Macroflux® Microprojection Array Patch Technology: A New and Efficient Approach for Intracutaneous Immunization

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Purpose. We evaluated the Macroflux® microprojection array patch technology as a novel system for intracutaneous delivery of protein antigens.

Methods. Macroflux® microprojection array systems (330-μm microprojection length, 190 microprojections/cm^2, 1- and 2-cm^2 area) were coated with a model protein antigen, ovalbumin (OVA), to produce a dry-film coating. After system application, microprojection penetration depth, OVA delivery, and comparative immune responses were evaluated in a hairless guinea pig model.

Results. Macroflux® microprojections penetrated into hairless guinea pig skin at an average depth of 100 μm with no projections deeper than 300 μm. Doses of 1 to 80 μg of OVA were delivered via 1- or 2-cm^2 systems by varying the coating solution concentration and wearing time. Delivery rates were as high as 20 μg in 5 s. In a prime and boost dose immune response study, OVA-coated Macroflux® was most comparable to equivalent doses injected intradermally. Higher antibody titers were observed when OVA was administered with the microprojection array or intradermally at low doses (1 and 5 μg). Macroflux® administration at 1- and 5-μg doses gave immune responses up to 50-fold greater than that observed after the same subcutaneous or intramuscular dose. Dry coating an adjuvant, glucosaminyl muramyl dipeptide, with OVA on the Macroflux® resulted in augmented antibody responses.

Conclusions. Macroflux® skin patch technology provides rapid and reproducible intracutaneous administration of dry-coated antigen. The depth of skin penetration targets skin immune cells; the quantity of antigen delivered can be controlled by formulation, patch wearing time, and system size. This novel needle-free patch technology may ultimately have broad applications for a wide variety of therapeutic vaccines to improve efficacy and convenience of use.

KEY WORDS: administration route; dry-film coating; immunization; Macroflux® microprojection array; vaccination.

INTRODUCTION

There is an increasing need for cost-effective, convenient, and efficient vaccine delivery systems. Although intramuscular (IM) and subcutaneous (SC) injections have been used traditionally, an intracutaneous delivery system would have significant benefits in enhancing the immune response if the delivery were reproducible and acceptable.

The skin is a major immunologic organ, with a dense network of potent antigen presenting cells, Langerhans cells (LCs), covering approximately 25% of the epidermal/dermal boundary (1–3). Foreign antigens that penetrate the skin's primary barrier (stratum corneum) are taken up by LCs, which migrate to draining lymph nodes, resulting in activation of antigen-specific immunity. In keeping up with this physiologic route of immunization, there is extensive literature documenting that intradermal (ID) immunization is more effective, especially at low antigen doses, than IM or SC administration (4–7). However, ID immunization can be technically challenging to administer, and only small volumes can be delivered. Consequently, there is a need for other more convenient and reproducible approaches for ID antigen delivery. However, so far, a practical and minimally invasive method for intracutaneous antigen delivery has not been fully developed.

The outermost "dead" layer of skin is the stratum corneum, the major barrier for transport of low molecular weight hydrophilic and macromolecules into the skin. In humans, the stratum corneum is about 10 to 20 μm in thickness, with the underlying epidermal layer ranging from 50 to 150 μm. Although preclinical studies for vaccine delivery generally have been performed with mice and rats, their stratum corneum and epidermis are significantly thinner than that of human skin. However, the hairless guinea pig (HGP) may be a better model because the anatomy of its skin is very similar to human skin (8,9). Skin permeation studies with hydrophilic and hydrophobic compounds indicate that HGP skin is closer to human skin than that of rats or hairless mice (8,10,11). In addition, the HGP has been used as an experimental model for a number of skin studies, including photodermatology (12), skin infection (13), wound healing (14), and skin irritation (15). Therefore, results obtained using the HGP model should be a reasonable basis for assessing intracutaneous delivery and skin tolerability and may be more relevant to human skin than other animal species. With respect to immunocompetence, the HGP is euthymic and has been used in vaccine (16,17) and contact sensitization studies (18,19).

The objective of these studies was to evaluate the performance of an antigen-coated microprojection array skin patch technology (Macroflux®), for intracutaneous antigen delivery in the HGP model. The Macroflux® skin patch comprises a titanium microprojection array with an adhesive patch backing. For vaccine delivery, a thin dry-film coating of a model protein antigen ovalbumin (OVA) is incorporated onto the surface of the microprojection array. Upon application of the patch, the microprojections penetrate into the skin at the target depth for intracutaneous delivery. These studies investigated the effects of microprojection penetration depth, antigen-coating formulation, patch-wearing time, and system size on antigen delivery into the skin, and compared the immune response relative to conventional needle injection routes of administration.

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MATERIALS AND METHODS

Animals

Outbred male and female euthymic HGPs were obtained from Biological Research Labs (Switzerland, strain ibm:GOHI-hr) and Charles River Labs (Michigan, strain IAF:HA-HO-hr). Animals were 250 to 1000 g. Animals were quarantined, individually housed, and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The research adhered to the *Principles of Laboratory Animal Care* (NIH publication #85-23, revised 1985).

Microprojection Array

Manufacturing

Microprojection arrays were produced using a controlled manufacturing process (20). The finished microprojection array is a titanium screen with a defined microprojection pattern, density, and length. The microprojection arrays used in these studies had a hexagonal close-packed pattern with 190 projections/cm^2. The length of each microprojection was 330 μm. Microprojection arrays used were 1 or 2 cm^2 in area (Fig. 1, A and B).

Ovalbumin and Glucosaminyl Muramyl Dipeptide (GMDP) Coating

Microprojection arrays were coated by immersion in a 1%, 5%, or 20% sterile aqueous solution of OVA (w/v, Grade V, Sigma Chemical Co., St. Louis, MO). Excess solution was removed by forced air and the arrays were air-dried for 1 h at ambient conditions. For studies that used fluorescein isothiocyanate (FITC)-labeled OVA (Molecular Probes, Portland, OR), the fluorescent compound alone was used for any coating solution containing 5% OVA or less. For OVA coating solutions at 20%, unlabeled OVA (15%) was mixed with FITC–OVA (5%). For studies using GMDP (Pharmitra, United Kingdom), microprojection arrays were immersed in an aqueous solution containing OVA (1%) and GMDP (10%).

The amount of OVA coated on the microprojection arrays was determined using FITC–OVA. Dry FITC–OVA coated on the device was extracted by immersing the device in 10 mL of boric acid (0.1 M, pH 9) for 1 h at room temperature in a glass scintillation vial. An aliquot of the extracted material was further diluted in boric acid for quantitation against known standards by luminescence spectrometry (excitation 494 nm, emission 520 nm). Microprojection arrays coated with FITC–OVA were also inspected visually by fluorescence microscopy before and after skin application.

Patch Assembly

After coating and drying, the microprojection array was affixed to an adhesive patch comprised of a low-density polyethylene backing with a polyisobutylene adhesive. The final systems had a total patch area of 8 cm^2 containing either a 1 or 2 cm^2 area microprojection array (Fig. 1A).

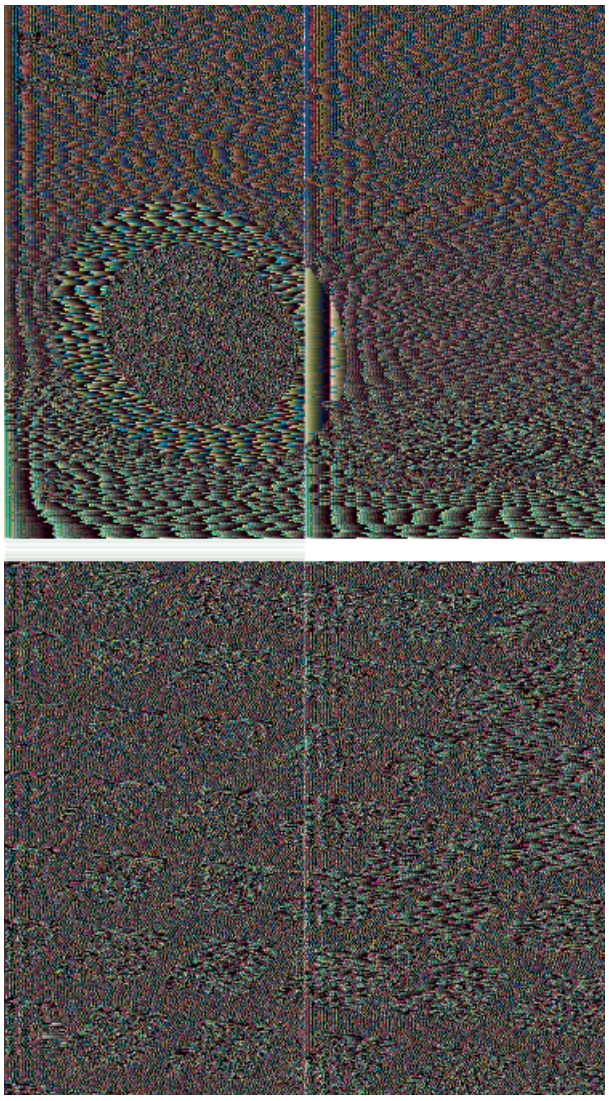


Fig. 1. (A) Schematic representation of the Macroflux® microprojection array integrated with an adhesive patch. (B) Scanning electron photomicrograph of an array of microprojections (330 μm length). For scale, a 25-gauge needle is shown adjacent to the array.

Patch Application

HGPs were anesthetized using a gas delivery system (isoflurane 3–3.5%, O_2 2–2.5 L/min) and skin treatment sites (lateral area of the thorax) were cleaned with isopropyl alcohol wipes (70%) and allowed to dry. The skin site was lightly stretched manually during the system application with an impact applicator. After application, the system was left on the skin for specified times. For devices left on skin for more than 5 s, the HGPs were wrapped with Vetwrap® (3M, St. Paul, MN) and individually housed.

Penetration Assessment

To evaluate the uniformity of skin penetration and microprojection penetration depth, the system was removed immediately after application to the HGP, and the skin site was dyed with a cotton swab imbibed with India ink. The dye was applied in a circular motion in two opposing directions for approximately 15 s. The excess dye was then wiped off with

gauze, and isopropyl alcohol wipes were used to remove any dye from the skin until only the pathways created by the microprojection array were visible. Subsequently, the HGPs were euthanized and the skin sites removed and frozen. Each frozen skin site was biopsied with one 8-m, biopsy punch. Biopsies were sectioned parallel to the skin surface, with the first section at 20 μm and the remainder at 50 μm. Then, the individual skin sections were mounted on microscope slides, and the dyed holes in each slice were counted. From these data and from the density of microprojections, the percentage of pathways that were dyed in a particular skin section was calculated and plotted as a function of depth. In some studies, skin sites were photographed using a video microscope system (Hi-Scope KH2200, Hirox Co., Japan).

Ovalbumin Skin Delivery Study

Dry-coated FITC–OVA microprojection arrays were applied as described above to HPG skin sites. After system removal, the treated skin sites were thoroughly washed with 70% isopropyl alcohol to remove any residual OVA on the skin surface. The HGPs were euthanized and 8-mm skin biopsies were taken. Each tissue sample was placed in a scintillation vial with 0.1 mL of deionized water. Hyamine hydroxide (0.9 mL, 1 M in methanol, JT Baker, Phillipsburg, NJ) was then added, and the samples were incubated overnight at 60°C. Thereafter, the dissolved material was further diluted with 2 mL of hyamine hydroxide:water (9:1), and fluorescence was quantitated by fluorimetry and compared with standards. Background control samples included untreated skin. A minimum replicate of three was used for each experimental condition.

Immunization Studies

Baseline blood samples were obtained from all animals before immunization. On the day of immunization, HGPs (n = 3–5/group) were anesthetized and the treatment sites were cleaned with 70% isopropyl alcohol and allowed to dry. For immunizations performed by needle injection, OVA was dissolved in saline and injected using 1-mL syringes with 25-gauge needles (Becton Dickinson, Franklin Lakes, NJ). ID and SC injections were performed on the dorsal–lateral area of HGPs. The quadriceps muscle of the hind leg was used for IM injection. Microprojection arrays containing dry-coated OVA (± GMDP) were applied as described above.

Each HGP received a primary immunization (Day 0) followed by a secondary booster immunization (new skin site) 4 weeks later with an identical article. After primary immunization, HGPs were anesthetized and blood was collected from the anterior vena cava. The serum samples were evaluated by immunoassay for the presence of anti-OVA antibodies.

Detection of Anti-OVA Antibodies

Sera from nonimmunized and immunized HGPs were tested for the presence of antibodies (IgG) to OVA by enzyme-linked immunosorbent assay. Briefly, 96-well polystyrene plates (Maxisorp, NUNC, Rochester, NY) were coated with 0.1 mL/well of OVA (10 μg/mL in 0.2 M Na bicarbonate/carbonate buffer, pH 9.6) and incubated overnight at 4°C. The plates were washed with phosphate-buffered saline (PBS)–Tween buffer then blocked with 200 μL of PBS/casein (0.5%)/Tween-20 (0.05%) buffer for 1 h at room temperature. Then, the plates were again washed and the test sera were added (100 μL/well at 2- to 5-fold serial dilutions, three replicates, 1 h room temperature). After washing, 100 μL peroxidase-conjugated goat anti-guinea pig IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was added and incubated for 1 h at room temperature. After incubation, the plates were washed, 100 μL of substrate (ABTS, Becton Dickinson, Franklin Lakes, NJ) was added, and they were incubated for 35 min at room temperature. Absorbance (405/490 nm) was measured using a SpectraMAX 250 (Molecular Devices Corporation, Sunnyvale, CA). The results are expressed as serum IgG titers defined as the inverse dilution of a sample from an immunized HGP that yields an OD equivalent to three standard deviations above the average OD generated from non-immunized control sera samples (n = 10).

Statistical Analysis

All results are presented as the mean with its associated standard error of the mean. Statistical analysis was performed by analysis of variance. A probability value of $P < 0.05$ was considered statistically significant.

RESULTS

Skin Response and Depth of Penetration

Macroflux® microprojection array skin patches were applied to HGP and were visually assessed for skin tolerability. When compared with untreated skin (Fig. 2A), responses after application varied from no detectable erythema to mild reactions that typically resolved within 24 h (Fig. 2B). No signs of edema, bleeding, or infection were evident. Evaluation of microprojection skin penetration using the India ink technique showed that greater than 95% of the microprojections penetrated through the stratum corneum barrier with a uniform penetration pattern (Fig. 2C). Skin biopsies taken from treated sites revealed that approximately 50% of the microprojections penetrated to the depth of about 100 μm with no microprojections penetrating deeper than 300 μm (Fig. 2D).

Protein Coating and Delivery

Increasing the concentration of OVA in the coating solution resulted in increased loading of OVA on the microprojection arrays. With a 1% OVA coating solution, the amount of OVA coated was approximately 7 $\mu g/cm^2$. Microprojection arrays coated with a 5% OVA coating solution contained ~42 $\mu g/cm^2$ dry-coated OVA, and those coated with a 20% OVA coating solution contained ~238 $\mu g/cm^2$ dry-coated OVA (Table I).

Observation by fluorescence microscopy revealed that the dry FITC–OVA coating was present as a thin amorphous glass. Surface coating was restricted to the microprojection side of the array (Fig. 3A) and both sides of each microprojection were coated (Fig. 3B). At the maximum OVA concentration used, the average calculated thickness was about 3 μm, which was consistent with microscopic observations. However, as can be observed (Fig. 3), there were local dif-

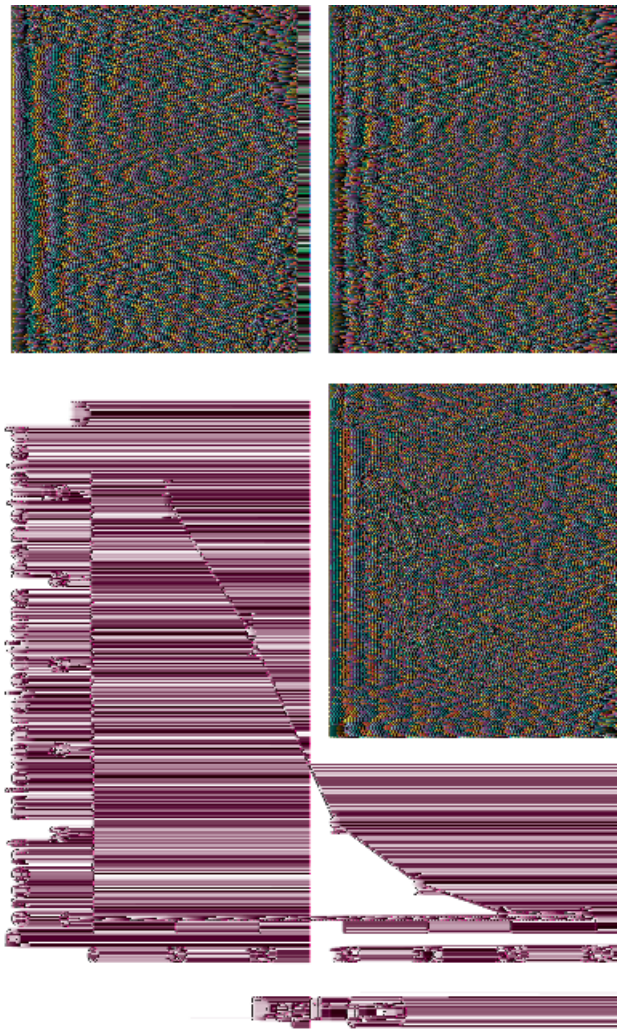


Fig. 2. Microprojection penetration in hairless guinea pig (HGP) skin. Microprojection array patch systems (non-coated) were applied to HGP skin and immediately removed. Skin sites were photographed (A) prior to system application, (B) 5 min after system removal, and (C) after staining with India ink. The penetration profile of the microprojections in HGP skin (D) was determined using the India ink staining and skin sectioning technique described in Materials and Methods.

ferences in coating thickness that were probably the result of a combination of surface tension of the solution and capillary effects. Overall, the coating was uniform on the entire array, and the coating process was reproducible, as demonstrated in Table I. In addition, after storage at ambient conditions (20°C, ~50% relative humidity), no significant degradation of OVA was found, as evaluated by reverse-phase high-performance liquid chromatography (data not shown).

OVA delivery from 2 cm^2 microprojection arrays coated with the three OVA concentrations was evaluated with systems applied on HGP skin for 5 s. These studies found that 1%, 5%, and 20% OVA coating solutions resulted in the delivery of an average of about 1, 6, and 10 $\mu g/cm^2$ of protein, respectively (Table I).

Using a 2 cm^2 device coated with a 20% OVA solution, the delivery of protein into the skin increased with longer application times (Fig. 4). A 5-s application delivered approximately 20 μg of OVA into the skin. A 30-min application delivered 50 μg of OVA, and a 1-h application delivered approximately 80 μg. The results indicate a linear relationship as a function of time versus amount delivered. Examination (fluorescent microscopy) of the microprojection arrays after application revealed that most of the dry coated-OVA was delivered from the tips of the microprojections.

Immunization

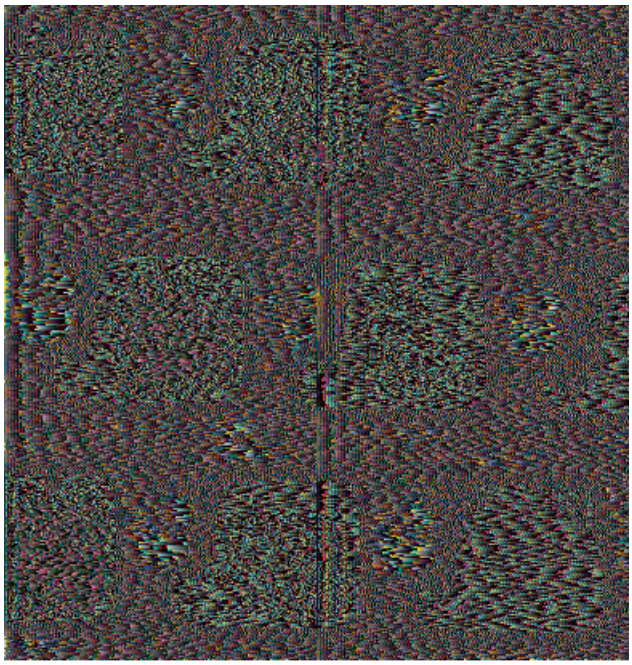
Studies were conducted to compare the immune response of OVA delivered from Macroflux® versus conventional ID, SC, and IM injection routes. Animals were divided into four treatment groups (n = 3–5/group) receiving 1, 5, 20, or 80 μg of OVA/group. Each HGP received a primary immunization. Four weeks thereafter, a booster immunization was performed under identical priming conditions. To determine the level of OVA-specific antibody (IgG) titers by enzyme-linked immunosorbent assay, serum was collected and analyzed from each animal at weekly intervals.

Relatively low levels of OVA-specific antibodies were observed 2 weeks after the primary immunization. Over the next 4 weeks, a general increase in antibody titer was observed among all treatment groups. The seroconversion rates increased with increasing antigen dose and with increasing time. All animals from all treatment groups had seroconverted after the booster immunization. As can be seen (Fig. 5), a dramatic increase in antibody titer was generated 1 week after booster administration. In general, peak antibody titers were observed 1 week after the booster immunization. Thereafter, antibody titers decreased until the next booster treatment was administered (data not shown). The kinetics of the antibody response to OVA using dry-coated OVA delivered by microprojection arrays was similar to that observed using an equivalent aqueous OVA dose by needle administration. In all treatment groups, an increase in the OVA dose resulted in an increase in OVA-specific antibody titers. Higher antigen

Table I. Amount of Ovalbumin Coated on Microprojection Arrays and Delivered into Hairless Guinea Pig Skin[a]

Ovalbumin-coating concentration (%)	Amount of ovalbumin coated on microprojection arrays ($\mu g/cm^2$; mean ± SEM)	Amount of ovalbumin delivered ($\mu g/cm^2$; mean ± SEM)
1	7.4 ± 0.6	0.9 ± 0.1
5	42.2 ± 1.9	5.8 ± 1.4
20	238 ± 20	9.9 ± 0.6

[a]Microprojection patch arrays (2 cm^2) were coated with fluorescein isothiocyanate-labeled ovalbumin. Arrays were applied on hairless guinea pigs (n = 3) for 5 s.



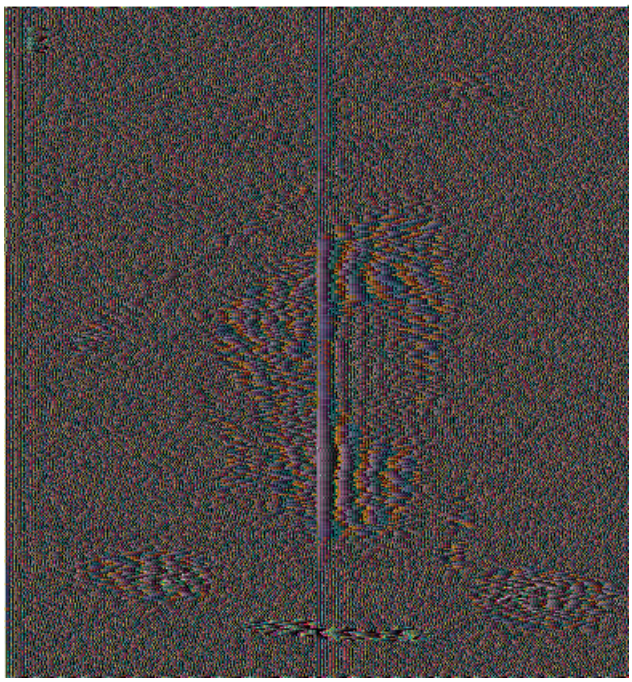


Fig. 3. Fluorescent photomicrograph of dry-coated fluorescein isothiocyanate-labeled ovalbumin on Macroflux® microprojection array. Photographs taken of microprojections facing in an upward direction (A) and an individual microprojection (B).

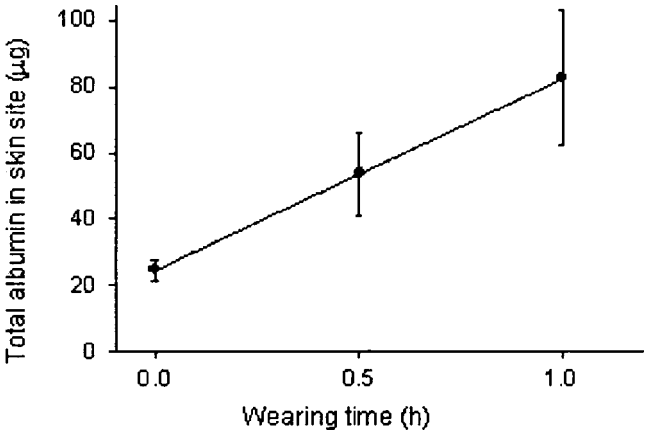


Fig. 4. Time course of ovalbumin (OVA) skin delivery from dry-coated OVA Macroflux® microprojection array. Microprojection arrays were coated with 20% fluorescein isothiocyanato-labeled OVA, and systems were applied to hairless guinea pig skin for the indicated periods. The OVA skin content (n = 3, mean ± SEM) was evaluated as described in Materials and Methods. The data point at time = 0 corresponds to a 5-s application time.

doses correlated with increased seroconversion rates after primary immunization (data not shown). With the exception of a few animals immunized with low doses of OVA (i.e., SC at 1 μg, IM at 1 and 5 μg), all other HGPs had detectable anti-OVA antibodies 2 weeks after the booster immunization.

Analysis of variance was performed to evaluate possible differences among the various treatment groups, analyzing antibody titers one week after the booster immunization. A significant dose–response effect was observed for all methods of antigen delivery. OVA-coated microprojection arrays resulted in antibody titers most similar to equivalent doses injected ID. At 1 and 5 μg of OVA, higher antibody titers were generated when the antigen was delivered using the microprojection array, or by ID administration, when compared to equivalent doses injected SC or IM. A 1-μg dose of OVA delivered by the microprojection array resulted in higher antibody levels compared with the SC (10-fold) or IM (50-fold) injection routes. A 5-μg dose of OVA delivered using the microprojection array resulted in a 24-fold greater response over IM needle administration. No difference was observed between Macroflux® and SC administration at this dose. At higher antigen doses of 20 and 80 μg OVA, no increase in antibody titers were observed using the microprojection array over SC or IM injection.

Studies were conducted to determine whether an adjuvant co-formulated with OVA and dry-coated onto the microprojection array could enhance the antibody responses. Immunization studies using microprojection arrays dry-coated with OVA and GMDP, delivering approximately 1 μg of OVA along with 15 μg GMDP, resulted in a significant increase in antibody titers over non-adjuvant controls. Significant increased antibody titers were also achieved when GMDP was co-delivered with OVA using parenteral injection. After IM administration, the increase in antibody titer was 160-fold as compared to 2.5, 13, and 15-fold following, ID, microprojection array, and SC administration, respectively (Fig. 6).

The dry-coated OVA microprojection arrays were well tolerated in the HGP. After primary immunization, erythema at the application site was mild and dissipated within 24 h. In addition, no signs of skin infection were observed in any of the animals either during the study or post study observation. After booster administration with the microprojection array or ID injection, moderate skin erythema and edema were observed. This skin reaction appeared rapidly and lasted a few days, suggesting an antigen-specific immunologic response.

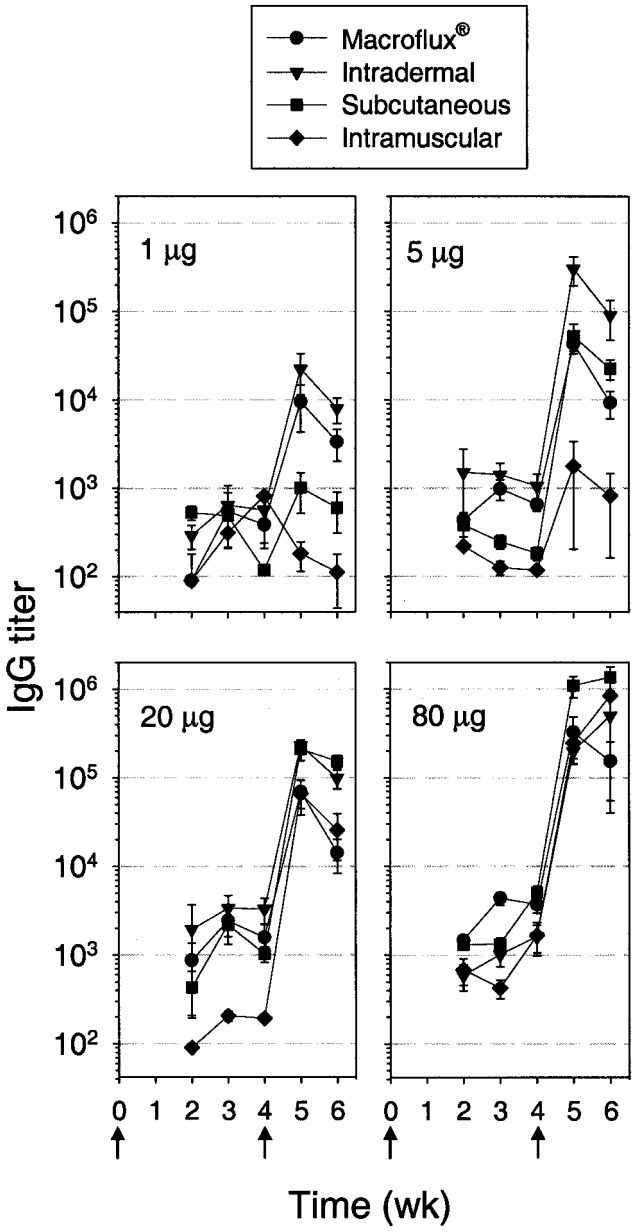


Fig. 5. Kinetics and magnitude of anti-OVA antibody (IgG) titers in HGPs (n = 3–5/group) immunized with dry-coated OVA microprojection arrays or by conventional ID, SC, or IM injection. Arrows indicated prime and boost. The conditions for delivery of OVA using microprojection arrays are as follows: for 1 μg OVA delivery, 1% OVA dry-coated onto a 1 cm^2 array and applied for 5 s; for 5 μg OVA delivery, 5% OVA dry-coated onto a 1 cm^2 array and applied for 5 s; for 20 μg OVA delivery, 20% OVA dry-coated onto a 2 cm^2 array and applied for 5 s; and for 80 μg OVA delivery, 20% OVA dry-coated onto a 2 cm^2 array and applied for 5 s.

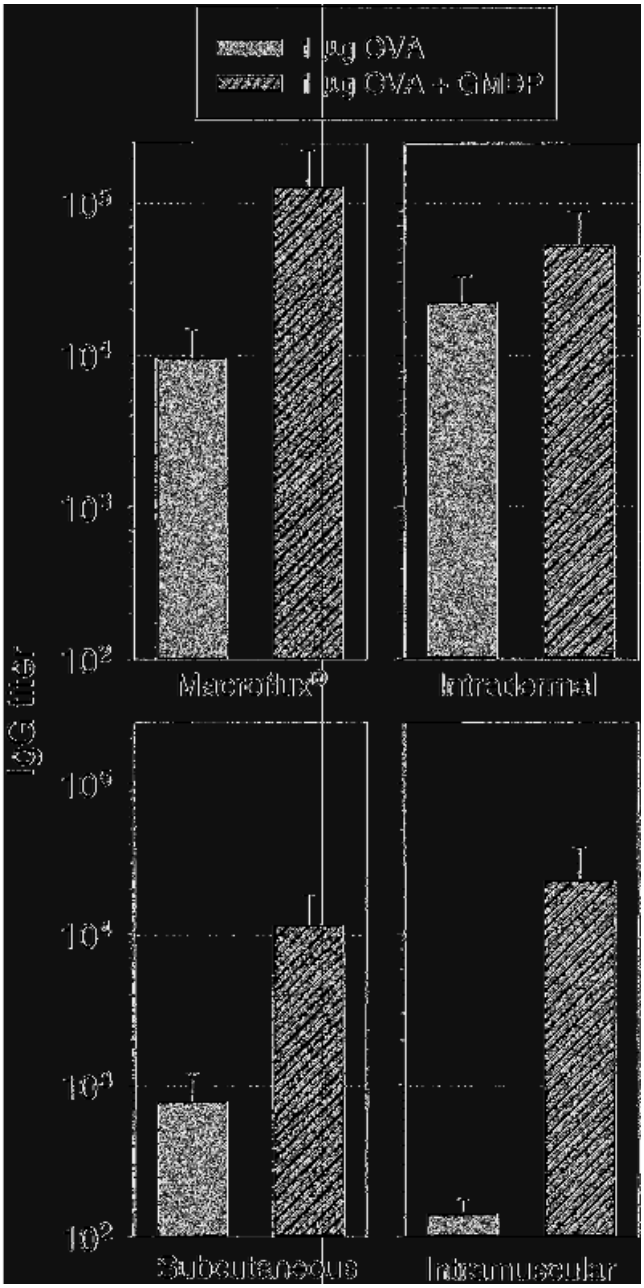


Fig. 6. Effect of GMDP on anti- OVA antibody response. HGPs were immunized to OVA with or without GMDP. For ID, SC, and IM immunization, HGPs received OVA (1 μg) with or without GMDP (15 μg). For immunization using the microprojection array, OVA (1%) with or without GMDP (10%) was dry-coated onto the array and applied to HGPs for 5 s. Antibody titers are shown one week following the booster immunization.

DISCUSSION

A number of studies have demonstrated that administration of antigens into the skin can lead to effective immune responses (21–24). ID needle administration allows direct access into the epidermal/dermal compartment. However, this method can be technically challenging and is not widely used in the clinical setting. A significant limitation of using the needle is the inability to precisely control the depth of penetration and to deliver the entire dose into the intracutaneous space. In contrast, our results demonstrated that the Macroflux® microprojection skin patch allows for reproducible control of skin penetration depth. The microprojection array penetrated uniformly across the entire treated skin surface area to an average depth of 100 μm. The range of penetration depths observed from the 330-μm array was probably due to the irregular surface of HGP skin and its viscoelastic properties. This variability can be reduced further by increasing skin tension during system application. Macroflux® microprojec-

tion skin patch systems appeared to be well tolerated. No indication of bleeding was observed, suggesting that the majority of the microprojections remained above the microcapillary bed, with minimal or no vascular damage. In addition, no signs of infection were observed.

The model antigen, OVA, is a relatively large protein (45 kDa) that should not readily cross the stratum corneum skin barrier. In spite of this, humoral and cell-mediated responses have been demonstrated using a topical OVA solution application in mice (25,26). Other laboratories have presented similar evidence in mice that antigen-specific immune responses can be generated using plasmid DNA, viral vectors, and other protein antigens topically applied to intact skin (22,27,28). In these studies, the amount of material actually delivered into the skin was not quantified. Further, the dose response effect was only observed in some cases and the skin contact time needed was hours or even days. Our results demonstrated that dry-coated OVA on the Macroflux® microprojection array could be delivered intracutaneously in a rapid and reproducible manner. The technology creates superficial pathways through the stratum corneum skin barrier and allows delivery of antigen into the epidermal/dermal compartment, which is rich in antigen-presenting cells. This enables the system to be efficient and effective for vaccine antigen-targeted delivery.

Control of intracutaneous OVA delivery by the microprojection array was achieved by varying the coating solution concentration, wearing time, and system size. The microprojection arrays used in these studies were dip-coated in an aqueous OVA solution. This process resulted in coating the entire surface of the microprojection side of the device. However, only a fraction of the total amount of antigen coated on the microprojection arrays was delivered into the skin (Table I). Further examination revealed that the majority of the protein was delivered predominantly from the tips of the microprojections. Improvements to increase antigen utilization are being explored. Because most compounds are more stable in a dry state, microprojection array technology has the potential to eliminate cold-chain storage. It is expected that these results will be applicable to other protein antigens. Future work will evaluate the stability of other relevant vaccine antigens.

Both primary and secondary antigen-specific antibody responses were generated using dry-coated OVA microprojection array skin patches. Qualitatively, the kinetics of the antibody responses was similar to that observed using conventional injection. At all antigen doses, the magnitude of the antibody response using dry-coated OVA microprojection array systems was equivalent to ID injection. However, 1- and 5-μg doses dry-coated OVA microprojection array systems produced 10- and 50-fold higher antibody titers than those observed after equivalent doses administered by SC or IM injection, respectively. Higher antigen doses of 20 and 80 μg using SC and IM injections resulted in similar antibody titer levels to using microprojection arrays or ID injection. The results observed using the Macroflux® microprojection skin patch to intracutaneously deliver antigen is consistent with a dose-sparing effect that has been seen in other immunization studies using the ID route (4–7,24).

The antibody response after delivery of a low antigen dose (1 μg) could be enhanced by co-delivery of the adjuvant GMDP. Delivery studies with OVA and GMDP dry-coated array demonstrated that the presence of the adjuvant did not significantly affect the amount of OVA delivered (data not shown). Although the amount of GMDP delivered into the skin using the microprojection array could not be directly quantified, we estimated that approximately 15 μg of GMDP was delivered into the skin based on mass transfer calculations. At this dose, GMDP boosted the antibody response regardless of the route of administration but the effect was greatest following IM administration. Despite the dramatic increase in antibody titers produced by GMDP using IM administration, the antibody titer levels were lower than that observed with microprojection array co-administration of GMDP and OVA. In addition, the antibody titers generated with microprojection arrays that delivered GMDP and OVA approaches the titer levels achieved with OVA doses of 20 μg or greater in the absence of GMDP, which demonstrates a significant dose-sparing effect. The difference in enhancement observed between microprojection array delivery and ID is not understood at this time but may be the result of subtle differences in antigen and adjuvant localization in the different layers of the skin after ID or microprojection array administration. Indeed, experiments have demonstrated that OVA localizes primarily in the epidermal layers after microprojection array delivery (data not shown). Such a preferred localization may result in increased exposure of relevant epidermal cells, such as Langerhans cells, to the adjuvant, which may trigger enhanced activation. Unfortunately, pinpointing the origin of these differences would be very difficult with OVA and GMDP because of their high water solubility and probable high diffusivity in tissues. Future studies will attempt to resolve this matter using a poorly diffusible antigen.

In conclusion, Macroflux® microprojection array patch technology allows bolus or short-duration administration of dry-coated antigen. The antigen dose can be controlled by formulation, patch wearing time, and system size. This novel skin patch technology may be broadly applicable to intracutaneous delivery of a wide variety of therapeutic vaccines to improve convenience and efficacy.

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