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Dose sparing enabled by skin immunization with influenza virus-like particle vaccine using microneedles

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ABSTRACT

To address the limitations of conventional influenza vaccine manufacturing and delivery, this study investigated administration of virus-like particle (VLP) influenza vaccine using a microneedle patch. The goal was to determine if skin immunization with influenza VLP vaccine using microneedles enables dose sparing. We found that low-dose influenza (A/PR/8/34 H1N1) VLP vaccination using microneedles was more immunogenic than low-dose intramuscular (IM) vaccination and similarly immunogenic as high-dose IM vaccination in a mouse model. With a 1 μg dose of vaccine, both routes showed similar immune responses and protective efficacy, with microneedle vaccination being more effective in inducing recall antibody responses in lungs and antibody secreting cells in bone marrow. With a low dose of vaccine (0.3 μg), microneedle vaccination induced significantly superior protective immunity, which included binding and functional antibodies as well as complete protection against a high dose lethal infection with A/PR/8/34 virus, whereas IM immunization provided only partial (40%) protection. Therefore, this study demonstrates that microneedle vaccination in the skin confers more effective protective immunity at a lower dose, thus providing vaccine dose-sparing effects.

1. Introduction

Influenza is a major threat to public health that is responsible for approximately 500,000 deaths worldwide each year [1]. Especially due to the emergence of influenza strains resistant to antiviral agents, vaccination is an indispensible method to prevent the spread of influenza [2,3]. Currently, the egg-based trivalent inactivated virus vaccine is broadly used for seasonal influenza vaccination campaigns, but it has several limitations including problems in mass production, egg allergy, and handling live influenza viruses [4].

To overcome these disadvantages, novel cell-based vaccines have been suggested. Virus-like particles (VLPs) without viral replication characteristics have been produced in mammalian and insect cell systems and large-scale bioprocesses for VLP production have been studied [5]. VLPs lack the RNA genome of the virus, which improves the safety of the vaccine [6]. Influenza VLP vaccines of various strains have conferred good protection from lethal influenza virus challenge [7–14].

The limitations of vaccine manufacturing could be further addressed by reducing the required dose and thereby reducing the amount of vaccine manufactured. In this study, we hypothesized that low-dose influenza VLP vaccination via the skin would be more immunogenic than low-dose IM vaccination and similarly immunogenic as high-dose IM vaccination. We tested this hypothesis using a vaccine dose that is three-fold lower than the high-dose vaccination. We propose this hypothesis because skin has two bone marrow-derived antigen-presenting cell types, i.e., Langerhans cells and dermal dendritic cells, which play a critical role in the immune system [15].

Increased immunogenicity has been demonstrated for a number of vaccines when given by intradermal (ID) injection compared to intramuscular (IM) injection. WHO recommends ID injection of rabies vaccine as a dose-sparing and, thereby, cost-saving approach [16]. Other vaccines, such as smallpox and tuberculosis (BCG), are also commonly administered ID, although not for dose-sparing purposes [17,18]. Recently, ID influenza vaccination was approved in Europe and shown to increase immunogenicity in the elderly at the same dose relative to IM injection [19,20].

Previous studies have assessed the dose-sparing potential of ID influenza vaccination and have reached different conclusions. A number of studies have compared regular-dose IM vaccination to low-dose ID vaccination and found similar immunogenicity, which suggested dose-sparing effects [21–26]. However, these reports have been criticized for lacking a low-dose IM vaccination comparison group, which would more clearly show the role of the ID route of administration. Others have included the low-dose IM comparator and did not show dose sparing associated with the ID route [27].

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Differences in the doses at which the comparisons were made may help explain these varied results.

Most previous studies assessing the dose-sparing potential of ID vaccination have used hypodermic needles, which are difficult and unreliable to use for ID injection [28]. To enable simple vaccination in the skin, we have developed patches containing antigen-coated microneedles that can be simply and painlessly inserted into the skin [29]. The vaccine then dissolves off the microneedles into the skin within minutes. Coated microneedles used in this way have been shown to enable induction of strong immune responses against influenza vaccines [30–35]. Dose-sparing of coated microneedles was demonstrated using ovalbumin as a model antigen [36,37]. Other types of microneedle systems have also been used for vaccination [20,23,38–41].

In this study, we determined the immunogenicity and protective efficacy of different doses of influenza VLP vaccine delivered to the skin using coated microneedles in comparison with IM vaccination. We found that microneedle vaccination in the skin with a low dose of influenza VLP vaccine induced comparable protection to IM immunization with a three-fold higher dose of influenza VLPs and much stronger protection compared to IM immunization at the same low dose. These findings indicate significant dose-sparing effects of microneedle vaccination in the skin.

2. Materials and methods

2.1. Preparation of microneedle vaccines and coating with VLP

Microneedle preparations and coatings were performed as previously described [30]. Rows of stainless-steel (SS304, 75 μm thickness, McMaster-Carr, Atlanta, GA) microneedles were produced by laser-cutting (Resonetics Maestro, Nashua, NH) (Fig. 1A). These microneedles were cleaned and electropolished in a bath containing a 6:3:1 mixture by volume of glycerin, phosphoric acid, and water to remove debris [42]. The dimensions of the microneedles were 700 μm in length, 160 μm in width at the base, and 50 μm in thickness. For a vaccine coating on microneedles, five-microneedle arrays were dipped six times using a coating device containing coating solution at room temperature and dried in ambient air [30]. The coating solution was composed of 1% (w/v) carboxymethylcellulose (CMC) sodium salt (Carbo-Mer, San Diego, CA), 0.5% (w/v) Lutrol F-68 NF (BASF, Mt. Olive, NJ), 15% (w/v) D-(+)-trehalose dihydrate (Sigma Aldrich, St. Louis, MO) and 0.75–2.5 mg/ml influenza VLPs in phosphate buffered saline (PBS). In order to determine the dose of VLPs coated on microneedles, vaccine-coated microneedles were incubated in PBS solution for 12 h at 4 °C and the amount of released protein was measured by a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). Microneedle arrays were imaged by bright-field microscopy (Olympus SZX12 stereo microscope, Tokyo, Japan) with a CCD camera (Leica DC 300, Leica Microsystems, Wetzlar, Germany). To image microneedle arrays after delivery, microneedles coated with influenza VLPs were inserted into mouse cadaver skin for 5 min and then were imaged.

2.2. Preparation of influenza virus and VLPs

A/PR/8/1934 (H1N1; A/PR8) influenza virus was cultivated in 10-day old embryonated hen's eggs and purified from allantoic fluid. The purified virus was inactivated by mixing the virus with formalin at a final concentration of 1:4000 (v/v) as described previously [14]. *Spodoptera frugiperda* Sf9 cells were maintained in suspension in serum-free SF900II medium (GIBCO-BRL, Carlsbad, CA). MDCK cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM). Influenza VLPs containing HA and M1 proteins derived from A/PR8 were prepared as described previously [14]. Briefly, the Sf9 insect cells were co-infected with recombinant baculoviruses expressing HA and M1 proteins at an infection multiplication of 2 and 1, respectively. Influenza VLPs in the culture supernatants were purified by using discontinuous sucrose gradient (15%, 30% and 60%) layers, and characterized by western blot and hemagglutination activity analysis [43]. The HA content was approximately 10% of total proteins of influenza VLPs determined as previously described [44]. For negative staining of VLPs for electron microscopy, sucrose gradient-purified VLPs were applied to a carbon-coated Formvar grid for 30 s as described previously [14]. The grid was immediately stained with 1% uranyl acetate and the samples were examined using a transmission electron microscope (H-7500, Hitachi, Pleasanton, CA).

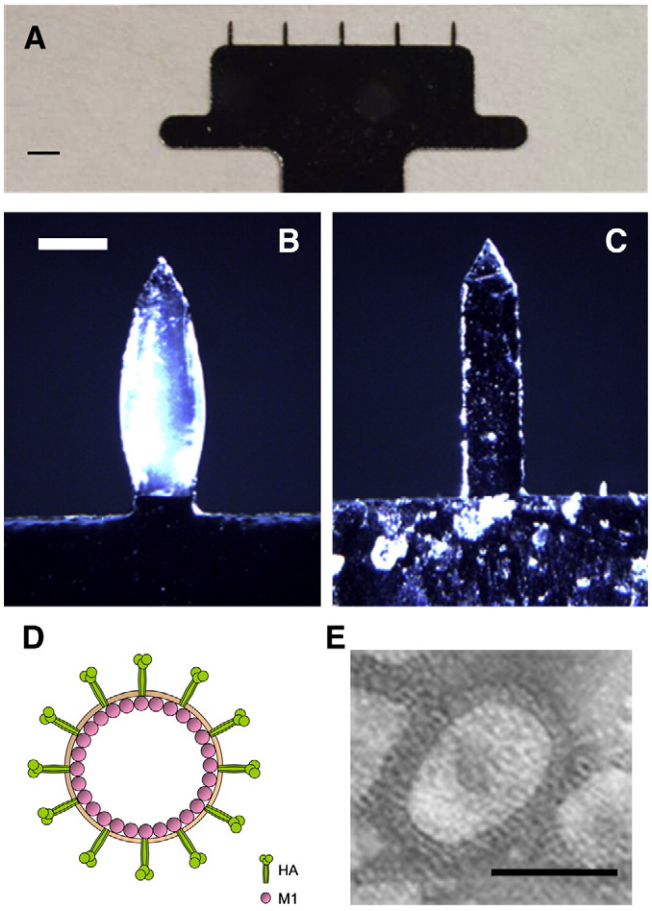

Fig. 1. Microneedles and virus-like particles (VLP) for vaccination. (A) Image of a five-microneedle array shown by bright-field microscopy (scale bar = 1 mm). (B) Microneedle coated with influenza virus-like particle vaccine and (C) microneedle after insertion into mouse skin for 10 min shown by bright-field microscopy (scale bar = 200 μm). (D) Schematic diagram of influenza VLPs containing hemagglutinin (HA) and matrix (M1) proteins. (E) Transmission electron micrographs of negatively stained influenza VLPs (scale bar = 100 nm).

2.3. Immunization and challenge infection

Female inbred BALB/c mice (Charles River, Wilmington, MA) aged 6 to 8 weeks were used. Groups of mice (12 mice per group) were immunized with a microneedle array coated with VLP vaccine at a dose of either 1 μg or 0.3 μg total VLP proteins for delivery to the skin or immunized by IM injection with intact vaccine (1 μg and 0.3 μg/100 μl) in the upper quadriceps muscles of mice (both legs, each with 50 μl).

The experimental groups included mice immunized at a high dose (1 μg) using microneedles (MN(H)) or IM injection (IM(H)) or at a low dose (0.3 μg) using microneedles (MN(L)) or IM injection (IM (L)). During microneedle delivery, mice were anesthetized with ketamine (110 mg/kg, Abbott Laboratories, Abbott Park, IL) mixed