Greater Collagen Deposition with the Microneedle Therapy System Than with Intense Pulsed Light

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BACKGROUND Intense pulsed light (IPL) and the microneedle therapy system (MTS) are currently available for the treatment of scars. Greater collagen deposition has been proposed as a mechanism for the treatment of scars.

OBJECTIVE To compare the effects of IPL and MTS on collagen deposition.

MATERIALS AND METHODS Fifty-four imprinting control region mice were divided into three groups: untreated controls, treatment with IPL, and treatment with MTS. A single pass of IPL 10.5 J/cm^2 and five passes (total 15 strokes) of MTS were performed three times every 2 weeks. Four weeks after the last treatment, skin thickness measurements using a caliper, microscopic examination, Western blot analysis for type I collagen, and enzyme-linked immunosorbent assay for total collagen content were performed.

RESULTS Measured using calipers, MTS, resulted in greater skin thickness than IPL that paralleled the dermal thickness of the biopsied specimens. MTS also increased expression levels of type I collagen and total collagen content more than IPL. IPL effects were superior to control.

CONCLUSION MTS increased collagen deposition more than IPL, and MTS might be more effective than IPL for scar treatment.

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Acne scarring is one of the most common and emotionally debilitating problems encountered in the field of dermatology. Treatment choice depends on the type of acne scar, with ablative or nonablative methods available. Because ablative methods such as dermabrasion and laser resurfacing cause significant postoperative changes in the skin and require significant healing time, nonablative therapy using lasers and light, such as intense pulsed light (IPL), have become popular treatment methods.[1] IPL devices contain a noncoherent filtered flashlamp that emits a broad wavelength spectrum of 560 to 1,200 nm, delivering a high peak in short pulses. Although IPL has been used for the treatment of telangiectasias,[2] photoepilation,[3] and lentigines,[4,5] it has also become increasingly popular as a nonablative skin rejuvenation treatment. Data indicating that IPL significantly increases procollagen I[6,7] and III, with a significant decrease in matrix metalloproteinase (MMP)-1 and MMP-2, support the beneficial effects of IPL on dermal collagen remodeling,[7] although IPL treatment requires trained and experienced personnel and expensive laser equipment.

Skin needling is an effective way to treat scars and wrinkles. Subcuticular undermining, termed subcision, involves inserting a needle into the skin and tunneling in various directions parallel to the skin surface.[8] Skin needling can also be accomplished by puncturing. Although the puncturing technique was developed for transdermal drug delivery,[9–11]

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puncturing or trepanation, referred to as needle dermabrasion, also improves scar appearance.[12] Treatment with skin needling breaks old collagen strands in scars and wrinkles, which might promote removal of damaged collagen growth and induce more collagen growth immediately under the epidermis.[13] Rolling with multiple microneedles over areas with acne scars increases the amount of collagen and elastin deposition.[13–15] The advantage is that the epidermis remains intact with the rolling microneedles, minimizing the risks induced by ablative methods such as laser resurfacing.[13] Moreover, microneedle therapy is a simple technique that shows immediate improvements.[13] We found that microneedle therapy significantly increased the ultrasound distance between the epidermis and dermosubcutaneous junction within 8 weeks in 17 patients with acne scars, showing good clinical effects (data not shown).

IPL and microneedle therapy have many advantages over ablative methods, but no comparison between the two techniques has been reported. We compared the effects of IPL and skin needling on collagen synthesis and skin thickness using mice as a model for the improvement of acne scars.

Materials and Methods

Animal Preparation

Fifty-four 10-week-old imprinting control region mice were used for the study. They were fed a standard diet and kept in a constant environment at the appropriate temperature (23°C) and humidity (25%) with a 12-hour light–dark cycle. The mice were divided into three groups: untreated control, treated with IPL (Ellipse Flex, Danish Dermatologic Development,), or treated with the microneedle therapy system (MTS, 0.5-mm Dermaroller, Clinical Resolution, Brea, CA). Before treatment, all mice were anesthetized with 2 mg/0.1 mL of Avertin intraperitoneally. Their backs were shaved with an electrical clipper and then denuded with 80% thioglycolic acid (Niclean Cream, Il Dong Pharm, Seoul, Korea).

Treatment

The dorsal skin of 18 mice was irradiated with a single IPL pass using a fluence of 10.5 J/cm^2 after application of cooled ultrasound gel. The wavelength of IPL ranged from 555 to 950 nm. Two pulses were used, with a duration of 2.5 ms and a delay of 10 ms. The dorsal skin of the other 18 mice received five passes (total 15 strokes) with the MTS. The MTS contains 192 needles separated by 2.5 mm. Needle thickness was 0.25 mm, tapered to a 0.125-mm apex. IPL and MTS were performed three times every 3 weeks.

Skin Thickness Measurement

Four weeks after the last treatment, the thickness of the dorsal skin was measured using calipers (Mituyoto, Mituyoto Corp., Kawasaki, Japan).

Microscopic Examination

Skin specimens from eight untreated controls and four of each treated group were obtained for microscopic examination using a 5-mm-diameter punch. The samples were fixed in 10% formalin and embedded in paraffin. The samples were then sectioned and stained with hematoxylin and eosin and Masson's trichrome. The distance between the epidermis and subcutaneous fat was measured in the eight control mice to compare with the skin thickness measured using calipers.

Western Blot Analysis

The biopsied skin specimens from 15 mice (5 per group) were homogenized in ice-cold homogenization buffer containing 50 mM Tri-base (pH 8.0), 150 mM sodium chloride, 2 mM ethylenediaminetetraacetic acid, 1% glycerol, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, and protease inhibitor (0.1 mM phenylmethylsulfonylfluoride, 5 g/mL of aprotinin, and 5 g/mL of leupeptin). Equal amounts of the extracted protein (30 g) were resolved using 8% to 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. After blocking with 5%

nonfat dry milk in Tris-buffer saline (pH 7.6), the membranes were incubated with an anti-COL1A1 antibody (goat polyclonal; sc-8784, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) that could detect precursor and mature collagen type 1 of mouse, rat, and human origin diluted 1:1,000 in bovine serum albumin overnight at 4°C. The membranes were further incubated with a mouse antigoat peroxidase conjugated antibody. The membranes were treated with an enhanced chemiluminescence solution (ECL kit; Amersham Life Sciences, Buckinghamshire, UK), and the signals were captured on an Image Reader (LAS-3000, Fuji Photo Film, Tokyo, Japan). To monitor the amount of protein loaded into each lane, the membranes were treated with a stripping buffer and reprobed with a monoclonal antibody against β-actin (Sigma-Aldrich, St. Louis, MO). The protein bands were analyzed using densitometry.

Enzyme-Linked Immunosorbent Assay

Total collagen content was determined for quantitative analysis. A Sircol Collagen kit (Biocolor Ltd., Belfast, Northern Ireland) was used according to the manufacturer's instructions. The dermal skin specimens of all mice were homogenized in 10 mL of 0.5 M acetic acid containing 1 mg of pepsin per 10 mg of tissue residue. The homogenized specimens were incubated for 24 hours at 4°C with gentle stirring. After centrifugation, 100 µL of the supernatant was added to 1 mL of Sircol dye reagent and mixed for 30 minutes to bind the reagent to collagen. After centrifugation, the pellet was resuspended in 1 mL of alkali reagent, and the absorbance was read using a spectrophotometer at 540 nm.The collagen concentration (µg/mL) was determined against a collagen standard.

Statistical Analysis

The data were analyzed using one-way analysis of variance, with sets of two groups compared using the Tukey-Kramer multiple comparison test. The correlation between caliper-measured skin thickness and microscopic dermal thickness was analyzed uisng the Spearman rank correlation test. Statistical analyses were performed using NCSS 2007 software (NCSS, Kaysville, UT). $p < .05$ was considered statistically significant. All data are expressed as means ± standard deviations.

Results

Correlation Between Caliper-Measured Skin Thickness and Microscopic Dermal Thickness

Caliper-measured skin thickness was compared with microscopic dermal thickness as the distance between the lower margin of the epidermis and the uppermost portion of the subcutaneous fat in biopsied samples from untreated mice. Caliper-measured skin thickness was approximately 2.4 times as great as microscopic dermal thickness (correlation coefficient = 0.845, $p < .05$) (Figure 1), so it was substituted for microscopic dermal thickness in further experiments.

Larger Increase in Skin Thickness with MTS Than IPL

The thickness of the dorsal skin was measured using calipers 4 weeks after the last treatment. The thickness of the IPL-treated, MTS-treated, and untreated groups ranged from 860 to 1,370 µm, 1,060 to 1,530 µm, and 800 to 1,100 µm,

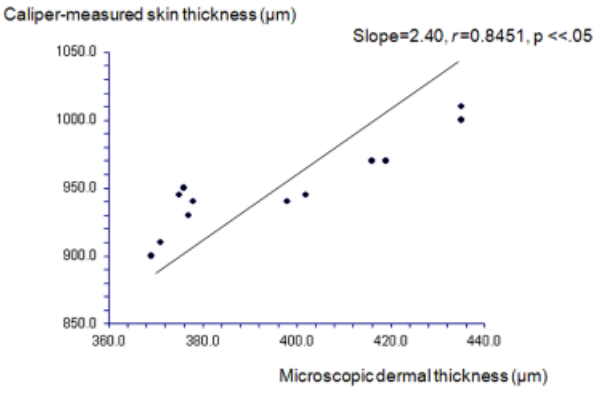

Figure 1. Skin thickness was measured using calipers in 12 untreated mice. The caliper-measured spots were biopsied for microscopic measurement of dermal thickness. The values measured by both methods correlated with each other (correlation coefficient = 0.845, $p < .05$), showing a slope of 2.40.

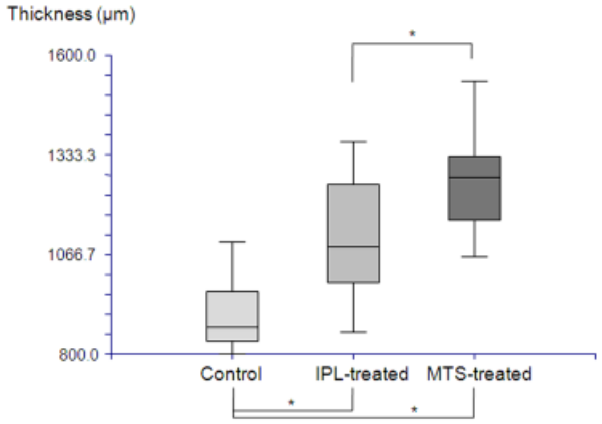

Figure 2. Caliper-measured skin thickness was analyzed in 54 mice divided into three groups (18 mice/group): untreated (control), treated with intense pulsed light (IPL), and treated with a microneedle therapy system (MTS). Values are expressed as means ± standard deviations. The multiple comparison tests showed all pairwise differences (*) between the means.

respectively, with a mean value of 1,100.0 ± 153.9, 1,266.1 ± 121.6, and 910.0 ± 90.9 μm, respectively ($p < .05$). All groups were different from the others (Figure 2).

MTS Treatment Increases Collagen More Than IPL

Both treatments increased dermal thickness, particularly MTS, and increased staining and thickness of collagen fiber in Masson's trichrome staining (Figure 3), suggesting a greater amount of collagen. Levels of type I collagen were examined in five samples from each group using Western blot analysis. Both treatments increased expression of the α1 chain of type I collagen more than the untreated group, with the MTS group higher than the IPL group ($p < .05$; Figure 4).

Collagen levels were quantitatively analyzed in all samples using enzyme-linked immunosorbent assay (ELISA). Collagen levels after IPL treatment ranged from 9.8 to 29.5 μg/mL (mean 19.2 ± 4.6 μg/mL) and after MTS treatment ranged from 12.9 to 53.3 μg/mL (mean 25.2 ± 9.5 μg/mL), whereas levels in untreated animals ranged from 7.5 to 20.0 μg/mL (mean 12.7 ± 3.3 μg/mL) ($p < .05$), with significant differences between all groups (Figure 5).

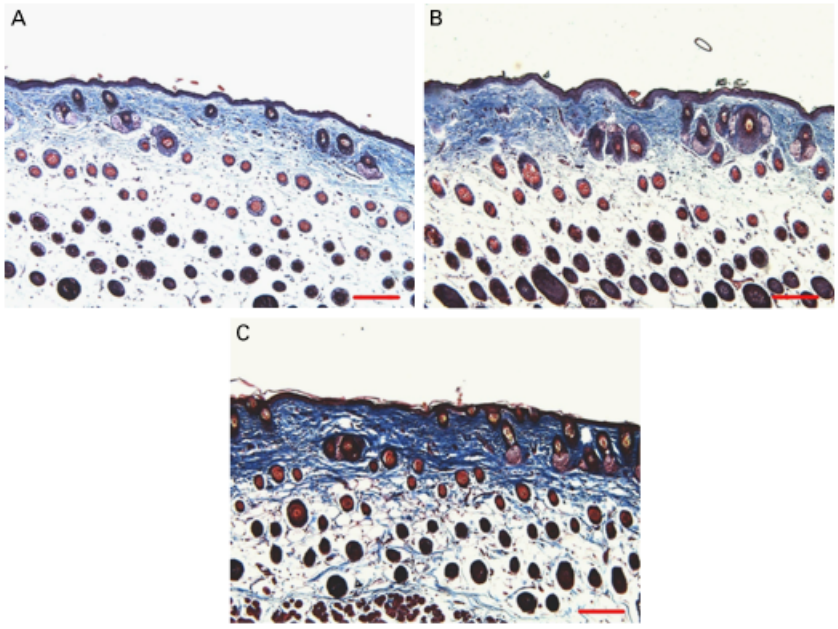

Figure 3. Microscopic evaluation with Masson's trichrome staining: (A) untreated control group, (B) group treated with intense pulsed light (IPL), (C) group treated with a microneedle therapy system (MTS). Dermal and collagen fiber thickness from the thinnest to thickest: untreated, IPL-treated, and MTS-treated. (original magnification × 100) (bar = 100 mm).

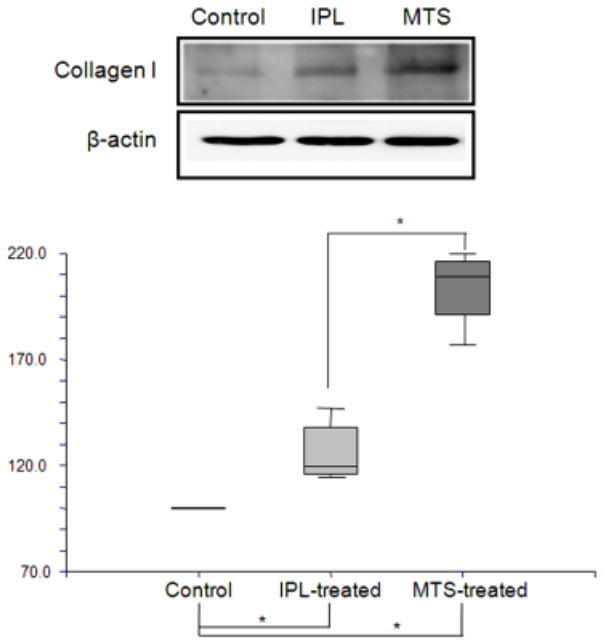

Figure 4. Western blot analysis of type I collagen: Expression of the α1 chain of type I collagen increased with treatments in the following order: untreated (control), treated with intense pulsed light (IPL), and treated with a microneedle therapy system (MTS) (*$p<.05$). Levels were significantly higher after MTS than IPL treatment (*$p<.05$).

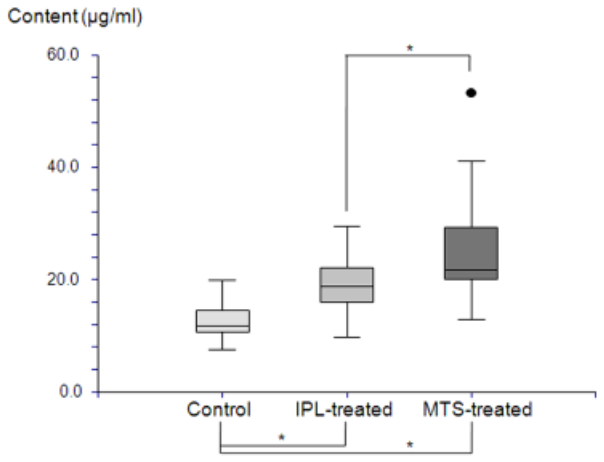

Figure 5. Total collagen content in untreated (control), treated with intense pulsed light (IPL), and treated with a microneedle therapy system (MTS). The data are expressed as means ± standard deviations ($n=18$). Multiple comparison tests showed significant differences (*) for all groups. The black dot in the MTS-treated group indicates a mild outlier, which is between more than 1.5 times and less than 3 times interquartile ranges from the 75th percentile.

Discussion

Because of the drawbacks of ablative methods, IPL and MTS are used for the treatment of scars and wrinkles and result in beneficial effects by inducing collagen remodeling. MTS induced larger increases in collagen deposition than IPL, although both treatments were effective.

MTS and IPL increased skin thickness as measured by calipers (Figure 2), with the biggest increases in the MTS-treated groups. Although microscopic dermal thickness is a measure of dermal thickness resulting from collagen change, it requires a skin biopsy and microscopic examination. However, microscopic dermal thickness correlated with changes in caliper-measured skin thickness, albeit at 2.4-fold thinner (Figure 1). Therefore, measuring skin thickness using calipers could replace measuring microscopic dermal thickness as a screening method.

Collagen represents the most abundant component of dermal connective tissue. Scar improvement involves collagen remodeling, including breaking old collagen strands and inducing collagen growth. Moreover, treatment of depressed acne scars with trichloroacetic acid increases collagen fiber density and elastic fiber fragmentation;[16] microneedles also increase collagen fibers and elastin deposition in acne scars.[14,15] ELISA with Sircol dye also showed a significant difference between the untreated and the treated groups as well as between the IPL-treated and the MTS-treated groups (Figure 5), indicating highest levels in the MTS-treated groups. There are seven different types of collagen in the dermis, with different compositions and antigenicity, although type I and type III are the main components of the reticular and papillary dermis, respectively. Other types of collagen in other regions, such as type II collagen in cartilage, type IV in the basement membrane, and type VII in the basement membrane and anchoring fibrils, may not be involved in scar improvement. Type I collagen is the prominent type in adult skin.[17,18] MTS increased type I collagen levels more

than IPL, as assessed using Western blot analysis, in the limited number of mice examined (Figure 4).

In summary, MTS increased collagen deposition in the dermis more than IPL, suggesting that MTS might be more effective than IPL for scar treatment.

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