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Performance and characteristics evaluation of a sodium hyaluronate-based microneedle patch for a transcutaneous drug delivery system

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ABSTRACT

The MicroHyala® microneedle (MN) patch was developed to provide a simple, safe, and effective drug delivery system. In this study, we examined the performance and characteristics of our fabricated MN patch to identify potential quality issues with future commercial application. Mechanical failure force analysis identified that the strength of the MN patch was affected by environmental humidity, because higher moisture levels weakened the strength of the MN. Incorporation of all-trans retinoic acid (ATRA) or ovalbumin (OVA) into the MN patch decreased the mechanical failure force by almost 50% of the strength of placebo (without drug) patches. ATRA-loaded MN patches displayed good stability after storage at 4 °C, with more than 90% and 85% of the drug remaining in the patch after 8 and 24 weeks of storage, respectively. Tetanus toxoid- and diphtheria toxoid-loaded MN patches stored for 12 months induced robust antigen-specific immune responses similar to the responses by freshly prepared MN patches. Fluorescence imaging findings suggested that prolonged antigen deposition was induced by MN-mediated fluorescein isothiocyanate-labeled (FITC)–OVA vaccination. Overall, although the strength of MN requires improvement, our developed MN patch appears to be an effective pharmaceutical product providing a simple, safe, and relatively painless approach.

1. Introduction

Transdermal drug delivery is an attractive administration option for small-molecule and macromolecule products, including vaccines, because of its accessibility, safety, painless drug administration, potential for self-administration, and avoidance of enzymatic degradation in the gastrointestinal tract or liver. However, the physical barrier of the stratum corneum, the outer layer of the skin, causes poor permeability across the skin and limits the bioavailability of macromolecules, thus limiting the progress of transdermal administration (Arora et al., 2008; Prausnitz, 2004; Prausnitz and Langer, 2008). To overcome this limitation, microneedle (MN) patches consisting of micron-scale needles assembled on a transdermal patch have been developed using fabrication technology of the microelectronics industry (Kim et al., 2012a; Prausnitz et al., 2009).

Although MN technology was first developed in the early 1970s, few studies demonstrated the its usefulness as a tool for transdermal drug delivery, until the advancement of microelectronics technology in the 1990s. MN fabrication technology has rapidly progressed since then. MNs have been made from silicon, metal, or polymer (Kim et al., 2012a; Kis et al., 2012). The majority of research papers published thus far used nondissolving MNs. Metal-based MNs coated with water-soluble formulations facilitate the successful delivery of agents such as insulin (Gill and Prausnitz, 2007), salmon calcitonin (Tas et al., 2012), parathyroid hormone (PTH; 1–34) (Ameri et al., 2010), hepatitis B surface antigen (Andrianov et al., 2009), inactivated influenza virus (Zhu et al., 2009), influenza virus-like particle (Quan et al., 2010), bacillus Calmette–Guérin (Hiraishi et al., 2011), and influenza virus hemagglutinin-DNA (Kim et al., 2012b; Song et al., 2012) into the skin.

Polymer MNs that dissolve in the skin have also been developed recently; these MNs display successful delivery and efficacy (Sullivan et al., 2010). Compared with metal-based MNs, polymer-based dissolving MNs have several potential advantages. Because polymer-based MNs dissolve completely in the skin, they cannot be

Abbreviations: MN, microneedle; ATRA, all-trans retinoic acid; TT, tetanus toxoid; DT, diphtheria toxoid; OV, ovalbumin; FITC–OVA, fluorescein isothiocyanate-labeled ovalbumin; HPLC, high-performance liquid chromatography; ERH, equilibrium relative humidity; ID, intradermal; PBS, phosphate-buffered saline; HSD, honestly significant difference; ANOVA, analysis of variance.

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intentionally reused, which may help prevent the transmission of blood-borne pathogens and diseases caused by reuse of needle and syringes in developing countries. In addition, they can completely eliminate biohazardous sharp waste after use and safety concerns such as fractured metal needles piercing the skin (Al-Zahrani et al., 2012).

In our earlier studies, we developed a sodium hyaluronate-based dissolving MN patch called MicroHyala®. Sodium hyaluronate is a component of skin tissue and is hydrophilic in nature; thus, it may be biocompatible with the skin and safe for exogenous material insertion, which has been verified by our recent study involving healthy human volunteers (submitted for publication). We also demonstrated that transcutaneous immunization using the MN patch induces immune responses against ovalbumin (OVA), adenoviral vectors, tetanus, diphtheria, malaria, and influenza, with comparable efficacy to traditional hypodermic needle-based immunization (Matsuo et al., 2012a,b). These safety and efficacy findings indicate that our developed MN patch is a promising delivery system. Considering its future commercial application, there are several critical attributes necessary for product quality. First, MNs need to be precisely inserted into the skin without mechanical failure in order to ensure drug delivery into the skin, which requires sufficient MN strength (Lee et al., 2008). Second, MNs should dissolve in the skin's interstitial fluid within no more than a few hours (hopefully within a few minutes) to minimize the patch application time; a shorter application time may be better from the point of view of patient compliance. Third, the stability of the drug loaded into the MN patch is an important factor for efficacy. Furthermore, thermostable vaccine formulations could facilitate increased vaccine coverage, especially in developing countries that lack an adequate healthcare infrastructure for cold-chain storage (Bell et al., 2001; Berhane et al., 2000).

In the present study, we investigated the performance and characteristics of a sodium hyaluronate-based MN patch. We evaluated the mechanical failure force of MNs and the dissolution characteristics of drugs from MNs. In addition, the stability performance of drug-loaded MN patches was assessed using all-trans retinoic acid (ATRA; vitamin A acid) and the tetanus toxoid (TT)/diphtheria toxoid (DT) divalent vaccine as model compounds. We also described the deposition of antigen in mouse skin by in vivo fluorescence imaging after MN administration using comparisons with traditional hypodermic needle-based intradermal (ID) administration.

2. Materials and methods

2.1. Animals

Six-week-old female Wistar–ST rats and 7- to 10-week-old female ICR mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). Seven- to nine-week-old female HR-1 hairless mice were purchased from SHIMIZU Laboratory Supplies Co., Ltd. (Kyoto, Japan). All animals were housed at the Osaka University animal facility. All animal studies were conducted in accordance with the guidelines provided by the Animal Care and Use Committee of Osaka University.

2.2. Fabrication of the dissolving MN patch

As described previously (Matsuo et al., 2012b), the dissolving MN patch was fabricated at CosMED Pharmaceutical Co. Ltd., (Kyoto, Japan) using micromolding technologies with sodium hyaluronate as the base material. Previously developed MN contained collagen. In this study, we used a collagen-free MH as collagen is suspected to induce inflammation in human. In brief, sodium hyaluronate (JP grade, Kikoman Biochemifa Company, Tokyo, Japan), dextran 70 (JP grade, Meito Sangyo, Nagoya, Aichi), and Polyvidone (JPE grade, BASF Japan, Tokyo, Japan) were dissolved in distilled water at a ratio of 11:8:1 and then mixed with ATRA (Sigma–Aldrich Inc., St. Louis, MO, USA), OVA (Sigma–Aldrich Inc.), fluorescein isothiocyanate-labeled–OVA (FITC–OVA; Molecular Probes, Eugene, OR, USA), or the TT/DT divalent vaccine (The Research Foundation for Microbial Diseases of Osaka University, Suita, Japan). The aqueous solution was casted onto micromolds and then dried in a desiccator at room temperature. The dissolving MN patches were obtained by removing them from the micromolds. Placebo dissolving MN patches were also fabricated in the same manner, without an active pharmaceutical ingredient. The MN patches contained more than 200 MNs/cm^2. To form the MN transcutaneous patch system, patches with an area of 0.8 cm^2 were fixed onto an adhesive film with a surface area of 2.3 cm^2. Hence, our dissolving MN patch system consisted of the MicroHyala® patch with MNs that were 200 (MH200), 300 (MH300), or 800 μm (MH800) in length.

2.3. Moisture conditioning and moisture content measurement of the MN patch

To condition the MN patch to different moisture contents, the patch was placed in desiccators containing Tri-Sorb molecular sieves (Süd-Chemie Performance Packaging, Colton, CA, USA) or a saturated solution of potassium acetate (Wako Pure Chemical, Osaka, Japan), magnesium chloride hexahydrate (Wako Pure Chemical), potassium carbonate (Wako Pure Chemical), or sodium chloride (Wako Pure Chemical). The desiccators were stored at room temperature (25 °C) for 1 week in an environment of 0, 22, 33, 44, or 75% relative humidity (RH) (Young, 1967). After removal of the MN patch from the desiccator, the endpoint moisture level was evaluated as a function of equilibrium relative humidity (ERH) using a water activity analyzer (HygroLab; Rotronic AG, Bassersdorf, Switzerland).

2.4. Measurement of mechanical failure force for MNs

The force necessary for mechanical MN fracture was measured using a TA-XT plus texture analyzer (StableMicro Systems, Surrey, UK). An MN patch was attached to a test station by double-sided adhesive tape. Axial force was then applied using a flathead 5-mm diameter stainless steel cylinder to move the cylinder at a rate of 0.6 and 1.1 mm/min for MH300 and MH800, respectively, and the trigger force was set at 0.049 N.

2.5. Quantification of ATRA loaded into the MN patch

To determine the amount of ATRA loaded into the MN patches, MNs were first removed from the base material using a razor. The removed MNs were soaked in distilled water followed by vortex mixing to completely dissolve them. Ethanol (Wako Pure Chemical) was added to the sample solution followed by vortex mixing, and the sample solution was then diluted with acetonitrile (Wako Pure Chemical). The sample solution was filtered through a membrane filter (0.45-μm diameter) and analyzed using a high-performance liquid chromatography (HPLC) method, as reported previously (Tashtoush et al., 2007). In brief, a D-2000 Elite HPLC system (Hitachi, Tokyo, Japan) was used. Chromatographic separation was performed using a reverse-phase Nucleosil 5 μm C18 100A column (250 mm × 4.6 mm; GL Science Inc., Tokyo, Japan). The mobile phase comprised 0.01% trifluoroacetic acid and acetonitrile (15:85, v/v) at a flow rate of 1 ml/min. The column temperature was 40 °C, and the detection wavelength was 342 nm. The concentration of ATRA in the sample solution was determined using a standard curve