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Skin permeability of compounds loaded within dissolving microneedles dependent on composition of sodium hyaluronate and carboxymethyl cellulose

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Abstract−Dissolving microneedles are transdermal delivery systems designed to mechanically penetrate the skin and fully dissolve in the skin in a minimally invasive manner. In this study, the skin permeability of compounds encapsulated in microneedles was controlled by changing the composition of microneedle materials. Sodium hyaluronate (SH) and carboxymethyl cellulose (CMC) were chosen as structural materials and amylopectin was used to increase the mechanical strength of microneedles. To determine the effect of microneedle composition on skin permeability, microneedle properties such as mechanical strength and solubility were investigated according to various compositions of SH and CMC. When the CMC fraction in the needle increased, the mechanical strength of the microneedle increased, leading to high skin permeability of rhodamine B, a model compound. Using microneedles, significantly higher skin permeability of niacinamide was also obtained. These results indicate that the microneedles developed in this study improved the skin permeability of compounds loaded in the needle, and the skin permeability could be tuned by changing the composition of microneedle materials.

Keywords: Dissolving Microneedles, Microneedle Composition, Skin Permeability, Sodium Hyaluronate, Carboxymethyl Cellulose

INTRODUCTION

The most common routes of administering drugs into the human body are either oral delivery or parenteral injection. Drug delivery via injection is highly efficient, but carries several limitations, such as bleeding, pain, risk of infection, and the need for trained personnel [1]. Although oral delivery can avoid the limitations of injection delivery, the bioavailability of orally delivered drugs is vastly reduced due to first-pass metabolism, which can be influenced by enzyme activities in the gastrointestinal tract or liver [2,3]. To solve problems of oral delivery and parenteral injection, transdermal drug delivery using microneedles has been developed. Microneedles are micron-scale needles that are designed to pierce across the stratum corneum, which is the outermost layer of the skin, and into the epidermis and/or superficial dermis to deliver compounds such as dugs and cosmetic ingredients into the skin for local or systemic administration. These minimally invasive devices are long enough to pierce through the permeability barrier, but short and thin enough to avoid causing pain [4-6]. Among various types of microneedles, dissolving microneedles have attracted great attention. Dissolving microneedles are made from water-soluble materials that encapsulate functional compounds within the needle matrix and fully dissolve upon insertion into the skin. The permeability of compounds loaded into microneedles is significantly higher because compounds are directly released into the skin after penetration, resulting in minimal to no loss. In addition, the fully dissolving matrix material of the microneedle leaves no biohazardous sharp waste after use [7-9]. For dissolving microneedle fabrication, proper material selection is very important because the skin permeability of compounds encapsulated within microneedles is closely related to the mechanical strength and dissolution of materials from which microneedles are made. Therefore, our goal was to control the skin permeability of compounds loaded within microneedles by changing the composition of materials from which the microneedle was made. Sodium hyaluronate (SH) and carboxymethyl cellulose (CMC) were chosen as structural materials since they have been used for dissolving microneedle materials due to their high biocompatibility and water-dissolubility. SH is a biocompatible polysaccharide that can be found abundantly in all tissues and body fluids, especially in the skin. It is widely used as a cosmetic ingredient in skin care products and as an injectable filler for soft tissue augmentation [10,11]. CMC is also a dissolvable, mechanically strong, and water soluble polysaccharide that can be intradermally injected with no toxic or inflammatory effects, which facilitates repetitive treatment for autoinflammatory diseases with relapsing-remitting nature [12,13].

A PDMS mold for dissolving microneedles was fabricated using a laser-writing technique instead of a photolithography technique, which is the general method for fabricating a mold for microneedles. The characteristics of the prepared microneedles, including mechanical and dissolution properties were investigated according to the composition of SH and CMC in the microneedle. Finally, *in vitro* skin permeation through the skin was determined with porcine skin and a Franz diffusion cell according to microneedle composition.

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EXPERIMENTAL

1. Materials

SH, CMC, AP, and niacinamide were purchased from Sigma-Aldrich. Polydimethylsiloxane (PDMS) and rhodamine B (RhB) were obtained from Dow Corning and Junsei Chemical, respectively. All chemicals are reagent grade.

2. Fabrication of Mold Using Laser-writing Process

The PDMS mold for the microneedle was prepared using a laser writer (PL-40K, Korea stamp) according to previously reported methods [14,15]. Briefly, PDMS substrate was prepared by mixing polymeric PDMS solution and hardener. This PDMS pre-solution was poured on a flat surface, and then kept in a vacuum desiccator to completely remove blisters. Finally, the substrate was heated to be hardened in a dry oven at 72 °C for 2 hours to achieve a hardened PDMS sheet. Laser beams were then shot at the flat PDMS sheet to create an array of cone-shaped holes. The size and shape of the holes could be adjusted by manipulating the parameters in the operating software for the laser writer. An array of 12 by 12 cone-shaped holes with a depth of 800-900 μm and a diameter of 400 μm in the PDMS sheet was made and this PDMS mold was cleaned in an ultrasonic cleanser with 70% ethanol.

3. Fabrication of Dissolving Microneedles

Dissolving microneedles having various compositions of SH, CMC, and AP were fabricated. SH, CMC, and AP were dissolved in deionized water with weight ratios of 0 : 5 :5 , 4 : 1 : 5, and 5 : 0 : 5 (SH : CMC : AP) and the solution was poured onto the PDMS mold previously fabricated with the laser writer. After placing in a desiccator, the solution on the mold was degassed under vacuum to completely fill the holes of the mold. After degassing for 4 hours, the microneedles were dried in an oven at 50 °C for 8 hours and then the needles were separated from the mold. 0.1 g of RhB or 0.035 g of niacinamide were mixed with 30 ml of microneedle pre-solution to prepare the microneedles containing RhB or niacinamide, respectively.

4. Characterization of Dissolving Microneedles

The mechanical properties of the microneedles were investigated with a custom-designed device. The device is designed so that a specific weight can be applied perpendicularly to simulate the situation where the microneedles are applied onto the skin. For each measurement, a designated weight was applied on the microneedles for 1 minute, after which the height of the microneedles was measured with a microscope. The amount of deformation was calculated by measuring changes in the height of the microneedles. Three different loads of 3, 5, and 10 N were tested. Two types of solubility of microneedles were examined. First, the water solubility of the needle was tested by immersing the needle portions of the microneedles in 5 ml of phosphate buffered saline (PBS, pH 7.0) and the remaining length of the microneedles was measured using microscopy. Second, the *in vitro* solubility of the microneedles was determined by inserting them into porcine skin (Medikinetics) and examining the remaining length of the microneedles.

5. *In Vitro* Skin Permeation Experiment

An *in vitro* skin permeation experiment with RhB, as a model compound, was carried out using Franz diffusion cells (FCDV-15, Labfine) and porcine skin with a thickness of 1.7-2.0 mm and a size of 2×2 cm^2. A diffusion cell consisted of a donor chamber and a receptor chamber with the skin positioned between the chambers. The absorption surface area of the skin was 0.785 cm^2 and the receptor chamber was filled with 5 ml of PBS. After equilibration of the porcine skin with the receptor phase, the dissolving microneedle containing RhB was applied to the surface of the skin. The receptor fluid was maintained in contact with the underside of the skin from the time of application until the end of the experiment when the receptor fluid was collected. The diffusion cell and the skin were maintained at a constant temperature of 36 °C and the receptor fluid was continuously agitated with a magnetic stirrer at 500 rpm. After 24 hours, the sample solution was taken from the receptor chamber and the RhB concentration was measured with a UV-Visible spectrophotometer (Varian Cary 100, Agilent) at 553 nm. For the *in vitro* skin permeation experiment with niacinamide, the method was the same except the niacinamide concentration was analyzed using HPLC (Agilent 1260, Agilent) after the receptor fluid was withdrawn. The HPLC was operated with an Agilent Zorbax Eclipse XDB (C18, 4.6×250 mm, 5 μm) column, a mobile phase of 75% 0.05M KH_2PO_4 solution and 25% methanol, and a UV detection wavelength of 263 nm. For control experiments, the same amount of RhB or niacinamide that was encapsulated in microneedles was directly applied on the skin without the microneedle and the permeated concentration of RhB or niacinamide was measured.

RESULTS AND DISCUSSION

1. Fabrication of PDMS Mold for Microneedles

Fig. 1 shows images of the PDMS mold prepared with the laser-writing process and the microneedles. The mold for the microneedles was fabricated by creating cone-shaped holes in the PDMS sheet using the laser-writer. The diameter and the depth of the cone-shaped holes were tuned by adjusting the laser parameters so that holes with a diameter between 200 and 600 μm and a depth between 300 and 1,200 μm could be fabricated. Fig. 1(a) shows the PDMS mold containing 12 by 12 holes whose diameter and depth were 400 μm and 800 μm, respectively. Fabrication of the mold with the laser-writing process reduces significantly the amount of time and cost compared to other microfabrication processes and does not involve the toxic and hazardous chemicals usually used in the photolithography and etching processes [16]. In addition, fabrication of non-vertical shapes like the cone-shaped hole with photolithography often requires many complicated fabrication steps. However, the laser-writing process can create cone-shaped holes with sharp edges via a simple one-step fabrication process. Therefore, the laser-writing process provides a quicker and safer method for rapid prototyping of microneedles than conventional microfabrication methods.

2. Fabrication of Dissolving Microneedles

Fig. 1(b) presents the optical images of prepared dissolving microneedles. The image shows that the shapes of the microneedles are relatively uniform, indicating that our laser writing and replica molding process was able to produce microneedles with relatively high uniformity. The geometry of the cone-shaped holes in the mold was faithfully reproduced so that the resulting microneed-

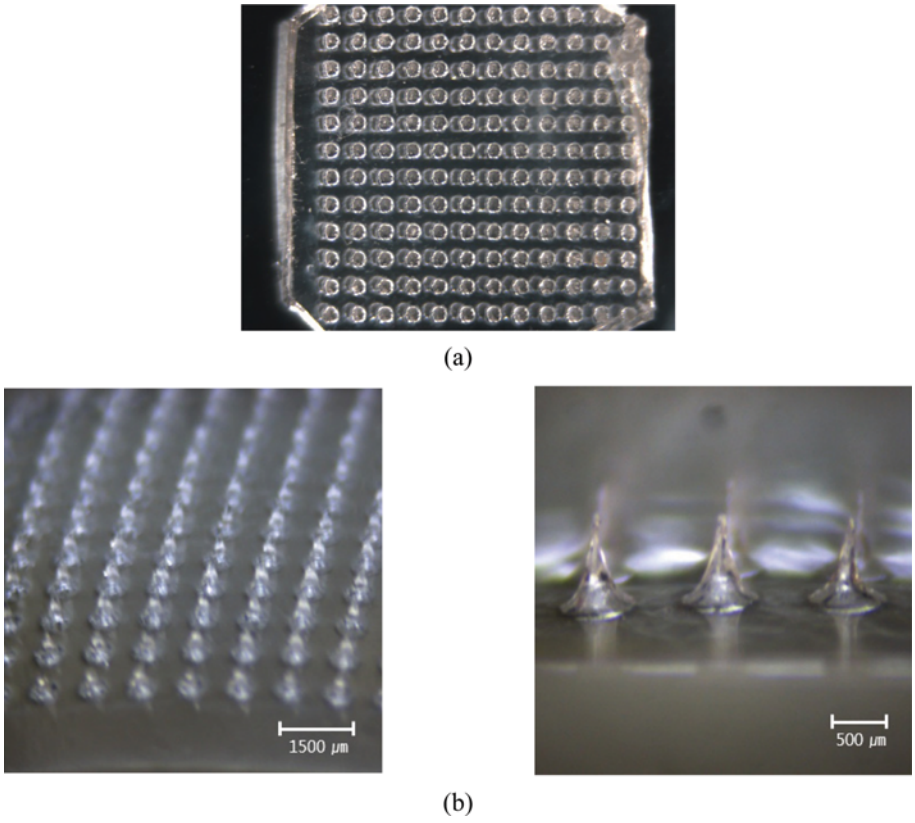


Fig. 1. Images of a PDMS mold prepared by the laser-writing process (a) and dissolving microneedles (b).

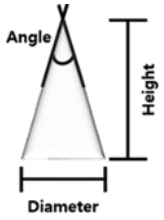
dle array had 144 needles, each with an average height of 800 μm and diameter of 400 μm at the base in an area of 2×2 cm^2. Fabrication of microneedles with SH, CMC, and AP does not involve toxic or harsh organic solvents that might degrade or contaminate the compounds encapsulated within the microneedles. Since many compounds in the pharmaceutical and cosmetic fields are generally very unstable to chemicals so that they can easily decompose into biologically inactive compounds, this fabrication method is suitable for encapsulating environment-sensitive compounds within the microneedles. To verify that the molding process was sufficiently consistent and accurate, yielding microneedles with consistent shapes and sizes, the coefficient of variation (CV) of heights, diameters, and angles of ten microneedles (SH : CMC : AP=4 : 1 : 5) in a single array and in three different arrays were calculated and are listed in Table 1. The CV is a standardized measure of dispersion of a probability distribution or frequency distribution and is defined as the ratio of the standard deviation to the mean. All of the calculated CVs were less than 5.9%, which indicates that the geometry of the needles was uniform both within a single microneedle array and between different arrays.

3. Characterization of Dissolving Microneedles

Microneedles are required to have minimum mechanical strength to penetrate into the skin without breaking. The solidity of the fabricated microneedles was tested by applying defined amounts of weight onto the microneedles in a perpendicular direction and calculating the remaining length ratio, defined as the ratio of the final height of the deformed microneedle to the height of the original microneedle. Microneedles made from SH and CMC alone were mechanically weak, with significant deformation even when the minimum weight of 3 N was applied (data not shown). Therefore, to increase the mechanical strength of the microneedles, we added AP to the microneedles. AP is a biocompatible polysaccha-

Table 1. Coefficient of variation (CV) of heights, diameters, and angles of microneedles (SH : CMC : AP=4 : 1 : 5) in a single array and three different arrays

	Coefficient of variation (%)		
	Height	Diameter	Angle
Microneedles in a single array	0.9	2.2	5.1
Microneedles in three different arrays	1.0	3.6	5.9



ride with a history of use in FDA-approval parenteral formulations, highly water soluble for rapid dissolution on the skin [17,18], and it is reported that adding AP increases the mechanical strength of microneedles [7]. Fig. 2 shows the remaining length ratio of microneedles after application of various loads. The results show that a higher fraction of CMC rendered microneedles stronger than SH, resulting in an increase of the remaining length ratio. As expected, when the load increased, the remaining length ratio of the needle decreased, indicating that there was more deformation of the needles. The relationship between microneedle composition and the mechanical strength of the needle can be used when it is necessary to control the mechanical properties of the needle for specific needs.

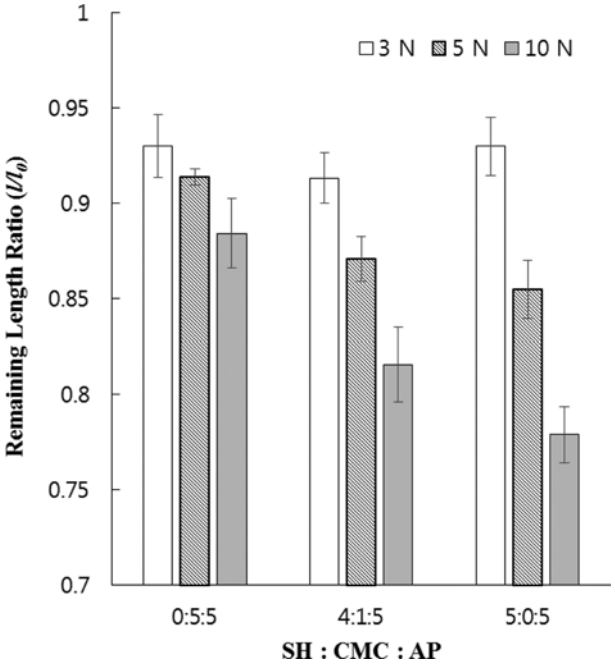


Fig. 2. Mechanical strength of microneedles as a function of SH, CMC, and AP composition (average±SD, n=3-5).

Water solubility of the microneedles was determined by immersing the needle portion of the microneedles in PBS for 10 seconds and calculating the remaining length ratio of the needle. Three different ratios of SH, CMC, and AP (0 : 5 : 5, 4 : 1 : 5, and 5 : 0 : 5) were tested and, as shown in Fig. 3, for microneedles with a 0 : 5 ratio of SH to CMC ratio, approximately 46% of the original structure remained after 10 seconds in PBS, and a higher fraction of SH resulted in lower dissolution, with about 73% of the original shape remaining in case of a 5 : 0 ratio of SH to CMC. The dissolution of microneedles in porcine skin was also investigated (Fig. 4), and we verified that increasing the composition of SH decreased the dissolution in both PBS and porcine skin, although there were fewer differences in dissolution of microneedles in PBS according to the composition compared to dissolving in porcine skin. However, the dissolving time of microneedles in porcine skin was significantly slower compared to the dissolving time in PBS. For a microneedle with a 4 : 1 ratio of SH to CMC, it took 8 minutes and 10 seconds to dissolve approximately 40% of the microneedle in the porcine skin and in PBS, respectively. These results indicated that SH can make microneedles less soluble in both PBS and porcine skin than CMC. It is important to control the dissolution of the microneedles since the release of compounds contained within the microneedles is mainly governed by the solubility of the needles. Thus, fast dissolution of the microneedle would result in a rapid release of

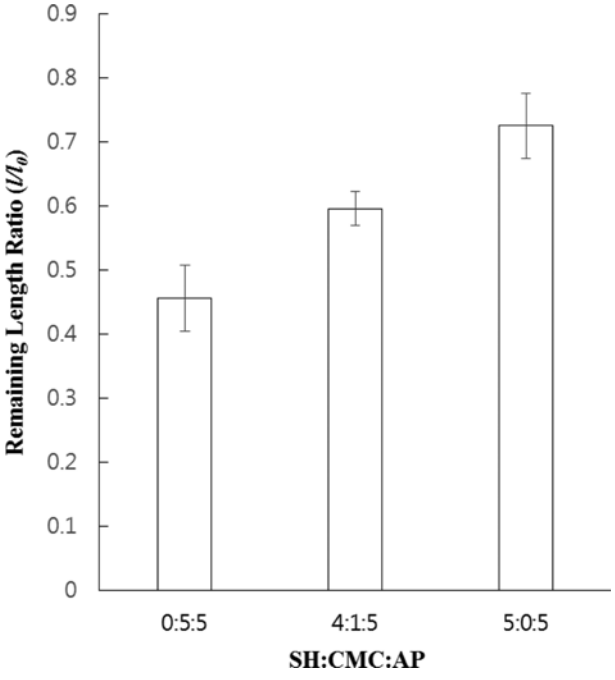


Fig. 3. Dissolution of microneedles as a function of SH, CMC, and AP composition. The microneedles were dissolved in PBS for 10 seconds (average±SD, n=3-5).

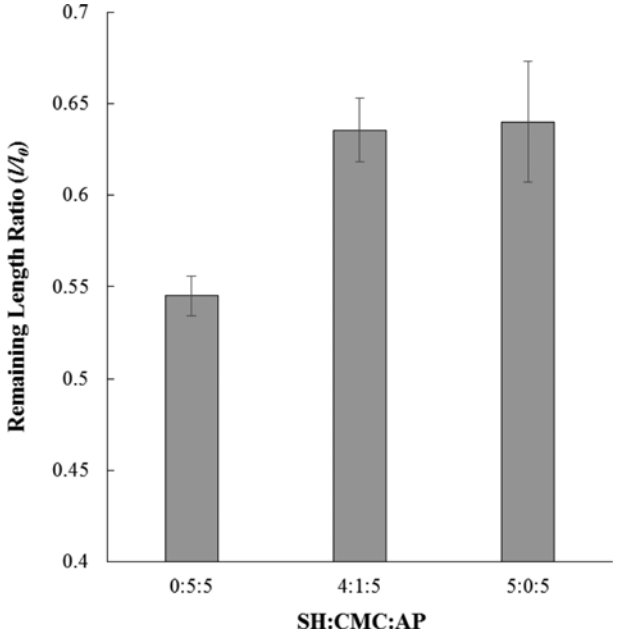


Fig. 4. Dissolution of microneedles as a function of SH, CMC, and AP composition. The microneedles were dissolved in porcine skin for 8 minutes (average±SD, n=3-5).

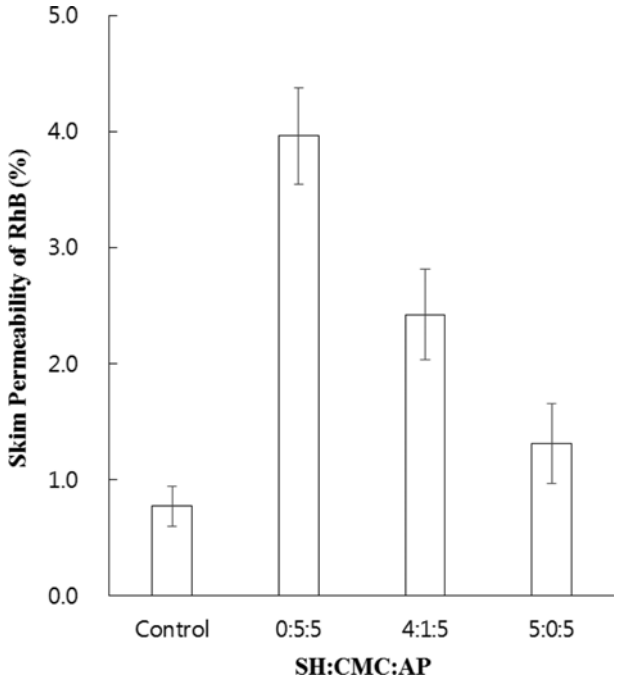


Fig. 5. Skin permeability of RhB through the porcine skin according to microneedle composition (average±SD, n=3-5).

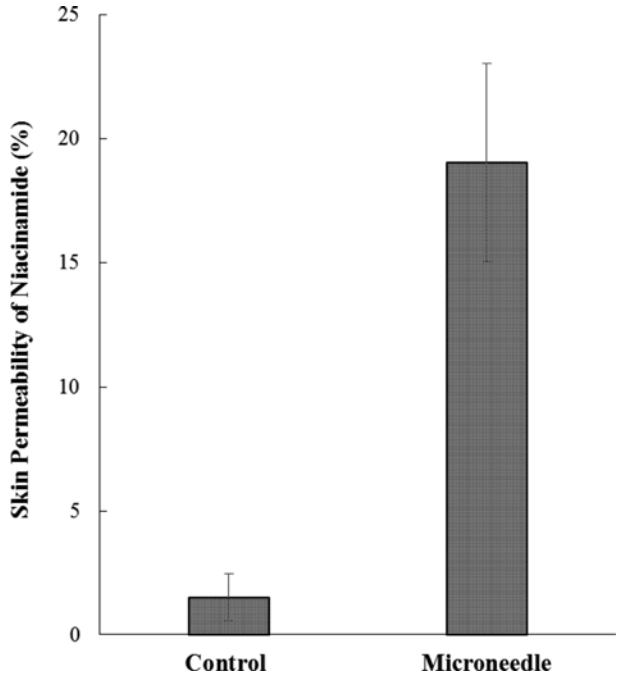


Fig. 6. Skin permeability of niacinamide through porcine skin with and without use of microneedles (average±SD, n=3-5).

encapsulated compounds, whereas slow dissolution would enable a more sustained release.

4. *In Vitro* Skin Permeation Analysis

To examine the effect of microneedle composition on skin permeation of compounds encapsulated within the needle, RhB was used as a model compound. RhB was loaded inside the microneedle during the fabrication process, and the microneedle was inserted into the porcine skin in the Franz diffusion cell to measure the concentration of RhB that diffused through the skin. For control experiments (skin permeability of RhB without the microneedle), the same amount of RhB that was contained within RhB-loaded microneedles was placed on the surface of the skin without the microneedle. In both cases, the amount of RhB in the receptor chamber was measured using a UV-Visible spectrophotometer at 553 nm. Fig. 5 shows the skin permeability of RhB through the porcine skin according to the microneedle composition. As RhB was delivered using microneedles, the skin permeability of RhB was greatly improved and there was a significant difference in the skin permeability of RhB according to microneedle composition. The CMC fraction in the needle increased the skin permeability of Rh B, which was related to the needle properties such as mechanical strength and solubility. As previously mentioned, when the CMC fraction in the needle increased, the mechanical strength of the microneedle increased while the solubility decreased. Therefore, the skin permeability of the microneedle was more related to the mechanical strength than the solubility, which can be explained in that harder microneedles could be inserted deeper in the skin leading to delivery of more compounds loaded in the needle.

One of the potential applications of the microneedle developed in this study is for enhancing skin permeation of cosmetic ingredients, since the effectiveness of traditional methods in administering cosmetic ingredients to the skin, such as creams and patches, is limited since the stratum corneum of the skin provides a significant barrier to the transport of those ingredients into the body. This microneedle array would be assembled into patches, which would offer simplicity of use and low cost, similar to conventional cosmetic patches, and have a significant impact on microneedle applications for the cosmetic industry. Therefore, we investigated the improvement of skin permeability of niacinamide using microneedles. Niacinamide, also known as vitamin B_3 or nicotinamide, has been reported to show various beneficial effects on the skin, such as suppression of melanosome transfer, leading to a reduction of cutaneous pigmentation, anti-inflammatory effects in acne, prevention of photocarcinogenesis, anti-aging effects, and reduction of transepidermal water loss. It also increases the synthesis of ceramides and other stratum corneum lipids with enhanced epidermal permeability barrier function [19,20]. Niacinamide was encapsulated inside the microneedle during the fabrication process, and the microneedle was inserted into the porcine skin in the Franz diffusion cell to measure the amount of niacinamide that diffused through the skin. Fig. 6 shows the permeation of niacinamide through the porcine skin with and without using the microneedles. The amount of niacinamide transported through the skin was measured using HPLC. When niacinamide was administered with the microneedle, significantly higher skin permeability was obtained.

CONCLUSIONS

This study presents the microfabrication of PDMS molds for microneedles, which is a very simple and efficient method compared to the conventional photolithography technique, and the

effect of microneedle compositions on skin permeability of compounds, such as drugs and cosmetic ingredients, encapsulated within the microneedles. The laser-writing process produced sufficiently consistent and accurate PDMS molds, yielding microneedles with consistent shapes and sizes. Changing the composition of SH and CMC, as microneedle materials, affected the microneedle properties such as mechanical strength and solubility, resulting in an influence on skin permeability. When the CMC fraction in the needle increased, the mechanical strength of the microneedle increased, while the solubility decreased. Using microneedles, significantly higher skin permeability of RhB and niacinamide was obtained compared to the control (direct application without the microneedle) and the skin permeability of RhB increased with the CMC fraction in the needle.

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