Histologic Study of Collagen and Stem Cells After Radiofrequency Treatment for Aging Skin

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BACKGROUND Monopolar radiofrequency (mRF) devices have been shown to be clinically effective for treating aging skin, but there are few histologic studies about the mechanisms.

OBJECTIVE To histologically analyze chronologic and quantitative change in collagens after mRF treatment to determine the mechanisms of the antiaging effect.

METHODS Five patients were enrolled in this study. Skin specimens were taken before and 1 and 3 months after treatment. Immunostaining was performed to determine change in type I and III collagen levels and stem and other cell counts in skin layers.

RESULTS In all cases, both types of collagen significantly increased after irradiation in the dermis (p < .05), and their changes were noticed uniformly in all layers. No significant change was noticed in stem and other cell counts.

CONCLUSIONS This 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.

The authors have indicated no significant interest with commercial supporters.

With beauty consciousness rising, increasing numbers of people visit medical institutions to seek treatment for wrinkles and skin laxity. Surgical treatments, such as face lifts, are required for achieving drastic improvement, but patients generally prefer less-invasive methods, and there are various procedures available, including botulin and hyaluronic acid injection and irradiation with lasers or other heat sources. Treatment with heat-confering devices is said to trigger dermal restoration as a result of continual tissue improvement. A radiofrequency (RF) irradiation device is one method used for tissue improvement.

The mechanisms of RF treatment are thought to be instant thermal contraction of collagen (immediate tightening) and a thermal injury recovery process that continues for several months (secondary tightening). It is estimated that secondary tightening is associated not only with thermal damage, but also with dermis remodeling promoted by other factors. There have been reports on adipose-derived stem cells and the influence they have, direct or indirect, on wound healing processes, as well as dermis fibroblasts. We were interested in the behavior of stem cells and their effects on skin after esthetic treatments.

This study was aimed to histologically analyze chronologic and quantitative change in collagen after treatment of facial skin using a monopolar RF (mRF) device, as well as the relationship between

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such changes and stem cells to determine the mechanisms of wrinkle and laxity alleviation.

**Patients**

Five patients (three male, two female; average age 42.6, range 30–47; skin type III to IV) treated between June 2012 and January 2013 were enrolled. We excluded individuals who had received facial treatments at esthetic salons or medical clinics within the previous 6 months, who clearly had skin disease other than photoaging, who had keloid diathesis, who were pregnant, who had conditions inhibiting RF treatment, or who had any underlying disease that might affect wound recovery. Excessive exposure to ultraviolet light, esthetic treatments, home peeling, and other skin care treatments were prohibited during treatment. All subjects were given a detailed explanation of the expected advantages and disadvantages of the procedure, such as treatment effect, possible complications of treatment and exploratory excision. All gave written consent to undergo the treatment. The Fujita Health University Ethics Committee authorized the study guidelines according to the WMA Declaration of Helsinki (1975), Ethical Principles for Medical Research Involving Human Subjects.

**Methods**

**Equipment Used**

We used a mRF device that creates an electric field under the treatment tip, using capacitive coupled treatment tip as an active electrode along with a return electrode (TheraCool, Thermage, Hayward, CA). Because 26°C below zero cryogen gas is released from the hand piece during treatment, the device can heat deep dermis and subcutaneous tissue without damaging skin surface.

**Treatment Protocol**

No anesthesia or precooling was performed. Using a 3-cm² tip, the appropriate location was evenly irradiated using the “multiple method” mode. RF output was regulated according to the pain that each patient felt. No cooling or antiinflammatory agents (steroid or nonsteroid) care was used after treatment. Subjects were instructed to avoid exposure to ultraviolet light.

**Outcome Assessments**

**Clinical Evaluation**

Using a skin image analysis system (VISIA; Canfield Imaging Systems, Fairfield, NJ), photographs were taken before treatment and 1 and 3 months after treatment. Two dermatologists judged improvement in skin condition as none, mild (improvement in skin texture without any improvement in rhytides), moderate (improvement in skin texture with lessening of rhytides), or excellent (improvement in skin texture with total resolution of rhytides). Patients subjectively evaluated the results.

**Histologic Evaluation**

Skin tissue specimens were taken three times horizontally from 2 cm outside the left outer canthus. For sample staining, we used hematoxylin and eosin staining and the indirect fluorescent antibody technique using type I collagen antibody (1:500; LB-1102; LSL, Tokyo, Japan), type III collagen antibody (1:500; LB-1102; LSL), and antibody to CD271 (1:100; MAB5386; Millipore, Billerica, MA), which is thought to be a dermal stem cell marker.

Samples were processed using the indirect fluorescent antibody technique to take photographs under a consistent exposure time. Mean fluorescence intensity (MFI)/10⁴/μm² was counted at five locations chosen at random from a 200-times-magnified image with appendages excluded. Based on the above measurements, average value and standard deviation (SD) were calculated of the amount of collagen contained in the images. Next, to determine total cell counts and stem cell counts, we used images with the nucleus stained blue and
CD271 stained red using the indirect fluorescent antibody technique. The 200-times-magnified image was longitudinally divided into four parts, from each of which blue-stained nuclei were counted to provide total number of cells. Nuclei whose circumference was stained red were also counted to provide a stem cell count. Based on measurements from four parts, the average value and SD were calculated. The increase rates were calculated with the assumption that the value before irradiation was “1” in each case.

**Statistical Analysis**

The Wilcoxon rank-sum test was used. Differences were considered statistically significant at $p < .05$.

**Adverse Events**

Immediately after irradiation, the treated skin of all cases showed erythema, which was temporary and disappeared the next day. No burns or postinflammatory pigment deposition were observed. No additional adverse events were noted.

**Results**

**Clinical Course**

All five cases received a one-time treatment and had a 3-month follow-up appointment. The device setting, based on patient pain and immediate reaction during treatment, ranged from 33 to 38 J/cm$^2$. Although it is difficult to determine from the pictures alone, skin texture improved, especially around the cheek, and the line from the cheek to the jaw seemed tightened in all cases (Figure 1, Table 1).

**HE Staining**

Collagen fibers became thicker throughout the dermis layer after treatment (Figure 2).

**Type I and III Collagen**

Average type I collagen calculated from the five cases increased in all layers, statistically significantly so in the superficial and middle layers. Figure 3A is a representative case (Patient 1). Type I collagen expression was enhanced after irradiation (Figure 3B).

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**Figure 1.** Clinical evaluation. Patient 1: (A) Left cheek: Fine wrinkles decreased and texture was improved. (B) Left jaw: Prominent change was not observed.
Type III collagen was similarly analyzed and increased statistically significantly in all but the superficial layer. Figure 4 shows type III collagen change and increase rates for all five cases.

**Total Cell Counts and Stem Cell Counts**

The changes of the total cell counts were not constant. Each layer showed an increase, but there was no statistically significant difference before and after treatment.

The number of CD271 antibody-positive cells was also calculated. A significant increase from baseline was observed only in the middle layer 3 months later. Figure 5 shows transition of total cell counts and CD271 antibody-positive stem cell counts, as well as stained pictures of the representative case (Patient 1).

**Discussion**

This is the first clinical study of the chronologic and quantitative transition of collagen and its relationship with stem cells after RF irradiation of the face.

Of the various devices that have been used to correct wrinkles and laxity, RF is thought to be highly effective and is frequently used worldwide, but despite many reports on the positive clinical effects, few histologic studies are available. The reason that RF treatment effects continue as long as several months is not known, so clarification of such mechanism is needed. First,

**TABLE 1. Clinical Evaluation**

<table>
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<th>Patient</th>
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<th>Improvement (patient)</th>
<th>Improvement (Doctor)</th>
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Figure 2. Hematoxylin and eosin staining original magnification ×200 (Patient 1). Increase in dermal collagen fibers was observed.
we analyzed chronologic and quantitative change in type I and III collagen and compared their change between skin layers. Both types of collagen had increased significantly at least 3 months after irradiation from pre-irradiation levels. These results indicate that type I and III collagen increased three-dimensionally and uniformly throughout the dermis at least 1 to 3 months after irradiation. Chin or cheek tightening could be attributable to increases in type I and III collagen, but a three-dimensional analysis device may be necessary for more-accurate demonstration of results because evaluation for laxity and texture improvement largely depends on sense of touch rather than pictures.

We analyzed dermal stem cells. Hasebe and colleagues used CD44, CD54, CD105, CD90, and CD271 as dermal stem cell markers. In particular, CD54 and CD271 were used for staining, because they were considered to have high growth and differentiation abilities. CD271 antibody-positive stem cells were analyzed, because they showed more positive results, but analysis of total cell count and CD271 antibody-positive stem cell count did not reveal significant change in any case. Cell counts did not change whereas almost all type I and III collagens increased significantly. No association for a change of collagens and cell counts was recognized. The amount of stem cells was thought not to affect an increase in collagens from these results.

There are many reports referring to adipose-derived stem cells (ADSCs) as a factor in influencing fibroblast activity by directly or indirectly stimu-
lating the wound healing process or dermal fibroblasts themselves.3,4 Additionally, p75NTR-positive (CD271-positive) cells, which are considered to be especially involved in comprehensive skin regeneration, are thought to exist in the epidermis and dermis in addition to subcutaneous adipose tissues,11 and there are reports on lasers that activate stem cell migration and proliferation in vitro.12,13 We predicted from these reports that collagen increase attributable to stem cell migration to the treated area triggered by RF stimulation, differentiation of stem cells into fibroblasts, and paracrine effects on fibroblasts caused secondary tightening after RF irradiation, but staining results indicated no quantitative change in CD271-positive stem cells and total cells, which meant that there was no differentiation into or proliferation of fibroblasts.

Regarding why there was no change in the amount of stem cells, we predicted the possibility that the time of the sampling was too late and the possibility that ADSC influenced the increase in collagens.

Further examination will be necessary to examine the possibility that indirect effects from stem cells enhanced collagen-producing capabilities of fibroblasts.

**Conclusions**

This study histologically demonstrated that type I and III collagen had significantly increased in the dermis after mRF treatment. The amount of stem cells did not affect the increase in collagens.

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![Figure 4](image-url)

**Figure 4.** (A) Type III collagen increased in all layers except the dermal superficial layer in Patient 4. Average indicates statistically significant increase in all layers. (B) Type III collagen was fluorescently stained green. Type III collagen in dermis increased its density (original magnification ×200).
Figure 5. Total cell and stem cell count. (A) Particular tendency was not observed in total cell count for each layer or patient and on average. (B) Stem cell count had neither consistent rules nor statistically significant difference. (C) Blue represents DAPI, red represents CD271. Significant change has not been observed in each layer (original magnification ×200).
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References


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