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Treatment of Hypertrophic Scars and Keloids With a Radiofrequency Device: A Study of Collagen Effects

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Background and Objective: To determine the efficacy and safety of the ThermoCool[®] TC radiofrequency system for treatment of hypertrophic and keloid scars and evaluate treatment associated collagen changes.

Materials and Methods: Six subjects with hypertrophic and four with keloid scars were treated with the ThermoCool[®] device: one-third of the scar received no treatment (control), one-third received one treatment and one-third received two treatments (4-week interval). Scars were graded before and then 12 and 24 weeks after treatment on symptoms, pigmentation, vascularity, pliability, and height. Biopsies were taken from four subjects with hypertrophic scars and evaluated with hematoxylin and eosin (H & E) staining, multiphoton microscopy, and pro-collagen I and III immunohistochemistry.

Results: No adverse treatment effects occurred. Clinical and H & E evaluation revealed no significant differences between control and treatment sites. Differences in collagen morphology were detected in some subjects. Increased collagen production (type III > type I) was observed, appeared to peak between 6 and 10 weeks post-treatment and had not returned to baseline even after 12 weeks.

Conclusion: Use of the Thermage radiofrequency device on hypertrophic scars resulted in collagen fibril morphology and production changes. ThermoCool[®] alone did not achieve clinical hypertrophic scar or keloid improvement. The collagen effects of this device should be evaluated further in order to optimize its therapeutic potential for all indications. *Lasers Surg. Med.* 37:343–349, 2005.

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Key words: multi photon excitation microscopy; Thermage; pro-collagen

INTRODUCTION

The ThermoCool TC system (Thermage, Inc., Hayward, CA) is a radiofrequency (RF) technology, which has FDA 510 K clearance for non-invasive treatment of facial wrinkles and rhytids. The ThermoCool system utilizes active and return electrodes on the skin [1]. Under the charged active electrode, an electric field is produced which is rapidly alternated, positive to negative. A dielectric is

used to couple capacitively the electrode to the skin and produce a uniform distribution of charge. Tissue ions and charged molecules within the electric field move and/or rotate and inherent resistance to this movement causes heat. Depth of treatment effect can be changed by altering electrode geometry, power delivered, delivery time, and cooling parameters. A cooling tip delivers cryogen spray to protect the epidermis.

The ThermoCool[®] system has been used for cheek [2], neck [3] and brow [4] lifting and for treatment of moderate to severe acne vulgaris [5]. Improvement has been noted for all of these indications and side effects were generally limited to transient erythema and edema.

Zelickson et al. [6] performed histologic ultrastructural analysis on biopsies from areas treated with the ThermoCool[®] system and demonstrated changes in collagen fibril morphology which were thought to be central to the therapeutic effect of this device. These collagen effects may provide an opportunity for alternative treatment applications and, as such, should be investigated further.

Hypertrophic and keloid scars are potential indications for the ThermoCool[®] that have not been explored, but for which this device may have therapeutic potential. Hypertrophic and keloid scars are a source of concern for many patients and a challenge for their physicians. Currently available treatments, including intralesional corticosteroids, bleomycin, excision, pulsed dye or CO₂ laser irradiation, cryotherapy, and radiation therapy [7–13], can achieve flattening in some cases, but complete removal is never obtained. Further, multiple treatments are required and adverse effects, including infections, hypoesthesia, necrosis, and dyspigmentation, may occur. Because of the limitations of currently available treatments, other options are sought.

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One reason that removal of hypertrophic and keloid scars remains difficult is that the pathogenesis of these lesions is not understood. Alterations in production of collagen types I and III have been reported, and several currently utilized scar treatments, including pulsed dye lasers and cryotherapy, affect collagen production [12–14].

It was hypothesized that treatment of hypertrophic and keloid scars with the ThermoCool® system may result in collagen remodeling (a change in the amount and type of collagen), which in turn may lead to scar improvement (Personal Communication, Karl Pope, Director of Research, Thermage, Inc.). The current study was a single-center, open-label, pilot study designed to determine the safety and efficacy of the ThermoCool® system for treatment of hypertrophic scars and keloids and to characterize the collagen effects of this therapy.

MATERIALS AND METHODS

The Institutional Review Board at the University of California, Irvine approved the research protocol. Subjects with non-facial hypertrophic or keloid scars were recruited for the study. Exclusion criteria included pregnancy, treatment of the scar in the last 4 weeks and presence of a pacemaker or automatic electronic defibrillator.

For each subject, three scar areas were selected for evaluation and designated control, one or two treatments areas. Care was taken to select areas with similar characteristics. In 7 of the 10 subjects, a single scar was divided into three equal areas. In 3 of the 10 subjects, 2 or 3 scars of similar characteristics were evaluated.

The control and test sites were assessed clinically pre-treatment and 12 and 24 weeks post-treatment. One of the investigators (KMK) and a research assistant evaluated the test sites in reference to five scar traits: symptoms [8], pigmentation, vascularity, pliability, and height [15] (Table 1). The five scar characteristics were summed to yield an overall scar assessment. Recent publications have documented the reliability of similar scar assessments [16,17]. At each visit, subjects were also monitored for any adverse effects including epidermal disruption, worsening of scarring, and skin discoloration.

A topical anesthetic (ELA-max 5% cream, Ferndale laboratories, Ferndale MI) was applied to treatment areas 1 hour before the procedure. All treatments were performed with the Thermage ThermoCool system. The ThermoCool system applies pre-cooling to the skin, RF energy at 6 MHz with concomitant cooling, and a post cool. The treatment area was 1.5 cm² and the treatment time for each spot was 1 second for RF delivery and 0.9 seconds for additional cooling. The amount of energy delivered varied according to a self-reported moderate pain threshold of each subject.

At the first treatment visit, starting from the subject's left, the first two-thirds of the scar received no treatment; the final one-third of the scar was treated. Four weeks later, patients made a second treatment visit. The first one-third of the scar area was not treated (this was left as the control site and received no treatment at either visit), the second

TABLE 1. Evaluated Scar Characteristics and Rating Scale

Scar trait	Rating scale
Symptoms	0 = none 1 = mild itch/burn 2 = moderate itch/burn 3 = severe itch/burn
Pigmentation	0 = color same as surrounding skin 1 = hypopigmentation 2 = hyperpigmentation
Vascularity	0 = normal 1 = pink 2 = red 3 = purple
Pliability	0 = normal 1 = supple: flexible with minimal resistance 2 = yielding: giving way to pressure with moderate resistance 3 = firm: solid, resistant to pressure 4 = banding: rope-like tissue 5 = contracture: permanent shortening of scar-producing deformity
Height	0 = normal 1 = <2 mm 2 = 2–5 mm 3 = >5 mm

one-third of the scar received a first treatment and the final one-third of the scar area received a second treatment.

ANOVA was used to compare the study sites (control, one and two treatments) at each time point (pre-treatment and 12 and 24 weeks post-treatment) and each site (control, one and two treatments) over the study period for each of the evaluated scar characteristics and the overall scar assessment score.

Four subjects with hypertrophic scars underwent 3 mm punch biopsies to each of the three study areas (control, one, two treatments). Biopsies were performed at different post-treatment points (1, 6, 10, or 12 weeks post-treatment) to provide an evaluation of collagen effects over time.

Biopsy specimens were placed in saline and imaged by multi photon excitation microscopy (MPM), a non-invasive optical tomography method for evaluation of collagen fibril structure. This technology uses an 800 nm femtosecond laser excitation source to interact with collagen, producing a second harmonic generation (SHG) signal, which allows selective visualization of the collagen matrix [18].

After MPM imaging, specimens were fixed in buffered 10% formalin, embedded in paraffin, cut into 6 µm thick sections and mounted onto albumin-coated slides for hematoxylin and eosin (H&E) staining.

For immunohistochemistry [19], paraffin blocks were cut into 4 µm sections. The sections were pretreated with 0.14 g trypsin digestion in 100 ml PBS at 37°C for 40 minutes. Anti-procollagen I and anti-procollagen III immunostaining was then performed. Two dermatologists counted stained and unstained fibroblasts in three fields using the

TABLE 2. Subject and Scar Characteristics

Subject number	Subject age (years)	Age of scar (years)	Scar site	Hypertrophic scar or keloid	Period between treatments and biopsy (weeks)
1	20	4	Knee	Hypertrophic	1
2	74	4	Chest	Hypertrophic	6
3	51	50	Abdomen	Hypertrophic	10
4	46	1	Shoulder	Hypertrophic	12
5	36	24	Back	Hypertrophic	N/A
6	60	4	Shoulder	Hypertrophic	N/A
7	38	15	Chest	Keloid	N/A
8	20	4	Chest	Keloid	N/A
9	26	<4 varied onset	Posterior neck	Keloid	N/A
10	20	2	Chest	Keloid	N/A

40 $\times$ objective and recorded the total number of stained and unstained fibroblasts observed.

RESULTS

Ten subjects were enrolled in the study: 6 with hypertrophic scars and 4 with keloids (Table 2). The average age of the hypertrophic scar subjects was 48.2 and that of the keloid subjects was 26.0. The scar duration ranged from 1 to 50 years for hypertrophic scars and 2–15 years for keloids. One subject with a keloid was lost to follow-up after the treatment visits. One additional subject with a keloid was evaluated at 12 weeks post-treatment but was lost to follow-up at 24 weeks post-treatment. Treatment levels ranged from $\times 2.0$ to $\times 6.0$ with an average of $\times 4.2$ (89 J).

Table 3 provides the summed evaluation scores for the control, one and two treatments test sites as determined 12 and 24 weeks post-treatment.

Scar symptoms did improve slightly over the 24 weeks study period (although the difference did not reach statistical significance) but there was no difference in improvement for control versus one treatment versus two treatment sites. No significant differences were noted among the three sites (control, one and two treatments) or in any one study site over time for any of the evaluated scar characteristics or for the overall scar assessment score. No adverse effects were observed.

Tissue Evaluation: Multi-Photon Microscopy

Subject 1, knee: biopsy 1 week-post treatment. Due to significant epidermal thickness, the samples were imaged from the dermal side. At the control site, a weak, unresolved second harmonic signal was detected (Fig. 1A,

top). At sites treated once or twice, imaging by means of SHG revealed thick collagen fibers with varied orientation (Fig. 1A, middle) and thick curved collagen sheets (Fig. 1A, bottom). No significant differences were noted among the three test sites.

Subject 2, chest: biopsy 6 weeks post-treatment. At the control site, very thick, well-organized collagen fibril sheets were resolved with a strong SHG signal. The individual fibers in the sheets were resolved (Fig. 1B, top). At the one treatment site, SHG signal from collagen was strong at the surface. Short fiber bundles with varying orientation were resolved (Fig. 1B, middle). At the two treatments site, collagen was clumped; individual fibers were not resolved. Treatment appeared to result in fiber shortening, but no significant difference was noted between the one and two treatment sites (Fig. 1B, bottom).

Subject 3, abdomen: biopsy 10 weeks post-treatment. At the control no SHG signal was generated (Fig. 1C, top). At the one treatment site an unresolved collagen signal was obtained from areas with a thinner epidermis (Fig. 1C, middle). At the two treatments site an SHG signal from unresolved collagen clumps was observed. At the surface, collagen fibers took on a thin thread-like appearance (Fig. 1C, bottom). A progression in light penetration depth was observed for the control (least penetration), one and two treatment specimens. The reason for this change was not clear but may have been secondary to varied epidermal thickness or collagen density (decreased density would allow greater light penetration).

Subject 4, shoulder: biopsy 12 weeks-post treatment. Control, one and two treatments: At ~ 60 – $80\ \mu\text{m}$ there was an unresolved collagen signal. Collagen fibers were ordered into a barrel-like structure with “crisscrossing” fiber bundles (Fig. 1D, top, middle, bottom, respectively). No significant differences were noted among the three test sites.

Histological Evaluation

Evaluation of H&E histology specimens revealed normal scar pathology with fibrosis and scattered inflammatory cells. Thickness and orientation of fibers and depth of the scar varied among the four subjects who underwent

TABLE 3. Summed Evaluation Scores (Hypertrophic/Keloid Scars) for Each Test Site at 12 and 24 Weeks Post the Final Treatment

	Control site	1 RX	2 RX
12 weeks	5.5/6.7	5.5/7.0	5.7/7.0
24 weeks	5.5/8.5	5.5/8.5	5.7/8.5

biopsies. No significant differences were noted between control, one and two treatment areas.

Procollagen I and III Immunofluorescence

Procollagen I and III immunofluorescence results are summarized in Table 4 and graphed in Figure 2a,b, respectively. Subjects 1 through 4 were biopsied at 1, 6, 10, and 12 weeks after the last treatment, respectively. Because of the small number of samples (1 at each time point) statistics were not performed. However, trends can be determined. Biopsies from subject 1 (1 week post-treatment) demonstrated no differences in collagen production between control and treated sites. In subject 2 (biopsied 6 weeks post-treatment), there was an increase in procollagen I and a more pronounced increase in procollagen III at the one treatment site but not in the two treatments site as compared to control. Subject 3 had an increase in procollagen I and procollagen III in the one treatment site but again no difference in the two treatments site as compared to control. Finally, in subject 4 at 12 weeks, procollagen III

was elevated as compared to control after one treatment. No difference was noted at the two treatments site.

DISCUSSION

No significant differences were found in the clinical assessments of pre- and post-treatment scars or between control, one and two treatments areas. Additional studies such as ultrasound assessment of scar thickness, cutometer evaluation of scar firmness, or spectrophotometer measurement of erythema and pigmentation may have revealed treatment-associated changes not appreciated by clinical assessment and could be considered for future studies.

Histological assessment of treated and non-treated areas, similarly did not demonstrate significant differences. A small number of biopsies (4) were performed in this study; however, MPM and immunohistochemistry evaluations did provide interesting information on the collagen effects of the Thermage device, supporting the idea that radiofrequency effects occur at a submicroscopic level.

Fig. 1. Multiphoton microscopy and H&E histology for (A) Subject 1, (B) Subject 2, (C) Subject 3, and (D) Subject 4.

Fig. 1. (Continued)

MPM is a unique imaging method that allows selective evaluation of collagen fibril morphology. In this study, significant variation was found in the collagen structure of the biopsies from the four subjects. This may be due to differences in body site, age of the scar and clinical scar characteristics. In two of the subjects (2 and 3), MPM detected SHG (collagen signal) differences between control and treatment specimens characterized as post-treatment fiber shortening or an increase in light penetration, which could result from fiber shortening. This adds additional information about ThermoCool[®] treatment collagen effects to the electron microscopy data provided by Zelickson et al., who reported scattered, isolated areas of alteration, including increased diameter, and a loss of distinct borders [6]. MPM did not detect significant differences in collagen between control and treatment areas in subjects 1 and 4. It is possible that no changes were present in these specimens, but it is also possible that there were local changes that went undetected as MPM is a form of optical tomography. Further biopsy evaluations are warranted.

Immunohistochemistry analysis of the biopsies also provided interesting information on procollagen levels. In areas that received one treatment, we observed no change in collagen production 1 week post-treatment, followed by an increase, which appeared to peak between 6 and 10 weeks and, for collagen III, had not returned to baseline by 12 weeks. At 6 weeks (subject 2) this effect was more pronounced for procollagen III as compared to procollagen I. Some authors have noted an increased collagen I/III ratio in keloids as a result of increased procollagen I production [14]. The greater procollagen III production that we observed might result in a normalization of this ratio; however, we did not observe clinical scar improvement. Other authors have theorized that collagen production and collagen degradation are both aberrant in keloids and scars and that normalization of the balance between these two processes is required for scar resolution [20].

However, the patterns of collagen production observed may help to explain clinical effects achieved with ThermoCool[®] for other indications such as wrinkle reduction and

TABLE 4. Ratio of Positive/Negative Fibroblasts for Procollagen I and Procollagen III Immunohistochemistry

	Subject 1, 1 week	Subject 2, 6 weeks	Subject 3, 10 weeks	Subject 4, 12 weeks
Procollagen I				
Control	4.6	2.5	5.5	27.3
1 Rx	4.6	23.6	44.5	28.5
2 Rx	5.7	12.4	3.5	1.5
Procollagen III				
Control	12.0	12.2	3.4	6.2
1 Rx	0.1	102.0	55.0	23.5
2 Rx	10.0	22.5	6.9	11.5

may provide information to help optimize treatment protocols. Clinical improvements associated with collagen production would not be present until more than a week post-treatment, and improvement may continue for more than 12 weeks. It is interesting that patients 2 and 3 showed procollagen production after one treatment but not after

two treatments. Perhaps a second treatment should not be performed until after 12 weeks or more, when collagen production has returned to baseline.

It is also important to note that ThermoCool[®] treatment protocols have been revised since initiation of this study. When the current study was designed and performed, a commonly utilized and recommended procedure was the implementation of maximum tolerated treatment fluences and a single pass. Subsequently, it has been determined that better treatment effects may be achieved using lower fluences and multiple passes. Collagen effects may be different with newer protocols and should be evaluated.

In conclusion, use of the Thermage radiofrequency device on hypertrophic and keloid scars resulted in changes in collagen fibril morphology and production. ThermoCool[®] alone did not achieve clinical scar improvement; however, the significant collagen effects of this device should be evaluated further in order to optimize its therapeutic potential.

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REFERENCES

1. Nelson JS, Majaron B, Kelly KM. What is nonablative photorejuvenation of human skin? *Sem Cut Med Surg* 2002; 21:238–250.
2. Alster TS, Tanzi EL. Treatment of prominent nasolabial folds and cheek laxity with a nonablative radiofrequency device. *Lasers Surg Med* 2003;15S:34.
3. Tanzi EL, Alster TS. Improvement of neck laxity with a nonablative radiofrequency device: A lifting experience. *Lasers Surg Med* 2003;15S:34.
4. Fitzpatrick RE, Gernoeum R, Goldberg D, Kaminer M, Kilmer S, Ruiz-Esparza J. First multi-center study of a new

Fig. 2. Ratio of positive/negative fibroblasts for (A) procollagen I and (B) procollagen III. Treatments were 1 month apart.

- non-ablative radio frequency device to tighten facial tissue. *Lasers Surg Med* 2003;15S:35.
5. Ruiz-Esparza J, Gomez JB. Nonablative radiofrequency for active acne vulgaris: The use of deep dermal heat in the treatment of moderate to severe acne vulgaris (thermotherapy): A report of 22 patients. *Dermatol Surg* 2003;29:333–339.
 6. Zelickson BD, Kist D, Bernstein E, Burns J, Kilmer S, Pope K. Histologic and ultrastructural evaluation of the effects of a radiofrequency based non-ablative dermal remodelling device. *Lasers Surg Med* 2003;15S:35.
 7. Nouri K, Jimenez GP, Harrison-Balestra C, Elgart GW. 585-nm pulsed dye laser in the treatment of surgical scars starts on the suture removal day. *Dermatol Surg* 2003;29:65–73.
 8. Alster T. Laser scar revision: Comparison study of 585 nm pulsed dye laser with and without intralesional corticosteroids. *Dermatol Surg* 2003;29:25–29.
 9. Nouri K, Lodha R. Scarring solutions. *Cosmetic Dermatology* 2003;16:49–56.
 10. Kelly AP. Keloids: Pathogenesis and treatment. *Cosmetic Dermatology* 2003;16:29–32.
 11. Har-Shai Y, Amar M, Sabo E. Intralesional cryotherapy for enhancing the involution of hypertrophic scars and keloids. *Plast Reconstr Surg* 2003;111:1841–1852.
 12. Kuo Yr, Jeng SF, Wang FS, et al. Flashlamp pulsed dye laser suppression of keloid proliferation through down-regulation of TGF-beta1 expression and extracellular matrix expression. *Lasers Surg Med* 2004;34:104–108.
 13. Dalkowski A, Fimmel S, Beutler C, Zouboulis ChC. Cryotherapy modifies synthetic activity and differentiation of keloidal fibroblasts in vitro. *Exp Dermatol* 2003;12:673–681.
 14. Friedman DW, Boyd CD, Mackenzie JW, Norton P, Olson RM, Deak SB. Regulation of collagen gene expression in keloids and hypertrophic scars. *J Surg Res* 1993;55:214–222.
 15. Baryza MJ, Baryza GA. The Vancouver scar scale: An administration tool and the interrater reliability. *J Care Rehab* 1995;16:535–538.
 16. van de Kar AL, Corion LU, Smeulders MJ, Draaijers LJ, van der Horst CM, van Zuijlen PP. Reliable and feasible evaluation of linear scars by the patient and observer scar assessment scale. *Plast Reconstr Surg* 2005;116:514–522.
 17. Li-Tsang CW, Lau JC, Chan CC. Prevalence of hypertrophic scar formation and its characteristics among the Chinese population. *Burns* 2005;31:610–616.
 18. Zoumi A, Yeh A, Tromberg BJ. Imaging cells and extracellular matrix in vivo by using second-harmonic generation and two-photon excited fluorescence. *Proc Natl Acad Sci USA* 2002;99:11014–11019.
 19. Nuutinen P, Reikki R, Parikka M, Salo T, Autio P, Risteli J, Oikarinen A. modulation of collagen synthesis and messenger RNA by continuous and intermittent use of topical hydrocortisone in human skin. *Br J Dermatol* 2003;148:39–45.
 20. Cohen IK, Diegelmann RF, Johnson ML. Effect of corticosteroids on collagen synthesis. *Surgery* 1977;82:15–20.