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Research paper

Transdermal delivery of relatively high molecular weight drugs using novel self-dissolving microneedle arrays fabricated from hyaluronic acid and their characteristics and safety after application to the skin

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ABSTRACT

The purpose of this study was to develop novel dissolving microneedle arrays fabricated from hyaluronic acid (HA) as a material and to improve the transdermal permeability of relatively high molecular weight drugs. In this study, fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4 kDa (FD4) was used as a model drug with a relatively high molecular weight. The microneedle arrays significantly increased transepidermal water loss (TEWL) and reduced transcutaneous electrical resistance (TER), indicating that they could puncture the skin and create drug permeation pathways successfully. Both TEWL and TER almost recovered to baseline levels in the microneedle array group, and relatively small pathways created by the microneedles rapidly recovered as compared with those created by a tape stripping treatment. These findings confirmed that the microneedle arrays were quite safe. Furthermore, we found that the transdermal permeability of FD4 using the microneedle arrays was much higher than that of the FD4 solution. Furthermore, we found that the microneedle arrays were much more effective for increasing the amount of FD4 accumulated in the skin.

These findings indicated that using novel microneedle arrays fabricated from HA is a very useful and effective strategy to improve the transdermal delivery of drugs, especially relatively high molecular weight drugs without seriously damaging the skin.

1. Introduction

As a novel minimally invasive approach, microneedle-mediated transdermal delivery has received increased attention in the recent years [1,2]. Needles with micrometer dimensions have been demonstrated to effectively penetrate the skin barrier of the stratum corneum and create efficient pathways for the delivery of small drugs, macromolecules and nanoparticles, as well as for fluid extraction [3–6]. Moreover, their sharp tips help prevent damaging the nerves that perceive pain [7,8]. Depending on the designed needle length, they can deliver drugs to a desired depth in the skin. Therefore, this novel transdermal approach for drug delivery using microneedle arrays has many of the advantages, and few of the disadvantages have been observed in both conventional injection needles and transdermal patches [9].

Previously, most microneedles have been fabricated from silicon [10–12], metal [13–15] and glass [16]. Since the loading amounts of drugs into these types of microneedles are quite limited, these needles have been used for the skin treatment before or after the application of a topical formulation or patch to facilitate the transdermal absorption of drugs [11,12,14]. Furthermore, due to the decrease in pore size over time after the removal of the needles, only small amounts of drugs can be delivered through the pathways created. In such cases, large amounts of drugs with high concentrations have to be used in order to obtain a detectable effect [17,18]. Moreover, this two-step application process is cumbersome for patients and prone to errors. To overcome these shortcomings and limitations, microneedles with drug-coated shaft surfaces have been developed recently [10,19–21]. Model drugs were uniformly coated on needle shafts, and coatings were rapidly

Abbreviations: HA, hyaluronic acid; FD4, fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4 kDa; TEWL, transepidermal water loss; TER, transcutaneous electrical resistance; H&E, hematoxylin and eosin; P.I.I., Primary Irritation Index.

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dissolved in the skin without wiping off on the skin surface [20,21]. However, these microneedles have been limited to small drug doses (micrograms) due to their inherently small volume and surface area. Moreover, there is a critical risk that microneedles can be accidentally broken and remain in the skin for a long period of time, similar to the cases of traditional microneedles fabricated from silicon, metal and glass.

Recently, attention has been paid to the use of microneedles fabricated from biocompatible and biodegradable polymers [22–24] and carbohydrates [25–29], which are free from the risk of complications. If left in the skin, these types of needles safely degrade and eventually disappear. They also have the potential for loading drugs into a matrix of needles and releasing them in the skin by biodegradation or dissolution in the interstitial fluid; a one-step application. However, it was reported that a heating step over 140 °C was needed to fabricate the microneedles composed of polylactic acid, polyglycolic acid, polylactic-co-glycolic acid, sugar, maltose and galactose [28,30,31]. In this case, heat-sensitive drugs, such as peptide and protein drugs, may undergo partial denaturation and lose their pharmacological activities. Thus, the fabrication of microneedles without any heating step is of practical importance. Based on these findings, Sullivan et al. fabricated microneedles at room temperature (23 °C) using photo-polymerization of a liquid monomer (vinyl pyrrolidone) to form polyvinylpyrrolidone microneedles that contain the lyophilized vaccine [32]. The absence of organic solvents and elevated temperatures seems to be notable advantages in preserving the stability of vaccines or other biomolecules. Most of the previous polymer needles have slow-degrading characteristics which can retain drugs in the skin for a long period of time. Therefore, they are suitable for sustained delivery of drugs. Unlike polymer microneedles, carbohydrate microneedles such as maltose and galactose are readily dissolved in the skin, and they are supposed to accomplish rapid drug delivery. However, it has been reported that galactose microneedles spontaneously dissolve due to hydrolysis even at 43% and 75% humidity, which negatively affects the ability to insert these needles into the skin [28].

With these findings in mind, we used hyaluronic acid (HA) to fabricate novel dissolving microneedle arrays in this study. HA is a water-soluble polymer of disaccharides, naturally found in many tissues of the body, such as skin, cartilage and the vitreous humor. In 2003, the FDA approved HA injections for filling soft tissue defects. Until now, HA was used as a common ingredient in cosmetics to minimize the appearance of facial lines and wrinkles, with effects typically lasting for different times according to the molecular weight of the injected HA. Moreover, HA has also been added to moisturizers, makeup and soap. Its high biocompatibility and other physical and chemical properties make it a suitable candidate for the fabrication of microneedles without any heating step. Furthermore, the mold-based fabrication process is relatively inexpensive and suitable for mass production. Based on these findings, we already prepared the HA-fabricated microneedle arrays containing alendronate and insulin and found that they were effective for improving the transdermal drug delivery [33,34]. However, the piercing and dissolution properties of the microneedle arrays in the skin were not fully examined. Furthermore, we did not confirm the safety of these arrays or the resulting irritation after their application to the skin.

In this study, therefore, we prepared HA-fabricated microneedle arrays containing fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4 kDa (FD4) as a model drug with relatively high molecular weight, and assessed both their disruption of the skin and safety by a primary skin irritation test. We also evaluated the transdermal delivery of FD4 using the microneedle arrays and compared it with the transdermal delivery of FD4 in a solution formulation *in vitro*.

2. Materials and methods

2.1. Materials

HA was kindly provided by Shiseido Co., Ltd. (Tokyo, Japan). FD4 was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Tissue-TeK® O.C.T. Compound was purchased from Sakura Finetek Japan Co., Ltd. (Tokyo, Japan). All other chemicals and reagents were of analytical reagent grade.

Male Wistar rats, weighing 220–270 g, were purchased from Shimizu Laboratory Supplies Co., Ltd. (Tokyo, Japan). All experiments were performed in accordance with the guidelines of the animal ethics committee at Kyoto Pharmaceutical University.

2.2. Fabrication of HA microneedle arrays

The microneedle arrays without any drug material (placebo), and containing blue dye or FD4, were fabricated by micromoulding technologies with HA as a base material. The fabrication process of microneedle arrays can be considered as transcription from the micromould with needle-shape in place. In detail, 15% HA solution was obtained by mixing well with distilled water. Blue dye or FD4 solution was added to the 15% HA solution and mixed well to prepare HA solution containing blue dye or FD4. 0.1 ml of the resulting HA solution containing blue dye or FD4 was placed on a 2 cm × 2 cm micromould at room temperature. After 2 h drying in desiccator, a 2 cm × 2 cm polyethylene terephthalate (PET) adhesive tape was attached on the base plate for reinforcing, then 0.1 ml of 20% HA solution was placed on the PET. After drying completely, a sheet of microneedle arrays containing blue dye or FD4 was obtained by peeling the mold off. Microneedle arrays containing blue dye or FD4 in circular area with a diameter of 10 mm were obtained by cutting the sheet with a punch.

Using this fabrication process, the resulting base plate below PET connected with needles was considered to consist of HA and blue dye or FD4 with same concentration as that in needles. In the case of microneedle arrays containing 5% (w/v) FD4, the total amount of FD4 is 250 μg, including 50 μg in needles and 200 μg in base plate.

2.3. Histological and microscopic analysis of HA microneedle arrays

Microneedle arrays were used for insertion into human cadaver skin and left in place for 5 min. Upon the removal of the microneedle arrays, the skin sample was subsequently embedded in OCT compound. The samples were then frozen in dry ice and acetone (−78 °C) and sectioned into 10-μm-thick slices using a cryomicrotome (Histostat Cryostat Microtome, Buffalo, NY, USA). The skin sections were stained with hematoxylin and eosin (H&E) and examined using a all-in-one microscope (BZ-8000, Keyence, Osaka, Japan) to observe the pathway created by the microneedle.

In another experiment, the diffusion of FD4 from the microneedle arrays in human cadaver skin was observed in order to assess the microneedle-media transdermal drug delivery. Microneedle arrays containing 5% (w/v) FD4 were inserted into human cadaver skin for 1 h. After removal, the skin sections were obtained by the same process mentioned above and examined by an all-in-one fluorescence microscope.

2.4. Visualization of micropores created by insertion of microneedle arrays

Microneedle arrays containing blue dye were applied to the rat skin and left in place for 1 h *in vivo*. After removal, pictures of the treated skin site were taken using a digital camera to confirm the uniformity of the insertion.