Skin Rejuvenation by Microneedle Fractional Radiofrequency Treatment in Asian Skin; Clinical and Histological Analysis

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Background: For skin rejuvenation, microneedle fractional radiofrequency (RF) is a recently developed minimally invasive method for delivering RF energy directly into the skin using microneedle.
Objective: We evaluated efficacy and safety of microneedle fractional RF for skin rejuvenation in Asian skin and also conducted immunohistochemical analysis before and after treatment.
Patients and Methods: Twenty-five females (mean age 54.2, Fitzpatrick skin phototypes III–IV) received three consecutive fractional RF treatment at 4-week intervals. Outcome assessments included standardized photography, physician's global assessment, patient's satisfaction scores, objective biophysical measurements, and histologic analysis including immunohistochemical staining.
Results: All patients showed clinical improvement on physician's global assessment and patient's satisfaction scores. Among objective biophysical measurements, improvement in hydration and skin roughness was noticed. Histologic examination revealed marked increase in dermal thickness, dermal collagen content and dermal fibrillin content. Side effects were minimal.
Limitations: The limitations are the small number of patients and lack of long-term follow-up.
Conclusion: Microneedle fractional RF is a safe and effective skin rejuvenation method in Asians. Lasers Surg. Med. 44:631–636, 2012. © 2012 Wiley Periodicals, Inc.

Key words: aging; microneedle fractional radiofrequency

INTRODUCTION

There are two biologically distinct aging processes affecting the skin. The one is intrinsic aging, which affects the skin by slow and irreversible tissue degeneration. The other is extrinsic aging, so called "photoaging," resulted from chronic ultraviolet (UV) exposure [1,2]. Various energy-based modalities including lasers have been used to reverse symptoms of aging. Although ablative lasers are effective traditional treatment for photodamaged skin rejuvenation, they have significant side effects such as prolonged erythema and post-inflammatory hyperpigmentation, which occur more commonly in dark-skinned patients. Non-ablative lasers are associated with less downtime, but the results seem to be limited and inconsistent [3–5]. Recently, fractional radiofrequency (RF) has revolutionized the field of skin rejuvenation [6]. Hantash et al. [7] introduced a minimally invasive device, microneedle fractional RF, which adopted microneedle therapy system (MTS) for delivering biopolar RF energy directly into the skin through microneedle. RF thermal lesions are fractionally generated within the reticular dermis. Fractional thermal injury to deep dermal collagen induces a vigorous wound healing process leading to dermal remodeling and the generation of new collagen, elastin and hyaluronic acid [7,8].

The present study was undertaken to evaluate in vivo efficacy of microneedle fractional RF for skin rejuvenation in Asians. In particular, this study used histologic quantitative assessment and diverse non-invasive skin measuring devices to objectively assess changes in biophysical properties of skin following microneedle fractional RF treatment.

METHODS

Patients

Twenty-five patients with various signs of aging such as visible lines, wrinkles, and widened pores were recruited for this prospective study. All patients ranged in ages from 41 to 64 years (mean 54.2 ± 3.21 years of age) and had skin types III and IV according to the Fitzpatrick scale. Exclusion criteria were: use of bleaching creams, history of any skin rejuvenation treatment within 6 months, history of keloid or active eczema. The study protocol and informed consent form were submitted and approved by the CHA University Institutional Review Board. All 25 patients were informed of the benefits, risks and possible complications of the treatment before enrollment, and informed consent was obtained from each patient.

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Description of Devices

We used a microneedle fractional RF device (Scarlet™, Viol Co., Korea), which has a disposable single-use treatment tip consisting of 5 non-insulated microneedle electrode pairs per the area of 10 mm^2, with the exposed electrode extending from 0.5 to 3 mm below the skin surface. Microneedles have a 0.3 mm outer diameter. These bipolar electrode pins form a closed circuit through the irradiated skin, delivering 2 MHz of conducted RF current to the skin. Adjustable RF voltage up to a maximum of 40 V can be delivered, in relation to the intensity (1–10) and conduction time (100–800 m seconds).

Treatment Protocols

Patients received three sessions of treatments every 4 weeks. Face was anesthetized using topical 4% lidocaine cream (LMX4, Ferndale Laboratories, Inc., Ferndale, MI) about 30 minutes before the procedure. The face was cleansed with a mild soap and 70% alcohol. As per the manufacturer's recommendation, full face was treated with a microneedle fractional RF device. The treatment parameters were determined based on the specific anatomical location and proximity of underlying bones. We altered the depth parameter according to underlying tissue thickness (periorbital area: 0.5 mm, forehead: 0.8 mm, chin and temple: 1 mm, and cheek: 2 mm). Treatment was delivered in a single, non-overlapping pass over the indicated area. An epidermal cooling device (CARESYS, Danil SMC, Korea) was used to relieve pain and erythema after the treatment. Subjects were allowed to apply sunscreen or emollients according to their usage before study entry.

Clinical Assessments

Patients were assessed at baseline and 4 weeks after the final treatment. Photographs by digital camera (Nikon D90, Tokyo, Japan) were obtained at each visit. For patients' self-assessment, patients answered questionnaires regarding efficacy and adverse events 12 weeks after commencement of the study. The patient satisfaction scale was as follows: 0 = not satisfied, 1 = somewhat satisfied, 2 = satisfied, 3 = very satisfied. In addition, patients were asked to report any adverse effects during the study. Objective clinical assessments were performed by two blinded dermatologists comparing before and after photos. Clinical assessments included wrinkles, pores, and overall improvement. We used a quartile grading scale on the evaluations (grade 0 = no improvement; grade 1, 1–25% = minimal improvement; grade 2, 26–50% = moderate improvement; grade 3, 51–75% = marked improvement; and grade 4, 75–100% = near total improvement).

Non-Invasive Objective Skin Hydration Measurements

Measurement of skin hydration was performed using a corneometer (CM825, CK Electronics, Köln, Germany). The skin measurements were performed at baseline and 4 weeks after the final treatment. Three corneometer readings were taken from the same malar areas and analyzed to determine changes in skin hydration.

Non-Invasive Objective Skin Color Measurements

Prior to all measurements, patients were acclimatized to the temperature (20°C)—and humidity (40%)—controlled room conditions and the instruments were calibrated according to the manufacturer's instructions. A narrow-band simple reflectance meter, Mexameter (MX18, CK Electronic, Köln, Germany), was used to quantitatively evaluate color changes after treatments. This instrument uses arrays of light-emitting diodes that emit light at three defined wavelengths: 568 (green), 660 (red), and 880 (infrared) nm. Melanin index (MI) and erythema index (EI) were measured in triplicate on the same malar area on each side of the face and mean values were used for analysis.

Non-Invasive Objective Wrinkle Measurements

To evaluate the effects of treatments on collagen regeneration, each patient's periorbital wrinkles were objectively measured using a skin replica and a microrelief instrument (Visiometer SV600, CK Electronic), at baseline and 4 weeks after the final treatment. Skin visiometer can measure skin roughness and the depth of furrows by measuring the light transmission through a very thin skin replica. The roughness parameters investigated in this study were R2 (maximum roughness) and R3 (average roughness).

Biopsy Specimens and Histologic Measurements

Paired 4 mm-punch biopsy specimens were obtained from each side of facial skin at baseline and at the end of treatment. Post-treatment biopsy specimens were taken near the previous biopsy site. Tissue samples were fixed in 10% buffered formalin, then embedded in paraffin. Standard hematoxylin-eosin and immunohistochemical staining, including procollagen-1 and fibrillin-1, were performed. Quantitative measurement of procollagen-1 was done using a analysis program (Image Pro Software Version 7.0, Media Cybernetics, MD).

Statistical Analysis

Student *t*-tests were carried out to compare interval changes in all parameters at each visit. Data were analyzed using SPSS software (version 12.0, SPSS, Inc., Chicago, IL). *P*-values of <0.05 were considered statistically significant.

RESULTS

Clinical Assessments

All 25 volunteers completed the study. Final follow-up visit to our institute was scheduled at 4 weeks after the final treatment. All patients showed clinical improvements after microneedle fractional RF treatment. Representative photographs showed improvements of wrinkles, pores, and overall skin appearance following microneedle fractional RF (Fig. 1). Patients' overall satisfaction scores

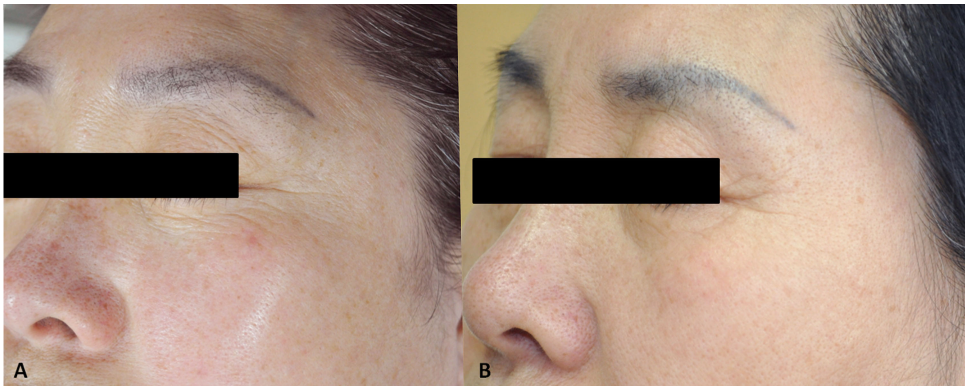

Fig. 1. Clinical photographs showed marked improvement of wrinkles after microneedle fractional RF treatment. **A**: At baseline, and (**B**) 4 weeks after the final treatment. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/lsm]

were 2.05 ± 0.25. The mean investigators' assessment scores for pores, wrinkles and overall improvement done at 4 weeks after the final treatment showed 2.44, 2.68, and 2.56, respectively. Fifty-six percent of patients had achieved more than 50% improvement in overall appearance (Fig. 2).

Non-invasive Objective Skin Measurements

The patients showed improvement in skin hydration scores obtained by Corneometer, demonstrating the efficacy of fractional RF on skin hydration. The mean skin hydration scores increased from 44.52 at baseline to 55.86 after the final treatment ($P < 0.05$; Fig. 3A). The mean melanin index (MI) and erythema index (EI) obtained by Mexameter decreased from 77.66 and 296.38 at baseline to 72.26 and 265.66 after the final session, respectively ($P > 0.05$; Fig. 3B,C). The mean R2 (maximum roughness) and R3 (average roughness) values measured by

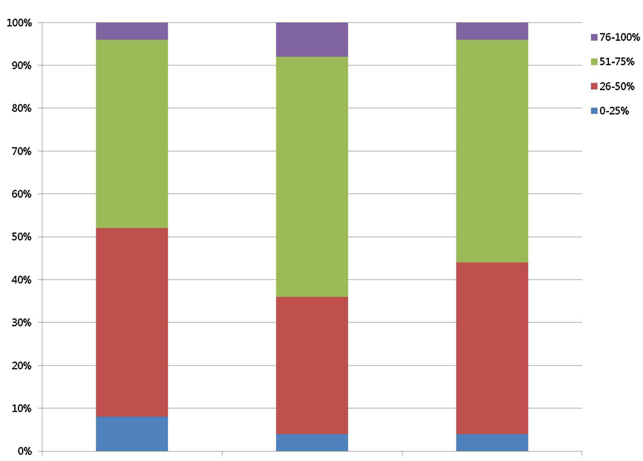

Fig. 2. Physician's assessment of pores, wrinkles, and overall improvement at 4 weeks after the final treatment. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/lsm]

Visiometer decreased from 0.63 and 1.00 at baseline to 0.55 and 0.85 after the final session (respectively $P < 0.05$; Fig. 3D,E).

Histologic Evaluation

Microscopic examination of hematoxylin-eosin stained sections showed dermal remodeling after microneedle fractional RF (Fig. 4A,B). A significant increase in dermal collagen content was observed at 4 weeks after three sessions of fractional RF compared to the baseline. Immunohistochemical staining for fibrillin-1 revealed only scant fibrillin-rich microfibrils along the dermoepidermal junction (DEJ) before treatment. However, post-treatment staining showed a significant increase in its density from DEJ to the deep dermis compared to the baseline (Fig. 4C,D).

Protocollagen Image Analysis

Immunohistochemical staining for procollagen-1 revealed narrow collagen bands from the basement membrane to upper dermis before treatment (Fig. 5A). The density of procollagen-1 increased at 4 weeks after the final treatment (Fig. 5B). Quantitative assessment of the density of procollagen-1 within 0.2 and 0.5 mm depths of dermis from the basement membrane showed significant increase at 4 weeks after three sessions of fractional RF compared to the baseline ($P < 0.05$; Fig. 5C).

Adverse Events

No serious adverse events were encountered. Mild pain and temporary erythema during and after procedures were well tolerated in all subjects. Other possible adverse events, including bruising, secondary infection, folliculitis, aggravation of erythema, scarring, and hyper/hypopigmentation, were not noted.

DISCUSSION

Skin aging is mediated by the influences of both natural aging process over time (intrinsic aging) and environmental factors (extrinsic aging) on its cellular and extra-

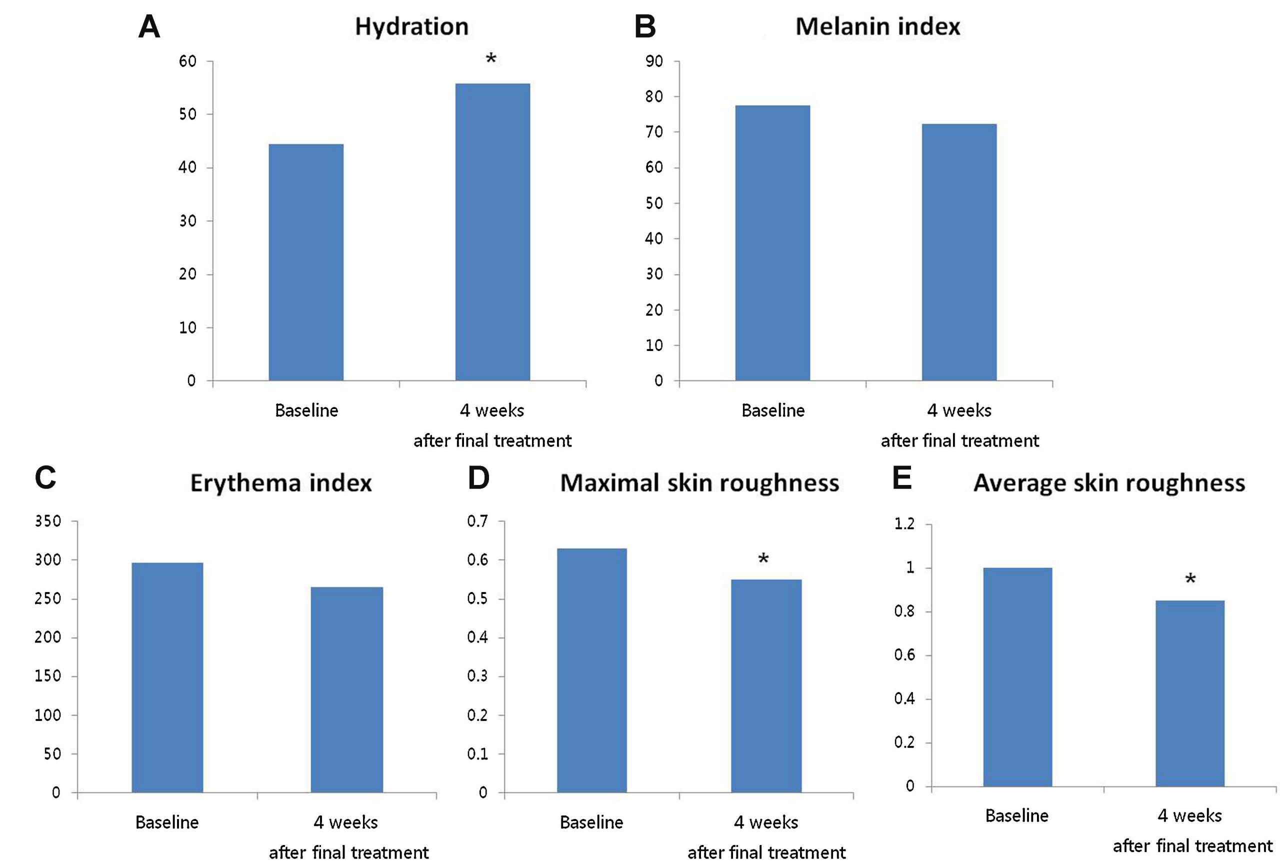

Fig. 3. Skin measurements by non-invasive objective evaluation methods. **A**: Objective measurement of hydration, (**B**) pigment, (**C**) erythema, and (**D**,**E**) wrinkles, (D) R2: maximum roughness, (E) R3: average roughness. $^{*}P < 0.05$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/lsm]

cellular components [1–2]. Although the treatment of skin aging has been focused on ablative laser resurfacing techniques, recently there is an increased interest in non-ablative techniques, which enables skin rejuvenation with minimal downtime and complications [3–5]. But post-inflammatory hyperpigmentation was observed in up to 40% of those who received non-ablative laser, especially in skin types III and IV [9–11].

RF is non-ionizing electromagnetic radiation in the frequency range of 3 kHz to 300 GHz. In contrast to most lasers that target specific chromophores, RF is chromophore-independent and depends on electrical properties of target tissue, and thus is expected to have better safety profiles for all skin types [6–8,12]. In 2008, FDA approved fractional RF to offer skin rejuvenation, which can achieve fractional and contiguous treatment patterns while sparing epidermis and key adnexal structures that contribute to rapid healing. Moreover, recently introduced microneedle fractional RF can control the depth of RF thermal zones and can induce tissue heating focused in the dermis with lower risk of adverse events such as post-inflammatory hyperpigmentation associated with epidermal injury. Unlike other microneedle fractional RF devices, Scarlet™ adopted non-insulated microneedle electrodes, which resulted in advantages regarding bleeding control during operation and a broad electric field in the dermis. As the thermal effect from RF devices is related to the impedance and conductivity of skin, the energy impact around microneedle electrode is narrower at the epidermal surface but wider in the dermis, in contrast to previous laser-based fractional systems [12–16].

In a study by Hruza et al. [17], subjects who received fractional RF treatments showed clinical improvement in skin texture by investigators' assessment, which was greater than 40%. In another study regarding facial photodamage in Asians, fractional RF treatments produced moderate (26–50%) and incremental improvements in skin smoothness and tightness [18]. These results of previous studies in clinical improvement of overall skin appearance are comparable with those of our study, which shows 26–75% improvement over baseline. In particular, this is the first study using microneedle fractional RF, to our knowledge, which showed statistically significant improvement both in degree of skin roughness measured by Visiometer and in histologic quantitative assessment of procollagen-1. In addition, erythema and melanin index showed improvement but without statistical significance.

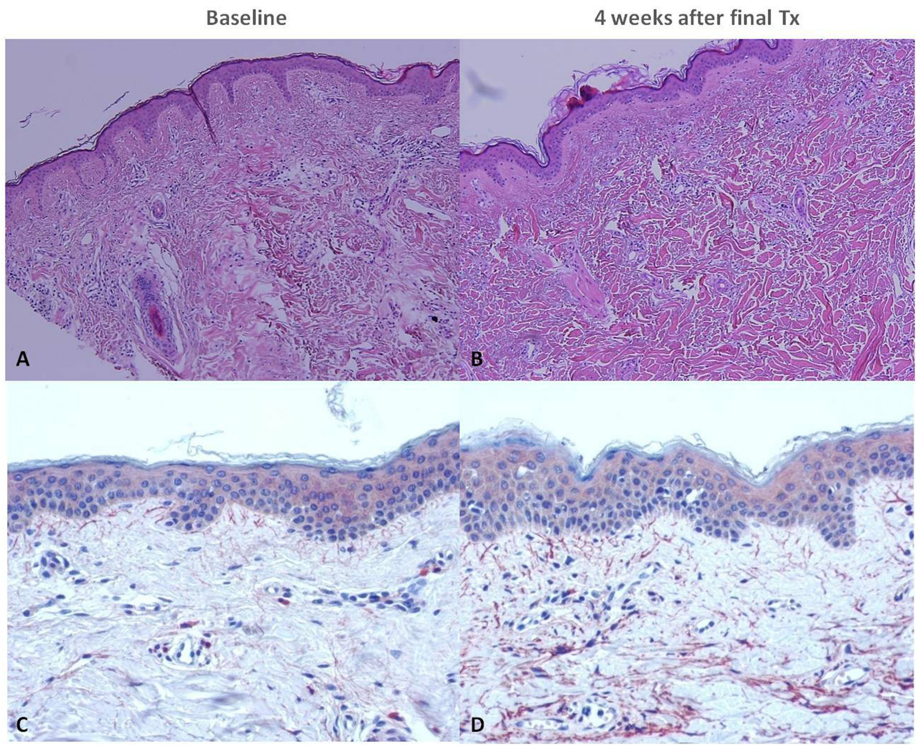

Fig. 4. Dermal remodeling and neocollagenesis after microneedle fractional RF in H&E stain (40×). **A**: At baseline, (**B**) 4 weeks after the final treatment. Immunohistochemical staining for fibrillin-1. **C**: At baseline, (**D**) 4 weeks after the final treatment. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/lsm]

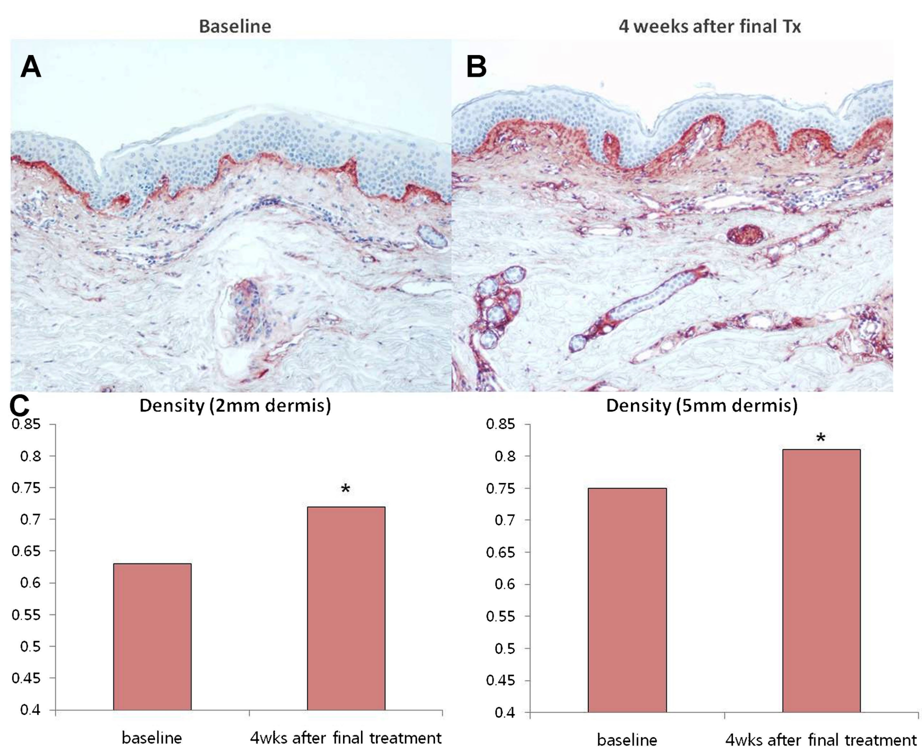

Fig. 5. **A**: Immunohistochemical staining for procollagen-1, (A) at baseline, (**B**) 4 weeks after the final treatment. **C**: Quantitative assessment of the density of protocollagen-1 within 0.2 mm and 0.5 mm of dermis from basement membrane. $^{*}P < 0.05$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/lsm]

Clinical improvement regarding skin pigmentation may be related to dermal remodeling mediated by fibroblasts, but more comprehensive studies would be required for further specific explanation [19].

Adverse events found in this study were limited to mild pain and temporary erythema during and after procedures. In general, incidence of side effects is lower with fractional RF than other non-ablative fractionated lasers. Some patients (up to 3–10%) may develop depressions, vesiculations or superficial burn after conventional RF. These are mainly attributed to uneven electrode contact with the skin, which is sometimes unavoidable because of uneven facial contours [11,16,18]. We altered the depth parameter according to underlying tissue thickness based on the previous study about skin thickness of Korean adults [14]. In areas such as temple and chin where underlying bones are close to the skin surface and applying high RF energy could be dangerous, we lowered RF energy and depth of needle. As high RF energy could result in superficial burn even in the case of microneedle fractional RF, appropriate parameters, including energy and depth, adjusted according to the cosmetic units of the face and proper contact of electrode to skin surface are important to reduce adverse event.

In conclusion, microneedle fractional bipolar RF treatment is effective for the treatment of photoaged Asian faces with darker skin types and poses little risk of post-inflammatory hyperpigmentation. The main limitations of this study are the small number of patients and lack of long-term follow-up after the final treatment. Further studies with more diverse treatment parameters and longer follow-ups are warranted to confirm the long-term efficacy and tolerability of fractional bipolar RF treatment.

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