that they are satisfied with the treatment time (4.1). No adverse events were reported.

Conclusions: Consistent clinical efficacy was confirmed across all the treated areas, together with high patient comfort and satisfaction. We conclude the device is a highly versatile solution that can deliver results across body parts and different indications.

KEYWORDS
body contouring, non-invasive, radiofrequency, skin laxity, ultrasound

1 | INTRODUCTION

Ever growing demand for safe and effective devices for noninvasive body skin tightening and reduction in fat has dramatically risen over the last decade. Various modalities have been developed to target subcutaneous tissue as well as deeper layers of adipocytes. These primarily include ultrasound, radiofrequency (RF), and various cooling and light-based devices.[1-3]

Radiofrequency has been used in medicine for many years to ablate tissue. Oscillating electrical current is created by the RF, which induces collisions between charged ions and molecules in the tissue, resulting in generation of heat.[4,5] The biological effects of

tissue heating vary, depending on the frequency used, depth of delivery, and selectivity achieved with skin cooling.

Radiofrequency can also be used to heat and destroy fat. Heating of adipocytes with RF increases adipocyte apoptosis as well as lipase-mediated enzymatic degradation of triglycerides into free fatty acids and glycerol.[6]

Ultrasound utilizes mechanical compression or sound waves above the audible range and is characterized by its frequency and intensity. Waves propagate through the tissue causing molecules to oscillate. This mechanical effect can translate into heat in a similar way to RF.

The appearance of the face and neck is profoundly affected during the aging process. There is decreased tissue elasticity coupled with changes in facial volume, that is, compounded by the effects of gravity.[7] RF treatment of skin produces temporary shrinkage of collagen fibers and stimulates new collagen and elastin production. The amount of tissue contraction and remodeling is dependent upon the maximum temperature reached, the length of time the temperature is maintained, and the conductivity and age of the tissue. RF mediated thermal stimulation of the dermal matrix comprised of collagen, elastin, and ground substances results in an immediate change in the helical structure of the collagen.[8,9]

The investigated device (BTL Exilis system, BTL Industries) combines RF and ultrasound in each of the system's two applicators designed for a wide range of facial and body treatments. The ultrasound component is intended to alter the impedance of the tissue, increase cell permeability, and allow for better penetration of the RF energy to deeper layers. The manufacturer has also recently adjusted the facial applicator tip, which now emits the energy in a 360° manner. This allows delivering more energy to the tissue and helps treat therapeutically problematic areas such as periorbital zone very close to the eyes.

It is the purpose of this study to investigate the clinical versatility of the device stemming from its novel design, as most published studies on the efficacy of noninvasive RF procedures are based on treating subjects on a single body area only.

2 | MATERIALS AND METHODS

Our study enrolled 29 female and 7 male subjects with 34 completed. Two subjects did not finish the treatments for reasons not related to the study. Subjects were between 33 and 60 years of age (average 43) who exhibited mild-to-moderate laxity in the face and/or abundant abdominal, thigh or arm fat deposits. Based on the presence and severity of their indications at baseline, subjects were divided into 4 groups: Group A (10 subjects) was treated for facial laxity, Group B (7 subjects) was treated for fat deposits in arms, Group C (8 subjects) was treated for fat on thighs, and Group D (9 subjects) was treated for abdominal fat.

All subjects received 4 treatments administered 7 (±2) days apart using the monopolar RF and ultrasound system. Standard treatment protocols were used and were as follows: 45 minutes per treatment with the starting energy setting of 90 units for facial skin laxity treatment (full face), 30 minutes per treatment with the starting energy setting of 80 units for arm fat, 30 minutes per treatment with the starting energy setting of 100 units for fat in thighs, and 20 minutes per treatment with the starting energy setting of 120 units for abdominal fat treatment. The power settings were titrated based on the subject's verbal response for heat tolerance.

The face treatment was administered as follows: (i) from frontal area to periorbital area, (ii) from submalar region to mandible, (iii) submentum to midline. The fat deposits treatment was administered using slow circular motion across the entire treated area. Temperature of the skin was maintained at 42-43°C during every treatment, monitored using an external infrared thermometer. No topical anesthetics or oral pain medications were used.

Subjects were consented and had their medical histories taken.

The primary objective was to assess treatment efficacy using blinded expert evaluation of digital images. Photographs of appropriate areas were taken, and hard copies were generated on a 4″ × 6″ sized paper for printing at 300dpi resolution or higher. Images were randomly re-numbered, and evaluators scored each photograph as "B" for BEFORE and "A" for AFTER. The evaluation was statistically analyzed.

The secondary objective was to validate clinical efficacy across all the treated areas based on subjective patient satisfaction. A 5-point Likert Scale survey was completed at the 3-month follow-up and included the following questions: (i) I was satisfied with the treatment results; (ii) I found the treatment comfortable; (iii) I was satisfied with the overall treatment time. Patients rated their level of agreement with these claims using the following possible answers: strongly agree (5) – agree (4) – undecided (3) – disagree (2) – strongly disagree (1).

3 | RESULTS

3.1 | Evaluation of photographs

Photo assessment by blinded expert graders resulted in a total recognition rate of 92.16% (weighted arithmetic mean). This represents a very low percentage of nonresponding patients. Images

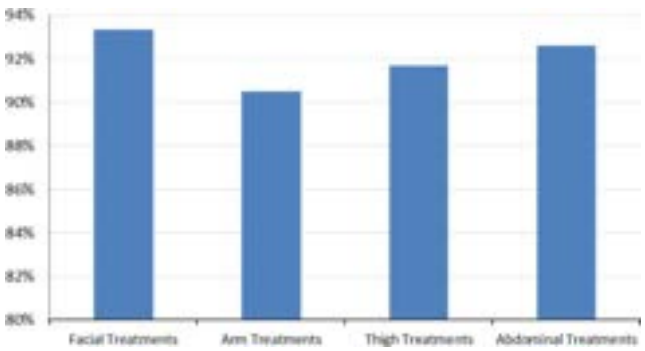

FIGURE 1 Recognition rate of aesthetic improvement in the treated area as per reviewers' evaluation

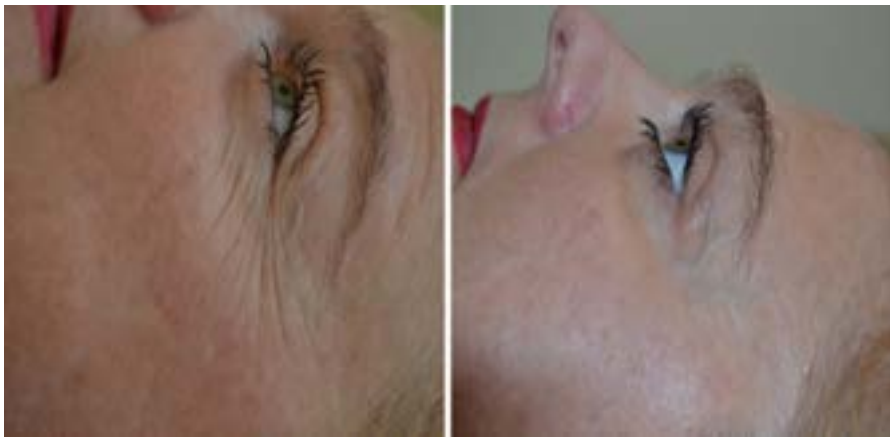

FIGURE 2 Example of patient images taken at baseline and 3 mo after the last treatment

FIGURE 3 Example of patient images taken at baseline and 3 mo after the last treatment

TABLE 1 Patient satisfaction questionnaire average scores (5 – strongly agree, 4 – agree, 3 – undecided, 2 – disagree, 1 – strongly disagree)

	Q1: I was satisfied with the treatment results	Q2: I found the treatment comfortable	Q3: I was satisfied with the overall treatment time
Group A – Facial treatments (10 subjects)	4.30 (±0.78)	4.20 (±0.75)	3.80 (±0.98)
Group B – Arm treatments (7 subjects)	4.00 (±1.07)	3.71 (±0.88)	4.14 (±0.35)
Group C – Thigh treatments (8 subjects)	4.13 (±1.05)	4.00 (±0.71)	4.13 (±0.78)
Group D – Abdominal treatments (9 subjects)	4.11 (±0.74)	4.22 (±0.63)	4.33 (±0.82)
Total study average	4.15 (±0.91)	4.06 (±0.76)	4.09 (±0.82)

taken at the baseline were compared to images taken 3 months after the last treatment, and blinded evaluators successfully recognized 93.33% of facial B&A photographs, 90.48% of arms B&A photographs, 91.67% of thighs B&A photographs, and 92.59% of abdominal B&A photographs (all arithmetic mean). See Figure 1. Of the 34 patients: In 79% of cases (27 subjects), all three evaluators recognized the pictures; in 18% of cases (6 subjects), two of three evaluators succeeded; images of one patient (thigh group) was only recognized by one evaluator. See Figures 2 and 3, for examples, of patient images.

3.2 | Patient satisfaction survey

Results obtained from patient questionnaire showed overall satisfaction with the therapy course and results. In general, the patients agreed (4.1) that they are satisfied with the therapy, agreed that the treatment is comfortable (4.1) and that they are satisfied with overall treatment time (4.1). The standard deviation across all the groups averaged ±0.83 points. This shows relatively high consistency of patients' responses. See Table 1 for detailed results.

3.3 | Safety

No adverse events were observed during the study. Several subjects reported side effects including temporary skin redness and/or mild swelling, which resolved within 1-2 hours after the treatment.

4 | CONCLUSION

Most studies on noninvasive skin tightening and body shaping present results after treating one specific body part of the enrolled

subjects. The goal of our study was to validate whether the novel investigated device delivers clinical versatility in terms efficacy, safety, and patient satisfaction when treating various indications across different body areas. We treated 34 subjects for facial skin laxity and body fat deposits and followed them for 3 months.

The treatment efficacy was assessed from pre and posttreatment photographs scored by three blinded evaluators. Statistical analysis of the study results has confirmed aesthetic improvement in the treated indications with a high rate of responding patients, and consistency among all the treated body parts and indications (none of the patient groups had the average recognition rate below 90%). All patients tolerated the treatments well with no significant posttreatment pain or clinical signs of skin damage. Efficacy was also confirmed by results from the patient satisfaction questionnaire. Patients noted comfortable treatments with overall satisfaction with the treatment results and treatment time. No adverse events during the 90-day follow-up were observed. We can thus conclude that both objectives of the study were met with success.

Treatments using the investigated device produce a reduction in skin laxity and fat deposits without any significant complications. The study showed a very low percentage of nonresponding subjects. During the treatments, we used maximum energy settings, which were still within the range recommended by the manufacturer. Despite this fact, our patients reported high levels of comfort and experienced no or very little side effects. It is unclear if such efficacy coupled with high comfort is a direct effect of the additional ultrasound component and/or the redesigned applicator tips. This should be investigated further in future studies.

DISCLOSURES

Dr. Chilukuri is a speaker/consultant for the following companies: Alastin, Allergan Aesthetics, BTL Industries, Cynosure Lasers, Eclipse Micropen, Emvera Lasers, Galderma Aesthetics, PCA Skin, Skin Medica, Suneva Asthetics, and Theravent Lasers. Dr. Fouque and Dr. Denjean have no conflicts of interest to declare.

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How to cite this article: Chilukuri S, Denjean D, Fouque L. Treating multiple body parts for skin laxity and fat deposits using a novel focused radiofrequency device with an ultrasound component: Safety and efficacy study. *J Cosmet Dermatol*. 2017;00:1–4. https://doi.org/10.1111/jocd.12448

Accepted: 29 March 2017

DOI: 10.1111/jocd.12348

ORIGINAL CONTRIBUTION

WILEY JCD Journal of Cosmetic Dermatology

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.

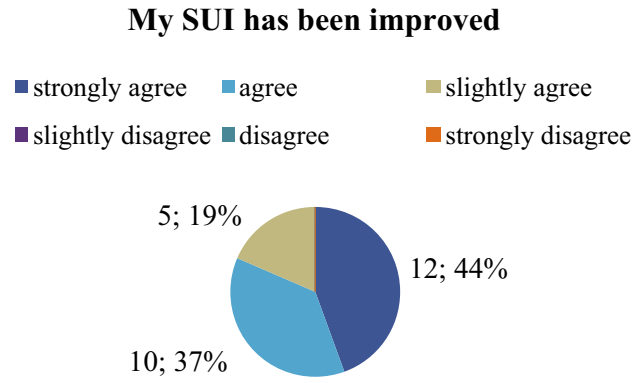

FIGURE 1 Stress urinary incontinence (SUI) improvement (Post-treatment)

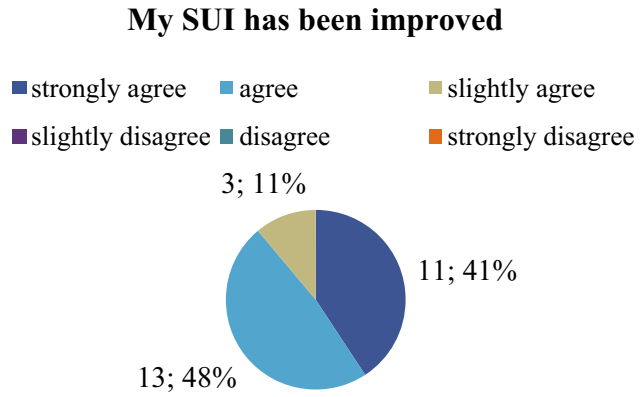

FIGURE 2 Stress urinary incontinence (SUI) improvement (1-month follow-up visit)

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.

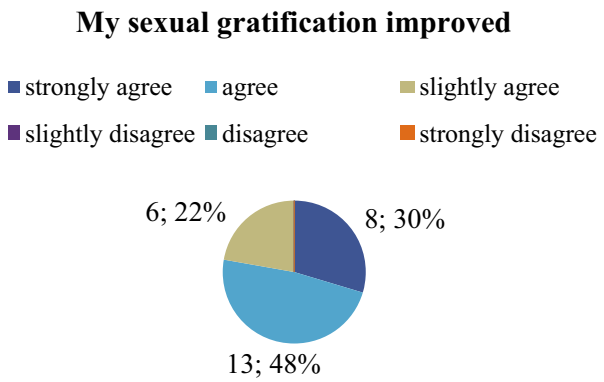

FIGURE 3 Sexual gratification improvement (Post-treatment)

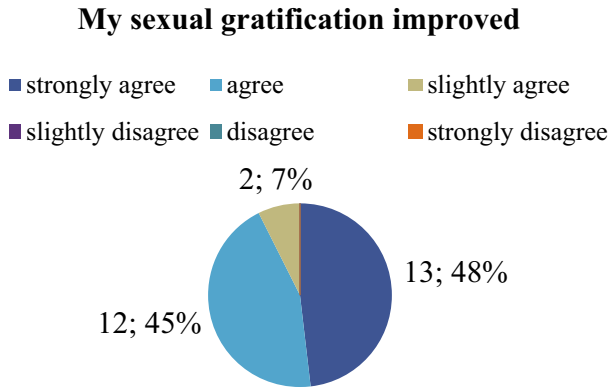

FIGURE 4 Sexual gratification improvement (1-month follow-up visit)

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.

ACKNOWLEDGMENTS

The authors have no commercial interest in BTL and received no compensation for this study. Shelena Lalji and Paula Lozanova have no relevant conflicts to declare.

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How to cite this article: Lalji S, Lozanova P. Evaluation of the safety and efficacy of a monopolar nonablative radiofrequency device for the improvement of vulvo-vaginal laxity and urinary incontinence. *J Cosmet Dermatol*. 2017;16:230–234. https://doi.org/10.1111/jocd.12348

Journal of Cosmetic and Laser Therapy

ISSN: 1476-4172 (Print) 1476-4180 (Online) Journal homepage: http://www.tandfonline.com/loi/ijcl20

Labial tissue rejuvenation and sexual function improvement using a novel noninvasive focused monopolar radio frequency device

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To cite this article: Zeljka Clark (2017): Labial tissue rejuvenation and sexual function improvement using a novel noninvasive focused monopolar radio frequency device, Journal of Cosmetic and Laser Therapy, DOI: 10.1080/14764172.2017.1368565

To link to this article: http://dx.doi.org/10.1080/14764172.2017.1368565

Accepted author version posted online: 30 Aug 2017.
Published online: 30 Aug 2017.

JOURNAL OF COSMETIC AND LASER THERAPY
https://doi.org/10.1080/14764172.2017.1368565

Labial tissue rejuvenation and sexual function improvement using a novel noninvasive focused monopolar radio frequency device

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ABSTRACT

Introduction: With aging, the vulvar tissue loses its vitality and elasticity due to collagen fibers fatigue. Such changes and functional characteristics of the external genitalia often cause negative psychological effects resulting in impeded sexual function. The objective of this study is to evaluate the safety and efficacy of a radio frequency (RF) device when used for treating labial laxity and for improvement of female sexual fuction. **Materials and Methods:** Using a monopolar RF device, 19 women received four once-a-week treatments. Images taken at the baseline and at the 1-month follow-up were evaluated for improvement in vulvar appearance on a scale of 0–3. The female sexual function index (FSFI) scores were calculated and compared between the baseline, the 1-month follow-up visit, and the 12-month follow-up visit. **Results:** Average improvement in the vulvar appearance according to the patients and the physician was 2.00 ± 0.58 and 1.79 ± 0.54, respectively. Both values represent "moderate change" according to the applied scale. The average FSFI increased by 9.79 ± 4.35 and 7.10 ± 5.17 when comparing the baseline to the 1-month and the 12-month follow-up, respectively. No adverse events were reported. **Discussion:** Efficacy and safety of the investigated device were proven. Longevity of results was proven by the 12-month follow-up.

ARTICLE HISTORY
Received 18 April 2017
Accepted 14 August 2017

KEYWORDS
FSFI; labia rejuvenation; Radio frequency; sexual function improvement; vulvar appearance

Introduction

Collagen is the major structural component of skin that histologically becomes disorganized into collagen fibrils and abnormal elastic material. This is primarily as a result of aging, pregnancy, and childbearing processes. With aging, the vulvar tissue loses its vitality, elasticity is reduced due to fatigue of the collagen fibers, and wrinkles start to appear. These changes are usually accompanied by women's reduced satisfaction with the esthetic appearance of her genitalia.

Regardless of age, education, or socioeconomic status, such anatomical appearance together with physiological and functional characteristics of the external female genitalia often causes negative psychological effects. Embarrassment and anxiety over sexual function leads many women to seek help in ameliorating these conditions and concerns.

One possible treatment solution is represented by surgical remodeling of vulvar tissue (labia minora, labia majora, mons pubis, perineum, and vaginal introitus). Reduction labiaplasty is used for sculpting of elongated or unequal labia minora, mostly in response to labia minora protruding beyond the labia major (1). Perineoplasty is a way to rejuvenate relaxed perineum and can also enhance the sagging labia majora and labia minora. Augmentation labiaplasty can provide esthetic improvement and rejuvenation of labia majora.

The main concerns with surgical procedures and ablative laser resurfacing relate to possible adverse events (infection, scarring, keloid formation, asymmetrical appearance of the surgically treated tissue) and associated downtime.

Skin rejuvenation options with a low risk of severe adverse events and without (or with very little) downtime include various non-ablative systems: vascular lasers, intense pulse light, infrared lasers and broadband light sources, radio frequency (RF) devices, photodynamic therapy, and fractional lasers. All these devices may be used to achieve restoration of youthful appearance and to improve sexual function and performance.

RF methods were first studied in 1949 for the treatment of skin laxity, resulting in significant improvement in skin tissue appearance (2). However, the application of RF to external female genitalia only began to emerge in the last decade. The RF-induced elevation of tissue temperature leads to a break-up of intermolecular cross links, stabilization of collagen helicoid structures, and thickening of collagen fibers. The mild inflammatory response of the treated tissue stimulates fibroblasts. As a result, new collagen and elastin fibers are produced as part of the natural healing response (3).

Various RF devices are currently available on the market, with monopolar units penetrating 20–25 mm and bipolar RF reaching depths of approximately 2–8 mm (4). Lordello et al (5) used a bipolar device on women with sagging labial tissue. The protocol consisted of eight 20-minutes weekly sessions, reaching a temperature of 39–41°C. All patients reported satisfaction with the treatment outcome regarding

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sexual function, arousal, and lubrication. Average female sexual function index (FSFI) score (5) increased from 25.66 ± 5.7 to 27.30 ± 5.5 (p = 0.379).

In 2016, a prospective cohort study on 17 women was conducted in Croatia using a monopolar RF device for labial tissue tightening and improvement of labial laxity (5). The investigators reported an average "moderate change" regarding improvement in vulvar appearance. Mean FSFI scores increased from 75% to 87%.

Materials and methods

Study cohort

This is a prospective, randomized, and controlled study which aims to evaluate the safety and efficacy of a monopolar RF treatment when applied to external female genitalia for treating labial laxity and to improve female sexual function.

We enrolled 19 healthy women aged 35–64 (average age 46.7 years) who had reported dissatisfaction with the esthetic appearance of their external genitalia before the commencement of the study. Fourteen of the 19 women had a history of at least one prior delivery. Clinical and demographic characteristics of the subjects are presented in Table 1. The cohort included four postmenopausal subjects, aged 55–64, who had never been exposed to any form of hormone replacement therapy.

Consent to the treatments was obtained prior to commencement, and a detailed medical history of each individual was taken. This included obstetric and gynecological details, and any previous surgeries in the genital area. Each subject was evaluated for contraindications that would disqualify her from receiving RF treatments, including but not limited to pacemakers, defibrillators, facial implants, intradermal fillers, pregnancy, breastfeeding, gynecological or skin lesions in the genital region, or malignancy (6).

The entire study was conducted in compliance with the WMA Declaration of Helsinki's ethical principles for medical research involving human subjects.

Treatment protocol

The treatment protocol consisted of four weekly sessions, each taking approximately 20 minutes. A monopolar RF device was used (BTL Exilis System, BTL Industries Inc.), with the starting energy set to 90 points and 100% duty factor. The energy was adjusted based on patient's feedback.

Thick hydrosoluble gel was applied to skin in the treated area prior to each treatment. The procedure was conducted using slow circular motions in a cranial–caudal direction, covering the mons pubis, labia majora, clitoris, perineum, and vaginal introitus. Light pressure was applied on the handpiece during the treatment.

All treatments were performed using the same starting setting and the same treatment technique, since monopolar RF application has been found to be safe for all skin types (7).

Table 1. Demographic and clinical profile of the study cohort

Personal history	*N*	Mean ± SD or %
Age (years)	19	47 ± 7.6
BMI (kg/m^2)	19	25.3 ± 5.2
Sexual activity in the last 6 months	16	84
Sexually inactive	3	16
Oral contraception	2	11
Menopausal	4	21
Birth history		
Caesarean section only	7	37
Vaginal delivery	5	26
Vaginal and caesarean	2	11
No pregnancies	5	26
Race/ethnicity		
Caucasian	12	63
African	5	26
Mixed	2	11

Outcome measures

Digital photographs of the treated area with patient in a litothomy position were taken before and after each treatment, 1 month after the last treatment, and 12 months after the last treatment. Lighting conditions were kept constant, and the same digital camera (12MP, HD) was used, placed exactly 30 cm from the genital area.

Both the patients and the physician evaluated the photographs, comparing the baseline images to the images taken 1 month after the final treatment. A 4-point scale system was used to record the improvements in vulvar appearance (0 – no improvement; 1 – mild improvement; 2 – moderate improvement; 3 – excellent improvement).

The FSFI questionnaire was completed by all subjects before the first session, 1 month after the last session, and 12 months after the last session.

All results were checked for statistical significance using the Student's t-test. Values of $p < 0.05$ were deemed statistically significant.

Any adverse events or side effects were recorded.

Results

All 19 women completed the full treatment protocol and the 1-month follow-up visit. Two patients did not undergo the 12-month follow-up visit due to personal circumstances not related to the study.

Sexual function

The following results are based on 15 out of 16 enrolled sexually active women who finished the study (one did not complete the 12-month follow-up visit). When comparing the baseline to the 1-month follow-up visit and the 12-month follow-up visit, the average improvement of sexual function as measured by the FSFI questionnaire was 9.79 ± 4.35 points and 7.10 ± 5.17 points, respectively. Both results show high statistical significance ($p < 0.001$). The average score increased from 22.59 ± 4.00 (baseline) to 32.38 ± 1.68 (1-month follow-up visit) and 29.69 ± 2.97 (12-month follow-up visit). This represents an improvement of FSFI from 63% to 90% and 83%, respectively. For details, see Table 2.

Statistically significant increase in FSFI scores was observed in five out of six FSFI dimensions at both follow-up visits. The improvement in these five dimensions averaged 1.94 points (32pp) and 1.44 points (24pp) 1 month and 12

Table 2. Change in the FSFI score 1 month and 12 months after the final treatment

Patient/Pregnancies/Deliveries	Sexual activity	Before	1-Month post Tx	12- Months post Tx	Change 1-Month post Tx	Change 12-Months post Tx
A. No pregnancies	YES	27.5	31.5	31.8	4.0	4.3
B. No pregnancies	YES	23.4	27.9	23.4	4.5	0
C. P2G1 – NVD and ectopic	YES	24	33.6	27	9.6	3
D. P2G2 – NVD	YES	18	31.5	30.3	13.5	12.3
E*. P2G2 0 NVD	YES			Didn't finish the study		
F. P2G2 – 2X C-Section	YES	24.5	30.9	29.2	6.4	4.7
G*. P2G2 – 2X C-Section	NO			Not sexually active		
H. P2G2 – 2X C-Section	YES	22.4	31.4	33.2	9	10.8
I. P1G1 – 1X C-Section	YES	21	34.8	29.9	13.8	8.9
J. No pregnancies	YES	24	33.1	33.9	9.1	9.9
K. No pregnancies	YES	30	33.9	31.8	3.9	1.8
L. P2G2 – 2X C-Section	YES	26	33.7	28.3	7.7	2.3
M. P3G3 – 3X C-Section	YES	13	33	33.3	20	20.3
N. P2G2 – NVD	YES	20	34.5	32.1	14.5	12.1
0. P3G3 – NVD	YES	23	32.2	28.6	9.2	5.6
P*. No pregnancies	NO			Not sexually active. Didn't finish the study		
Q. P3G3 – 2X NVD 1 X C-Section	YES	23.6	32	26.8	8.4	3.2
R. P1G1 – 1X C-Section	YES	18.4	31.7	25.7	13.3	7.3
S*. P4G4 – 3X NVD 1X C-Section	NO			Not sexually active		
Mean ± SD		22.59 ± 4.00	32.38 ± 1.68	29.69 ± 2.97	9.79 ± 4.35	7.10 ± 5.17

*Key: E – 3 deaths to immediate family in the last 12 months
G – Marriage problems
P – Subject reported a sexual problem with her husband and did not think she should continue with the survey
S – Client lost her husband before starting the study

Table 3. Changes in the sexual function

FSFI Dimension	Score range	Pre Tx	1-Month post Tx	*P* value	12-Months post Tx	*P* value	Improvement (pre to 1-month Post)	Improvement (pre to 12-months Post)
Desire	(1–5)	3.15 ± 0.90	4.68 ± 0.93	<0.001	4.44 ± 1.05	<0.001	1.53 ± 1.12	1.29 ± 0.95
Arousal	(0–5)	3.43 ± 1.23	5.33 ± 0.43	<0.001	4.86 ± 0.47	<0.005	1.90 ± 1.25	1.43 ± 1.31
Lubrication	(0–5)	3.25 ± 1.23	5.47 ± 0.68	<0.001	4.68 ± 1.64	<0.05	2.21 ± 1.42	1.43 ± 1.84
Orgasm	(0–5)	3.41 ± 0.95	5.49 ± 0.47	<0.001	5.09 ± 0.88	<0.001	2.08 ± 1.08	1.68 ± 1.38
Satisfaction	(0/1–5)	3.55 ± 0.95	5.52 ± 0.70	<0.001	4.96 ± 0.87	<0.05	1.97 ± 1.17	1.41 ± 1.35
Pain	(0–5)	5.80 ± 0.75	5.89 ± 0.23	>0.05	5.65 ± 0.65	>0.05	0.09 ± 0.58	−0.15 ± 0.40
TOTAL	(2–36)	22.59 ± 4.00	32.38 ± 1.68	<0.001	29.69 ± 2.97	<0.001	9.79 ± 4.35	7.10 ± 5.17

Data are mean ± SD

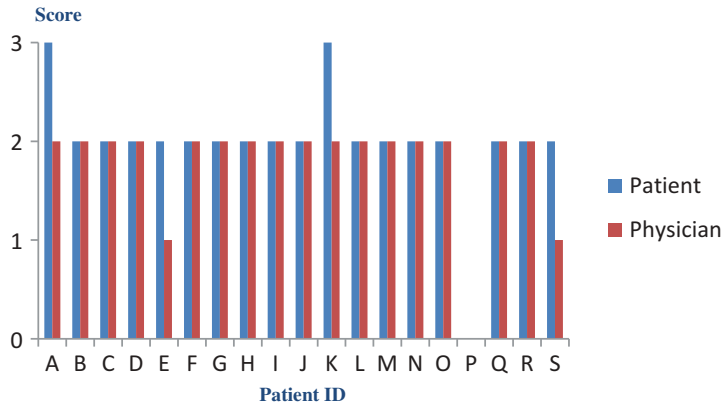
months after the final treatment, respectively. "Pain" is the only dimension in which there were no statistically significant changes.

Evaluation of digital images

The average score based on the patients' responses was 2.00 ± 0.58, with a slightly lower average score of 1.79 ± 0.54 calculated based on the physician's responses. Both results represent "moderate improvement" according to the applied scale. The data are shown in Figure 1. Photographs taken at the baseline were compared to those taken 1 month after the final treatment for all 19 study subjects. Samples of the images are available in Figure 2.

Eighteen out of 19 patients (94.7%) reported "moderate" or "excellent" improvement in vulvar appearance. One woman who later decided to quit the study had reported no change.

The physician also identified at least mild improvement on 18 (94.7%) women, with "moderate" or "excellent" improvement on 16 (84.2%) subjects.



0 - No Improvement; 1 – Mild Improvement; 2 – Moderate Improvement; 3 – Excellent Improvement

Figure 1. Improvement in vulvar appearance based on evaluation of digital images.

Figure 2. Examples of digital images taken at the baseline and at the 1-month follow-up visit.

Safety

No adverse events or aggravation were observed.

Discussion

The use of RF for treating facial and body skin laxity has been well described and evidenced in the last decade. However, the application of RF to treat labial skin laxity and improve sexual function is rather new. Therefore, clinical studies of this kind are essential in order to assess the real efficacy of this treatment modality.

All patients in our study reported high levels of satisfaction regarding improvement in the appearance of their genitalia. A significant change in appearance occurred as soon as after the first treatment, with maximum improvement observed after the second treatment. The third and the fourth sessions caused further improvements, but of a lesser magnitude.

With respect to sexual function, all sexually active subjects reported improvement after the treatments. All FSFS dimensions except for "Pain" showed statistical significant score increase, and the changes followed the same patterns as those observed in genital appearance. The best results were seen in better lubrication, ease of reaching orgasm during intercourse, and overall satisfaction with sexual life. No significant improvement in the "Pain" dimension was observed, nor expected. Subjects with increased tightness as a result of the treatments are likely to experience more intensive sensations during sexual intercourse. The overall increase in sexual function was also significant at the 12-months follow-up, proving the effects to be long lasting.

Our results suggest that one or two treatments may be sufficient to achieve a very reasonable level of improvement in the treated conditions; however, some patients felt that they would benefit from more than four sessions. The FSFI changes exceeded the results achieved by previous studies (5,8), suggesting the investigated device is a highly efficient modality for treating impeded female sexual function.

The results of this clinical study prove that the investigated monopolar RF device provides an effective and safe alternative to existing treatment methods for improving labial laxity and sexual function. Negative side effects of RF treatment are extremely rare.

Declaration of interests

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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ISSN: 1545 9616

JOURNAL OF DRUGS IN DERMATOLOGY

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DRUGS • DEVICES • METHODS

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

David McDaniel MD, Robert Weiss MD, Margaret Weiss MD, Chris Mazur BS, and Charmaine Griffin CCRP

J Drugs in Dermatol. 2014;13(9):1112-1117.

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.

J Drugs Dermatol. 2014;13(9):1112-1117.

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).

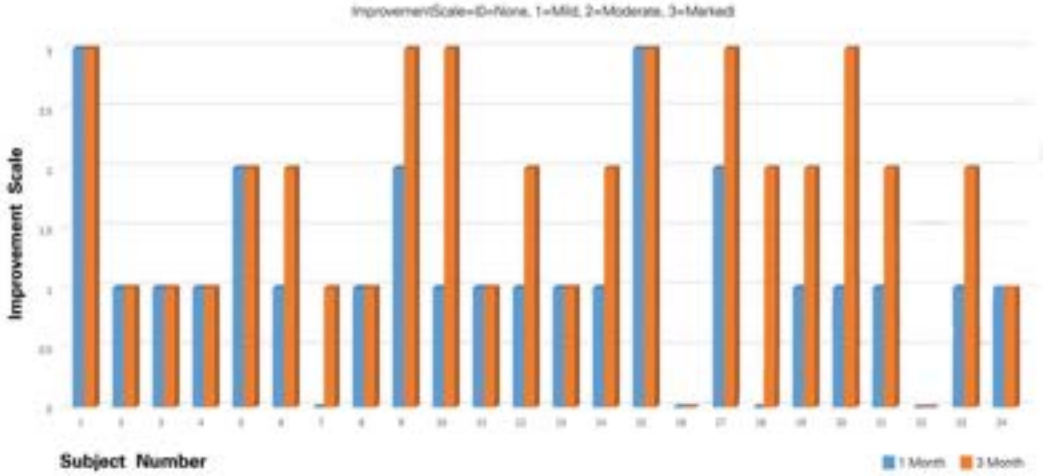
MATERIALS AND METHODS

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.

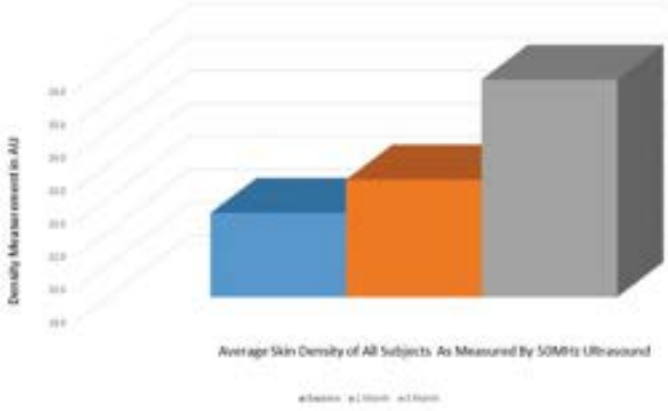
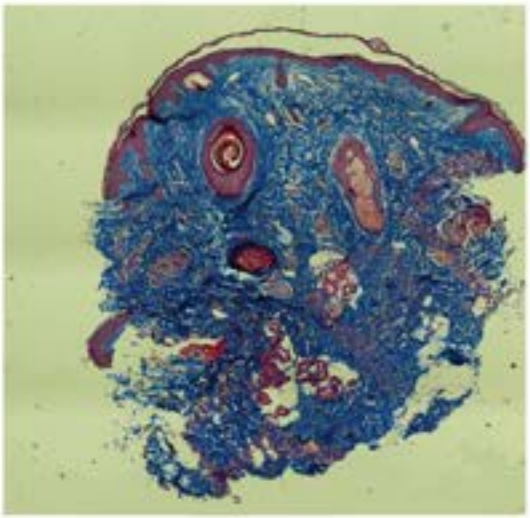


FIGURE 3a. Masson staining in one subject demonstrates increased collagen (blue) at 3 months post treatment.

Pre



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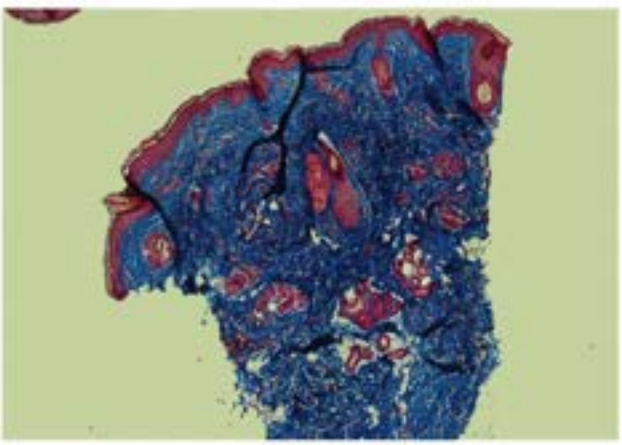
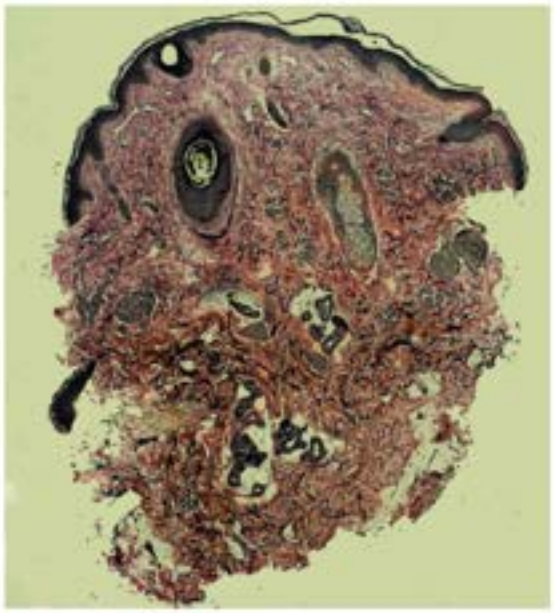


FIGURE 3b. Verhoeff staining in one subject demonstrates increased elastin fibers (black), with more uniform pattern and distribution at 3 months.

Pre



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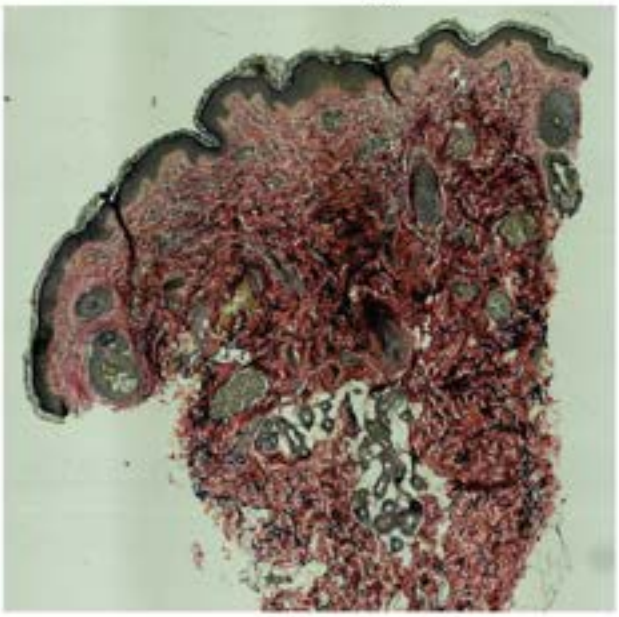
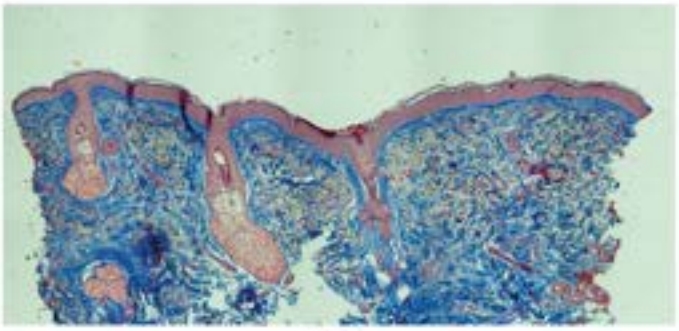


dermis in two of the four subjects. Two subject's full thickness images are presented below (Figures 3a, b, and 4a, b). Clinical images of these same subjects demonstrated significant clinical improvement, consistent with the histological findings (Figures 5 and 6). There were minimal changes in the 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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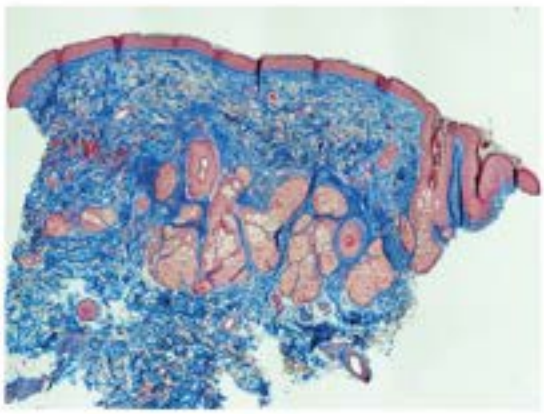

FIGURE 4b. Verhoeff staining in one subject demonstrates increased elastin fibers (black), with more uniform pattern and distribution at 3 months.

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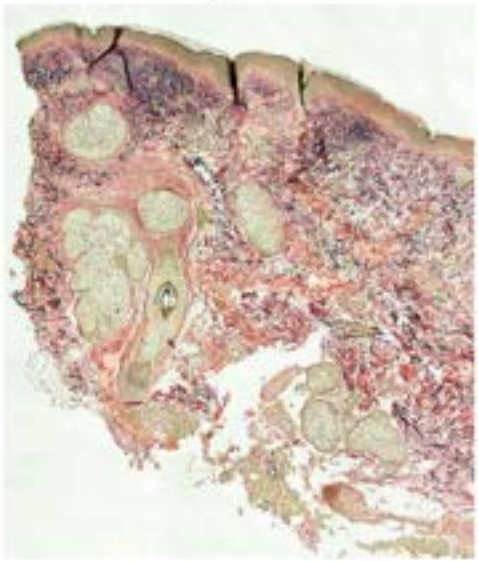

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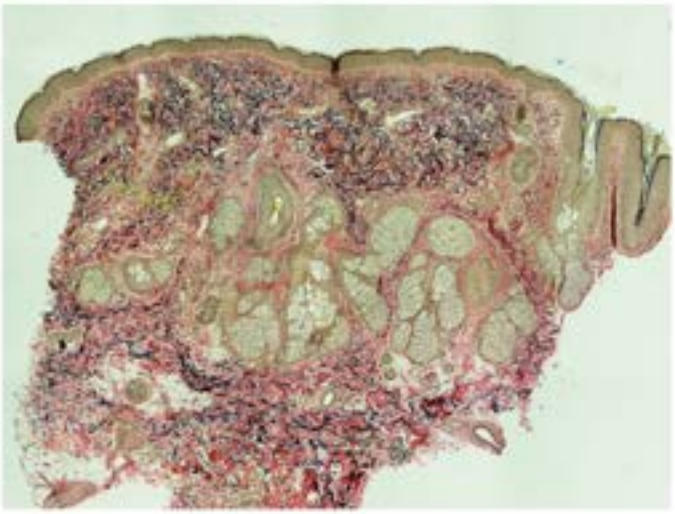


the others). Tightening around the jawline, under the chin and neck are clearly demonstrated (Figure 7).

CONCLUSION

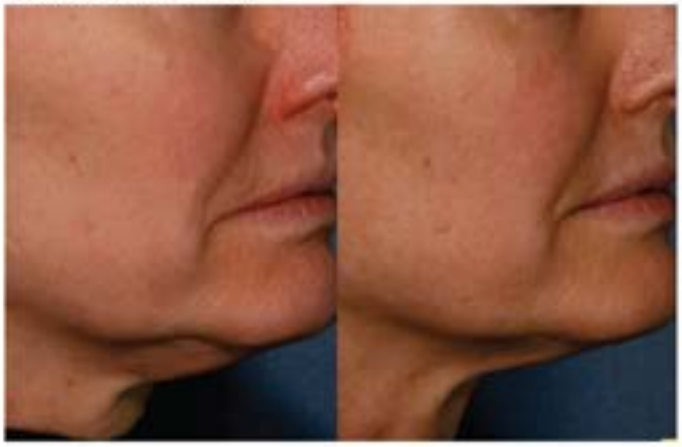
This study demonstrates that a novel monopolar radiofrequency device with increased fluence and continuous impedance monitoring used with a new two treatment protocol achieves measurable clinical benefits. The data supports previous statements that mention by maintaining a lower skin temperature for 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.

ACKNOWLEGMENTS

The authors would like to thank Robert A. Frazier Jr. MD of Dominion Pathology Laboratories, Norfolk, VA for processing the histologic specimens.

DISCLOSURES

Dr. Robert Weiss and Dr. Margaret Weiss received honoraria, research grants and equipment for speaking and work as an investigator for BTL. No stock, equity or royalties are received. Dr. David McDaniel received financial support for studies/research/consulting/medical advisory board from BTL as well as equipment loan and technical assistance from the BTL engineering team in Prague. No stock, equity or royalties are received.

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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.

J Drugs Dermatol. 2014;13(11):1336-1340.

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]

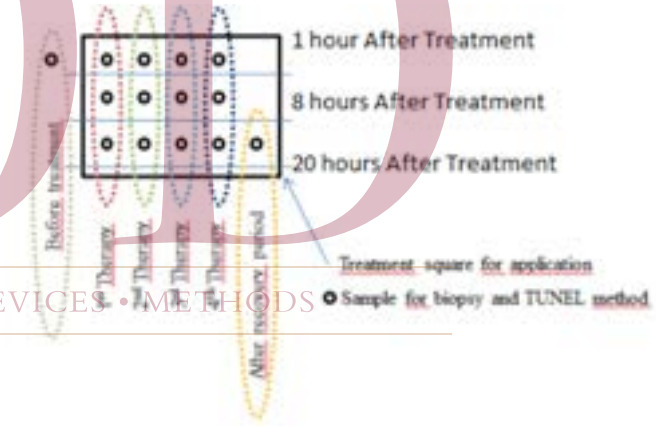
"Induction of the death of adipocytes through apoptosis is emerging as a promising strategy for the prevention and treatment of excess fat due to the destruction of adipocytes via this mechanism, resulting in reduced body fat."

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.

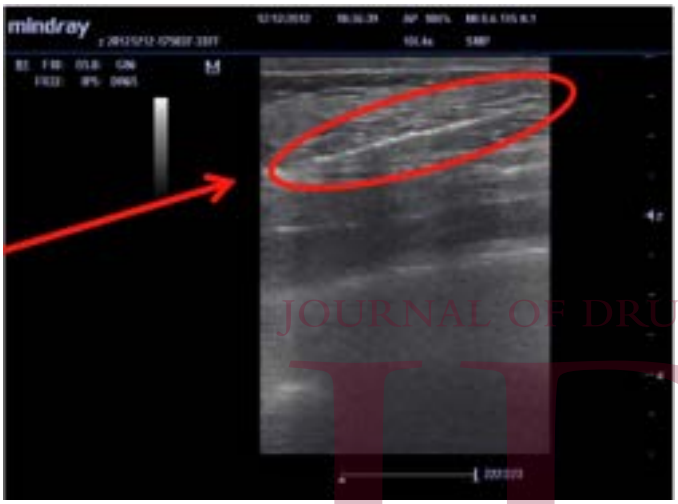
Clinical Procedures

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.

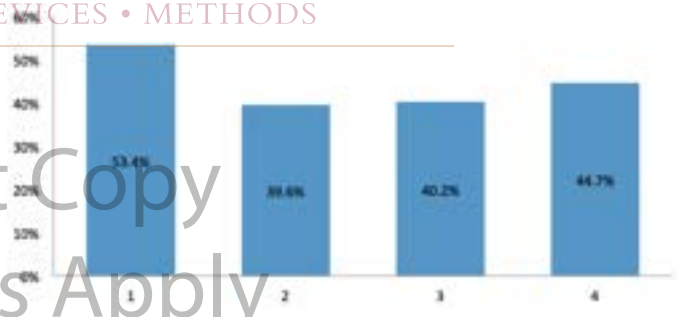


TABLE 1.

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.

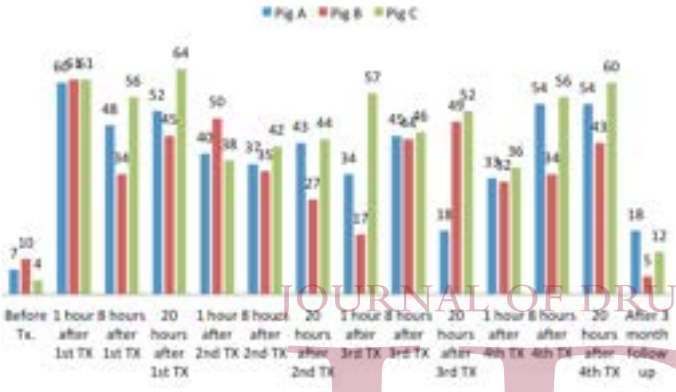


TABLE 2.

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.

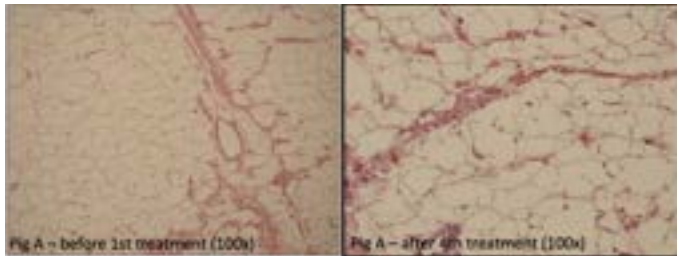


FIGURE 8. Thermal probe position verification using ultrasound measurement.

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.

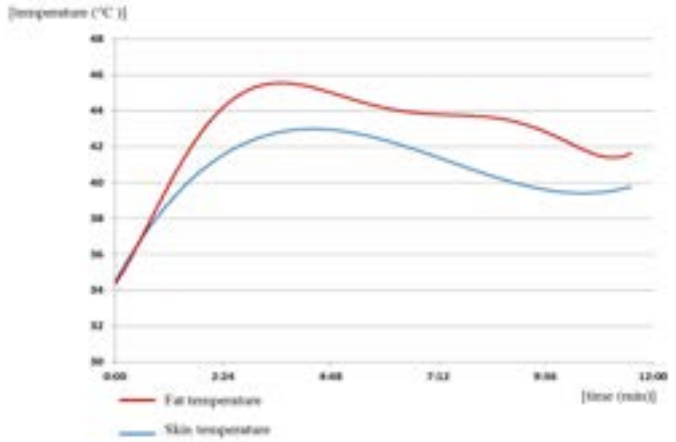
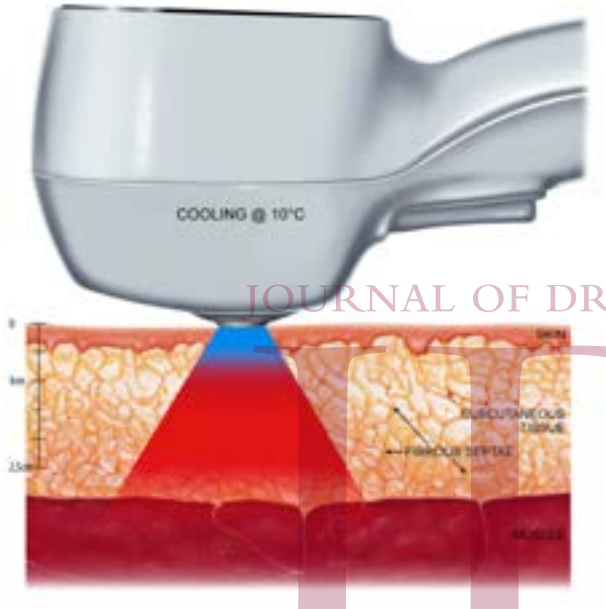


FIGURE 10. Cooling with corresponding depth of heat penetration.



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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JOURNAL OF DRUGS IN DERMATOLOGY

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DRUGS • DEVICES • METHODS

"DEEP HEATING" NONINVASIVE SKIN TIGHTENING DEVICES: REVIEW OF EFFECTIVENESS AND PATIENT SATISFACTION

Suneel Chilukuri MD FAAD FASDS and Jason Lupton MD

J Drugs in Dermatol

"Deep Heating" Noninvasive Skin Tightening Devices: Review of Effectiveness and Patient Satisfaction

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ABSTRACT

Non-surgical aesthetic devices intended for treatment of lax and loose skin have gained popularity due to their ability to non-invasively improve patient's aesthetic condition and its low side effect profile. This study is intended to review available peer reviewed literature about Ultherapy, ThermaCool, and Exilis Ultra 360 non-invasive skin tightening devices to compare their treatment efficacy and patient subjective satisfaction.

J Drugs Dermatol. 2017;16(12):611-615.

INTRODUCTION

Aesthetic improvement in the appearance of facial wrinkles, redundant facial, neck, or body laxity is a major feature of aging. Monopolar radiofrequency (RF) and ultrasound sources became a treatment of choice for non-ablative tissue tightening by volumetric tissue heating of the deep dermis.

Non-ablative radiofrequency devices have gained popularity because of their ability to offer improvement of skin laxity without the postoperative recovery or financial burden of surgical procedures. It remains in demand secondary to its lower side effect profile and remarkably short post procedural downtime.

This continuing shift away from ablative and invasive aesthetic procedures continues to be driven largely by patient and clinician preferences.[1] According to the American Society Aesthetic Plastic Surgery, 526,000 non-surgical skin tightening procedures (annual growth of 11 %) were performed in 2016 in the United States.[2]

The aim of this clinical paper is to review available literature for selected aesthetic devices utilizing deep tissue heating (Ultherapy, ThermaCool, Exilis Ultra 360). The data reported herein are based on a retrospective review of peer-reviewed clinical studies. Aforementioned devices are evaluated for safety and efficacy. In everyday practice, patient's perceived improvement typically outweighs the practitioner's scoring. Therefore, most of the clinical studies utilize subjective patient satisfaction scores.

DISCUSSION

Ultherapy (Ulthera, Merz North America, NC)

The Ultherapy procedure is indicated for use in: lifting skin on the neck, on the eyebrow and under the chin as well as improving lines and wrinkles on the décolletage.

The first aesthetic use of high intensity focused ultrasound (HIFU) was introduced in 2008 and it was FDA cleared for brow-lifting a year later. Currently, the microfocused ultrasound (MFU) is being used for non-invasive tissue remodeling.

Currently available transducers emit frequencies of 10.0 MHz, 7.0 MHz, and 4.0 MHz with focal depths of 1.5 mm, 3.0 mm, and 4.5 mm, respectively. The higher energy transducers allow energy deposition in smaller anatomical regions. The ultrasound beam is focused to a point less than 1 mm^3 in size below the skin surface (in the superficial muscular aponeurotic system) to form "thermal coagulation points".[3] Temperature inside of such points is increased to 65°C. Superficial layer of skin remains un-affected. This results in immediate collagen contraction and initiates collagen synthesis. The device incorporates automatic ultrasound imaging of the tissue for controlled energy delivery and acoustic coupling of the probe. The treatment zone is 25 mm and 14 mm in length, for the standard and the narrow transducers, respectively. Treatment is administered in a "stamping" manner.

A prospective cohort study[4] described results of facial treatment with the 4 MHz and 7 MHz transducers. At 90 days, 30 patients (86%) showed clinically significant brow-lift with a 1.7 mm mean elevation of the eyebrow. Fabi et al[5] treated 70 patients on the neck. Quantitative assessment indicated that 72.9% of subjects achieved a visible tissue lift of > 20.0 mm^2 in the sub-mental area. Three months after, the improvement was still visible for 68.6% of patients treated in sub-mental and neck area, and for 67% of patients treated on face and neck.

The long-term efficacy was studied also by Fabi et al.[6] At 180 days, physician GAIS score revealed that 77.7% patients achieved improvement in the face and upper neck area, while

patient evaluation resulted in 77.8% improvement. Blinded reviewers assessed photographs with an average score of 67%.

Park et al[7] treated 20 patients with approximately 420 shots each spread among the supraorbital, zygomatic, infra-orbital, periorbital, cheek, pre-auricular, and jawline areas. Physician's GAIS scale evaluation (improvement: 0- none, 1- mild, 2- mild/moderate, 3- moderate, 4- severe) showed 0.9 overall improvement after 90 days, and it stayed unchanged when re-evaluated at 180 days. Patient satisfaction score was 3.80 and 3.65 at 3 months and 6 months, respectively (1- not satisfied; 2- somewhat satisfied; 3- satisfied; 4- very satisfied; 5- extremely satisfied).

Another study investigated improved efficacy when multiple treatment passes had been used.[8] Neck and face were targeted using the 4 MHz transducer followed by the 7 MHz transducer on 10 patients. Clinicians reported 80% improvement at 90 days, with 20% patients showing no change. Patients reported 90% improvement by self assessment, but the overall outcome was in most cases described as mild or moderate (N=7). The mean pain score was 3.9 ± 1.66 (range, 2-7) on the VIS. No patient reported pain at the follow up.

Oni et al[9] performed a large Ulthera sponsored study, evaluating improvement in lower face/neck appearance in 93 patients treated with 4 MHz and 7 MHz transducers. At 90 days, 65.6% patients reported their satisfaction with results, the remaining 34.4% saw no improvement. According to masked evaluators, improvement of skin laxity occurred in 54 patients (58.1%). In 16 patients (17.2%) there was no change, and in 23 patients (24.7%) their condition worsened. On a 0-10 scale, the average pain scores were between 5.68 and 6.53 for submandibular region (5- moderate pain, 6- increasing discomfort, 7- significant discomfort).

MFU was also studied for décolletage lifting and rhytids.[10] At 90 days, 96 % patients showed improvement according to PGAIS score, 1 patient showed no results. According to SGAIS score, 100% of patients noticed some kind of aesthetic improvement, all of them were very satisfied (37.5%) or satisfied (62.5%) with provided treatment. At 180 days, they observed a decrease in all aspects of the outcomes. PGAIS decreased to 86 % and SGAIS decreased to 95%. The mean midclavicular-to-nipple distance decreased from 20.9 cm to 19.5 cm at the end of the follow up.

The most common post procedural findings were tenderness, edema, erythema, bruising, numbness, and welts. In a 2014 clinical study on Ultherapy's safety profile,[11] most unexpected AEs that happen in <0.4% of cases include pain, nerve irritation, numbness/paresthesia, lumps, swelling, tingling, itchiness, redness, hives/rash, headaches, swollen throat, and could be attributed to incorrect treatment techniques or they are classified as unrelated to the treatment. Gutowski[12] reported only mild side effects which resolved within 7 days, another study[13] reported side effects which lasted up to 3 months (skin pigment changes, neuropathic pain, bruising). To overcome pain-related side effects, topical or oral anesthetics were used in numerous studies, improving the somatic experience during the procedure.[5,8,10,12,14]

Thermage ThermaCool (Solta Medical, San Francisco, CA)

The ThermaCool procedure is indicated for use in: Dermatologic and general surgical procedures for electrocoagulation and hemostasis; non-invasive treatment of periorbital wrinkles and rhytids including upper and lower eyelids; and non-invasive treatment of wrinkles and rhytids.

The system is made up of several components that allow delivery of electromagnetic energy to the skin through a single-use treatment tip, which cools down its surface while the RF energy is being delivered. Energy settings are determined based on anatomy of the treated area. Treatment tips come in various sizes, currently 0.25 cm^2, 1.0 cm^2, 1.5 cm^2, and 3.0 cm^2. Tip heats up the dermis to temperatures of 65–75°C, causing collagen denaturation while the epidermis is kept at 40°C. The cooling is provided by a continuous application of cryogen spray onto the inner surface of the tip membrane.

Initial studies showed modest results, particularly in improvement of wrinkle scores of the face, neck, and brow. These studies demonstrated that outcomes were more significant in younger patients and when treating larger surface area with increased number of treatments. Clinical results improved over time as 4-month scores were statistically higher than the 1-month scores. Areas less responsive to treatments included jowls, mandibular ridge, and neck. Because of significant pain, anesthesia and oral pain medication was needed.[15,16] A long-term study of the skin tightening effect confirmed that multiple treatments might be beneficial to patients as evidenced by Suh et al.,[17] where 8 patients were observed over 6 years after having an average of 4 sessions over that period.

Fitzpatrick et al[18] investigated periorbital tightening on 86 subjects. Review of photographs showed improvement in 83% cases, with 14% patients seeing no change, and 3% patients worsened. The same evaluation method showed lifting of eyebrows in 62% cases. Patients were satisfied or very satisfied with the treatment outcome in 50% of cases, with 49% patients claiming their appearance improved. According to subjective comfort rating, only approximately 7.5% of treatments (counted for both sides of face) were painless, the remaining 92.5% patients reported mild/moderate/severe/intolerable pain.

Fritz et al[19] treated one group with single treatment (N_1=11) and the second group with two treatments one month apart (N_2 = 9), to evaluate the outcome of multiple treatments to

nasolabial fold improvement. Patients who received two treatments showed higher rate of improvement in self-assessment rating. The overall change noted by physicians and patients was modest, reaching the maximum of 14-16% improvement. Three patients reported less than 10 % of overall improvement. All patients experienced mild or mild-to-modest erythema.

Another study[20] focused on cheek and neck laxity treatment found a 35% to 40% subjective improvement of nasolabial and melolabial folds appearance, and 30% to 35% subjective improvement of neck laxity after one treatment session. Patients described the procedure as moderately uncomfortable.

A multi-center study[21] evaluated low-fluence algorithm intended for facial laxity treatment. At 4 months, 95% of patients showed improvement. Most patients (65%) had reported good improvement (range, 26-50%) or very good improvement (range, 51-75%). Five percent of patients showed no improvement. Results were similar at 6 months when the number of patients with no improvement increased to 8 %. Subjective satisfaction was 78% at 4 months, and decreased to 70% at 6 months post treatment.

Short-lasting post procedural findings, such as erythema and edema, are reported in the majority of patients.[22] Edwards et al[23] reported that erythema lasted less than 24 hours in 50% patients; 1 patient had edema that lasted beyond one week. Weiss et al. performed a large-scale retrospective study[24] and identified some of the rarer side effects of crusting, oozing, scarring, bruising, pigment alteration, nerve damage, texture change, atrophy, burns, and prolonged swelling, pain, or erythema. There was one case of fat atrophy causing a small depression on the cheek, and one superficial linear crust. These resolved in 3.5 months and one week, respectively.

A degree of pain reported without pain management interventions in the earlier studies was severe (6 of 10 on a 1–10 pain scale)[17] and vibration was added to modify pain fiber recruitment.[25] Also the application of topical anesthetics is recommended prior to the treatment to increase tolerability.[16,18,20,21]

Exilis Ultra 360 (BTL Industries, Boston, MA)

The procedure is FDA cleared for use in: non-invasive dermatologic and general surgical procedures for non-invasive treatment of wrinkles and rhytids, to provide a temporary reduction in the appearance of cellulite.

The system is the latest generation of device based on the Exilis platform. As such the review also includes all evidence relating to the older generations of the device. It is a monopolar RF device with ultrasound component, and a number of built-in safety features, including integrated Peltier cooling. The system has 2 types of different hand applicators, one designed mainly for the face, and one for the body. During the treatment, the temperature in the treated tissue is raised to 40-45°C while the handpiece is in continuous motion so that areas of skin with the most laxity can be specifically targeted. When targeting deeper layers, the skin is cooled and protected, allowing the heat to reach deeper. Any spikes in RF delivery are automatically eliminated due to constant energy flow monitoring. This virtually eliminates the risk of burns. As such, it allows use of higher power, which then leads to shorter treatment times.

Efficacy of the system on collagen remodeling was first studied by stereological analysis in a veterinary study, which showed large-scale increase of collagen (P=0.018) in the treated area.[26] The subjects received 4 treatments. Based on evaluation of 54 histological samples of epidermis and dermal-epidermal junction, the collagen content in the tissue increased from 9% to 26% (288% increase) at the 3-month follow-up.

Weiss and McDaniel[27] confirmed that modified 2-treatment only protocol is well tolerated by subjects, and produces significant subjective as well as objective improvement. Three months post treatments, 92% of patients showed improvement in skin laxity based on evaluation of photographs. No adverse events were reported. Objectively, skin density increased by 19% at 3 months. Biopsies showed increase in dermal collagen and elastin fibers which correlated with subjective patient evaluation.

A recent study[28] proved a high degree of versatility of the system when evaluating efficacy on multiple body parts. Patients (N=34) were divided according to their indication, and were treated for laxity on face, arms, as well as for fat in thighs and abdomen. Four 30-minute treatments were applied. Independent evaluators recognized patient baseline photographs from the 3-month follow-up in 92 % cases, with all groups scoring above 90 % (the highest on facial photographs with 93%, the lowest on arm photographs with 90.5%). On average, 8% patients showed no response. Patients satisfaction averaged 4.15 on a given scale (5- Strong satisfaction to 1- Strong dissatisfaction), and they agreed that the treatment was comfortable with average score of 4.06 (5- Strongly agree, 1- Strongly disagree). There was no post-treatment pain or skin damage.

The efficacy for fat treatments was described by McDaniel et al.[29] The study proved that the unit can selectively heat fat, causing apoptosis of adipocytes. The skin surface remained intact, while subcutaneous fat showed apoptotic index increase from 7% to an average of 44% after the last treatment. Study also proved safety through histological analysis, blood chemistry, and hematology samples.

The efficacy has also been investigated when treating laxity of female intimate parts. A study published in Lasers and Surgery

TABLE 1.

Comparative Summary of Non-Invasive Skin Tightening Devices			
	Ultherapy	ThermaCool	Exilis Ultra 360
Manufacturer	Merz (NC)	Solta Medical (San Francisco, CA)	BTL Industries Inc. (Boston, MA)
Technology	Microfocused Ultrasound	Monopolar RF	Monopolar RF
Mechanism of action	Collagen denaturation and subsequent synthesis[9]	Collagen remodelling[18]	Collagen remodelling; increase of elastin and collagen fibres (small applicator)[26], fat apoptosis (large applicator)[29]
Treatment Time (Full Face in min)	60-85[9]*	60-120[15]*	45[28]
Number of Treatments	1-2[9]	1-2[15]	2-4[27,28]
Therapeutic Temperatures (°C)	65	65-75	40-45
Anesthetics	YES	YES	NO
Clinical Efficacy	58.1-96.0 %[5-11]	47-95 %[18,19,20]	89-93 %[28,31]
Patient Satisfaction	65.6-95.0 %[5-11]	53-78 %[19,21,22]	77-95 %[28,30,32]
Pain (0-10 score)	3.9-6.53[9,10]	6[18]	No data
Non-responsive Patients	14 - 20.0 %[9-11]	5 - 14 %[19,22]	3 - 8 %[28,31]
Worsening of Patient's Condition	24.7 %[10]	2.5 %[19]	No Data
Serious Adverse Events	Rare	Rare	None

*Including anesthesia

in Medicine[30] presents an average 2.9 point (of maximum 4) improvement in vulvar appearance after 4 treatments, accompanied by increased sexual function measured by FSFI score from initial 75% to 87%. No adverse events were reported. Later on, vaginal treatments have also been studied by other investigators.[31,32]

In general, post procedural findings included only mild erythema which is also considered the therapeutic endpoint of a proper treatment. No adverse events, nor long-lasting side effects have been reported.

CONCLUSION

A summary of most important disciplines is in Table 1, comparing aspects crucial from both the physician's and patient's perspective. The data is based on available peer-reviewed trials. Quantitative comparison is stated in percentages due to non-uniform approaches to efficacy and safety evaluation in the respective studies.

All 3 devices have solid clinical evidence behind and proved efficacy in tissue laxity treatment. Exact clinical efficacy varies among the devices and also seems to be dependent upon the study design, treated body part and selected outcome measures. Some of the studies have been sponsored by the manufacturers (Oni et al., White et al., Polder et al., etc.), thus leaving room for bias.

Two of the devices leverage higher therapeutic temperatures to achieve results at a smaller number of treatments. This seems to be paid off by increased patient discomfort and the need to use anesthetics, which also prolongs the overall time needed for each session. Exilis Ultra 360 protocol consists of 4 treatments, but shows higher patient comfort. The efficacy of two-treatment protocol was also investigated and proved by Weiss et al. More evidence is likely to appear over time, but as of now there seems to be no clear correlation between the 3 different therapeutic temperatures and clinical efficacy.

None of the studies investigated in more detail how the patient profile influences clinical results. Number of non-responding patients is comparable for Exilis Ultra 360 and ThermaCool devices, ranging from 3% to 8%. Ultherapy studies show slightly higher percentage of non-responders, which was reported by Oni et al. (17.2%), Lee et al. (20%), or Fabi et al. (14%). Oni et al. also reported that after Ultherapy treatment, for 24.7% patients the post-treatment outcome was evaluated as worse against the baseline. Additional research is needed to allow for proper expectation management in patients.

This retrospective review primarily focused on patient satisfaction and treatment efficacy based on comparison of validated peer reviewed articles of three non-surgical skin tightening devices. Despite the slight differences in principles of mechanism

of action, all reviewed devices proved their therapeutic effect on tissue tightening. While the Ultherapy device is specialized mostly on the face, neck and décolletage area, the ThermaCool, and Exilis Ultra 360 are widely used to treat different body parts. The highest rate of versatility offers the Exilis Ultra 360 device, which can be used for treating additional areas such as back, hands, bra fat, forearms, thighs, and female intimate parts.

DISCLOSURES

Dr. Chilukuri is a speaker/consultant for the following companies: Alastin, Allergan Aesthetics, BTL Industries, Cynosure Lasers, Eclipse Micropen, Emvera Lasers, Galderma Aesthetics, PCA Skin, Skin Medica, Suneva Asthetics, and Theravent Lasers. Dr. Lupton has no conflicts of interest to declare.

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RADIOFREQUENCY VOLUMETRIC HEATING FOR VAGINAL LAXITY TREATMENT: EFFECT ON SEXUAL SATISFACTION.

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Presented at ISSWSH Annual Meeting 2018, February 8-11, 2018, San Diego, CA, USA
Study abstract has been published in the Journal of Sexual Medicine June 18, Supplement 2

ABSTRACT

Introduction:
Stretching of the vaginal tissue caused by natural factors as aging or childbirth is the primary cause of vaginal laxity and loss of sexual satisfaction in women. Such medical condition can lead to physical and/or psychological health problems that impact relationship happiness. This prospective study investigates the treatment of vaginal looseness and sexual dissatisfaction with a noninvasive radiofrequency device.

Materials and methods:
We enrolled 51 patients (average age 44.43±9.85 years) who reported vaginal looseness symptoms and sexual dissatisfaction. They received 3 treatments with the EXILIS ULTRA 360 device (BTL Industries, Boston MA, USA) spaced by one week. Each session consisted of separate consecutive intravaginal and extravaginal application using the disposable tips. Therapy parameters were set according to the manufacturer's recommended protocol. Patient sexual satisfaction was evaluated by the Female Sexual Distress Scale-Revised questionnaire (FSDS-R) and the degree of vaginal looseness was measured by the Vaginal Laxity Questionnaire (VLQ) at the baseline and 90 days post treatments. Patient comfort during each procedure was assessed using a Visual Analogue Scale (VAS). Signed informed consents were obtained from all patients.

Results:
The average FSDS-R score improved ($p<0.001$) by 72.79% from 15.60±9.22 to 4.24±5.71 points, with 93.33% of patients showing improvement in sexual satisfaction. Over 55% of patients reported complete absence of any dysfunction 90 days post treatments. VLQ showed vaginal tightness improvement in almost 96% of patients ($p<0.001$) with the average increase of 2.63±1.41 points (1-7 scale). At the follow-up, all patients uniformly reported they don't feel any degree of vaginal laxity at all (VLQ≥4). Some level of discomfort was reported in 2.65% of treatments, with the rounded average VAS discomfort score reaching zero.

Conclusion:
The EXILIS ULTRA 360 proved safe and effective for treatment of vaginal looseness which resulted in a significant improvement of sexual life satisfaction.

INTRODUCTION

With advancing life most women face an increasing looseness of vaginal tissue architecture referred to as vaginal laxity. It's associated with the effects of aging[1], weakening of connective tissue[2], and trauma to the pelvic muscles caused by childbirth[3]. All these factors contribute to an overall sexual dysfunction which may lead to long-term physical and psychological consequences. The exact prevalence of vaginal laxity symptoms is unknown, yet it's generally considered a highly underreported condition as stated by over 80% of the International Urogynecological Association members[4]. If untreated, vaginal laxity can significantly impact patient's self-esteem and quality of life[4]. It's linked with a decreased sexual sensation during intercourse, impeded sexual life satisfaction and dysfunctional relationships[5-7]. Laxity is often observed together with urinary incontinence symptoms or chronic changes in pelvic muscles organization[6,8].

Vaginal looseness can be addressed by exercise-based non-surgical therapies such as the Kegels or through a pelvic floor therapy, but recently various energy-based devices have become widely popular which include minimally invasive fractional CO_2 laser ablation and non-ablative radiofrequency (RF) heating[5,6,9,10]. These technologies

allow relatively comfortable and safe treatments focused on the vaginal area, in contrast with a more painful surgery which may often lead to an increased risk of dyspareunia[11]. Surgical interventions are used to perform reconstructive changes of the vaginal area[12] while the energy-based devices rely on natural reparation of laxity through stimulation of collagen/elastin fibers regeneration, contraction of collagen, neovascularization and improved lubrication[13].

This study evaluates the efficacy of RF volumetric heating for treatment of vaginal laxity and sexual dysfunction in women reporting vaginal looseness symptoms.

METHODS

In total 51 sexually active women (all of whom reported vaginal laxity and/or sexual life dissatisfaction) were enrolled into a single-arm prospective study. See Table 1 for detailed data. The following exclusion criteria were applied: age<25, sexual inactivity, active gynecologic problems (bleeding, injury, infection or pain), metal IUD, current pregnancy, or any contraindication to RF energy treatments. Written informed consents were obtained from all participants.

Patients (N)	51
Age (avg±SD)	44.43±9.85
BMI (avg±SD)	28.80±5.66
Natural deliveries	
(N)	74
(Avg±SD)	1.45±1.12
Number of natural deliveries (N/%)	
0	14 (27.45%)
1	11 (21.57%)
2	16 (31.37%)
3	9 (17.65%)
4+	1 (1.96%)
C-Section	
(N)	27
(Avg±SD)	0.53±0.92
Number of C-Sections (N/%)	
0	35 (68.63%)
1	8 (15.69%)
2	6 (11.76%)
3	1 (1.96%)
4+	1 (1.96%)

Table 1. Demographic and clinical data.

Subjects were treated in 3 sessions across 3 weeks. Each session comprised both an intra-vaginal and an extra-vaginal application using the EXILIS ULTRA 360 device (BTL Industries, Boston MA). The one-off disposable applicator tips were used.

The Female Sexual Distress Scale-Revised questionnaire (FSDS-R) was used to assess changes in patents' confidence about their sexuality. A 7-point scale Vaginal Laxity Questionnaire (VLQ) was used to evaluate changes in patients' subjective perception of vaginal laxity/looseness. All evaluations were made at the baseline and 90 days post-treatments. Immediately after each session a 0-10 Visual Analogue Scale (VAS) was used to determine the level of patient's treatment discomfort. Paired t-test (α=5%) was used to determine the statistical significance of results.

Results

No anesthetics were required. The procedures were well tolerated when 94.12% of patients (N=48) didn't report any pain at all with regards to the RF delivery throughout the 3 sessions, while in 2.65% of procedures the patient reported certain level of discomfort (avg. score 0.085 out of 10). No adverse events were reported.

The results presented herein include FSDS-R data from 45 subjects (four subjects had a score of 0 already at the baseline; two subjects were lost to follow-up) and VLQ data from 49 subjects (two subjects were lost to follow-up). The average FSDS-R score improved by 72.79 % from 15.60±9.22 (baseline) to 4.24±5.71 (follow-up) ($p<0.001$) with statistically significant improvement in 12 out of 13 questions. In total 42 subjects (93.33%) reported improvement, with 25 subjects (55.55%) reaching a zero FSDS-R score at the follow-up, indicating a total absence of any sexual dysfunction. Three patients didn't respond to the treatments and either reported no improvement (2.22%) or worsening of the initial condition (4.44%). See Table 2 for detailed data of patient's score improvement in each question.

The average VLQ score improved by 76.33% (2.63±1.41) from 3.45±1.26 at the baseline (corresponding to slight looseness/neither tightness nor looseness) to 6.08±0.72 at the follow-up (corresponding to moderate tightness) ($p<0.001$). In total 47 (95.92%) patients reported some level of improvement while 2 (4.08%) patients didn't feel any change. The percentage of subjects who reported perception of vaginal laxity improved from 57.14% (baseline) to 0% (follow-up). At the follow up, 97.96% of patients reached some degree of tightness. See Table 3 for detailed data.

FSDS-R QUESTION	AVERAGE SCORE			PATIENTS IMPROVED (%)
	PRE-TX	3-M FU	P-VALUE	
1) How often do you feel distressed about your sex life?	1.56	0.44	<0.001	75.56%
2) How often did you feel unhappy about your sexual relationship?	1.47	0.44	<0.001	71.11%
3) How often did you feel guilty about your sexual difficulties?	1.33	0.36	<0.001	62.22%
4) How often did you feel frustrated by your sexual problems?	1.31	0.29	<0.001	68.89%
5) How often did you feel stressed about sex?	1.33	0.36	<0.001	64.44%
6) How often did you feel inferior because of sexual problems?	1.02	0.29	<0.001	55.56%
7) How often did you feel worried about sex?	0.98	0.22	<0.001	51.11%
8) How often did you feel sexually inadequate?	1.24	0.29	<0.001	62.22%
9) How often did you feel regrets about your sexuality?	0.78	0.27	<0.01	42.22%
10) How often did you feel embarrassed about sexual problems?	1.04	0.29	<0.001	55.56%
11) How often did you feel dissatisfied with your sex life?	1.47	0.36	<0.001	68.89%
12) How often did you feel angry about your sex?	0.44	0.27	>0.05	22.22%
13) How often did you feel bothered by low desire?	1.62	0.38	<0.001	71.11%
TOTAL STUDY AVERAGE AND OVERAL IPROVEMENT	**15.60**	**4.24**	**<0.001**	

Table 2. FSDS-R questionnaire evaluation.

SCORE	INTERPRETATION	PRE-TX		3M FOLLOW-UP	
		Patients (N)	Patients (%)	Patients (N)	Patients (%)
1	Very loose	2	4,08%	0	0,00%
2	Moderately loose	9	18,37%	0	0,00%
3	Slightly loose	17	34,69%	0	0,00%
4	Neither tight/loose	11	22,45%	1	2,04%
5	Slightly tight	6	12,24%	8	16,33%
6	Moderately tight	4	8,16%	26	53,06%
7	Very tight	0	0,00%	14	28,57%

Table 3. Evaluation of vaginal laxity.

Discussion

This study evaluated non-invasive RF treatments of sexually active women after childbirth affected by sexual dysfunction. The physiological response to the heating is described as a re-activation of fibroblast cells leading to neocollagenesis and neoelastogenesis. This concept was proven in both animal and human studies when treating the skin[14,15], as well as after treating the vaginal tissue[16].

RF heating for treating vaginal laxity and sexual functions was already documented by previous authors[6,7,10,17]. The data presented herein show significant improvement in all FSDS-R dimensions ($p<0.01$) except for question 12 ($p>0.05$) which can be attributed to the low baseline score limiting any improvement potential. Overall the results suggest a substantial improvement in patients' sexual intercourse satisfaction. The average FSDS-R improvement exceeds what Sekiguchi et al have reported and is comparable to results published by Torre and Miller, while the latter study included 22 treatments over 45 days and reported a lower percentage of patients responding to the therapy.

The subjects treated in this study reported on average much less severe vaginal laxity before treatments than what is shown in previous publications[6,7,10,17], yet the average VLQ improvement post-treatments reaches similar values. This suggests that the treatments are highly effective for a wide spectrum of women, including those with only a mild or a less sever condition. The relatively higher average pre-treatment VLQ score may also help justify why close to 98% of the treated patients reported some degree of vaginal tightness at the follow-up. The arbitrarily small average discomfort score is consistent with previous studies on RF vaginal application except Sekiguchi et al.

Although previous authors have confirmed that patients preserve the improvement 6 and 12 months after similar RF-based treatments, future research might be necessary to validate such hypothesis for the investigated device, as well as to confirm its efficacy in a sham-controlled study.

Conclusion

The investigated device is safe and effective for treating sexual dysfunction and vaginal laxity in post-pubertal women non-invasively.

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