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ORIGINAL CONTRIBUTION

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Efficacy and safety of a new microneedle patch for skin brightening: A Randomized, split-face, single-blind study

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Summary

Background: Although microneedles are one of the best transdermal drug delivery systems for active compounds, few clinical trials have examined the safety and efficacy of brightening microneedle patches.

Aims: To determine the efficacy and safety of a newly developed whitening microneedle patch.

Patients/Methods: A split-face study was designed for efficacy assessment with 34 Korean women applying the tested product (a whitening microneedle patch) on one cheek and a control whitening essence on the other. We objectively measured changes in melanin index values and skin brightness by mexameter and chromameter. Each participant also used global assessment to determine skin whitening. In addition, 55 participants were selected for primary skin irritation tests and repeated insult patch tests for safety assessments.

Results: Mean skin brightness and melanin indexes improved ($P<.05$) 4 weeks and 8 weeks after product use in both the whitening patch and whitening essence groups. Significant differences ($P<.05$) were observed between the whitening patch and whitening essence groups 8 weeks after use. Global assessment by participants showed moderate cosmetic outcomes for both the whitening patch and whitening essence groups. No adverse effects were reported, and primary irritation and human repeated insult patch tests revealed no irritation from the test product.

Conclusions: A newly developed microneedle patch was effective and safe for skin brightening and would be a promising functional cosmetic product.

KEYWORDS
clinical research, microneedle patch, skin brightening

1 | INTRODUCTION

Most people want bright and clean skin. During the last decade, skin whitening products have been the largest continually growing segment of the skincare market, especially in Asia. With a naturally higher skin hydration level, Asian skin is particularly prone to hyperpigmentation or hypopigmentation disorders and general unevenness of skin tone with age rather than wrinkles.

Skin whitening or lightening agents are cosmetics that can be classified as cosmeceuticals. They have drug-like benefits as melanin-producing processes of the skin are disturbed. Several approaches have been used to find agents that inhibit the catalytic activity of

tyrosinase and disrupt the synthesis and release of melanin. Tyrosinase inhibition is still the most common strategy adopted to achieve skin whitening, but agents acting upstream or downstream of complex melanogenesis pathway also exist.[1]

Minimally invasive microneedling treatment of the epidermal barrier improves the absorbability of the skin for topically applied compounds. In particular, the stratum corneum is perforated, and cell layers below the epidermis (stratum granulosum, stratum spinosum, stratum basale) as well as the upper cell layer of the dermis (stratum papillare) are activated by the irritation from the needle. This cell activation leads to better circulation and stimulates metabolism in the upper skin layers. Thus, cosmetic products can more effectively cross the epidermal barrier, increasing the uptake of the substance.[2,3] Skin depigmentation agents are the best candidates for this dissolving microneedle technology because active compounds can be effectively delivered to melanocytes where the melanin is synthesized.[4] Here, we report a novel clinical study of the efficacy and safety of a newly developed, dissolving microneedle patch with whitening agents combined with hyaluronic acid (HA) solution.

2 | MATERIALS AND METHODS

2.1 | Materials

Microneedle arrays were fabricated by Small Lab Co. Ltd. (Daejeon, Korea). A HA polymer solution was prepared by mixing 18.4% HA (Bloomage Freda Biopharm Co. Ltd., Jinan, China) and 1.6% lactose (Sigma-Aldrich, St. Louis, USA) in distilled water. Ingredients for skin depigmentation were combined with HA solutions in concentrations shown in Table 1. All ingredients were obtained from Sigma-Aldrich.

The fabrication of the microneedle patch is based on combination of a micromolding technology and conventional manufacturing process of medical adhesive bandage as shown in Figure 1. The HA hydrogels were injected onto a micromold and compressed with 5 tons for 30 seconds. The compressed hydrogel sheet on the micromold was dried at 50°C. After 1 hour, the HA microneedle arrays were collected from the molds and processed to an eye-patch shape by die cutting. The eye-shaped microneedle array was attached onto the adhesive back surface of the preshaped hydrocolloid band from T&L Co., Ltd (Anseong, Korea). This hydrocolloid band consisting discontinuous phase (sodium alginate) and continuous phase (polyisobutane rubber) has been widely used for the wound care system to absorb exudate and secure the wound site. The resulting microneedle eye patch (Figure 2E) was packed into an aluminum pouch and sterilized by gamma radiation (15 kGy).

TABLE 1 Final concentrations of the whitening patch system

Ingredient	Patch (%)
Melatonin	0.01
Arbutin	2.00
Tranexamic acid	1.00
Niacinamide	2.00

As shown in Figure 2, the microneedle eye patches had 196 microneedles/cm^2 at 1 mm-intervals and were pyramid shaped with 200 μm×200 μm base area and 250 μm height. By microscopic observation, HA microneedle patches dissolved by more than 70% within 30 minutes and completely after 1 hour when applied on skin (data not shown).

Participants suitable for inclusion and exclusion criteria who agreed to the study applied the test product (the microneedle patch and whitening essence) twice a week for 8 weeks. They applied each product to one cheek on a cleansed face. Half the face received the whitening patch system and the other half a whitening essence system. The microneedle patch was attached to the middle portion of a pigmentation area and taken off the face after 30 minutes. Participants pumped essence one to two times (about 4 g), then spread it on a symmetrical pigmentation spot. Test areas were measured before product use and 2, 4, and 8 weeks afterward.

3 | SUBJECTS

Thirty-four volunteers (aged 30-49 with Fitzpatrick skin type III-V) were enrolled after being approved by the Institutional Review Board of Chung-Ang University Hospital. Written informed consent was obtained from all participants after the risks, and benefits of the procedure were explained in detail. Enrolled participants could freely terminate their participation at any time. Participants were excluded if they reported a history of active cutaneous inflammation, systemic allergies, hypersensitivity to cosmetics/drugs/sunlight, or any chemical peeling or laser treatments within 3 months.

3.1 | Efficacy evaluation

Prior to measurement, participants rested in a 20-25°C, 40%-60% humidity area for 30 minutes after washing the test area. The same researcher took objective measurements and measured the same area each time.

Skin brightness was evaluated by Chromameter CM700d (Konica Minolta, Japan) before and after product use. With this instrument, the skin surface is illuminated by a pulsed xenon arc lamp. The light reflected perpendicular to the surface is collected for a tristimulus color analysis at 450, 560, and 600 nm, using the L*a*b* color system. Among them, L* parameter expresses color brightness (varying between a value of 100 for a white surface and 0 for a black surface).[5] The cheek area was measured three times and the average used as evaluation data. An increase in L* represented improved skin brightness. Skin melanin (melanin index, MI) was measured by a narrow-band reflectance spectrophotometer, Mexameter MX18 (Courage-Khazaka electronic GmbH, Germany) on a hyperpigmented area. In this instrument, 16 light-emitting diodes arranged circularly emit light at three defined wavelengths 568, 660, and 880 nm. A photodetector measures the light reflected by the skin. A melanin index is computed from the intensity of the absorbed and the reflected light at, respectively, 660 and 880 nm.[5]

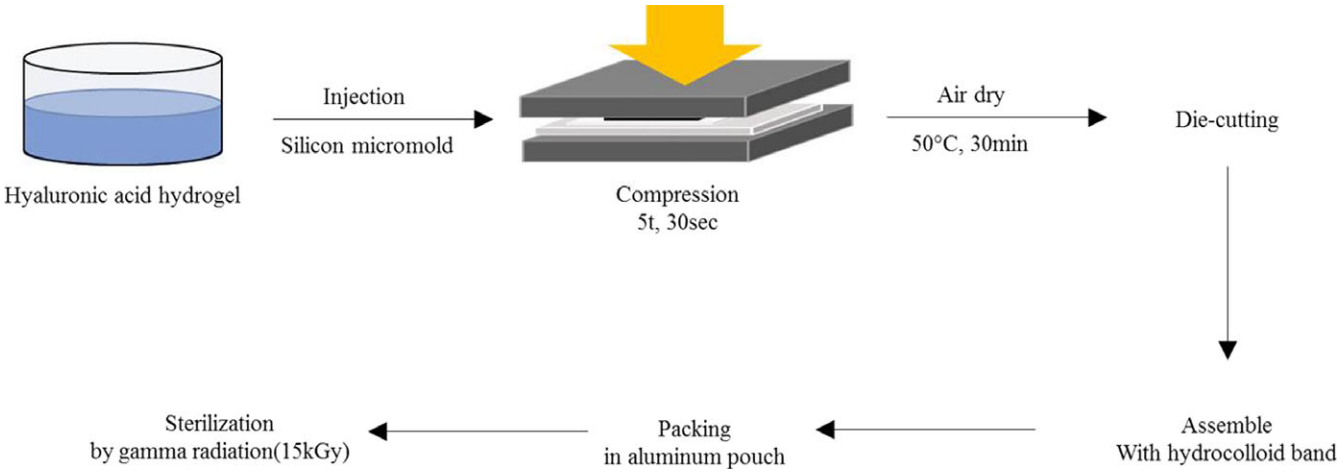


FIGURE 1 Schematic diagram of the hyaluronic acid (HA) microneedle eye-patch fabrication

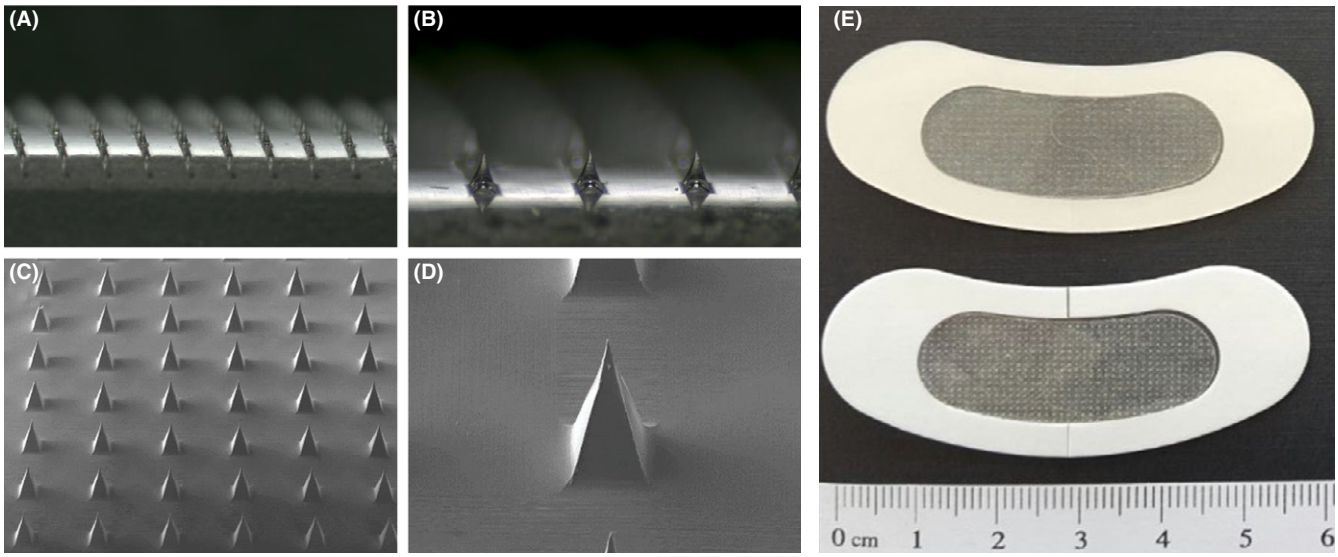


FIGURE 2 Stereomicroscopic images (A and B) and field-emission scanning electron microscopic images (C and D) of the hyaluronic acid (HA) microneedle arrays. (E) Photograph of HA microneedle eye patch containing skin depigmentation ingredients

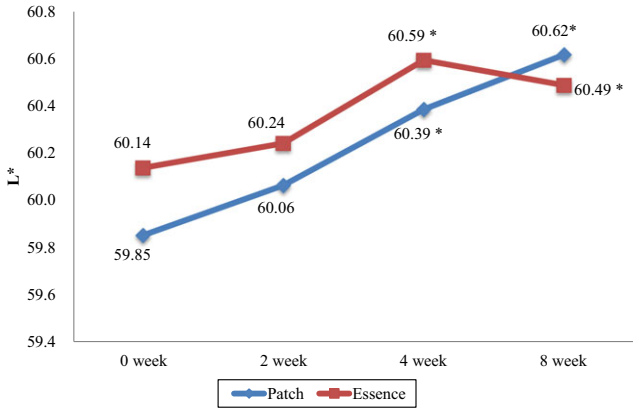
Participants took surveys about improvements in skin whitening after test product use with five stages: very good (4), good (3), moderate (2), bad (1), and very bad (0). The efficacy of the test product was evaluated by percentages of answers from participants.

3.2 | Safety assessment

The safety of the test product was evaluated at every visit for all participants. Adverse effects throughout the study period were recorded. In addition, before the study, primary irritation tests and human repeated insult patch tests (HRIPTs) of the whitening microneedle patch were performed with 55 participants aged 20-50 who met inclusion and exclusion criteria. All signed a research compliance agreement and were given a unique number. The primary irritation test was conducted for 48 and 72 hours using Frosch and Kligman's method[6] and in accordance with Cosmetic, Toiletry, and Fragrance Association guidelines.[7] The upper arm of each subject was the test site. After the area was washed with 70% ethanol and dried, a whitening microneedle patch and a control patch were applied for 48 hours. Skin response was evaluated as: 000-0.25=no irritation; 0.26-1.00=mild irritation; 1.01-2.50=moderate irritation; and 2.51-4.00=severe irritation.

3.3 | Statistical analysis

The statistical analysis package SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used to evaluate the efficacy of test product for skin changes. Significance level was set as 5% for *P*-values. Probability was rounded to three decimal places. For before and after comparisons, parametric tests and repeated measures ANOVA were used. In comparisons between groups, change rates were calculated and compared to before product use. Parametric tests and Independent samples *t*-tests were used for analysis.



* : $P<0.05$ by repeated measures ANOVA, post hoc Bonferroni correction

FIGURE 3 Skin brightness (L*). A significant increase was seen in probability (*$P<.05$) 4 and 8 weeks after product use in both the patch and essence groups

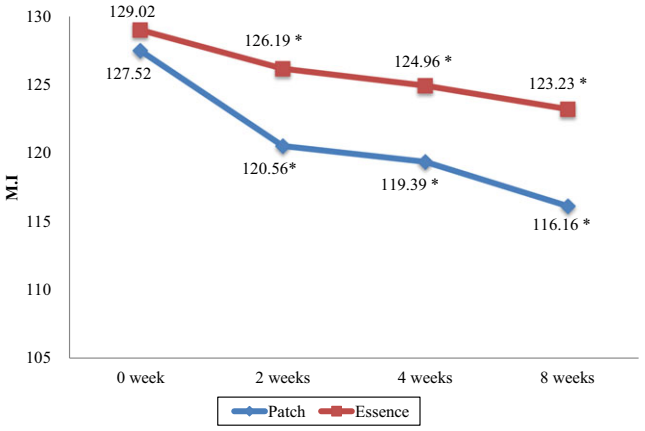


FIGURE 4 Skin melanin (melanin index, MI). A significant decrease in probability was seen (*$P<.05$) 2, 4, and 8 weeks after product use in both the patch and essence groups.

4 | RESULTS

The average age of the 34 participants was 40.41 years with 14 in their 30 seconds and 20 in their 40 seconds. All were women. One was withdrawn due to follow-up loss, for a total of 33 participants. To evaluate skin brightness changes with test products, skin brightness (L*) was measured before product use and 2, 4, and 8 weeks afterward. With the whitening patch, L* was 59.85±1.99 before, 60.06±2.06 2 weeks after, 60.39±2.08 4 weeks after, and 60.62±2.00 8 weeks after product use. With whitening essence, L* was 60.14±1.85 before, 60.24±1.91 2 weeks after, 60.59±1.90 4 weeks after, and 60.49±1.82 8 weeks after product use. A significant increase was seen in probability ($P<.05$) at 4 and 8 weeks after product use in both groups. A significant difference was seen at 8 weeks after product use in population comparisons (Figure 3).

To evaluate skin melanin changes with test products, skin MI was measured before product use and 2, 4, and 8 weeks afterward. With the whitening patch, MI was 127.52±23.92 before, 120.56±24.69 2 weeks after, 119.39±22.68 4 weeks after, and 116.16±21.25 8 weeks after product use. With whitening essence, MI was 129.02±23.20 before, 126.19±23.55 2 weeks after, 124.96±21.73 4 weeks after, and 123.23±20.12 8 weeks after product use. To evaluate skin melanin changes precisely and significantly, repeated measures ANOVA (with post hoc Bonferroni correction), a parametric test, and independent sample *t*-tests, also a parametric test, were used to test normality for test products. A significant decrease in probability ($P<.05$) was seen at 2, 4, and 8 weeks after product use in both groups. A significant difference was seen at 2, 4, and 8 weeks after product use in population comparisons (Figure 4).

Improvements in skin whitening after product use were determined from participant surveys. Results were calculated as percentages with 97.0% answering more than "moderate" for "skin whitening effects" for both whitening patch and essence groups (Table 2). Photographs of participants who applied the whitening patch and essence are in Figure 5. Dotted red line marks the area of the patch application. Pigmented spots gradually improved from baseline to 8 weeks for both whitening patch and essence groups. Pigmented lesions in areas of whitening patches showed slightly more improvement than the essence group.

No adverse effects such as itching, stinging, erythema, or swelling were observed during the application period of the test product. No skin abnormality was detected in physical examinations. The primary irritation tests and HRIPTs showed no irritation from the whitening microneedle patch (0.05 skin irritation index for the primary irritation tests and 0.07 for HRIPTs).

5 | DISCUSSION

Microneedle (MN) technology was first introduced for drug delivery many decades ago, but tremendous advances were achieved in the 1990s when fabrication technology enabled the manufacture of diverse MN types. These types include solid MNs for skin

TABLE 2 Global assessment score by patients with 97.0% responding that they experienced greater than moderate "skin whitening effects" in both whitening patches and whitening essence groups

	Number of subjects (percentage, %)						
	4*	3*	2*	1*	0*	Average	Standard deviation
Whitening patch	2 (6.1)	14 (42.4)	16 (48.5)	1 (3.0)	0	2.52	0.67
Whitening essence	1 (3.0)	11 (33.3)	20 (60.6)	1 (3.0)	0	2.36	0.60

4*, Very good; 3*,Good; 2*, Moderate; 1*, Bad; 0*, Very bad.

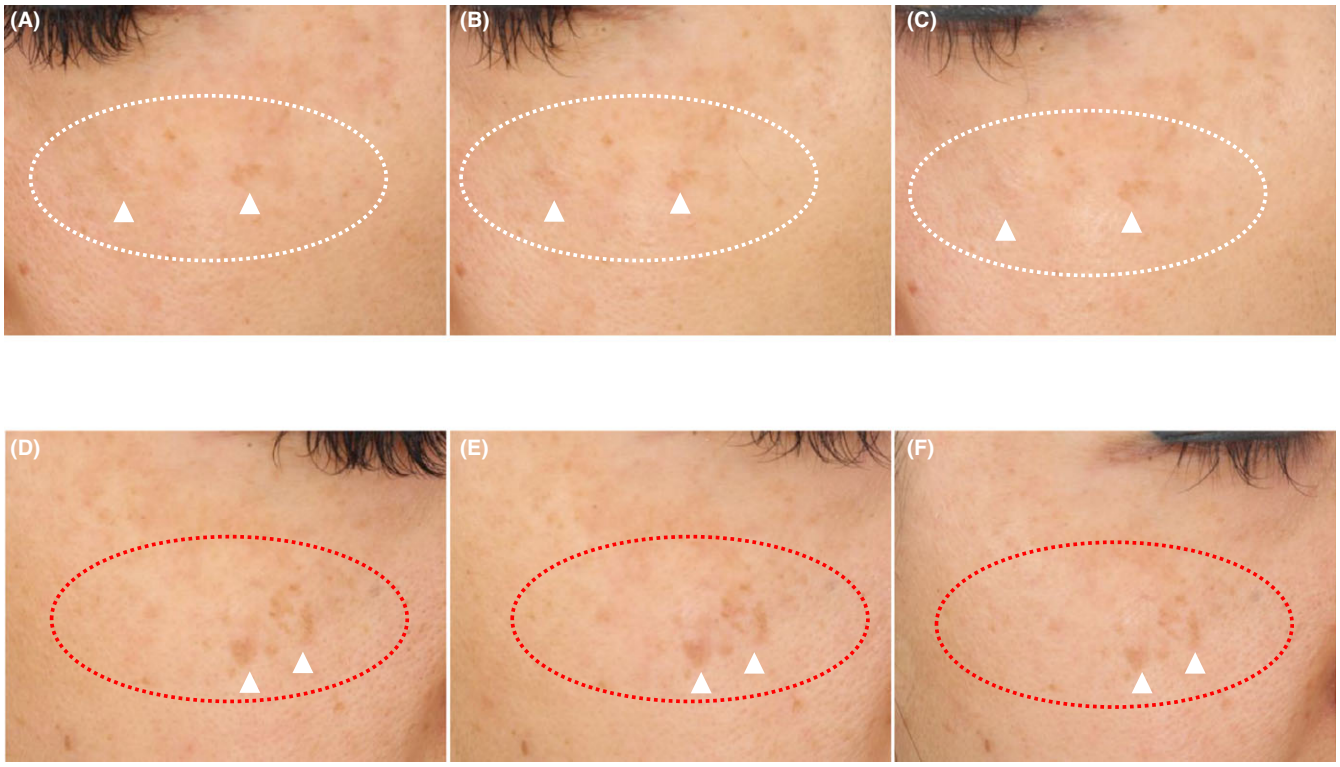


FIGURE 5 Clinical photographs. Area of whitening patch application, dotted white line, A-C; essence application, dotted red line, D-F. For both areas, pigmented spots in the test group gradually disappeared from 0 weeks (A and D) to 4 (B and E) and 8 weeks (C and F). Arrowhead indicated pigmented spots with moderate improvement.

pretreatment to increase permeability, coated MNs with drugs that dissolve in the skin, polymer MNs containing drugs that dissolve fully with polymers in the skin (called dissolving MNs or DMNs), and hollow needles for drug infusion into the skin. Compared with solid MNs, polymer-based DMNs have several advantages. As polymer-based DMNs dissolve completely in the skin, they cannot be reused, which prevents transmission of bloodborne pathogens and disease by cross-contamination. DMNs made of HA might be biocompatible with skin and safe for material penetration into the skin, because HA is a component of skin tissue.[8]

Hyaluronic acid is an endogenous component of the extracellular matrix, with collagen and elastin. Hyaluronic acid is a linear, polyanionic polysaccharide with repeating disaccharide units composed of β-glucuronic acid and N-acetylglucosamine. It decreases in the skin upon aging and is critical for tissue rejuvenation. HA-based gels are injected intradermally in augmentation therapy for the face and are the most widely used main component of filler injections and potential compounds in skin rejuvenation.[9,10] Hyaluronic acid has been studied as a suitable candidate for MN formulation and delivery of incorporated active pharmaceutical ingredients. One example is MicroHyala®, which was granted FDA approval in 2004.[11] MicroHyala® was developed as a patch containing HA that releases active ingredients into the skin to treat wrinkles. The salt form of HA, sodium hyaluronate, is the base material for the microneedle patch. The viscoelastic properties of HA, with its excellent biocompatibility and nonimmunogenicity, make it an ideal candidate for investigations of MN-based cosmetic, medical, and pharmaceutical products. Hyaluronic acid MNs were used to demonstrate that transcutaneous immunization induces an immune response against exogenous proteins, viral vectors, toxins, and virus particles. Safety and efficacy results with HA MNs indicate that these drug delivery tools are promising for vaccines, drugs, and cosmetic ingredients.[12–14]

Melanin is essential in protecting human skin against UV radiation, but overproduction of melanin is a major consequence of the UV damage and aging process that induces pigmentation disorders such as freckles and senile lentigo. Melanin inhibition is a desirable effect for various areas of the cosmetic industry for achieving skin whitening and lessening the aging appearance.

The newly developed HA DMN patch contained melatonin, arbutin, niacinamide, and tranexamic acid for skin depigmentation. Melatonin and its metabolites are produced endogenously in human epidermis and can affect melanocyte and melanoma behavior. Testing their phenotypic effects in normal human melanocytes showed that melatonin and its metabolites inhibit tyrosinase activity and cell growth and inhibit DNA synthesis dose dependently.[15] Melatonin can penetrate the stratum corneum and build a depot due to its distinct lipophilic chemical structure.[16] Arbutin is used as a whitening agent in cosmetic products. Arbutin inhibits melanin production in B16 cells induced with alpha-MSH and decreases tyrosinase activity. The hyperpigmentation effects of alpha-MSH are abrogated by addition of arbutin to brownish guinea pig and human skin tissues.[17] Studies note that topical niacinamide is extremely well tolerated by facial skin and provides beneficial effects to aging skin such as improved barrier function, and decreased appearance of facial

photoaging signs such as texture, pore size, and hyperpigmented spots.[18,19] Oral, topical, or intralesional tranexamic acid are used to treat melasma. Although the mechanism of action of tranexamic acid remains unclear, tranexamic acid is suggested to inhibit melanin synthesis by interfering with the interaction between melanocytes and keratinocytes. A study found that suppression of ET-1 could be a mechanism of action of TA on melasma.[20]

The safety and efficacy of cosmetic products are essential because of long-term use by consumers. Although all raw materials for the fabrication of DMNs are safe, interactions among raw materials may induce side effects. DMNs containing active compounds must be evaluated for cosmetic use as the final products can cause side effects such as irritation or allergy reaction. In this clinical study, we found that an HA DMN patch did not result in any irritation reactions in primary irritation, HRIPT, and brightening efficacy tests. The HA DMN patch showed improvements in brightness, comparing before and after use, and showed better performance than a topically applicable formulation.

Although we did not compare treatment groups with an untreated control group, using the HA DMN patch with active whitening agents may improve skin tone and pigmentation more effectively than a formulation with the same active ingredients. Further studies with long-term follow-up would be informative regarding the course of cutaneous pigmentation of treated and nontreated skin using the HA DMN whitening patch.

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CONFLICT OF INTEREST

None declared.

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