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THERAPEUTIC HOTLINE

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

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

KEYWORDS: collagen, radiofrequency, tightening

Introduction

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

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

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

Materials and methods

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

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

Treatment procedure

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

Collagen analysis

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

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

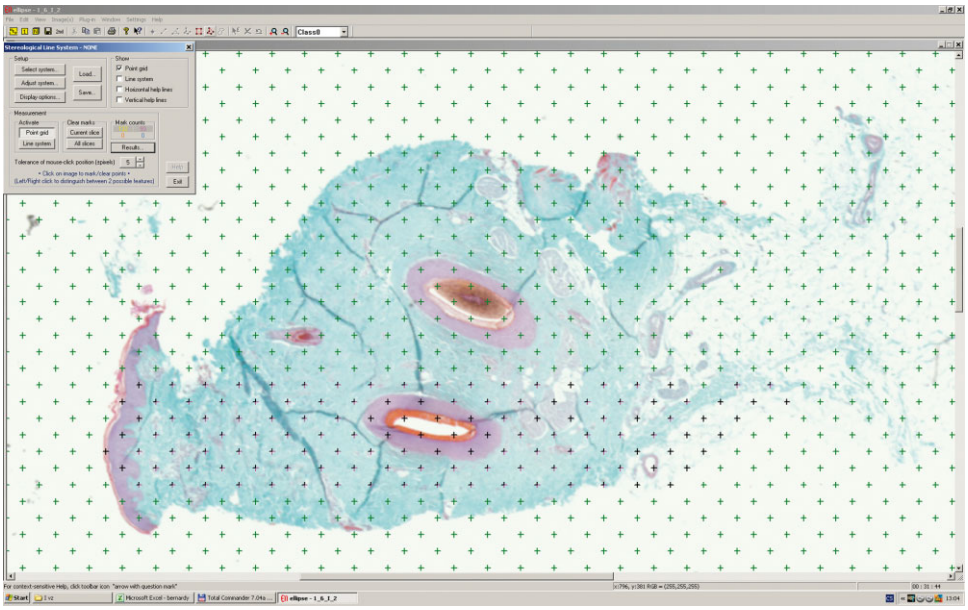

FIG. 1. Stereological analysis.

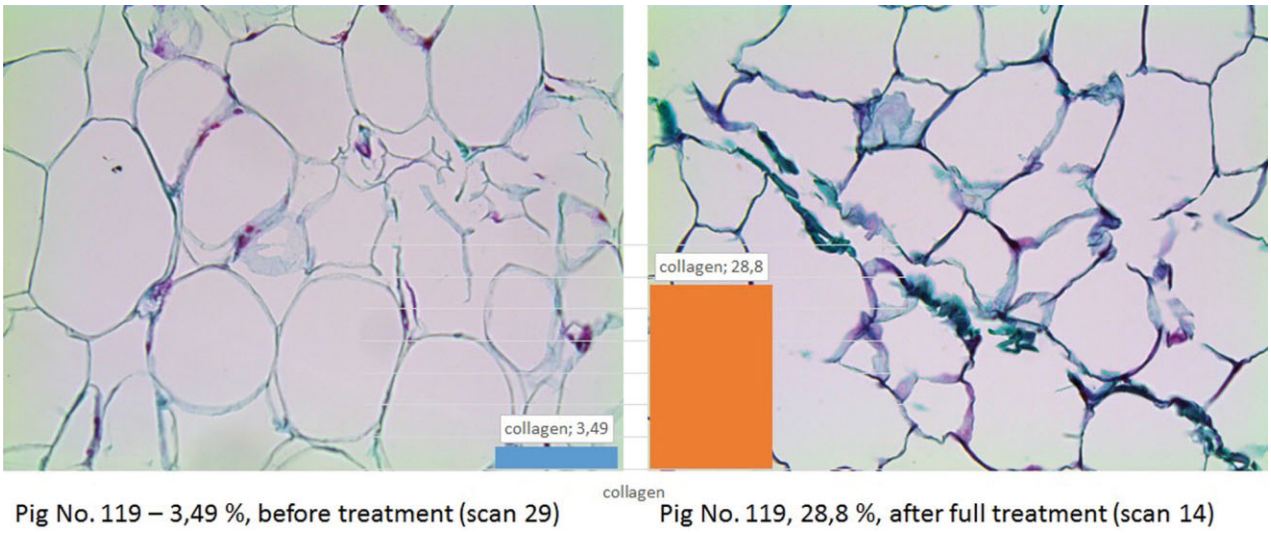

FIG. 2. Results of stereological analysis.

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

Results

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

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

Discussion

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

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

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

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

Conclusion

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

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