Assessment of the permeation of antigen protein conjugated nanoparticles and live bacteria through microneedle-treated mouse skin

Amrit Kumar, Xinran Li, Michael A. Sandovol, B. Leticia Rodriguez, Brian R. Sloat, and Zhengrong Cui
Pharmaceutics Division, College of Pharmacy, Dell Pediatric Research Institute, The University of Texas at Austin, Austin, TX 78712

PURPOSE
Transdermal route is an attractive alternative for oral and hypodermic route but it is currently limited to a very small group of drugs. The successful delivery of large hydrophilic molecules, such as proteins, across the skin is a big challenge. To address this issue, microneedle technology has been developed, which can disrupt the stratum corneum on the micron scale and hence can be used to enhance the drug transportation for the delivery of large molecular weight and hydrophilic compounds across the skin. One of the very attractive applications of the microneedle technology is in vaccine delivery. It is assumed that microchannels created through the stratum corneum will readily facilitate the intra-epidermal penetration of antigens to generate a strong immune response.

New generation vaccines based on recombinant DNA technology are not very immunogenic and needs a vaccine adjuvant to be strongly immunogenic. Data from numerous studies have confirmed that many polymeric or solid lipid nanoparticles as a vaccine antigen carrier have potent adjuvant activity.

The present study was designed to evaluate the extent to which pre-treatment with microneedles can enhance the skin permeation of a protein antigen conjugated onto solid lipid nanoparticles. Moreover, the permeation of live bacteria through mouse skin pre-treated with microneedles was also evaluated to study the potential risk of microbial infections.

METHODS
Microneedles were prepared from lecithin/glycerol-monostearate-in-water emulsions. Thiolated OVA was conjugated as an antigen onto the surface of prepared nanoparticles. Mouse skin was treated with microneedle rollers of different needle lengths to study the permeation of ovalbumin nanoparticles in vitro and the ability for the ovalbumin nanoparticles to induce immune responses in vivo. Immunization with subcutaneously administered ovalbumin nanoparticles were considered as a positive control. Mouse skin was pre-treated with microneedle rollers before hamster showed the permeation of live Escherichia coli. TEWL of microneedle pretreated mice skins was measured using a vaporometer.

RESULTS
Microscopic view of microneedle pretreated mice skin after staining with methylene blue solution

Fig. 1. Magnified microscopic view of mouse skin after treatment with a 21 G hypodermic needle (A) or microneedle rollers with different needle lengths (200 μm (B), 500 μm (C) and 1000 μm (D)). The skin was stained with methylene blue solution. The distance between the bars in A is 1 mm. All photos were taken under the same magnification.

The permeation of the OVA-conjugated nanoparticles through the skin

Fig. 2. Permeation of fluorescent-OVA-NPs (A), fluorescent-OVA (B), or fluorescent-NPs (OVA-free) (C) through mouse skin treated with microneedles of different lengths (200, 500, and 1000 μm) or untreated. Data shown are means ± S.E.M. (n=5).

Microneedle treatment reversibly increased the transepidermal water loss from the treated skin area

Fig. 5. TEWL values of the skin at different time points after treatment with microneedles of different lengths (200, 500, and 1000 μm). PT = prior to treatment with microneedles. Ctrl = untreated. Data shown are means ± S.E.M. (n=4).

The micropores created by microneedle rollers on the skin permit the permeation of live bacteria through the skin

Fig. 6. Permeation of E. coli DH5α through skin treated with microneedle rollers. (A) Number of bacteria, indicated by bacterial CFU, permeated through the micropores created by microneedles of different lengths (200, 500, and 1000 μm) on an area of 0.64 cm². As controls, intact skin or skin with a single pore created by a 21 G hypodermic needle (HND) were used. (B) Number of bacteria (CFU) permeated through micropores created by microneedles of 1000 μm at different time after the microneedle treatment. All numbers were after 4 h of permeation. Data shown are mean ± S.D. (n = 3).

CONCLUSIONS
Microneedle-mediated skin immunization with antigens carried by nanoparticles can potentially induce strong immune responses. The risk of bacterial infection associated with microneedle treatment is not greater than that with a hypodermic needle injection.

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