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Micro-fractional epidermal powder delivery for improved skin vaccination

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ABSTRACT

Skin vaccination has gained increasing attention in the last two decades due to its improved potency compared to intramuscular vaccination. Yet, the technical difficulty and frequent local reactions hamper its broad application in the clinic. In the current study, micro-fractional epidermal powder delivery (EPD) is developed to facilitate skin vaccination and minimize local adverse effects. EPD is based on ablative fractional laser or microneedle treatment of the skin to generate microchannel (MC) arrays in the epidermis followed by topical application of powder drug/vaccine-coated array patches to deliver drug/vaccine into the skin. The novel EPD delivered more than 80% sulforhodamine b (SRB) and model antigen ovalbumin (OVA) into murine, swine, and human skin within 1 h. EPD of OVA induced anti-OVA antibody titer at a level comparable to intradermal (ID) injection and was much more efficient than tape stripping in both delivery efficiency and immune responses. Strikingly, the micro-fractional delivery significantly reduced local side effects of LPS/CpG adjuvant and BCG vaccine, leading to complete skin recovery. In contrast, ID injection induced severe local reactions that persisted for weeks. While reducing local reactogenicity, EPD of OVA/LPS/CpG and BCG vaccine generated a comparable humoral immune response to ID injection. EPD of vaccinia virus encoding OVA induced significantly higher and long-lasting interferon γ-secreting CD8+ T cells than ID injection. In conclusion, EPD represents a promising technology for needle-free, painless skin vaccination with reduced local reactogenicity and at least sustained immunogenicity.

1. Introduction

Vaccination plays a crucial role in global public health. Due to the convenience of injection, the majority of vaccines are delivered into the muscular tissue. Yet, it has long been recognized that vaccines delivered into the skin elicit more potent immune responses, at least partly attributed to the abundant antigen-presenting cells (APCs) within the skin [1–5]. Currently, three vaccines including Bacillus Calmette-Guérin (BCG) vaccine, rabies vaccine, and seasonal influenza vaccine, are approved for intradermal (ID) injection. Unfortunately, ID injection of these vaccines induced frequent local reactions. For instance, the live-attenuated *Mycobacterium bovis* BCG vaccine induces severe local reactions that end with permanent scars in more than 90% vaccinees [6]. Rabies vaccine was recently approved for ID injection to spare vaccine doses and reduce costs because ID injection of one-fifth dose of rabies vaccine induced a comparable anti-rabies antibody titer to full dose intramuscular (IM) vaccination [7]. Yet, ID rabies vaccination induces much higher rates of erythema, pruritus, and other local reactions than IM vaccination [7]. Very recently, a reduced dose of seasonal influenza vaccine (9 μg instead of 15 μg) was approved for ID injection by a newly developed ID microinjection system [8]. ID influenza vaccination induced an overall 30–75% erythema, induration, edema, and pruritus at local injection site, while IM injection induced <10% of these local reactions [8]. More frequent and severe local reactions following ID vaccination might breach the integrity of the skin and increase local infection risk. The unpleasant local reactions also reduce patient compliance, limiting the acceptance of the highly immunogenic route of vaccination in the clinic.

ID vaccination-associated local reactions also hamper the incorporation of adjuvants to further boost skin vaccination because the majority of adjuvants induce strong local reactions following ID injection [9,10]. The most widely used aluminum salt-based alum adjuvant, the recently approved AS04 adjuvant by adsorbing toll-like receptor 4 (TLR4) agonist monophosphoryl lipid A (MPL) onto alum, and squalene-based oil-in-water emulsion adjuvant MF59 are not suitable for skin vaccination due to the long-term deposition and persistent local reactions at the injection site, as we have previously shown [9,10].

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Novel skin delivery technologies capable of minimizing vaccine/adjuvant-induced local reactions while sustaining vaccine immunogenicity are highly demanded to explore the full potential of skin vaccination. In this study, we present a micro-fractional epidermal powder delivery, hereafter abbreviated as EPD, to meet the above needs. EPD is based on ablative fractional laser (AFL) or microneedle (MN) treatment of the skin to generate microchannel (MC) arrays in the epidermis followed by topical application of powder vaccine-coated array patches to deliver vaccines into the skin. Interstitial fluid is expected to be drawn into each MC after laser or MN treatment, where topically applied vaccine powders would be dissolved and diffused from patches into MCs followed by entry into the surrounding tissue. Laser- and MN-generated MCs will be recovered in days because each MC is so small that it can be healed by surrounding normal skin [11–14]. This full repairing capacity forms the basis of cosmetic laser resurfacing and MN dermabrasion. Application of the micro-fractional repair concept to skin vaccination resulted in minimization of vaccine/adjuvant-induced local reactions without compromising vaccine immunogenicity and adjuvant potency. In this study, we found that EPD could efficiently deliver a variety of molecules, such as small molecule drugs, various types of vaccines and adjuvants, into the skin and profoundly reduce local side effects of a combinatorial lipopolysaccharide (LPS) and CpG adjuvant (LPS/CpG) and BCG vaccine, while inducing comparable immune responses to ID injection.

2. Materials and methods

2.1. Animals and human skin samples

BALB/c mice (6–8 weeks old) were purchased from Charles River Laboratories. Yorkshire pigs (~4 months old) were obtained from Tufts. Animals were housed in the animal facilities of Massachusetts General Hospital (MGH) and anesthetized for hair removal, laser and MN treatment, and patch application. Freshly excised human skin samples from plastic surgery patients were obtained from the Department of Dermatology of MGH. All animal procedures were approved by the Institutional Animal Care and Use Committees of MGH and human skin study was approved by the Institutional Review Boards (IRBs) of MGH.

2.2. Reagents and laser device

Sulforhodamine B (SRB), OVA (Grade V), and LPS were purchased from Sigma (St. Louis, MO). Alexa Fluor 647-conjugated OVA (AF647-OVA) was obtained from Invitrogen (Carlsbad, CA). Murine-specific CpG 1826 and pig-specific CpG 2007 were obtained from Invivogen (San Diego, CA). BCG vaccine, which contains 1-8 $\times 10^8$ colony forming units of BCG, was obtained from MGH Pharmacy. Vaccinia virus encoding OVA cDNA (VV-OVA) with a stock of 0.5–1 $\times 10^9$ pfu/ml was a kind gift of Dr. Chance John Luckey (Brigham and Women's Hospital, Boston, MA). An UltraPulse Fractional CO_2 Laser (Lumenis Inc., Yorkneam, Israel) with laser energy at 5.0 mJ and skin coverage at 5% was used to generate MC arrays in the skin surface. Sham laser treatment had the same procedure except that the laser was not activated.

2.3. Powder array patch coating

Powder array patch coating was illustrated in Fig. 1. SRB was directly coated without prior treatment, while lyophilized OVA, LPS/CpG, OVA/LPS/CpG, VV-OVA were crushed into fine powders by pressing the lyophilized powders across the frosted ends of microscope slides. To prepare BCG vaccine patches, BCG vaccine was quickly taken from 4 °C and fractionally coated without prior treatment and used instantly.

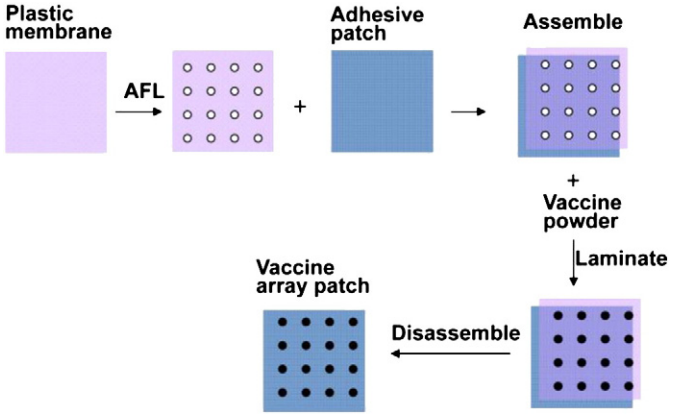

Fig. 1. Illustration of powder array patch coating. A plastic membrane was exposed to laser illumination (35 mJ, 5%) to generate a 4 × 4 array of microholes in ~2 × 2 mm^2 area, each with a measured diameter of ~189 μm. The membrane was topically layered onto an adhesive patch (3M). Vaccine powders were poured onto the membrane/patch assembly and pushed to fill the microholes. Non-adherent powders were removed before disassembly of the plastic membrane/adhesive patch assembly to obtain powder vaccine coated array patches.

2.4. Topical patch application and ID injection

One 9 × 9 array patch was cut into four 4 × 4 array patches for topical delivery. The remaining 1 × 8 and 1 × 9 array patches were used to quantify the coating amount as shown below. To evaluate the delivery efficiency, a 4 × 4 MC array in a 2 × 2 mm^2 area was generated followed by topical application of SRB- or OVA-coated 4 × 4 array patches onto laser- or sham-treated skin, or tape-stripped skin. The same tape stripping for 15 strokes (tape (1, 15)) and different tape stripping for 6 strokes by changing the tape every other stroke (tape (3, 6)) were used in the current study. To evaluate local reactogenicity, four 9 × 9 MC array in 2 × 2 cm^2 area were generated followed by topical application of four 8 × 8 LPS/CpG-coated array patches. Patch-coated OVA, LPS/CpG, and BCG vaccine were extracted into phosphate buffered saline (PBS) for ID injection to ensure the same vaccine and adjuvant doses were delivered in both groups.

2.5. Quantification of transcutaneous delivery

SRB delivered into the skin and remaining on the patch were quantified following skin homogenization and patch extraction as previously described [13]. The delivery efficiency will be calculated as the relative SRB amount in the skin to that in skin plus patch. To calculate OVA delivery efficiency, OVA amount coated on the patch before delivery and remaining on the patch after delivery was measured by a bicinchoninic acid (BCA) protein assay (Pierce). The difference between patch coated and patch remaining reflected the delivered OVA amount, by which the delivery efficiency was calculated.

2.6. Immunogenicity and local reactogenicity

Antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) by coating 100 μg/ml OVA or 50 μg/ml BCG vaccine. Cellular immune responses were evaluated by intracellular cytokine staining and flow cytometry. In brief, peripheral blood mononuclear cells (PBMCs) were isolated and stimulated overnight with OVA (100 μg/ml) in the presence of 4 μg/ml anti-CD28 antibody (37.51). Golgi-plug was added 5 h before harvesting and cells were stained with PerCP/Cy5.5-anti-CD8 (53-6.7) and FITC-anti-IFNγ (XMG1.2) antibodies followed by flow cytometry analysis. Local reactions were monitored daily for at least 2 weeks. Pictures of local reactions were taken by a NIKON D5100 camera. In some studies, skin samples were dissected and subjected to