Histological and Ultrastructural Evaluation of the Effects of a Radiofrequency-Based Nonablative Dermal Remodeling Device

A Pilot Study

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**Background:** Many light- and laser-based systems are used to reduce cutaneous wrinkles, and some have been shown to stimulate dermal collagen production. Using the ThermaCool TC radiofrequency device to treat bovine tendon and human abdominal skin, we documented the cutaneous effects of a radiofrequency-based system for nonablative treatment.

**Observations:** Electron microscopy of bovine tendon treated at varied heat and cooling settings revealed collagen fibrils with increased diameter and loss of distinct borders as deep as 6 mm. Human skin treated at varied heat and cooling settings and examined by means of routine light microscopy demonstrated no significant changes in the epidermis or dermal ground substance immediately after treatment; there was scattered mild perivascular and periadnexal inflammation. Three and 8 weeks after treatment, no observable changes were noted. Ultrastructural analysis, however, disclosed isolated, scattered areas of collagen fibrils with increased diameter and loss of distinct borders. In addition, Northern blot analysis demonstrated an increase in collagen type I messenger RNA steady-state expression.

**Conclusions:** Our findings suggest that collagen fibril contraction occurs immediately after treatment and gives rise to tissue contraction and thermally mediated wounding, which induces new collagen production.

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METHODS

BOVINE TENDON

Fresh bovine tendon was obtained from a local butcher and kept well hydrated before treatment. The treatment was performed within 48 hours after procurement of the tendon. The tendon was placed in a dish with isotonic sodium chloride solution and a return electrode, which was connected to the RF generator. The 1-cm² treatment tip of the ThermaCool TC system (ThermaCare, Inc, Hayward, Calif) was then used to treat the tendon at the settings shown in the Table.

After treatment, biopsy specimens of the tissue were placed in cold glutaraldehyde. The samples were then processed as previously described for transmission electron microscopy.13

ABDOMINOPLASTY SKIN

Two female patient volunteers who were scheduled for elective abdominoplasty gave informed consent for RF treatment and successive biopsy specimens in the region of skin to be excised during the subsequent abdominoplasty. Each subject had 8 treatment areas (grids) tattooed in a 2 × 2 pattern of 1-cm² squares.

The volunteers were at least 18 years of age and not pregnant, had no history of collagen vascular disease, and were not using anti-inflammatory medications. This study was conducted under a protocol approved by an investigation review committee.

The first subject had 4 of the 8 grids treated with 95 J using a 1-cm² treatment tip. The energy was delivered for 2.1 seconds. Cooling cryogen was delivered as follows: 23 milliseconds during 1 second of precooling, 33 milliseconds during 2.1 seconds of on-time cooling, and 33 milliseconds during 3.1 seconds of postcooling. Control biopsy and treatment biopsy specimens were obtained 2 days, 7 days, 3 weeks, and 8 weeks after treatment and were examined by means of electron microscopy and Northern blot analysis.

The second subject was involved in a dose-response study. Three pairs of grids were treated with different energy levels. The treatment energies for the 3 pairs were 104, 133, and 181 J using a 1-cm² treatment tip and an energy delivery time of 2.3 seconds. Cooling cryogen was delivered as follows: 23 milliseconds during 1 second of precooling, 36 milliseconds during 2.3 seconds of on-time cooling, and 33 milliseconds during 3 seconds of postcooling. Biopsy specimens were obtained before treatment, within 45 minutes after treatment, and 8 weeks after treatment. They were processed and examined using routine techniques for light histology and electron microscopy.

MECHANISM OF TISSUE HEATING WITH RF

The RF system places an active and a return electrode on the skin. As the active electrode builds up a charge, it produces an electric field underneath itself. The charge is then rapidly changed from positive to negative. To produce a uniform distribution of charge across the electrode face, a dielectric, a nonconducting material, is used to capacitively couple the energy to the skin. The size and strength of the electric field is dependent on the geometry of the electrode and the power delivered through the tip. Ions and charged molecules in the tissue within the electric field move or rotate. The inherent resistance (ohms) to the movement of these ions and molecules in tissue causes heat (joules). The resistance and the heat produced are tissue dependent. It is possible to change the depth of treatment by changing the electrode geometry, power delivered, delivery time, and cooling variables.

LIGHT AND ELECTRON MICROSCOPY ANALYSIS

Punch biopsy specimens were routinely processed for light and electron microscopy. The percentage of change was determined by measuring the area that the changed collagen fibrils occupied compared with that of the unchanged fibrils in electron micrographs.

STATISTICAL ANALYSIS OF PERCENTAGE OF COLLAGEN FIBER CHANGE BY DEPTH AND ENERGY

The percentage of collagen fiber change was calculated for 3 energy levels and 5 dermal depths. We compared the results using 2-way analysis of variance without replication, so the depth × energy interaction was used as the error term for the comparisons. Pairwise comparisons of energy levels were performed using the Tukey multiple comparisons procedure.

NORTHERN BLOT ANALYSIS

The RF-treated tissue and untreated control tissue from patient 1 was examined by means of Northern blot analysis. Briefly, total RNA was isolated from frozen biopsy specimens as previously described.14-16 Total RNA was isolated from the whole biopsy specimen, and 4.9 µg of total RNA was used for each sample.

RESULTS

BOVINE TENDON

Electron microscopic analysis revealed significant enlargement of the collagen fibrils with loss of electron density and sharp borders (Figure 1). These alterations were observed throughout the treated area, with healthy collagen fibrils adjacent to the altered ones. This change was observed to occur as deep as 6 mm. Without cooling, 100% of the collagen fibrils within the first 1 mm of tissue were affected. The percentage of affected fibrils decreased to 60% affected at 1 to 2 mm, 10% at 2 to 3 mm, 5% at 3 to 4 mm, and about 1% at 4 to 6 mm. When medium cooling was added, the percentage of altered fibrils was 10% at 0 to 1 mm, 30% at 1 to 2 mm, and 1% at 2 to 3 mm, and no alterations were observed at 4 to 6 mm. When high cooling was added, the percentage of alteration in fibrils was 0% at 0 to 1 mm, 1% at 1 to 2 mm, 20% at 2 to 3 mm, 20% at 3 to 4 mm, 10% at 4 to 5 mm, and 1% at 5 to 6 mm (Figure 2).

SKIN BIOPSY RESULTS

Light Microscopy

Subject 1. In this time course study, the epidermis and dermis of the treated biopsy specimens looked similar to
those of the control specimens at all time points. The finding that was not seen in the control tissue was a mild superficial perivascular infiltrate that was present at day 2 in treated specimens and not detected at any other time point. No significant change in the elastic tissue was found during examination by means of elastic tissue stains.

**Subject 2.** In the dose-response evaluation immediately and at 8 weeks after treatment, the epidermis and dermal ground substance appeared normal at all settings. Evaluation of the superficial capillary plexus showed no signs of endothelial swelling within 45 minutes after treatment. However, a mild perivascular and perifollicular inflammation was noted to be greater than that seen in the control specimen after treatment with 104 and 181 J (Figure 3). This inflammatory response was not seen at 8 weeks. Findings in the deeper dermis were unremarkable.

**Electron Microscopy**

Immediately after treatment, ultrastructural analysis disclosed scattered diffuse changes in the collagen fibril architecture. There was an observable increase in size and a loss of distinct borders among the collagen fibrils, with some areas showing the fibrils to be merged together with no discernable borders (Figure 4). The change was noted throughout the middermis in patchy areas. No other significant changes were noted in the epidermis or dermal adnexal structures.

The dose-response study showed altered collagen fibril changes that peaked at 3 to 4 mm in depth (Figure 5). The percentage of fiber change for 5 dermal depths averaged 9% at 104 J, 8% at 133 J, and 18% at 181 J. An overall significant difference among energy levels was found with a \( P \) value of .02. Pairwise comparisons show the 181-J energy level had a significantly greater percentage of change than the 104- and 133-J levels at \( P = .03 \) and \( P = .02 \), respectively. No significant difference among depths was found. With increasing energy, the protective effect of epidermal cooling could be overcome. The time course study showed a higher percentage of changes in collagen fibrils immediately after treatment that were slowly repaired during the next 8 weeks. A mild inflammatory infiltrate was found, indicating a wound-healing response.

**Northern Blot Analysis**

Treated skin demonstrated an elevation of collagen messenger RNA (mRNA) expression compared with untreated skin, after correction with 7 Svedberg units (S) ribosomal RNA as standards (Figure 6). Collagen type
I mRNA steady-state expression measured 2.4-fold that of the untreated control on day 2, and 1.7-fold that of the control 1 week after treatment. Three and 8 weeks after treatment, mRNA levels were slightly below those of the control.

**COMMENT**

This pilot study shows the immediate and short-term effects of a novel RF-based device designed for nonablative skin treatment. The most dramatic responses are detected with electron microscopy in skin and tendon samples immediately after treatment. The morphological alterations of collagen fibrils include increased diameter and loss of distinct edges. These changes are similar to those detected in studies involving rat tendon and corneal tissue. Small areas of focal changes were scattered throughout the dermis. The cooling device for this system appears to protect the epidermis from thermal injury within the variables used in this study. The breakage of intramolecular bonds in the collagen fibril appears after the tissue reaches a certain threshold of heating. The observation that the collagen changes are not seen in graduated effects may be explained by variations in the heat susceptibility of different types or in the ages of collagen distributed in tissue to uniform temperature conditions. It is also possible that selective heating of nonhomogeneous tissue structures may cause these changes.

In the bovine tendon model, with more homogeneous collagen than in skin, a more uniform and graduated pattern of collagen heat damage was observed than in human skin. The effect is more irregular in the skin compared with the tendon, which most likely reflects the less homogeneous tissue in the skin or the pattern of new collagen formation within skin vs tendon. This finding is somewhat similar to what has been detected in bovine tendon and skin treated with a carbon dioxide laser. In these ablative therapies, some normal-appearing fibrils are found adjacent to partially denatured fibrils. However, the overall change is much more uniform.

The shape of the electric field is dependent on the geometry of the electrode and the tissue to which it is applied. The effect of the electric field on tissue is dependent on the strength of the electric field and the resistance of the tissue to that field. In a homogeneous tissue such as tendon, the field decreased uniformly from the surface. The location of the peak temperature is then a result of the cooling in combination with the heating. In nonhomogeneous media, the electric field is less uniform. In areas that have high resistance, fewer molecules will move and less heat will be produced when near low-resistance areas. Microenvironments undoubtedly exist within tissue, but their extent and severity are still unknown. If we assume that the surrounding tissue is 10°C cooler than the denatured tissue (35°C vs 65°C), it would take about 0.1 milliseconds to drop the difference in temperature to the surrounding tissue for an area of the denatured collagen on the order of 5 µm in diameter. How such a small volume of tissue could maintain a temperature 10°C hotter during a 2.3-second energy delivery and how the types and ages of collagen in the dermis are distributed are not understood at present. However, these microenvironments may exist, and if coupled with different melting points for younger vs older collagen, they may account for the seemingly random distribution of denatured collagen sites within normal collagen regions.

Clinically, the collagen fibril contraction effect is used to shorten tendons in the shoulder capsule and tighten the joint. In the tendon, the collagen fibrils run in a parallel direction, so the shortening effect acts synergistically in one direction to give the desired effect. In the cor-
nea, pinpoint thermal inputs are placed in the diameter of the cornea to create a ring of contraction at the edge and change the shape of the central cornea.20,21 The arrangement of collagen in the skin is quite different in that it is arranged in bundles aligned in every direction. Thus, the clinical contraction will cause a 360° tightening effect from the point source of the heating. This contraction was not clearly detected immediately after treatments. Wall et al22 demonstrated that tendon tissue contracts by 35% when 100% of the collagen was denatured. In our human tissue samples, approximately 10% of the tissue collagen was denatured, as seen with transmission electron microscopy. If the same percentage of shrinkage to collagen damage holds in skin, then a 3.5% shrinkage in skin might be seen. A forehead 6 cm high would produce a 2-mm contraction in the skin, which has been demonstrated in clinical studies.23

A secondary effect detected is that of new collagen production. This treatment causes a thermal injury in the dermis, which most likely stimulates a wound-healing response. This may lead to an increase in new skin formation and thus collagen production. In this study, we show some very suggestive evidence by demonstrating increased collagen gene expression in samples from 2 patients. We are not aware at present of any similar studies using this method to evaluate collagen synthesis after laser therapy. This technique has been used to show a similar increase in elastin mRNA in sun-damaged skin compared with control skin.15 Regeneration of the extracellular matrix, as detected by means of histological and in vitro methods, often occurs in response to laser, alpha hydroxy acid, or retinoid treatment. Increased collagen type I mRNA steady-state expression in this small sample of treatment sites suggests this type of remodeling. The fact that whole biopsy specimens were used may have underestimated the percentage of increase. Further studies in larger patient populations are needed to confirm these findings. Evaluating different tissue depths to demonstrate local changes and changes in other mRNAs as well as in the extracellular matrix components themselves should help to further explain the mechanisms behind the clinical improvements noted after RF treatment. In addition, these studies will help define the heating and cooling variables used in future treatments.

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