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Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system

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Abstract

Immunization to the model antigen ovalbumin was investigated using a novel intracutaneous delivery system consisting of antigen-coated microneedle arrays. The influence of the following parameters on the resulting immune responses was investigated: depth of vaccine delivery, dose of vaccine delivered, density of microneedles on the array, and area of application. The immune response was found to be dose dependent, and mostly independent of depth of delivery, density of microneedles, or area of application. Our studies show that the shortest, most tolerable microneedle arrays can be used for achieving consistent and high antibody titers. Overall, the microneedle array proves to be a very versatile delivery technology, allowing easy and reproducible antigen delivery to skin for efficient vaccination without the use of a needle and syringe.

Keywords: Ovalbumin; Immunization; Microneedle array; Intracutaneous

1. Introduction

The skin represents the first immunological defense barrier to outside injury and has evolved into a major immunocompetent organ [1,2]. Foreign agents and antigens that penetrate the outermost stratum corneum encounter a dense network of potent antigen-presenting cells, the epidermal Langerhans cells and the dermal dendritic cells [3]. These cells readily take up foreign antigens, migrate to the draining lymph node to present antigen fragments to resting T lymphocytes, and initiate antigen-specific immune responses. The skin, therefore, is an attractive target site for vaccine delivery, allowing the most effective immunization with the least amount of antigen [4–6]. However, consistent and reproducible intradermal (ID) immunization is technically challenging and only relatively small volumes can be delivered. Therefore, vaccines are traditionally given by intramuscular (IM) or subcutaneous (SC) injection. A robust, practical, cost-effective, convenient, and efficient intracutaneous antigen delivery technology will have broad applications in the field, especially if the delivery can be achieved in a minimally invasive and painless fashion.

Approaches in this direction include the firing of powdered vaccines into the skin through the use of helium gas [7,8] requiring complex devices and vaccine formulations, and the use of vaccine patches, which are topically applied to the skin [9–11]. This completely noninvasive topical immunization uses well-established technology for drug delivery. However, as this approach has advanced into initial clinical trials, relatively low immune responses have been induced with high doses of potent immunogens [12]. Protein antigens delivered by a simple patch were shown to induce significant systemic immune responses in humans, but only in the presence of bacterial adjuvants such as heat-labile enterotoxin [13]. We have previously described a novel and efficient approach for minimally invasive intracutaneous immunization, the Macroflux® (MF) microneedle array patch technol-

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ogy [14]. The MF skin patch comprises a titanium microneedle array with an adhesive patch backing. For immunization, a thin film coating of a protein antigen is incorporated onto the surface of the microneedle array. Upon application of the patch, the microneedles penetrate into the skin and provide rapid and reproducible intracutaneous administration of the coated antigen, deep enough to target skin immune cells and to elicit potent antibody responses to the model antigen ovalbumin (OVA) in the hairless guinea pig (HGP) model [15].

Comparison of the immune response following intracutaneous microneedle array versus conventional syringe/needle ID or IM administration with different OVA doses was studied previously and demonstrated that the maximum differences (up to about a 100-fold increase using intracutaneous microneedle or ID versus IM) were observed at low (1 μg) antigen dose [15]. The objective of the current studies was to further investigate and evaluate the performance of this novel delivery system. To evaluate vaccination efficacy, we determined the influence of the following parameters on the resulting immune responses: depth of vaccine delivery, dose of vaccine delivered, density of microneedles on the array, and skin surface area to which a given dose is applied. We also investigated whether these delivery parameters resulted in differences in skin tolerability. Ovalbumin, a well-characterized antigen, was employed as a model for these immunization studies. The influence of depth and dose of antigen delivery was investigated using three microneedle array designs with identical low microneedle density (140 cm^{-2}) and shape, but increased microneedle lengths of 225, 400, and 600 μm. All three designs were used to deliver three antigen doses: 0.5, 5, and 25 μg per array. A high-density design (657 cm^{-2}, microneedle length: 225 μm) was used to deliver the same doses. These treatment groups addressed the influence of microneedle density on the immune response for the most shallow delivery depth, which is the most desirable design from a tolerability standpoint. These studies were performed using a 2 cm^2 microneedle array. To determine whether a given dose delivered to a larger area results in differences in the immune response, a low-density microneedle design loaded with 50% dose was administered on two sites of the animal, yielding a delivery of a given dose to twice the skin area. The vaccine delivery route that is currently most dominant, IM injection, was used as a reference to compare the efficacy of immunization to microneedle array delivery.

2. Materials and methods

2.1. Animals

Outbred 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 500–800 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).

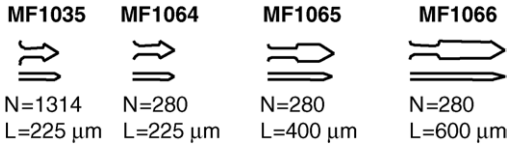

Fig. 1. Schematic representation of the microneedles used in this study. The top and the bottom drawings show the front and the side views of the microneedles, respectively. *N* indicates the number of microneedles per 2 cm^2 array, and *L* is the length of the microneedles. The maximum width of all microneedle designs (shown in the top drawings) is 100 μm, and the thickness (bottom drawings) is 35 μm.

2.2. Microneedle arrays and coating

Microneedle arrays were produced using photo/chemical etching, and formed using a controlled manufacturing process [16]. The finished microneedle array was a titanium screen with a defined microneedle pattern, shape, density, and length. The microneedle arrays used in these studies were 2 cm^2 in area, and had a hexagonal-close-packed pattern with the following microneedle densities and lengths: MF1035, 1314 microneedles per array (high density), 225 μm microneedle length; MF1064, 280 microneedles per array (low density), 225 μm microneedle length; MF1065, 280 microneedles per array (low density), 400 μm microneedle length; MF1066, 280 microneedles per array (low density), 600 μm microneedle length (Fig. 1). All microneedles were about 35 μm thick (side view) and presented a maximal width (front view) of 100 μm.

Coating was performed in ambient conditions (22 °C, 45% relative humidity) using an apparatus that limited application of the drug to the tip of the microneedles [17]. The number of coatings and the concentration of OVA (Grade V, purity ≥98% (agarose gel electrophoresis), Sigma Chemical Co., St. Louis, MO (Cat #A5503, Lot #71K7028)) in the coating formulation were used to control the amount of antigen coated onto the microneedles. To obtain multiple coatings, the tips of the microneedles were repeatedly immersed into the coating solution with a drying time of 5 s between coatings. Three different antigen coating amounts, 0.5 μg (low), 5 μg (medium), and 25 μg (high), were targeted for the four microneedle array designs. In addition, array MF1064 was also coated with half the low, medium, and high doses. To obtain a high loading dose, up to 12 coatings were needed using a 20 wt.% OVA formulation, while to obtain a low loading dose, only 1 coating was necessary using a 2 wt.% OVA formulation. Arrays coated with radiolabeled OVA (45 μCi ^{14}C OVA/mL solution) (American Radiolabeled Chemicals Inc., MO) were used in studies to determine the dose coated, the dose delivered, and the delivery depth. Fluorescein (Sigma Chemical Co., St. Louis, MO) was used at 5×10^{-3} M as