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ORIGINAL ARTICLE

Ex vivo study of the home-use TriPollar RF device using an experimental human skin model

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Abstract

Background: A wide variety of professional radio frequency (RF) aesthetic treatments for anti-aging are available aiming at skin tightening. A new home-use RF device for facial treatments has recently been developed based on TriPollar™ technology. *Objective*: To evaluate the mechanism of the new home-use device, in the process of collagen remodeling, using an ex vivo skin model. *Methods*: Human skin samples were collected in order to evaluate the anti-aging effect of a home-use device for facial treatments on an ex vivo human skin model. Skin tightening was evaluated by dermal histology, quantitative analysis of collagen fibers and dosage of collagen synthesis. *Results*: Significant collagen remodeling following RF treatment with the device was found in the superficial and mid-deep dermis. Biochemical measurement of newly synthesized collagen showed an increase of 41% in the treated samples as compared to UV-aged control samples. *Conclusions*: The new home-use device has been demonstrated to affect significant collagen remodeling, in terms of the structural and biochemical improvement of dermal collagen on treated skin samples.

Key words: *anti-aging, collagen, ex vivo, home-use, radio frequency, remodeling, TriPollar*

Introduction

Fine lines, wrinkles and facial skin texture have been constant concerns for aging people from the beginning of documented history. The majority of psychosocial surveys reveal that this concern is reasonable and related to self-esteem and age-related discrimination, especially in women. Poor self-image is associated with fewer preventive health behaviors, such as regular exercise and a healthy diet. Such far-reaching implications help explain the ongoing quest to develop better treatments to delay the signs of aging.

In looking at the biological mechanisms which lead to fine lines, wrinkles and changing texture, both intrinsic and extrinsic factors are at work simultaneously in aging skin. The process of intrinsic skin aging follows a similar route to that of most internal organs, with factors including unavoidable changes in hormone levels, particularly estrogen, as well as declines in metabolic activity and cell regeneration. Extrinsic skin aging is caused primarily by UV exposure, environmental pollution and smoking. As a result, the thickness of the epidermis and the cellular turnover rate of both the epidermis and the stratum corneum declines and epidermal unity is altered. Dermis becomes thinner as major structural molecules including collagen, elastin and glycosaminoglycans decrease in amount. Age-related changes in inter- and intra-cellular signaling lead to decreases in collagen synthesis. These changes work together to compromise skin's elasticity, firmness and structure, which contribute to areas of collapse and irregularity, and ultimately appear as fine lines and wrinkles (1).

In recent years, skin anti-aging research has advanced at a rapid pace and non-invasive treatments such as lasers, intense pulsed light (IPL), infrared (IR) and radio frequency (RF) were developed scientifically to treat the signs of aging at professional clinics (2). RF treatments are gaining more and more acceptance by physicians owing to their internal safety and

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efficacy. Unlike light-based treatments, RF is suitable for all skin types and colors (3).

RF energy is a form of electromagnetic energy. When applied to tissues, rapidly oscillating electromagnetic fields cause movement of charged particles within the tissue and the resultant molecular motion generates heat. During the past few years this source of heat has been extensively used in aesthetic clinics worldwide as a means of shrinking redundant or lax connective tissues through the mechanism of collagen denaturation (4). Heated fibroblasts are also implicated in new collagen formation and subsequent tissue remodeling which can also contribute to the final cosmetic result. Based on these mechanisms, large, high-power RF systems have been effectively used by dermatologists and aesthetic surgeons for indications such as skin tightening, wrinkle removal, body contouring and treatment of cellulite on various body areas including the face, neck, arms, abdomen, buttocks and thighs (5). In these systems, RF energy is either applied to tissue between two points on the tip of a probe (bipolar) or between a single electrode tip and a grounding plate (monopolar) (6). A new RF technology, however, employs a unique TriPollar™ design (regen™, apollo™; Pollogen Ltd) which is based on multiple electrodes with a proprietary sequence of current modulation between these electrodes. This technique has been clinically demonstrated to be highly effective in focusing RF power deep into dermal layers while maintaining safe epidermal skin temperatures (7).

The first home-use RF device for facial skin tightening (Ultragen, Israel) has recently been developed based on TriPollar RF technology. The system is indicated for the treatment of skin laxity, to reduce fine lines and wrinkles and improve skin texture. Based on prior clinical experience with the technology in professional clinics, it is expected to be safe and effective in reversing the signs of skin aging on all skin types, even when self-applied at home.

This ex vivo study was designed to investigate the anti-aging effect of the new home-use TriPollar RF device by using human skin samples submitted to experimental aging with UV exposure.

Materials and methods

The study was performed on eight human skin samples harvested from different donors who underwent abdominoplasty (four samples) and cervical-facial lifts (four samples). Each sample was sectioned into three parts. One part was left as the control while the other two parts were immediately subjected to a UV aging process accomplished by exposure to UVA (365 nm) at a fluence of 12 J/cm^2 together with UVB (312 nm) at 2 J/cm^2 from a UV radiation source (Vilbert-Lourmat, France). Such UV exposure has previously been shown to induce premature structural and cellular skin photoaging effects on fibroblasts and dermal collagen in human skin explants (8–10).

Of the two UV-aged skin sample parts, one was again left for control purposes while the other immediately underwent TriPollar STOP™ (Ultragen) treatment for 5 minutes.

All skin samples were then cut and placed in inserts which were positioned in culture wells at 37°C in a sterilizer with 5% CO_2. A survival medium, which approximates in vivo metabolic conditions, was added at the bottom of the wells, enabling a slow diffusion between the compartments through a 12-μm porous membrane. The survival medium was changed three times a week. Skin samples were maintained alive for 10 days.

Analysis of all skin samples was based on histological quantification of collagen by computerized image analysis and biochemical measurement of newly synthesized collagen.

Skin fragments were fixed in Bouin's solution and embedded in paraffin. Serial sections 4-μm thick were obtained and stained with a picric acid solution containing 0.1% Sirius red. Morphometric quantification, using a Leitz microscope at ×160 magnification, was performed on computerized images to determine the percentage of superficial to mid-dermal cross-sectional area occupied by collagen.

Newly synthesized collagen was retrieved from the skin samples, after specific fixation of the Sirius red colorant (Sircol Collagen Assay; Interchim), by placing the samples overnight at 4°C in 0.5 M acetic acid solution containing pepsin (6 mg/ml). Acid soluble newly synthesized collagen was then quantified by a 540-nm spectrocolorimetric method in units of μg/ml of tissue sample.

Mean values and standard deviations were calculated for each parameter. The statistical significance of changes recorded in the parameters was determined with paired Student's t-test ($p < 0.05$).

Three groups were compared: control samples (not aged and not treated); aged only samples; and aged and treated samples.

Results

Histological quantification of collagen shows a significant diminution of superficial to mid-dermis collagen following UV aging (Figure 1). In UV-aged skin samples, the cross-sectional area occupied by collagen was measured to be 56.6% compared with

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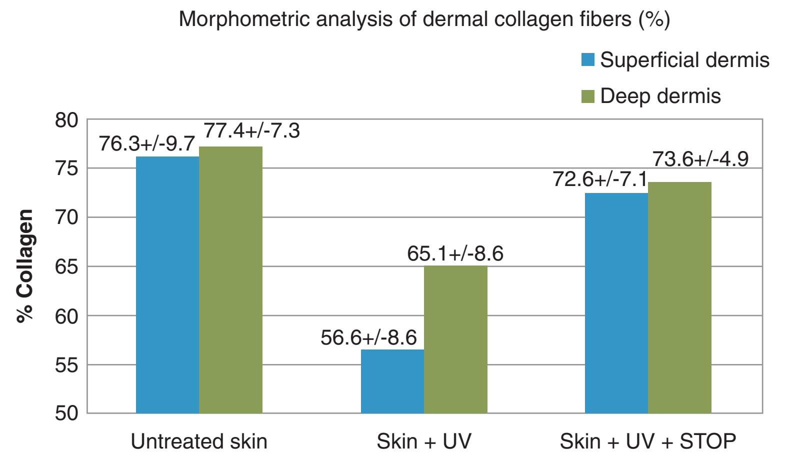

Figure 1. Histological quantification of collagen.

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76.3% in the non-aged samples ($p = 4.10^{-5}$). On the aged and treated samples, dermal collagen was measured to be 72.6% ($p = 10^{-6}$), indicating significant neocollagenesis following treatment with the home-use device.

In the mid to deep dermis collagen area, the percentage was measured to be 77.4% in the control samples, 65.06% ($p = 0.003$) in the UV-aged samples and 73.65% ($p = 0.012$) in the UV-aged and TriPollar-treated samples.

The increase in collagen following RF treatment is particularly prominent in all analyzed skin samples as a dense collagen strip disposed on the basal membrane (Figure 2).

Results of the biochemical measurement of newly synthesized collagen are presented in Figure 3. In the UV-aged samples, a slight decrease from 13.1 μg/mg to 12.1 μg/mg was found but this was not statistically significant. Following RF treatment, newly synthesized collagen was measured as 17.1 μg/mg, a dramatic, statistically significant increase over control skin samples ($p = 0.049$).

Discussion

Investigations of the clinical and histopathological effects of non-ablative treatments on dermal structures have been extensively conducted in past years. Studies have focused on understanding the mechanism of professional treatments based on optical or RF treatments done for the purpose of achieving tissue tightening for wrinkle removal. Alam et al. (11) published a review of histology and tissue effects following non-ablative laser and light treatments stating that though the analytic methodologies employed were limited, some generalizations can be drawn from these studies. Thermal injury most likely affects the vasculature, which initiates a cascade of inflammatory events that includes fibroblastic proliferation and apparent up-regulation of collagen expression. Weeks to months after a series of non-ablative treatments, collagen deposition is increased and assumes a horizontal orientation parallel to the plane of the epidermis with dermal thickening reported in some of the cases.

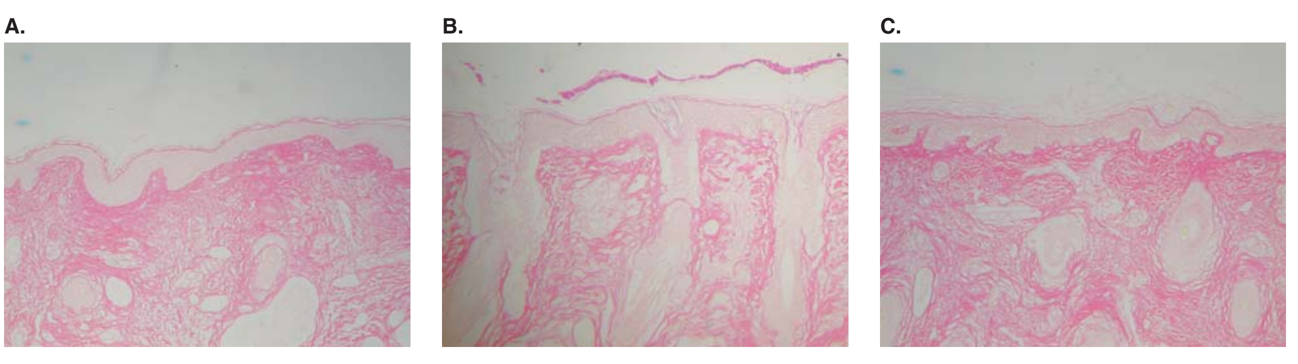

Figure 2. Histology of skin fragments from facial lifts – dermal collagen stained with Sirius red (magnification: ×10; 1 cm = 66 μm). (A) Untreated skin. (B) UV-exposed skin. Note the presence of a fragmentation and thinning of collagen fibers in the superficial dermis and in the upper level of the middle dermis. (C) UV exposure + device treatment. Note the repair of collagen fibers in the superficial dermis with appearance of a thick collagen strip on the basal membrane.

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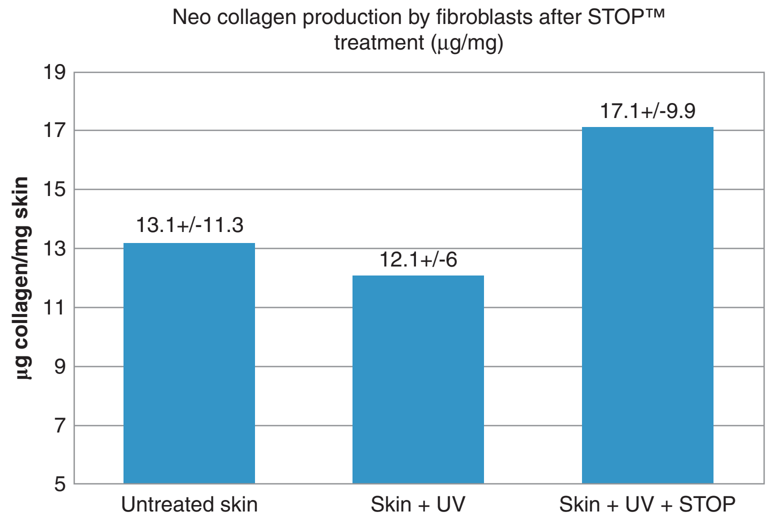

Figure 3. Biochemical measurement of newly synthesized collagen.

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The first RF device indicated for skin tightening was initially studied using a standard guinea pig model (12). Shallow dermal heating at the papillary dermis level or deeper heating of the subcutaneous fat was achieved with the monopolar RF device. The results showed that heating the dermal layer of the skin is associated with collagen denaturation and subsequent thickening and shortening of collagen fibrils. This is followed by a period of increased fibroblast activity and neo-collagen formation over a period of several months. Histological changes associated with new collagen formation in the dermis were noted in the treatment areas where significant skin contraction was observed.

The ex vivo method presented in the current study to obtain premature aging of human skin samples in order to investigate the reparative effects of various anti-aging agents has previously been reported (8–10). It allows quantitative analysis of the effects of anti-aging agents and devices on the fibrous components of the dermis in conditions which reproduce those of human skin in vivo. The same ex vivo method was previously used to evaluate the anti-aging effect of two professional devices based on the TriPollar RF technology (13,14).

In this study, the ex vivo human skin model was used to study the effect of a home-use device indicated for skin tightening. Results demonstrated significant collagen remodeling following RF treatment with the new home-use device.

In the superficial dermis, collagen content increased by 28.3% following a single TriPollar treatment while in the deep dermis it increased by 13.2%. Biochemical measurement of newly synthesized collagen showed an increase of 41% in the treated samples as compared with the UV-aged control samples.

Of particular interest is the observation that on all treated skin sample histologies a dense collagen strip disposed on the basal membrane was prominently apparent. The diminution of collagen during aging, particularly in this area, is responsible for the accentuation of wrinkles, so significant neocollagenesis in this area should lead to marked improvement in the appearance of the skin following treatment.

A clinical study in which female subjects used the device at home, demonstrated the safety and efficacy of the home-use RF device for facial skin tightening. Consistent application of the treatment, maintained a tighter and more supple skin with a significant reduction of fine lines and wrinkles (15).

Summary

Based on our previously reported ex vivo human skin model for the study of aging and anti-aging effects on dermal collagen, the new RF home-use device has been demonstrated to affect significant collagen remodeling on treated skin samples. Results were most prominent on the superficial to mid-dermis, where collagen depletion due to aging and photodamage presents the appearance of rhytids and wrinkles.

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