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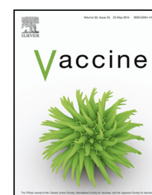

Fabrication of cell culture-derived influenza vaccine dissolvable microstructures and evaluation of immunogenicity in guinea pigs

Amanda Bonificio[a], Esi Ghartey-Tagoe[b], Simona Gallorini[c], Barbara Baudner[c], Guohua Chen[b], Parminder Singh[b], Derek T. O'Hagan[a], Sushma Kommareddy[a,*]

[a] Vaccines Research, Novartis Vaccines, Cambridge, MA 02139, USA
[b] Corium International, Inc., Menlo Park, CA 94025, USA
[c] Vaccines Research, Novartis Vaccines, Siena 53100, Italy

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ABSTRACT

Microstructure patches provide an opportunity for simple, effective, and safe vaccine administration, while achieving the desired immune response. We have evaluated the MicroCor® transdermal system for cell culture-derived trivalent influenza vaccine administration. Influenza monovalent purified bulk vaccines (monobulks) (H1N1, H3N2, B) were concentrated by tangential flow filtration, lyophilized, and formulated with biocompatible excipients to form the microstructure array dissolvable tips. Standard single radial immunodiffusion (SRID) determined that the influenza antigens retained potency through the formulation and microstructure array fabrication processes. Array stability was evaluated for storage in both refrigerated and room temperature conditions. Microstructure mechanical strength was confirmed by application to excised pig skin, resulting in successful skin penetration and tip dissolution within 5 min of microstructure insertion. Guinea pigs immunized with influenza vaccine-loaded microstructures had hemagglutinin inhibition (HI) and IgG titers comparable to those obtained by intramuscular injection. After two immunizations, serum HI titers for all immunized groups were greater than 40 (>4-fold higher than the untreated group). These data demonstrate the feasibility for the development of skin delivery technologies that are compatible with cell culture-derived influenza vaccines.

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1. Introduction

Vaccination against seasonal influenza is recommended to protect the individual from influenza infection. Trivalent seasonal influenza vaccines contain antigens from each of three strains: two type A influenza subtypes (H1N1 and H3N2) and one type B. Recently, a quadrivalent seasonal vaccine containing a second B strain has been introduced to improve coverage and provide broader protection [1]. Subunit influenza vaccines have historically been derived from viruses grown and harvested in chicken eggs, but recently new production techniques utilizing mammalian cell lines have emerged as an alternative with additional benefits. Optaflu®, marketed by Novartis Vaccines, is an influenza subunit vaccine that is grown and harvested in Madin–Darby canine kidney (MDCK) cells, significantly reducing the time for production compared to egg-derived vaccines [2,3]. This product was licensed in the United States in 2012 and is marketed as Flucelvax [4].

Influenza vaccines are traditionally given *via* the intramuscular route, resulting in pain on injection and require trained healthcare personnel for administration as well as disposal of biohazardous sharps. In the past few years, microstructure patch technologies for delivering influenza vaccine have emerged as a pain-free delivery system to improve the ease of vaccine administration while exploiting the active immune cells in the skin to elicit a strong immune response [5–7]. Alternative patch architectures for skin delivery include coated solid microstructures, solid hollow microstructures, and drug encapsulated in dissolvable microstructures. For example, inactivated influenza virus coated on solid microstructures elicited a strong immune response in mice [8]. In addition, an influenza virus-like particle (VLP) coated on solid microstructures generated stronger immune responses as compared to intramuscular administration [9,10]. Other studies suggest that the quality of the memory response after immunization using microstructures may be superior to intramuscular vaccination [11,12], and that skin vaccination can induce long-lasting immune responses [13,14].

Corium has developed MicroCor®, a fully integrated transdermal delivery system (TDS) incorporating vaccine(s) in a solid matrix of biodegradable microstructures that rapidly dissolve upon

* Corresponding author. Tel.: +1 617 871 3966.
E-mail address: sushma.kommareddy@novartis.com (S. Kommareddy).

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penetrating the skin. The MicroCor TDS is a drug/device combination product that incorporates a microstructure array (MSA) consisting of dissolvable tips containing the antigen of interest and an applicator device in a single piece integrated system. The microstructure array consists of a dissolvable vaccine-in-tip (VIT) layer and a non-dissolving, polymer-based backing layer that connects and supports the MSA tips in the patch. This technology offers all the benefits of pain-free, simplified administration, with the added advantage that the microstructures dissolve completely, thus eliminating the need for biohazardous sharps disposal. This technology has been successfully evaluated for transdermal delivery of a number of large molecules, including recombinant protective antigen from Bacillus anthracis (rPA) and human parathyroid hormone (1–34) [15–17].

Here we evaluate the MicroCor transdermal system for delivery of trivalent seasonal influenza cell-culture derived antigens. Antigens were concentrated by tangential flow filtration (TFF), lyophilized, and formulated with excipients to prepare a liquid formulation that was used to form the microstructure tips. The MSAs were characterized for skin penetration and for antigen stability in refrigerated and at room temperature conditions. Immunogenicity was evaluated in guinea pigs for serum hemagglutinin inhibition (HI) titers and specific IgG antibodies by ELISA and compared to that of intramuscular delivery.

2. Materials

Seasonal cell-culture monobulks (purified antigen of monovalent strains) A/Brisbane/59/2007 (H1N1, 2009–2010 season), A/Victoria/210/2009 (H3N2, 2011–2012 season), and B/Brisbane/60/2008 (B, 2011–2012 season) were obtained from Novartis Vaccines and Diagnostics, Marburg, Germany. Modified polyethersulfone 115 cm^2 hollow fiber filters (P-D1-100E-100-01 N) were obtained from Spectrum Labs (Rancho Dominguez, California, USA). 1× PBS (137 mM Sodium chloride, 1.4 mM Monopotassium phosphate, 4.3 mM Disodium phosphate, 2.7 mM Potassium Chloride, Endotoxin-Free) (P0300) for TFF equilibration and microstructure array extraction was obtained from Teknova (Hollister, California, USA). D-Sorbitol (85529) was obtained from Sigma (St. Louis, Missouri, USA). Maxisorp plates were obtained from Nunc (Rochester, New York, USA), and alkaline phosphatase-conjugated goat anti-guinea pig IgG was obtained from Sigma (St. Louis, Missouri, USA). The following materials were used to prepare the liquid VIT formulation: Dextran 70, USP obtained from Pharmacosmos (Holbaek, Denmark), D-sorbitol, NF obtained from EMD Millipore (Darmstasdt, Germany), and 10 mM phosphate buffered saline (138 mM sodium chloride, 2.7 mM potassium chloride) (P3813), pH 7.4 obtained from Sigma–Aldrich (St. Louis, Missouri, USA). The PBS powder was dissolved in Milli-Q water and filtered with a 0.2 μm bottle top filter system (430049) from Corning (Corning, New York, USA). Sterile water for injection, USP, which was used to reconstitute the lyophilized antigens, was obtained from Hospira (Lake Forest, Illinois, USA). For the non-dissolvable backing layer, poly(lactic-*co*-glycolic acid) (PLGA) was obtained from Durect (Birmingham, Alabama, USA) and acetonitrile was obtained from J.T. Baker (Center Valley, Pennsylvania, USA). Polydimethylsiloxane (PDMS), used to make the microstructure array molds, was obtained from Nusil (Carpinteria, California, USA).

3. Methods

3.1. Concentration of HA by tangential flow filtration

Influenza monobulks were concentrated by tangential flow filtration (TFF) as described previously [18]. The antigens were concentrated using the KrosFlo system (Spectrum Labs) with 100 kD cutoff modified polyethersulfone (mPES) 115 cm^2 hollow fiber filters. The filters were connected by size 14 Masterflex tubing to the KrosFlo pump and the digital pressure monitor. Polysulfone pressure transducers were connected either in-flow or in a T-junction to the inlet, outlet and permeate tubing, with a pressure regulator connected on the outlet/retentate tubing. The system was equilibrated for 20–30 min with PBS, leaving the permeate line open at a flow rate of 60 mL/min. Concentration was carried out at an operating flow rate of 120 mL/min and a pre-determined transmembrane pressure (TMP) of 8.0 psig. At the completion of the process, the TMP was slowly reduced to zero, the permeate line was closed, and the flow was stopped. The concentrate in the hold-up volume was collected by reversing the direction of flow.

3.2. Lyophilization of concentrated HA

Vaccine concentrates were lyophilized using a Virtis Advantage EL freeze dryer (SP Scientific). Sorbitol (3% w/v) was added as a cryoprotectant and the antigens were frozen at −50 °C for 4 h. The temperature was then increased to −34 °C over 90 min and the vacuum was lowered to 10 mTorr to initiate primary drying. After 28 h of primary drying, the temperature was ramped up to −5 °C over 130 min to initiate secondary drying, and secondary drying was continued for 10 h. After completion of the drying steps, the temperature was maintained at 5 °C until the samples were removed from the lyophilizer. The lyophilized antigen vials were backfilled with nitrogen gas and stored at 2–8 °C until further use.

3.3. Measurement of HA and protein content

Hemagglutinin (HA) content was determined by single radial immunodiffusion (SRID), the standard release assay for influenza vaccines [10]. Antigens were analyzed for stability and integrity by SRID after concentration and MSA fabrication as described previously [18]. Total protein concentrations were also measured to assess the yield after antigen concentration. Protein content in samples was determined by colorimetric estimation using bicinchoninic acid (BCA) reagent with bovine serum albumin as a standard as described previously [18].

3.4. Fabrication of flu vaccine microstructure arrays

The concentrated and lyophilized influenza antigens were individually reconstituted in water for injection overnight at 2–8 °C and then combined (Fig. 1a, Steps 1 and 2). The trivalent antigen mixture was then formulated in PBS, pH 7.4, with the water-soluble polysaccharide Dextran 70 and sorbitol to achieve 0.38% w/w H3N2, 0.19% w/w H1N1, and 0.19% w/w B in the final formulation (Fig. 1a, Step 3).

Negative molds of a machined microstructure array master were made using cured PDMS. To fabricate flu vaccine MSAs, liquid formulation was cast to fill the cavities of the silicone-based negative molds. The mold was dried under controlled temperature conditions (20 °C–35 °C) for approximately one hour to form the drug-in-tip (DIT), or in this case vaccine-in-tip (VIT), layer (Fig. 1b, Step 1). A solution of PLGA in acetonitrile was then cast on top of the dried VIT layer to form a non-water soluble backing layer that connects and supports the VIT layer microstructure tips (Fig. 1b, Step 2). The PLGA backing layer was dried for 30 min at room temperature followed by 90 min at 45 °C. The joined VIT and PLGA layers were removed from the mold (Fig. 1b, Step 3), using a polycarbonate film laminated to the PLGA backing with adhesive, to reveal the final MSA (Fig. 1b, Step 4).

At this point, circular MSAs of the desired size, either 11 mm (area = 0.95 cm^2) or 16 mm (area = 2 cm^2) in diameter, were die-cut from the larger array and inspected under a stereomicroscope

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