RESEARCH ARTICLE

Dissolvable Microneedle Patches for the Delivery of Cell-Culture-Derived Influenza Vaccine Antigens

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ABSTRACT: Microneedle patches are gaining increasing attention as an alternative approach for the delivery of vaccines. In this study, a licensed seasonal influenza vaccine from 2007 to 2008 was fabricated into dissolvable microneedles using TheraJect's microneedle technology (VaxMat). The tips of the microneedles were made of antigens mixed with trehalose and sodium carboxymethyl cellulose. The patches containing $15\,\mu g$ per strain of the influenza antigen were characterized extensively to confirm the stability of the antigen following fabrication into microneedles. The presence of excipients and very low concentrations of the vaccine on the microneedle patches made it challenging to characterize using the conventional single radial immunodiffusion analysis. Novel techniques such as capture enzyme-linked immunosorbent assay and enzyme digestion followed by mass spectroscopy were used to characterize the antigens on the microneedle patches. The *in vivo* studies in mice upon microneedle administration show immunogenicity against monovalent H1N1 at doses 0.1 and $1\,\mu g$ and trivalent vaccine at a dose of $1\,\mu g$. The initial data from the mouse studies is promising and indicates the potential use of microneedle technology for the delivery of influenza vaccine. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Influenza virus is one of the most important pathogens causing serious respiratory diseases resulting in seasonal epidemics and occasional worldwide pandemics. Seasonal influenza each year is caused by new viruses that slightly differ in surface proteins and account for approximately 500,000 deaths worldwide, with majority of deaths in the age group 65 and above. Pandemic influenza is caused when antigenically new viruses emerge, resulting in, for example, almost 40 million deaths (1918 pandemic flu).[1,2] Currently, antiviral drugs are used to treat the condition; however, increase in viral resistance to these drugs is very common. Hence, vaccination is considered the best approach to prevent or control both seasonal and pandemic outbreaks.

Licensed influenza vaccines contain subunits or inactivated split influenza virus, which is administered by injection intramuscularly or subcutaneously. These routes of administration, however, are painful and need trained personnel to ensure reproducible administration of the vaccine. To overcome this limitation, several new vaccine strategies such as intranasal delivery and microneedle technologies including intradermal needles have been evaluated.[3,4] Flumist by Medimmune for intranasal administration and IDFlu by Sanofi/BD for intradermal administration of influenza vaccine are already approved. With the recent advances in microfabrication technology, a wide variety of microneedles are available: solid microneedles made from polymeric or metallic microstructures with the drug substance coated on the surface, dissolvable microneedles in which the drug substance is formulated with the microneedle excipients to form the tip of the microneedle structure,
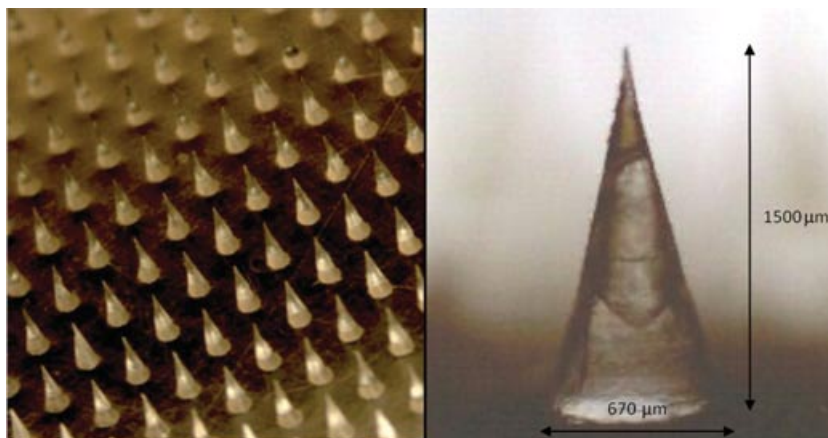
Figure 1. Image of the microneedle patch at low and high magnifications. The tip of the microneedles is formed by a mixture of antigens and sodium carboxymethyl cellulose.

and hollow metallic microneedles to deliver liquid formulations.[5–9] These microneedle technologies target skin where Langerhans and dermal dendritic cells are recruited to present the antigens to the immune system. Vaccine delivery using microneedles may be preferred for several reasons—mainly the route of vaccination being pain free, ease of administration, and possibility of self-administration.

The microneedle technology as we understand today has been evaluated for the delivery of influenza vaccine. Prausnitz et al.[10] have been studying coating and stabilization of inactivated influenza virus on metallic microneedles, which are fabricated from stainless steel by laser cutting and electropolishing. In a recent study, the same group evaluated dissolvable microneedles made by photopolymerization of polyvinylpyrrolidone (PVP) with lyophilized, inactivated influenza virus. In a separate study, Kendall and coworkers[6] have shown the proof of concept studies by fabricating commercially available, egg-derived subunit vaccine Fluvax 2008 into dissolvable microneedles using carboxymethylcellulose. Up to now, the attempts to characterize the antigen content within the microneedle patches by functional assays such as single radial immunodiffusion (SRID) have been limited in the literature. In this study, we use licensed purified subunit vaccine and use novel techniques to overcome the limitations of characterizing antigens on the microneedle patches.

The current study evaluates dissolvable microneedle patches as an alternative approach for the delivery of next-generation influenza subunit vaccine. The latest development facilitates production of influenza vaccine in cell lines as substrates for the growth of influenza virus. The cell-based vaccines have the potential to reduce production times by avoiding procurement of millions of eggs, bypassing the steps needed for stabilization of virus for production in eggs, and facilitating storage of cells by freezing.[11] Using the cell-based manufacturing technology, Novartis has demonstrated successful production of influenza vaccine (currently marketed in Europe as Optaflu®) in Madin–Darby canine kidney (MDCK) cells, cutting short weeks of time needed for production with traditional vaccine technologies.[12–14] Flu cell culture (FCC) vaccine together with dissolvable microneedle technology can simplify the production and administration of influenza vaccine in the case of a pandemic.

For the first time, a licensed cell-culture-derived influenza vaccine is fabricated into dissolvable microneedles using TheraJect's (Fremont, California) microneedle technology, characterized for antigen stability and evaluated in an *in vivo* mice model for immunogenicity. The antigens formulated into microneedle patches are tested in a mouse model for immunogenicity, hemagglutination inhibition (HI), and microneutralization titers in addition to the antibody titers are used to evaluate the immune response.

MATERIALS AND METHODS

Materials

Seasonal influenza antigen monobulks A/Solomon (H1N1), A/Wisconsin (H3N2), and B/Malaysia were obtained from the manufacturing facility of Novartis Vaccines and Diagnostics (Marburg, Germany). Sodium deoxycholate dry powder (D6750), trichloroacetic acid (TCA; T8657), para-nitrophenylphosphate (pNPP) substrate (cat. #P7998), and streptavidin (S2890) were obtained from Sigma. Antibodies were obtained from National Institute for Biological Standards and Control (NIBSC) and BALB/c mice were from Charles River Laboratories.

Fabrication of Microneedle Patches

The seasonal flu antigens were fabricated into microneedle patches of size 1 cm² by TheraJect, Inc. The

vaccine solution was adjusted to the target concentration with deionized water and trehalose, poured into a silicone negative mold (cone shape, 1500 μm height, 670 μm diameter of base) with microneedle-shaped cavities, centrifuged (model 2–6; Sigma) for 5 min at 3500 rpm, and air-dried for several hours. Sodium carboxylmethyl cellulose gel (8%, w/v) was cast over the vaccine film and dried overnight. The dried film from the mold was cut into 1-cm-diameter discs. The formulated microneedle patches were stored at 5°C prior to administration. Patches containing flu antigen with 15 μg of hemagglutinin (HA) of each antigen (human dose) were made for characterization studies with 44 microneedles per unit area. Patches, each containing 29 microneedles—monovalent H1N1 at doses 1 and 0.1 μg of HA and trivalent vaccine at 0.1 μg of HA—were used for testing immunogenicity in mice.

Analysis of Influenza Antigens by Enzyme Digestion and Mass Spectroscopy

Enzyme digestion followed by mass spectroscopy was used for the analysis of influenza antigens in the microneedles. Each patch was dissolved in a total of 1 mL sterile water in an eppendorf vial. Hundred microliter of 0.5% (w/v) sodium deoxycholate was added to the samples, which were allowed to sit at room temperature (RT) for 10 min. After incubation, 80 μL of 60% (v/v) TCA was added to the sample, vortexed, and centrifuged for 20 min at 12,000 rpm at RT. The supernatant was discarded and the protein at the bottom of the vial was resuspended in Tris buffer and analyzed. The suspended proteins were then trypsin-digested before subjecting to mass spectroscopy. While analyzing the antigens, the sequence of each antigen was searched in the National Center for Biotechnology Information database and compared for maximum sequence coverage. The peptides in high confidence were identified for the quantification of dissolved patches against the standards of antigen stocks at the same concentration. As a negative control, the undigested antigens were analyzed for the presence of peptides. The dissolved microneedle placebo spiked with the influenza antigens was used as a positive control, from which the proteins were TCA-precipitated, trypsin-digested, and analyzed for peptides by mass spectroscopy.

Antigen Content by Capture Enzyme-Linked Immunosorbent Assay

The capture enzyme-linked immunosorbent assay (ELISA) was used to determine the antigen content in the microneedle patches. High-binding 96-well plates were coated with 50 μg/mL solution of *Galanthus nivalis* agglutinin –lectin in 1× phosphate-buffered saline (PBS), Ca^{2+}/Mg^{2+}-free, overnight at 20°C. The plates were washed and treated with blocking buffer [10 mM Tris–HCl with 3% (w/v) sucrose, 1% (w/v) bovine serum albumin (BSA), and 150 mM sodium chloride]. Serial dilutions of dissolved patches and monobulk standards were made in dilution buffer and added to the coated plates. The plates were incubated at 37°C for 30 min. In a separate process, immunoglobulin G (IgG) antibodies were separated from the sheep sera of NIBSC standards specific for the strains, labeled with biotin, and purified to separate any unreacted biotin. The concentration of the biotin-labeled, strain-specific antibodies (biotin–IgG) required in the assay was optimized for each strain. Biotin–IgG was added at optimized concentrations and incubated at 37°C for 30 min. The unbound antigens were washed off and incubated at 37°C with streptavidin-conjugated alkaline phosphatase at 1 μg/mL. The plates were washed and then the enzyme substrate pNPP was added and incubated for 60 min at RT in dark. The antigen content of the samples was quantified by measuring absorbance at 450 nm. The assay was carried out in multiple plates to analyze antigens from different strains. Because of the limited availability of the NIBSC antibodies, only two of the three antigens were analyzed.

Immunization Studies in Mice

In vivo experiments were carried out in 8–10 weeks old female BALB/c mice with 10 mice per group. Twenty-four hours prior to immunization, the hair of the BALB/c mice was clipped with an electric cutter for application of microneedle patches. On the day of immunization, mice were anesthetized using isoflurane and the microneedle patches were pressed into the skin and held in position for 15 min. Two immunizations, a prime and a boost, were carried out 4 weeks apart and sera samples were collected before the first immunization and 2 weeks after each immunization.

The antigen from the 2007–2008 seasonal influenza vaccine A/Solomon (H1N1) was fabricated into microneedles at 0.1 and 1 μg dose of HA per patch. Individual serum samples from the bleeds were analyzed for total IgG titers by ELISA and HI titers by HI assay, and pooled serum samples were analyzed for microneutralization titers. In the second set of animal experiments, the trivalent antigens from the 2007–2008 seasonal influenza vaccine A/Solomon (H1N1), A/Wisconsin (H3N2), and B/Malaysia were fabricated into microneedles at a dose of 0.1 μg of HA per patch. The two immunizations were carried out 4 weeks apart and sera samples were collected before the first immunization and 2 weeks after second immunization. The pooled sera samples from the bleeds were analyzed for HA titers by hemagglutination assay.

Evaluation of Serum Antibody and HI Titers

Titration of HA-specific total IgG was performed on individual/pooled sera collected 2 weeks after each immunization. Maxisorp plates (Nunc, Roskilde, Denmark) were coated overnight at 2°C–8°C with 0.2 µg/well of H1N1, H3N2, or B antigens in PBS and blocked for 1 h at RT with 300 µL of 3% PVP. Serum samples and serum standard were initially diluted (1:5000–1:20,000 in PBS, 1% BSA, 0.05% Tween 20), transferred into coated–blocked plates, and serially diluted. Antigen-specific IgG was revealed with alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma Chemical Company, St. Louis, Missouri). Antibody titres are respective dilutions that gave an optical density (OD) higher than the mean plus five times the standard deviation of the average OD obtained in the preimmune sera. The titres were normalized with respect to the reference serum assayed in parallel. Geometric mean titres (GMT) 2 weeks after each immunization (post 1 and post 2) of 10 mice per group were calculated.

The HI assay was carried out on individual/pooled sera collected 2 weeks after each immunization. To inactivate nonspecific inhibitors in serum samples, aliquots of sera were treated with receptor-destroying enzyme prior to being tested with a final serum dilution of 1:10 (starting dilution for the assays). Samples were serially diluted twofold into V-bottom 96-well microtiter plates. Briefly, 25 µL of twofold serially diluted samples were incubated with 25 µL of strain-specific influenza antigen (whole virus, containing four HA units) for 60 min at RT. A 0.5% (v/v) suspension of red blood cells obtained from adult turkeys were added and the mixture was incubated for another 60 min. Reactions were followed through visual inspection: A red dot formation indicates a positive reaction (inhibition) and a diffuse patch of cells indicates a negative reaction (hemagglutination). As a negative control, serum samples of mice immunized with buffer were tested in parallel. Serum response to vaccine antigens was considered positive if a rise in antibody titers greater than fourfold as compared with the background was detectable. All sera were run in duplicate. The HI titer is defined as the serum dilution, in which the last complete agglutination inhibition occurs. GMT of ten mice per group are shown.

Microneutralization Titers

In order to perform the virus neutralization assay, the serum samples were pooled and heat-inactivated for 30 min at 56°C. The starting dilution was 1:40 and the samples were serially diluted threefold in Ultra-MDCK medium in 96-well plates. Fifty microliter of A/Solomon (300 TCID50) wild-type virus was added to each of the wells and incubated at 37°C for 60 min in a 5% CO_2 atmosphere. All the sera samples were run in duplicate. Subsequently, 100 µL of serum dilution and virus were added to MDCK cell plated in a 96-well/plate at a concentration of 20,000 cells/well. The plates were incubated for 20 h at 37° C in a 5% CO_2 atmosphere. After the incubation, the cells were fixed in the presence of 2% paraformaldehyde (15 min at RT), permeabilized, and stained with a primary antibody anti-M/anti-nucleoprotein fluorescein isothiocyanate (FITC) conjugated and with a secondary antibody anti-FITC peroxidase conjugated. After washing, the substrate was added to the cells and the plates were read at 450 nm on a plate reader. The neutralization titer was calculated as the sera dilution at which 80% of the cells were protected against virus infection. The titer is expressed as IC80 and calculated using a four-parameter curve fitting.

RESULTS

Enzyme Digestion and Mass Spectroscopy

To overcome the challenges in the detection of influenza antigens within the microneedle matrix, we developed an indirect method of analysis by trypsin digestion of the precipitated antigens from the dissolved matrix followed by peptide mapping using mass spectroscopy. The trypsin-digested peptides from the flu antigens were identified (Table 1) and the resulting peak areas from of the high-confidence peptide fragments were quantified by comparison against the controls; dissolved placebo matrix was spiked with trivalent antigens at 15 µg each. The percentage recovery of the placebo patch spiked with

Table 1. List of Peptide Fragments Used for Identification of Antigens in Microneedle Patches by Enzyme Digestion Followed by Mass Spectroscopy

H1N1 Solomon	H3N2 Wisconsin	B/Malaysia
LCLLK	AYSNCYPYDVPDYASLR	VSILHEVRPVTSGCFPIMHDR
FEIFPK	LNWLTQLK	QLPNLLR
NLLWLTGK	HQNSEGIGQAADLK	GILLPQK
ALYHTENAYVSVVSSHYSR	WDLFVER	AIGNCPIWVK
GFGSGIINSNAPMDECDAK	CDNACIGSIR	NLNSLSELEVK

Table 2. Antigen Content in Microneedle Patches Determined by Capture ELISA

Sample	A/Solomon/3/2006 (H1N1) (µg of HA)	A/Wisconsin/67/2005 (H3N2) (µg of HA)
Dissolved placebo spiked with flu antigens	12.6 ± 0.9	12.3 ± 0.9
Dissolved human dose patch	17.1 ± 0.6	9.0 ± 1.4

Only two of the three antigens were analyzed because of the limited availability of labeled NIBSC antibodies.

antigens A/Solomon, A/Wisconsin, and B/Malaysia was found to be at 51%, 48%, and 49%, respectively. In the case of human dose patch, the percentage recovery of the three antigens, A/Solomon, A/Wisconsin, and B/Malaysia, was at 45%, 41%, and 55%. The relative percentage recovery was calculated by comparing with the recovery of the placebo spiked and was found to be 89%, 85%, and 110%, respectively. This technique, though semiquantitative, confirms the presence of the three strains of flu antigens in the microneedle patch.

Antigen Content by ELISA

Capture ELISA was used to determine the antigen content in the human dose microneedle patches. Influenza monobulks characterized for HA content by SRID were used as standards. Trivalent-antigen-fabricated microneedle patch and dissolved placebo patch spiked with 15 µg of antigens were tested at different serial dilutions on separate plates for the estimation of HA content of A/Solomon and A/Wisconsin. The antigen recovery from the human dose patch and placebo patch spiked with antigens was calculated by comparing against the antigens alone, which were run as trivalent. The dissolved placebo patch spiked with antigens showed 12.6 µg of HA for A/Solomon and 12.3 µg of HA for A/Wisconsin, whereas the dissolved human dose patch resulted in 17.1 and 9.0 µg of HA for A/Solomon and A/Wisconsin, respectively (Table 2). The monovalent antigen containing microneedle patches prepared for challenge study were found to contain A/Solomon at doses of 0.16 and 1.37 µg, respectively. The assay confirms both specificity and functional activity of the antigens fabricated into the microneedle patches. When compared with the SRID, the capture ELISA assay has improved sensitivity limits and has no interference from the excipients used in the patch. The results indi-

cate that the antigens have survived the fabrication process.

Immunization of Mice with Monovalent and Trivalent Vaccine

The first set of *in vivo* experiments was carried out in BALB/c mice using monovalent A/Solomon (H1N1) vaccine. Sera samples from this study were analyzed for HI, ELISA (IgG), and microneutralization titers and the data are shown in Table 3. At the end of first immunization (prime), patch administration resulted in HI titers of 80 and 160 at doses 0.1 and 1.0 µg, respectively, with corresponding IgG titers of 99 and 412. In comparison, intramuscular administration resulted in HI titers of 10 at both doses and IgG titers of 2 and 9 at doses 0.1 and 1.0 µg, respectively. After the second administration (boost), mice immunized intramuscularly elicited higher HI and antibody titers when compared with mice immunized via microneedle patches. At doses of 0.1 and 1 µg, intramuscular immunization of the antigen A/Solomon resulted in HI titers of 1112 and 2036, and patch immunization resulted in titers of 864 and 883. Total IgG antibody titers were 3530 and 6891 for flu antigen administered intramuscularly and 2513 and 3703 for flu antigen administered via microneedle patches at doses 0.1 and 1.0 µg, respectively. Following two immunizations, HI titers of all treated groups were found to be greater than 40, indicating greater than fourfold increase in immune response when compared with the untreated group.

Microneutralization titers were tested on the pooled sera samples drawn 2 weeks after the boost. The dilution of sera that has 80% protection against viral infection was determined. As shown in Table 3, patch administration of A/Solomon antigen resulted in titers of 339 and 181 at doses of 0.1 and 1.0 µg, respectively. The intramuscular injection of flu

Table 3. IgG, Hemagglutination Inhibition, and Microneutralization Titers of Sera Drawn 2 Weeks After Second Immunization with Monovalent A/Solomon Influenza Vaccine

	ELISA (IgG Titers)	HI Titers	Microneutralization Titers
Flu liquid (0.1 µg) IM	3530 ± 2394	1112 ± 762	468
Flu patch (0.1 µg)	2513 ± 1487	864 ± 305	339
Flu liquid (1 µg) IM	6891 ± 4263	2036 ± 839	478
Flu patch (1 µg)	3703 ± 4311	883 ± 543	181
Untreated	2 ± 0	26 ± 8	11

Microneutralization titers were determined using pooled sera.

antigen at both doses of 0.1 and 1 μg resulted in neutralization titers of 468 and 478, respectively. Overall, all the treated groups at doses of 0.1 and 1 μg had positive neutralization titers when compared with the untreated control group.

In the second set of *in vivo* experiments, trivalent antigens from 2007–2008 seasonal influenza vaccine fabricated into microneedles at 0.1 μg of HA per patch were used to test immunogenicity in mice. The sera drawn 2 weeks after immunization (boost) were analyzed for strain-specific HI titers (Table 4). The trivalent vaccine injected intramuscularly at 0.1 μg per mice resulted in HI titers of 139 for A/Solomon, 141 for A/Wisconsin, and 139 for B/Malaysia. The microneedle patch administration resulted in HI titers of 103 for A/Solomon, 125 for A/Wisconsin, and 320 for B/Malaysia. In both cases, intramuscular and microneedle patch administration, the HI titers for all three strains were 10-fold greater than the control group, indicating immunogenicity in mice.

DISCUSSION

Most of the injectable vaccines are administered intramuscularly or subcutaneously, with the exception of rabies, bacillus Calmette–Guerin, and IDFlu (Sanofi/BD), which are administered intradermally. Previously approved or clinically evaluated intradermal microneedle devices for the administration of influenza antigens are hollow microneedles, which require skilled personnel for the administration of the vaccine.[15–17] In a recent study, Prausnitz and coworkers[7] investigated the use of dissolvable microneedles for the delivery of inactivated influenza virus and compared the immune responses generated using metallic microneedle delivery system. The dissolvable microneedle technology in combination with inactivated influenza virus was shown to offer better immune response with the delivery system made by photopolymerization of PVP with lyophilized inactivated virus.[18] Raphael et al.,[6] in their proof of concept studies, have shown the efficacy of egg-derived subunit vaccine Fluvax delivered transdermally using nanopatch, a densely packed dissolving microprojection array. All these studies use either inactivated virus or egg-derived subunit vaccine, which

need longer lead times for the production of vaccine in chicken eggs.

So far there has been very limited information on the characterization of influenza antigens fabricated into microneedles. In the case of dissolvable microneedles such as the ones that are used in this study, the presence of excipients, mainly trehalose and carboxymethyl cellulose, interfere by increasing the viscosity of the dissolved microneedle solution, thereby making it difficult to analyze the samples by SRID assay. To overcome the limitation of detecting the antigens within the microneedle matrix by conventional assays, we have used capture ELISA to detect and quantify the antigens. The assay employs the interaction of glycoproteins on the surface of influenza antigens with the lectin coated on the ELISA plates; the bound antigen was detected using the purified and labeled NIBSC antibodies with little or no interference from patch excipients. The antigen content in the human dose patches and monovalent antigen in the human dose patches was quantified successfully using capture ELISA, which has better sensitivity than SRID. In addition, all three antigens in the dissolved matrix were detected by the technique of peptide mapping by mass spectroscopy. The technique of peptide mapping identifies and confirms the presence of antigens, whereas capture ELISA confirms the quantity and integrity of the antigens in the microneedle patches.

In the *in vivo* studies, we have shown the ability of microneedles to deliver monovalent influenza antigen H1N1 and trivalent seasonal vaccine. Immunization using monovalent vaccine A/Solomon (H1N1), microneedle patches resulted in HI and IgG antibody titers at the end of the first immunization, indicating the potential use of this approach for an early immune response. Following boost, the difference between the means of HI titers by intramuscular and patch administration is statistically significant by two-tail *t*-test (p value of 0.03 for 0.1 μg dose and p value of 0.005 for 1.0 μg dose); however, the mean difference between the HI titers of two groups is less than twofold. In the second set of *in vivo* studies, we have shown immunogenicity toward trivalent influenza vaccine delivered via microneedle patches at a dose of 0.1 μg. The HI titers from this study show strain-specific induction of antibodies upon immunization with microneedle patches and are found to be comparable to that of intramuscular immunization. In conclusion, the study demonstrates the integrity and immunogenicity of the influenza antigens upon fabrication into microneedles. The immunologic data from the microneedle immunizations is encouraging and indicates potential for delivery of both pandemic and seasonal vaccine.

The microneedle technology, in general, has been shown to be less invasive and less painful than the traditional needles[4]; however, achieving accurate and

Table 4. Hemagglutination Inhibition Titers of Pooled Sera Samples Drawn 2 Weeks After Second Immunization with Trivalent Influenza Vaccine

	H1N1 Solomon	H3N2 Wisconsin	B/Malaysia
Flu liquid (0.1 μg) IM	139	141	160
Flu patch (0.1 μg)	103	125	320
Untreated	<10	<10	<10

reliable dosing is the key. In this study, we have shown the proof of concept for delivery of unadjuvanted cell culture vaccine using simple dissolvable microneedle patches. Although influenza is used as a model antigen for delivery through skin, this study shows the feasibility for delivery of purified subunit vaccines or combination products. The data indicates that dissolvable microneedle patch can be used to load human doses of influenza vaccine; however, the size and number of microneedles on the array need further optimization for use in humans. In addition, the use of adjuvants needs to be evaluated to further improve the efficacy and functional applicability. To reap the potential benefits, acceptability of the microneedle technology by patient population is important for successful translation from research to clinical practice.

CONCLUSIONS

Overall, this study demonstrates the integrity and immunogenicity of the influenza antigens upon fabrication into microneedles. The immunologic data from the microneedle immunizations are encouraging and indicate potential for patch-mediated delivery of influenza vaccine. The dissolvable microneedle technology, if improved, can offer a possibility of self-administration of vaccines that could save time and money.

ACKNOWLEDGMENTS

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