ORIGINAL ARTICLE

Facial Skin Barrier Function Recovery After Microneedle Transdermal Delivery Treatment

Tae Y. Han, MD, PhD,* Kui Y. Park, MD, PhD,† Ji Y. Ahn, MD, PhD,‡ Seo W. Kim, MD,* Hye J. Jung, MD,‡ and Beom J. Kim, MD, PhD†

BACKGROUND Microneedle treatment is currently used in the cosmetic industry for several skin conditions. Despite their extensive use, there is lack of sufficient data on the safety of microneedles.

OBJECTIVE To investigate the degree of acute skin damage and the time required for facial skin barrier function to recover using different microneedle lengths and numbers of applications.

MATERIALS AND METHODS Each side of a volunteer's face was randomly treated with one of the following treatments: five applications of 0.15-mm microneedles, five applications of 0.25-mm microneedles, 10 applications of 0.15-mm microneedles, or 10 applications of 0.25-mm microneedles. Transepidermal water loss, stratum corneum hydration, and skin erythema were measured at baseline, immediately after treatment, 4 hours after treatment, and 8 hours after treatment and at 24-hour intervals for 3 days.

RESULTS Prompt recovery of barrier function (within 72 hours) was observed after microneedle treatment.

CONCLUSION Microneedle treatment is simple and inexpensive, and the skin barrier disruption it causes resolves quickly. Therefore, it can serve as an effective physical method of enhancing transdermal delivery of medications for the treatment of many cosmetic and dermatological conditions.

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The skin is an attractive site for drug delivery, but the stratum corneum, the upper part of the epidermis, is a barrier to the transport of most compounds.[1] Several enhancement methods such as intraepidermal administration, iontophoresis, ultrasound, electroporation, chemical enhancers, and microneedles have been employed to overcome this barrier.[2–11] Although chemical enhancers have been widely investigated for enhancing drug permeation levels, they tend to disrupt the lipid bilayers of the stratum corneum. Physical enhancement methods have also been used.[12]

Microneedle technology, as a physical enhancement method, offers a minimally invasive and painless route of drug administration because microneedles are too short to reach dermal nerves or blood vessels.[8,13,14] Another advantage is that microneedle use is probably associated with low microbial ingress.[15] This technology is currently being used in the cosmetic industry to treat several skin conditions such as dyspigmentation, wrinkles, acne, burn-related scars, and large pores and is also a part of collagen induction therapy.[16–19] Despite their extensive use, data on the safety of microneedles are insufficient.

In this study, we measured the degree of superficial barrier-related skin damage and the time required for facial skin barrier function to recover using different microneedle lengths and application numbers on the faces of volunteers. The face is the site

*Department of Dermatology, Eulji General Hospital, College of Medicine, Eulji University, Seoul, Korea; †National Medical Center, Seoul, Korea; ‡College of Medicine, Chung-Ang University, Seoul, Korea

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where most microneedle therapies have been performed. We used a number of noninvasive bioengineering methods, including an evaporimeter, a corneometry, and a colorimeter.

Materials and Methods

Volunteers

Seventeen healthy, nonsmoking volunteers (12 women, 5 men) aged 21 to 33 (mean age 26.9) with no preexisting skin conditions and Fitzpatrick skin types III or IV participated in the study. They were asked not to apply any cosmetic formulations to the face, to refrain from drinking coffee and tea during the study period, to avoid sun exposure, and to wash their face with a provided cleanser. The Medical Ethical Committee of the National Medical Center approved the study.

Microneedles

The microneedle rollers used in this study were supplied by DTS Lab Inc. (Disk Microneedles Therapy System Lab, Inc., Seoul, South Korea). Two different lengths of microneedle rollers were used: 0.15 and 0.25 mm. All types of Disk Microneedle Rollers have 60 circular arrays of nine needles each (total 540 needles) in a cylindrical assembly (20-mm diameter, 18.2-mm length).

Experimental Procedure

To compare the effect of microneedle length and number of applications, each side of the volunteers' faces (total 34 sides) was randomly treated with one of the following treatments: five applications of 0.15-mm microneedles (8 sides), five applications of 0.25-mm microneedles (8 sides), 10 applications of 0.15-mm microneedles (9 sides), and 10 applications of 0.25-mm microneedles (9 sides). A computer-generated randomization list was prepared. Written treatment protocols based on previously described randomization lists were used to assign patients to different groups. Choices were placed in sealed envelopes and opened by the physician. A single investigator treated all patients with the same forces and directions. This experiment was always performed in the morning, between 8 a.m. and 10 a.m.

Measurements

Transepidermal water loss (TEWL), stratum corneum hydration, and skin erythema were measured at baseline, immediately after treatment, 4 hours after treatment, 8 hours after treatment, and at 24-hour intervals for 3 days (days 1–3). Measurements were taken from the point where the horizontal line from the angle of the mouth crosses the vertical line from the outer corner of the eye. TEWL was measured using an evaporimeter (Tewameter 210; Courage and Khazaka, Köln, Germany) in a sealed room devoid of air flow and direct sunlight.

Stratum corneum hydration measurements were taken using a Corneometer CM 820 (Courage and Khazaka), which measures the electrical capacitance of the skin as an indicator of stratum corneum hydration. Capacitance was digitally recorded in arbitrary units and not in SI units [capacitance (μF) or conductance (μS)]. Values expressed in arbitrary units were positively correlated with epidermal hydration. A colorimeter (Chromometer CR-300; Minolta Camera, Osaka, Japan) was used to measure the amount of erythema, indicated as a*. A standard white plate (Minolta Camera) was used to correct the a* values.

All measurements were performed in a room where the temperature was kept at 23°C and relative humidity at 50% to 60%. Volunteers adapted to the test room ambient conditions for at least 30 minutes before each measurement. Separate investigators performed barrier measurements and microneedle treatments.

Statistical Analysis

Two-way repeated-measures analysis of variance (ANOVA-2) was used to compare the two groups (5 vs 10 times, 0.15 vs 0.25 mm) with respect to TEWL, stratum corneum hydration, and skin erythema

values after being divided by baseline values over time. For significant data ($p < .05$) from ANOVA-2, independent *t*-tests were performed at a significance level of $p < .05/6$ using the Bonferroni method.

Results

Transepidermal Water Loss

Ratios of TEWL at baseline to those at each time point after treatment using the 0.15- and 0.25-mm microneedles are provided in Figure 1A. ANOVA-2 was used to compare the TEWL ratios between the two groups. A significant interaction was observed between the TEWL ratio and needle length ($p < .05$). The result of an independent *t*-test conducted after the ANOVA-2 revealed that only the TEWL ratio computed immediately after treatment differed significantly between the two groups ($p = .003$). Until 24 hours, the mean TEWL ratio was higher in the 0.25-mm microneedle group than in the 0.15-mm microneedle group. It took 8 hours for TEWL to recover to the baseline value in the 0.15-mm microneedle group and 24 hours in the 0.25-mm microneedle group.

The TEWL ratios for the group that received five applications and the group that received 10 applications are shown in Figure 1B. A significant interaction was observed between the TEWL ratios and the number of applications ($p < .05$). The post-test revealed that there was a significant difference in the TEWL ratio between the two groups immediately after ($p = .006$) and 4 hours after $p = .007$) treatment. Until 24 hours, the mean TEWL ratio was higher in the group that received 10 applications than in the group that received five. It took 8 hours for the TEWL ratio to recover to baseline values in the five-application group and 24 in 10 10-application group.

Stratum Corneum Hydration

The ratios of capacitance at baseline to those at each time point after treatment with the 0.15- and 0.25-mm microneedles are provided in Figure 2A. Mean

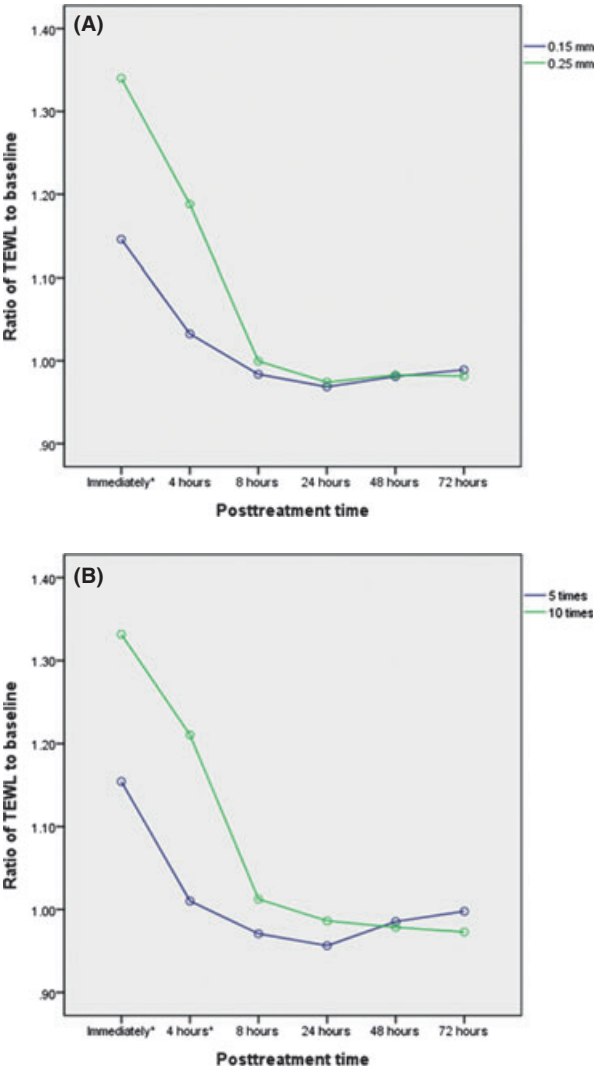

Figure 1. The ratios of TEWL at baseline to those at different time points after treatment with the (A) 0.15- and 0.25-mm microneedle and (B) in the five- and 10-application groups (*$p < .05/6$).

capacitance ratios at all measurement points in the 0.15-mm microneedle group were higher than those in the 0.25-mm microneedle group, although this was not a statistically significant difference. The recovery times to baseline values were 24 hours in the 0.15-mm microneedle group and 48 hours in 0.25-mm microneedle group.

Comparisons of the capacitance ratios at each time point between the five-application group and the 10-application group are shown in Figure 2B. There were no significant differences between the

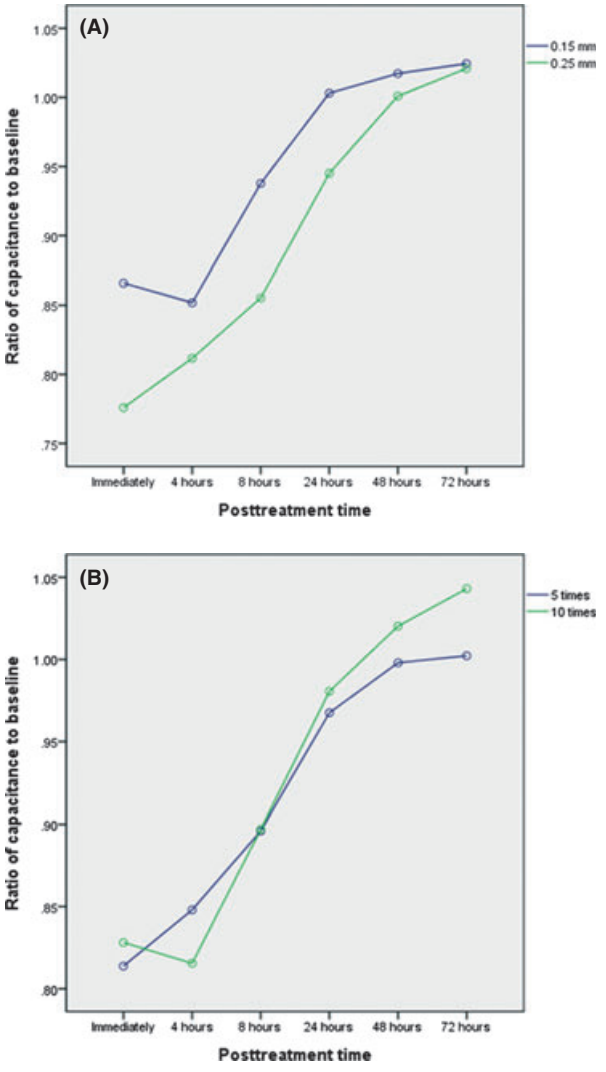

Figure 2. (A) The ratios of capacitance at baseline to those at different time points after treatment using the 0.15- and 0.25-mm microneedles. (B) Comparison of the ratios of capacitance at baseline to those at different time points after treatment between the five- and 10-application groups.

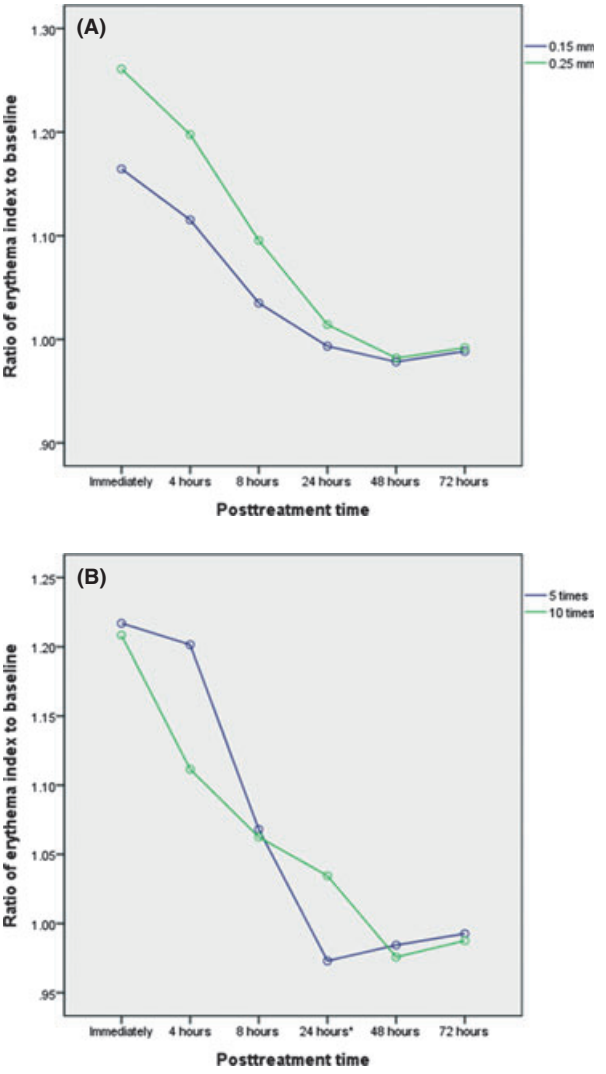

Figure 3. Comparison of the ratio of the erythema index at baseline to those at different time points (A) between the 0.15- and 0.25-mm microneedle length groups and (B) between the five- and 10-application groups (*$p < .05/6$).

two groups. The recovery times to baseline values were 48 hours in the five-application group and 72 hours in the 10-application group.

Skin Erythema

The ratio of the erythema index at baseline with those at each time point after treatment of the 0.15-mm microneedle group and the 0.25-mm microneedle group are compared in Figure 3A. The mean erythema index ratios were higher in the 0.25-mm microneedle group than in the 0.15-mm microneedle group at every measurement point, although these differences were not statistically significant. The recovery times to baseline values were 24 hours in the 0.15-mm microneedle group and 48 hours in the 0.25-mm microneedle group.

Comparisons of the erythema index ratios between the five-application group and the 10-application group are shown in Figure 3B. A significant interaction was observed between the erythema index ratio and number of applications ($p < .05$).

The post-test revealed that there was a significant difference in erythema index ratio between the two groups only 24 hours after treatment ($p = .002$). Twenty-four hours after treatment, the mean erythema index ratio was higher in the 10-application group than in the five-application group. The recovery time to baseline values was 24 hours in the five-application group and 48 hours in the 10-application group.

Discussion

Microneedle treatment has recently gained considerable attention for cosmetic use as well as for transdermal drug delivery, because it can induce collagen neogenesis and has certain advantages over laser resurfacing. Unlike with ablative laser treatments, the epidermis remains intact and is not damaged during microneedle therapy. Because of this, microneedle treatment can be safely repeated if needed.[17,20] Despite its cosmetic popularity and wide use, there are no published studies on the physiologic changes that microneedle treatments on the face induce, and most microneedle studies have focused on enhanced drug delivery across the skin. In this study, we aimed to assess skin barrier damage and its recovery after microneedle treatment on the face. For this purpose, we used microneedle treatment regimens with varying microneedle length and number of applications.

First, the influence of the microneedle arrays on TEWL was assessed. The 0.25-mm microneedles had a significantly higher TEWL ratio than the 0.15-mm microneedles but only immediately after the treatment. The recovery time to baseline values was 8 hours in the 0.15-mm microneedle group and 24 hours in the 0.25-mm microneedle group. The TEWL ratio was also compared based on the number of applications (5 vs 10 treatments). The TEWL ratio was significantly higher in the 10-application group immediately after and 4 hours after treatment. The recovery time to baseline values was 8 hours in the five-application group and 24 hours in the 10-application group, indicating that TEWL normalizes within 24 hours regardless of needle length (0.15 or 0.25 mm) or the number of applications (5 or 10 times). A significant difference based on needle length and number of applications was observed only several hours after treatment.

In a previous study, Bal and colleagues used microneedle arrays with varied lengths on the ventral forearms of 18 human volunteers.[1] The effects varied according to needle length, with TEWL and redness after treatment with 0.4-mm microneedle arrays significantly higher than those after treatment with 0.2-mm microneedle arrays, and microneedle irritation lasted less than 2 hours after both treatments.[1] We assume the reason our recovery time was longer than in the previous study was that there was a difference in the number of applications and the anatomical location. Bal and colleagues administered only one or two applications on the volunteers' ventral forearms.[1] The TEWL and capacitance values of the face and forearm are different, which is most likely due to the difference in the thickness of the stratum corneum and other innate properties that are location specific. Overall, the face is more prone to show changes in TEWL and capacitance than the trunk or extremities after acute barrier damage.[21] With stratum corneum hydration, no significant difference was observed based on needle length or number of applications. Recovery time was 24 to 72 hours in each of the different groups.

Skin erythema index, a sign of irritation from the needles, was measured using a chromometer. Erythema is one of the fundamental markers of inflammation. In response to barrier disruption, keratinocytes produce a variety of cytokines, of which interleukin-1 alpha (IL-1α) is the most important. Preformed and active IL-1α is already present in resting keratinocytes, and its release stimulates the release of additional IL-1α and other cytokines such as IL-8, IL-6, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor alpha.[22–24] This cytokine cascade leads to

dermal vasodilation and cellular infiltration in the epidermis, which directs the restoration of the skin barrier function.[25,26] Physical barrier disruption by tape stripping or ultraviolet radiation is also known to lead to the release of IL-1α and the resulting inflammation reaction. It may therefore be possible that microneedles also induce inflammatory reactions,[27,28] although in our study, erythema normalized within 24 to 48 hours in all groups. In addition, no significant difference was observed in the erythema index ratios between the different needle length groups. A significantly higher erythema index ratio was seen only 24 hours after treatment in the 10-application group than in the five-application group.

Our study has methodologic limitations. Because we used only two superficial needle lengths, additional studies with longer needles, such as 1.5 to 2.0 mm, are needed. Those lengths would be more suitable for evaluating skin barrier recovery after microneedling collagen induction therapy. Another limitation was our evaluation method. Although these noninvasive methods are reasonable, a histologic evaluation with biopsy would have been more definitive.

Although most cosmetic procedures using microneedle rollers are performed on the face, to our knowledge, there have been no studies to investigate the safety of microneedle roller treatment on the face. In this study, we observed prompt recovery of barrier function (within 72 hours) after microneedle treatment with lengths of microneedles that are widely used, 0.15 and 0.25 mm, applied five or 10 times. In conclusion, microneedle treatment is a simple and inexpensive treatment that only transiently disrupts the skin barrier function. It can serve as an effective physical method to enhance transdermal delivery for many cosmetic and dermatologic conditions.

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Address correspondence and reprint requests to:
Beom Joon Kim, MD, PhD, Department of Dermatology, Chung-Ang University Hospital, 224–1 Heukseok-dong, Dongjak-gu, Seoul 156–755, South Korea, or e-mail: beomjoon@unitel.co.kr