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# Development of a novel microneedle platform for biomarker assessment of atopic dermatitis patients

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## Abstract

**Background:** Atopic dermatitis (AD) is a chronic inflammatory skin disease whose pathogenesis, cause, and treatment have been extensively studied. The association of AD with Th2 cytokines is well known; therefore, the analysis of this association is crucial for the diagnosis and treatment of AD. This study aimed to present a new method for measuring protein biomarkers in patients with AD, before and after treatment, using minimally invasive microneedles.

**Materials and methods:** First, hyaluronic acid-loaded microneedle patches (HA-MNs) for skin sample collection were fabricated. Next, after Institutional Review Board approval, 20 patients with AD were recruited and skin samples were taken before and after treatment using four different sampling techniques: (1) tape stripping, (2) hydrocolloid patches, (3) hollow microneedles, and (4) HA-MNs. Lastly, proteins were isolated from the collected samples, and AD-related biomarkers were analyzed by enzyme-linked immunosorbent assay.

**Results:** Proteins were successfully extracted from the skin samples collected by tape stripping, hydrocolloid patches, and HA-MNs, except hollow microneedles. Interleukin (IL)-4, IL-13, and interferon-$\gamma$ were detected in the HA-MNs only. By comparing the biomarker level correlation before and after treatment and the improvement score of the patients, we observed a significant negative correlation between IL-4 and IL-13 with an improvement in AD symptoms.

**Conclusion:** Overall, our results verified that HA-MNs can be used to effectively analyze protein levels of biomarkers from skin metabolites of patients with AD and can be applied to monitor the treatment progress of patients with AD in a minimally invasive manner.

KEYWORDS
atopic dermatitis, biomarkers, hyaluronic acid, microneedle, skin sampling

## 1 | INTRODUCTION

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease caused by defects in the immune system and skin barrier, genetic conditions, and environmental factors.[1,2] Immune abnormalities are typically caused by a combination of innate and adaptive immune responses in dendritic cells, T cells, and other cells, resulting in an immune response through the release of various inflammatory reactants, such as interleukin (IL)-4, IL-13, and interferon (IFN)-$\gamma$.[3–7]

Skin samples collected from lesions are required to study the pathological mechanism of diseases. Currently, three method categories exist for obtaining skin samples: (i) invasive methods, such as skin biopsies; (ii) non-invasive methods, such as tape stripping; and (iii) minimally invasive methods, such as microneedling.

Skin biopsies have been extensively used to analyze biomarkers associated with AD to determine its effects, predict prognosis, and characterize the pathophysiology of AD.[8–11] However, skin biopsies are painful and typically result in scarring. Moreover, there is a risk of infection, and skilled surgeons are required for the procedure. For this reason, invasive methods to obtain skin samples are not highly effective for detecting the specific factors in human skin and hinder the development of new technologies or drugs.[12–17]

A non-invasive technique using tape stripping has been proposed to examine skin expression of biomarkers in the stratum corneum. However, because AD typically occurs in the stratum corneum and epidermal and dermal layers as an abnormality of the immune system, it is important to collect samples from both the epidermal and dermal layers.[8,12,13,16,18–20] Because tape stripping cannot be used to investigate the dermis, a new technique is required that is minimally invasive and allows for sample collection from both epidermal and dermal layers.

Microneedles (MNs), which are characterized by micrometer-scale needles, have also been actively studied as a transdermal delivery method for drugs and vaccines.[16,21–27] Moreover, minimally invasive sampling methods using MNs have recently emerged to extract interstitial fluid (ISF) and cells from the skin for monitoring biomarkers or glucose using solid, hollow, and swellable MN patches.[28–31] Specifically, biodegradable MNs have garnered considerable attention for the sampling of skin metabolites because of their biocompatibility and minimal skin damage during use.

Here, we demonstrate a new technique for the minimally invasive sampling of skin metabolites using a biodegradable hyaluronic acid-loaded MN patches (HA-MNs) and the management of biomarkers in AD patients. We hypothesized that HA-MNs would represent a novel method for analyzing skin biomarkers to replace invasive skin biopsies or tape stripping.

## 2 | MATERIALS AND METHODS

### 2.1 | Fabrication of biocompatible HA-MNs

Biocompatible HA-MNs were fabricated using the droplet extension method described in a previous paper.[32] Briefly, after spotting a viscous biocompatible polymer material on the bottom layer of the patch, the same biocompatible polymer material was spotted on the bottom layer of the patch positioned on another substrate. Then, the other substrate was inverted and brought into contact with the spotted polymer material. Thereafter, the two substrates were placed a certain distance apart so that the contacting viscous polymer material was stretched, and after the tensioning process was completed, blowing was performed to adjust the shape in the tensioned state. Finally, the middle part was cut so that the same MNs were formed on the two different substrates.

### 2.2 | Study subjects

Written informed consent was obtained from 20 volunteers with AD, including 10 patients with lichenified lesions, in accordance with a protocol approved by the Yonsei University College of Medicine Institutional Review Board (number: 1-2018-0073, registered date: 18 January 2019, enrolled date: 5 March 2019). Patients with AD were diagnosed by dermatologists at the Atopic Dermatitis Special Clinic of Severance Hospital (Seoul, Korea) using the Hanifin & Rajka diagnostic criteria.[33] At the time of sample collection, Eczema Area and Severity Index (EASI) scores, SCORing Atopic Dermatitis (SCORAD), and the degree of symptoms were recorded for the AD patients. Skin metabolite samples were acquired using D-Squame tape strips (tapes; CuDerm), hollow MNs (Incyto, Cheonan), hydrocolloid sheet patches (blank patches; Raphas), and HA-MNs (Raphas) at week 0 (first visit) and week 2 (second visit) after appropriate treatment administered by the dermatologist. All experiments involving humans were performed in accordance with the Declaration of Helsinki. The average patient age and sex, total immunoglobulin E (IgE) level, and disease severity are listed in Table 1, and the total list of each patient's age, sex, initial total IgE, initial SCORAD, initial EASI, and degree of symptoms is included in Table S1.

### 2.3 | Skin application of tape and MN patches

Twenty consecutive D-Squame tape strips (22 mm diameter) were collected from the lesional skin of the patients with AD. Hollow MNs, blank patches, and HA-MNs were also applied to the lesional skin of these patients for 10 min. The collected skin metabolites were stored individually in 60 mm dishes at −80°C prior to processing.

**TABLE 1** Demographics and clinical characteristics of patients with atopic dermatitis (AD).

| Characteristics | Subjects (mean ± SD) |
|---|---|
| Total AD patients | 20 |
| Age (years) | 29.95 ± 8.92 |
| Sex | |
| Male | 12 |
| Female | 8 |
| Total IgE | 1952.11 ± 1762.70 |
| Initial EASI | 15.31 ± 12.47 |
| Severity (SCORAD index) | |
| Mild (<15 points) | 0 |
| Moderate (15–40 points) | 12 |
| Severe (>40 points) | 8 |

Abbreviations: EASI, Eczema Area and Severity Index; IgE, immunoglobulin E; SCORAD, SCORing Atopic Dermatitis; SD, standard deviation.

### 2.4 | Protein extraction

To extract proteins from the skin metabolites of the tape, hollow MNs, blank patches, and HA-MNs, each sample was scraped into 1% sodium dodecyl sulfate (SDS) buffer[34,35] (Thermo Fisher Scientific). The protein extracts were then centrifuged to clear the debris and frozen at −80°C for processing.

### 2.5 | Total protein quantification

To quantify the concentration of total proteins in the skin metabolite extracts, a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific) was used according to the manufacturer's protocol.

### 2.6 | Enzyme-linked immunosorbent assay

Human IL-4, IL-13, and IFN-$\gamma$ enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) were used to determine the levels of IL-4, IL-13, and IFN-$\gamma$ in undiluted samples of the skin metabolites following the manufacturer's instructions.

### 2.7 | Statistical analysis

All assays were performed in duplicate, and the data are presented as the mean ± standard deviation. Statistical analyses were conducted using IBM SPSS Statistics 25.0 (Armonk). Pearson's correlation coefficient was used to analyze whether the data were statistically correlated. The data were analyzed by one-way analysis of variance, and the significance of the differences was determined using the unpaired two-tailed *t*-test or the Tukey–Kramer test. Statistical significance was set at $p < 0.05$.

## 3 | RESULTS

### 3.1 | MN patch design

Biocompatible HA-MNs (each 160 array, 1 mm base width, 650 $\mu$m height) were fabricated using the droplet extension method[32] by drying a pharmaceutical-grade HA solution on top of a hydrocolloid patch (Figure 1A). When HA-MNs are applied to the skin at constant pressure, they penetrate the stratum corneum, and skin metabolites around the epidermis and dermis attach to the surface of the MNs (Figure 1B1). Upon removal from the skin, HA-MNs were dissolved, and proteins in the skin metabolites were collected for experimental analysis (Figure 1B2,B3).

### 3.2 | Use of an HA-MN for extracting skin metabolites

To optimize the protein extraction method from the HA-MNs, we first attempted to find an appropriate extraction method based on other protein extraction methods. In the non-invasive tape stripping method, 0.1% SDS or radioimmunoprecipitation assay (RIPA) buffer is used as the extraction buffer and then vortexed or scraped using a scraper to extract the proteins.

Therefore, we compared the 0.1% SDS versus RIPA buffer methods and vortex versus scraping methods and quantified the final protein concentration using the BCA assay. The 0.1% SDS buffer and scraping methods were selected because they extracted the highest quantity of protein (Figure S1A).

Next, to determine the molecular weight of HA required to load the MN patch, we constructed two different types of MN patches, low molecular weight (LMW) HA-MNs (110 kDa) and high molecular weight (HMW) HA-MNs (1000 kDa). After applying and detaching the patches for a certain amount of time, we analyzed the morphology and length of the MNs using a polarized microscope. We observed that LMW HA shortened rapidly compared with the HMW HA (Figure S1B,C). In addition, to determine whether the molecular weight of HA affects protein analysis, we obtained skin samples using LMW HA-MNs and HMW HA-MNs from patients in their 20s and 60s and checked the level of pro-collagen 1 protein, a representative skin-aging marker. Both HA-MNs showed increased pro-collagen 1 levels in the patients in their 20s compared with those in their 60s, but more target proteins could be extracted from the HMW HA-MNs (Figure S1D). Therefore, HMW HA-MNs were selected to obtain further skin samples.

### 3.3 | Comparison of net protein concentration between sampling tools

We conducted a test to establish the optimal time conditions for applying HA-MNs for protein extraction from the skin samples. HA-MNs were applied to the skin samples for 1, 5, and 10 min,

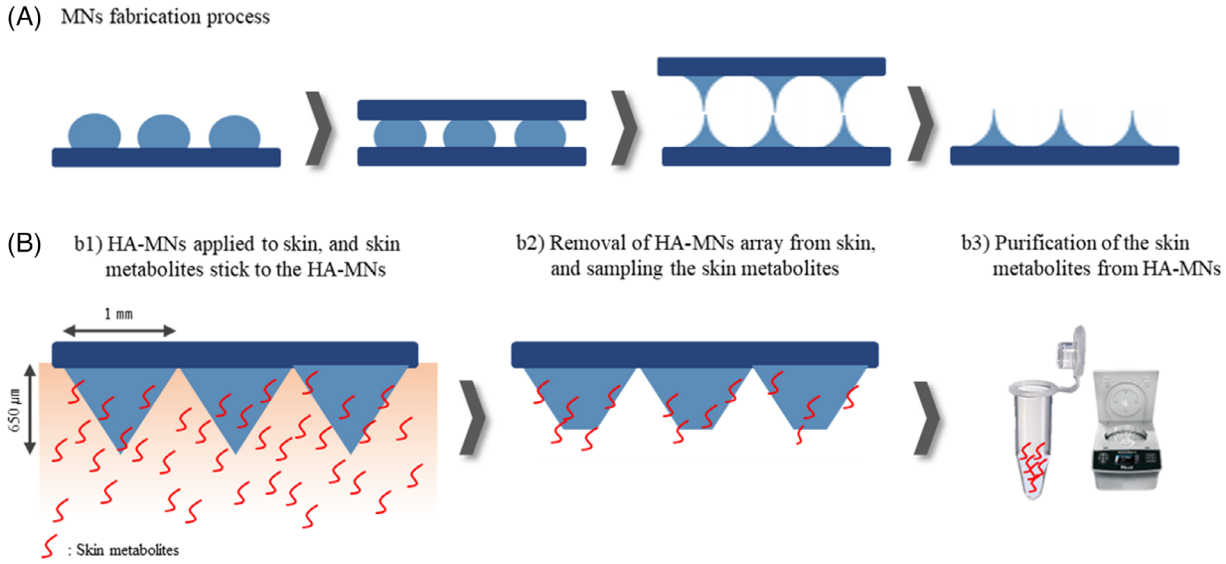


**FIGURE 1** Representative schematic of hyaluronic acid-loaded microneedle patch (HA-MN) platform for skin metabolite sampling. (A) HA-MN fabrication process using droplet extension manufacturing technology. (B) Schematic illustration of mechanisms for HA-MN sampling of skin metabolites.

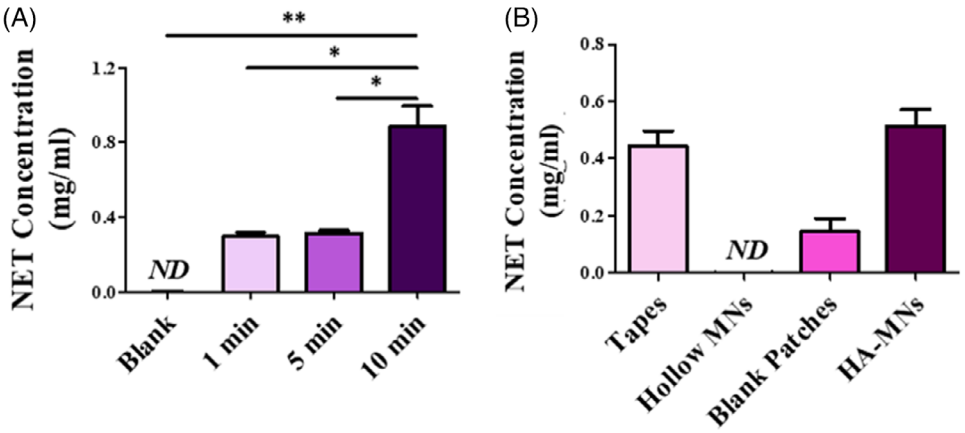


**FIGURE 2** Enhancement of protein sampling using minimally invasive tools. (A) Net protein concentration according to attachment time series of hyaluronic acid-loaded microneedle patches (HA-MNs). (B) Protein quantification for each method of skin metabolite sampling used in place of skin biopsies. Results are displayed as mean ± standard error of the mean of experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, ND: not detected.

and the concentration of the net protein was determined. As the highest net protein yield was observed at 10 min after application, the ideal HA-MN application time was determined to be 10 min (Figure 2A).

Twenty adults with moderate-to-severe AD were selected for this study to test our selected method (mean age 29.95 years; 12 men and eight women; mean EASI: 15.31) (Table 1). The appropriate treatment was applied to all the patients with AD, and lesional skin samples were collected before and 2 weeks after treatment. Skin samples were obtained using four different sampling tools: (i) a tape, (ii) a blank patch consisting of a hydrocolloid patch without MNs (Figure S2A), (iii) a hollow MN with a micro-sized cross-sectional area inside the MNs (Figure S2B), and (iv) HA-MN (Figure S2C).

The net protein concentrations of the samples were analyzed using the BCA assay. Proteins were successfully obtained from the skin samples with all the sampling tools except for the hollow MNs, and we determined that the highest net protein concentration was obtained from the HA-MNs (Figure 2B).

### 3.4 | Detection of Th1 and Th2 AD disease status using skin metabolites obtained from HA-MNs

To check the treatment progress of the patients with AD, proteins in the skin metabolites obtained by each of the three sampling tools were analyzed before and after treatment. The protein levels of biomarkers,

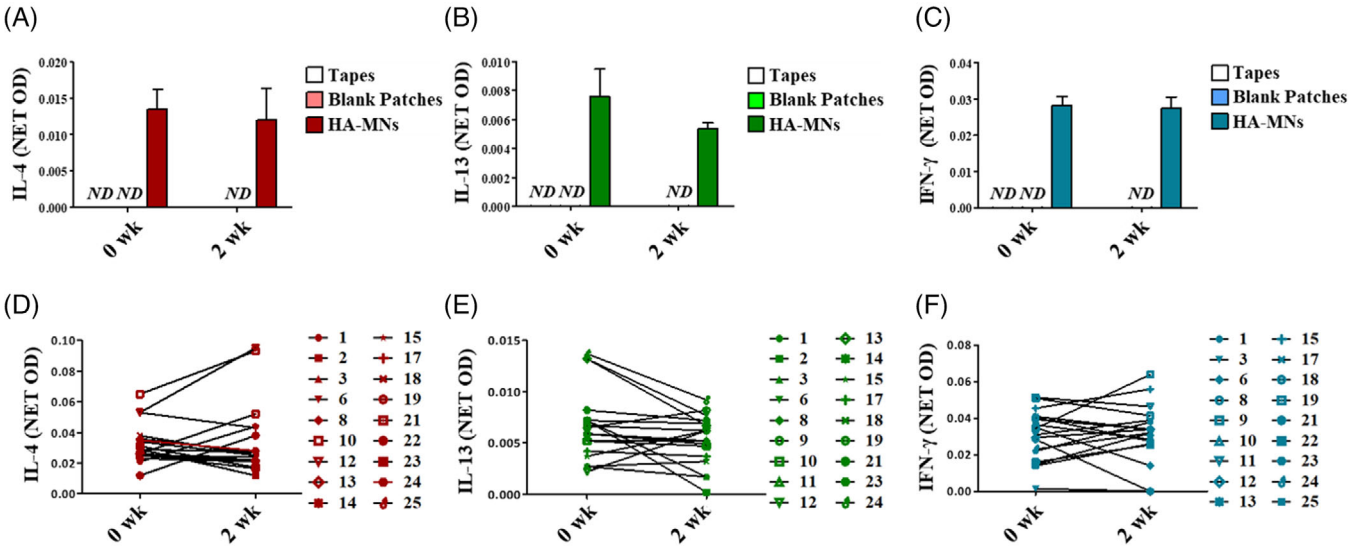


**FIGURE 3** Cytokine sampling from atopic dermatitis (AD) lesional skin using hyaluronic acid-loaded microneedle patches (HA-MNs). Net expression of cytokines, interleukin (IL)-4 (A), IL-13 (B), and interferon (IFN)-$\gamma$ (C) in skin metabolites collected before and after treatment of AD patients using tape strip, blank patch, and HA-MN methods. Net level of cytokines, IL-4 (D), IL-13 (E), and IFN-$\gamma$ (F) in each subject before and after treatment of AD patients in skin metabolites collected using HA-MNs. The results are displayed as mean ± standard error of the mean.

IL-4, IL-13, and IFN-$\gamma$, which are representative cytokines involved in the pathogenesis of AD, were confirmed. The expression of IL-4, IL-13, and IFN-$\gamma$ was not detected in the protein collected from the tape strips and blank patches; however, it was detected in the proteins collected from HA-MNs (Figure 3A–C).

By analyzing the protein obtained from HA-MNs, we further confirmed that the level of IL-4 decreased after treatment compared with before treatment (Figure 3A,D). In particular, the IL-13 level in the HA-MN-extracted protein significantly decreased after treatment (Figure 3B,E). Conversely, the expression of IFN-$\gamma$ in the proteins extracted from HA-MNs did not show a significant difference before and after treatment (Figure 3C,F).

Overall, these results suggest that HA-MNs are a minimally invasive skin sampling method that can be used to effectively determine the number of specific protein biomarkers in a skin sample, and it can thus be used to monitor the treatment progress of patients with AD.

### 3.5 | Correlation between AD symptom improvement and Th2 cytokines

At the second week of treatment, symptom improvement in the patients with AD was examined and judged by two dermatologists. Scores of +1 and −1 were assigned to patients who did not show a worsening of symptoms and those who did, respectively. The differences in IL-4 (ΔIL-4), IL-13 (ΔIL-13), and IFN-$\gamma$ (ΔIFN-$\gamma$) were also calculated by subtracting the values before and after treatment. We then analyzed the relationship between ΔIL-4, ΔIL-13, or ΔIFN-$\gamma$ and the clinical improvement score of AD symptoms using Spearman correlation analysis. The most significant negative correlations were observed between the net levels of IL-4 and IL-13 and the improvement score; these are depicted in Figure 4A,B. Additionally, no correlation was observed between the net expression of IFN-$\gamma$ and the improvement score (Figure 4C). Based on these results, we confirmed that, in addition to providing a minimally invasive sampling method, HA-MNs can be used to monitor the treatment progress of patients with AD.

## 4 | DISCUSSION

In molecular analysis for diagnosis or treatment evaluation of AD, Th2 cytokines are key factors because the increase in levels of Th2-related cytokines is a specific feature of AD. Th2 biomarkers correlated positively with disease severity and negatively with barrier products such as loricrin and filaggrin.[36,37] In treatment, dupilumab, an IL-4 receptor alpha antagonist, blocked IL-4 and IL-13 signaling and showed significant improvement of clinical AD symptoms compared with those in the placebo group. Transcriptome and serum profiles also showed a reduction in the level of type 2 inflammation-related biomarkers.[38,39]

Proteomic analysis in AD showed that skin protein analysis differs greatly from blood analysis.[40] For decades, the pathogenesis of AD has been well described in lesional skin obtained through punch biopsies, but because it is invasive and can cause scarring, non-invasive tape stripping has emerged as a better alternative. However, studies on the application of tape stripping for the collection of skin samples have reported the ineffectiveness of this method for reflecting changes in the dermis layer, where most inflammatory reactions of AD occur, as the collection area of the samples is limited to the epidermis.[13,16,17,34,35,41–44]

Several recent papers have reported the detection of IL-4 by analyzing multiplex immunoassay and RNA sequencing in samples collected

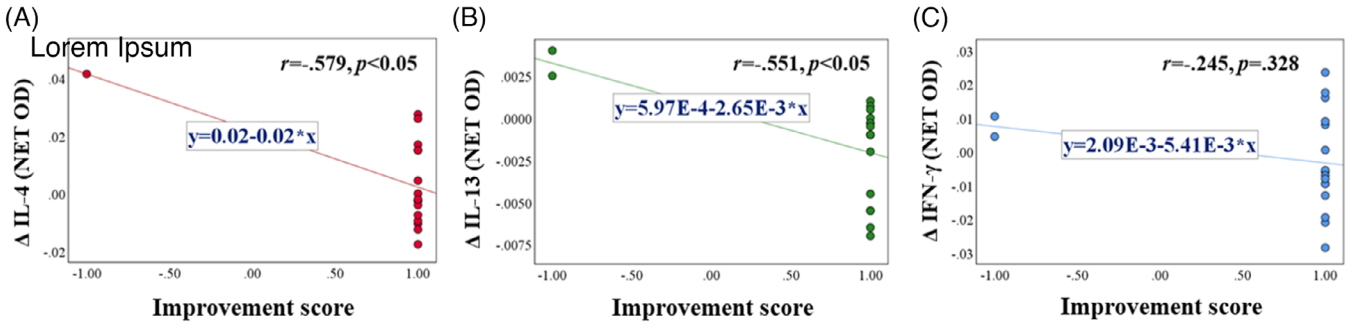


**FIGURE 4** Correlation analysis between cytokines extracted from hyaluronic acid-loaded microneedle patches (HA-MNs) and improvement score of atopic dermatitis (AD) symptoms. Spearman correlation plots with improvement score before and after treatment of AD patients and protein expression of difference in interleukin (ΔIL)-4 (A), ΔIL-13 (B), and difference in interferon (ΔIFN)-$\gamma$ (C) from HA-MNs. *p*, associated *p*-value; *r*, Spearman correlation coefficient.

by tape stripping.[45,46] However, in our study, IL-4 was not detected by ELISA in samples collected through 20 consecutive tape stripping. This discrepancy may be due to differences in sensitivity between sample analysis methods. However, since the tape stripping method can usually collect samples in the stratum corneum only, its effectiveness may be limited in detecting cytokines and chemokines secreted mainly in the dermis. Olesen et al.[47] have been reported that up to full stratum corneum can be collected through 20 consecutive tape stripping. Some references also supported that AD biomarkers secreted from the dermis are difficult to detect by the tape stripping method.[12]

Concerning the penetration depth of HA-MNs, the average depth of the epidermis is about 76.9–267.4 $\mu$m,[48] and the HA-MNs used in this study is 650 $\mu$m. So, it is sufficiently penetrable into papillary dermis except the soles and palms. In our previous study, we have been confirmed that HA-MNs with a length of 250–300 $\mu$m sufficiently penetrated the papillary dermis (data not shown). Other studies using MNs made of biodegradable materials also suggested that MNs with a length of 600–700 $\mu$m penetrate into the papillary dermis.[49,50]

Here, we present a novel minimally invasive system using biocompatible HA-MNs to analyze biomarkers and observe the treatment response of AD. This new platform enables the minimally invasive collection of skin metabolites from the entire skin layer by complementing the limitations of punch biopsies, tape stripping, and dermatological clinical assessments; thus, a longitudinal study of skin diseases such as AD can be quantitatively analyzed in a minimally invasive manner. The key to this platform is the optimized design of the HA-MN and the method for protein extraction.

Solid or hollow MNs made of silicon or metal are the most commonly applied patches for ISF extraction. This type of MN creates a tunnel on the skin to collect ISF using capillary force or a vacuum; however, this method poses a potential risk to the subject if MNs break inside the skin during extraction.[25,51,52] Therefore, we produced an ideal MN patch using HA, a candidate material suitable for extracting samples from the skin because of its biocompatibility and ability to attract a large amount of moisture[53]; we used this to collect skin samples efficiently while minimizing skin damage in patients with AD.

HA-MNs have been used for the extraction of ISF,[25,54] as well as drug or antibody transfer.[55,56] In this study, we first demonstrated the effectiveness of this method for extracting skin metabolites and analyzing biomarkers and the treatment response of patients with AD. Because each study using HA-MNs used different molecular weights, LMW and HMW of HA were compared, and we determined that a higher concentration of protein was extracted using a higher HA molecular weight. In addition, among the various time conditions and methods for extracting proteins, the optimal conditions were determined for extracting high concentrations of protein. Using this optimal method, we successfully extracted skin metabolites by applying HA-MNs before and 2 weeks after treatment in patients with AD and observed a significant correlation between changes in the levels of protein biomarkers IL-4, IL-13, and IFN-$\gamma$ and the dermatological assessments of symptom improvement.

Regarding sampling using HA, metabolites that are dissolved in moisture are likely to be detected well, but lipids may not be easily detected. Although this study alone cannot clarify the differences depending on the type of metabolite, our other study, which conducted RNA microarray analysis using HA-MNs, showed that several ceramide-related genes (CERS1, CERS2, CERS3, CERS4, CERS5, CERS6, SMPD, DEGS1, and DEGS2) were well detected. There were differences in ceramide-related genes between dry subjects and moist subjects, and CERS6, DEGS1, and DEGS2 showed statistical differences (data not shown). However, further study is needed to clarify the difference in the detection of type of the metabolite using HA-MNs.

## 5 | CONCLUSIONS

In summary, the conventional methods for obtaining skin samples are typically invasive and limited to specific sampling sites. In this study, we developed a novel platform for skin sampling to measure skin biomarkers in a minimally invasive manner using the biocompatible HA-MNs, and then we applied this platform to studies that observed protein levels of cytokines and treatment responses in patients with AD to verify its usefulness. These findings suggest that the highly effective and

novel platform presented in this study can be used as a minimally invasive alternative to traditional methods for assessing skin biomarkers. Based on the success of the development of this platform, we expect that it will significantly facilitate studies of biomarker analysis, pathogenesis, and drug development, as well as the analyses of treatment progress for longitudinal studies in various skin diseases.

## CONFLICT OF INTEREST STATEMENT

KHL, JDK, DHJ, and KHL* have submitted patents that have been or may be licensed to Raphas Co. Ltd. JDK and DHJ share the equivalents of Raphas Co. Ltd. DHJ is the chief executive officer of Raphas Co. Ltd. KHL* reports serving as an advisory committee for Raphas Co. Ltd. KHL is an employee of Raphas Co. Ltd. The interests of these authors did not influence academic fairness in conducting this study, analyzing results, or writing this paper. The other authors declare they have no potential conflicts of interest.

## FUNDING INFORMATION

The authors received no specific funding for this work.

## DATA AVAILABILITY STATEMENT

All data that support the findings of this study are available from the corresponding authors upon a reasonable request.

## ETHICS STATEMENT

The study protocol was approved by the Yonsei University College of Medicine Institutional Review Board (number: 1-2018-0073, registered date: 18 January 2019, enrolled date: 5 March 2019). The study was carried out in accordance with the approved protocol, and all participants provided written informed consent.

## CONSENT FOR PUBLICATION

We received consent from all participants involved in this study. The consent forms will be provided upon request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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