Microneedle Electrode Array for Electroporation of Skin for Gene Therapy

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ABSTRACT SUMMARY

To electroporate epidermal cells to increase gene transfection for DNA vaccines, we designed and fabricated a microneedle array with electrical functionality. This microneedle array was mechanically strong enough to penetrate human skin *in vivo* and was able to electroporate red blood cells as an *in vitro* model.

INTRODUCTION

One approach to gene therapy is to deliver DNA vaccines to cells in the skin's outer layer of epidermis. Due to the presence of Langerhans dendritic cells, DNA vaccines administered to skin can elicit a strong immune response [1]. This method of gene therapy is limited in part by the need to improve DNA delivery into cells for increased gene expression in the skin.

Electroporation is known to increase gene transfection based on extensive *in vitro* work and more recent success *in vivo* [2]. To improve on previous work, the challenge in this study was to develop a method to locally cause electroporation of epidermal cells using a device appropriate for eventual clinical use.

Our collaborator, Cyto Pulse Sciences, is addressing this challenge by adapting microneedles developed for transdermal drug delivery to serve as microelectrodes for minimally invasive, highly-localized electroporation of the epidermis (Figure 1) [3]. As envisioned by Cyto Pulse Sciences, microneedles could serve two functions for gene therapy of skin. By coating with DNA, microneedles could first serve as a vehicle to deliver DNA to epidermis. While microneedles remain inserted in skin, they could subsequently serve as microelectrodes to locally electroporate epidermal cells. Previous studies have shown that microneedles can be painlessly inserted into skin. Moreover, the close spacing of the microneedle electrodes means that the large electric fields needed for electroporation can be achieved at relatively low voltages.

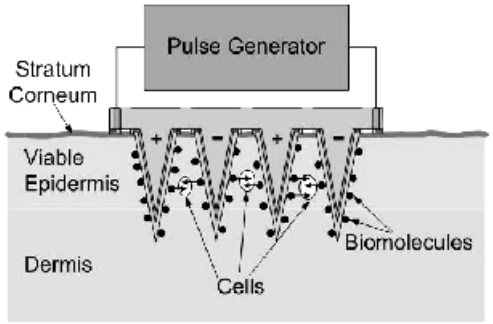

Figure 1. *Conceptual use of microneedle electrode arrays to electroporate cells in epidermis and thereby increase expression of DNA vaccines [3].*

EXPERIMENTAL METHODS

Microneedle electrode arrays were made by first fabricating master structures, from which replicates were molded and then made electrically active. To make a molding master structure, SU-8 was spun on a glass substrate bearing an array mask pattern, baked, and then exposed from the backside to form the tapered needle structure [4,5]. Needles were sharpened by RIE etching. A PDMS (polydimethylsiloxane) mold was then copied from the master. A PMMA (polymethylmethacrylate) microneedle array was formed by solvent-casting, and then released from the mold.

To achieve electrical functionality, a Ti/Cu seed layer was deposited on the PMMA array and patterned by excimer laser to electrically isolate adjacent rows. A 25 μm thick Ni layer was electroplated on the patterned seed layer to enhance structural rigidity. A backside electrical connection to the array was formed by backside etching of a hole and forming electrical connection through the hole.

To test mechanical strength, a microneedle array was inserted and removed by hand from the skin of a human subject (with IRB approval), after which Gentian Violet was applied to the skin for 2 min and then washed off to stain only the sites of microneedle insertion into skin.

Electrical functionality of microneedles was examined *in vitro* by electroporation of a 300 μl bovine red blood cell suspension. After applying 5-ms pulses of 20 – 100 V using the microneedle electrode array, cell lysis by electroporation was assayed by measuring hemoglobin release. After centrifuging electroporated cells, hemoglobin concentration in the supernatant was measured by UV absorption at 575 nm.

RESULTS AND DISCUSSION

After fabricating master structures using cleanroom microfabrication technology, PMMA replicates were formed by solvent casting into PDMS molds (Figure 2). The needles were arrange in a 16 x 16 array (i.e., 256

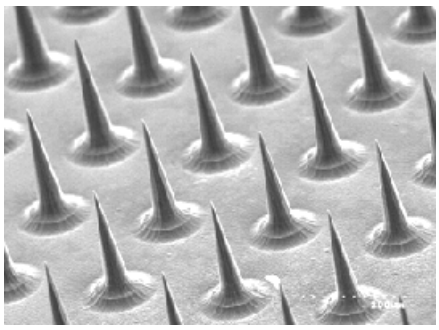

Figure 2. *PMMA microneedle array before adding electrical functionality.*

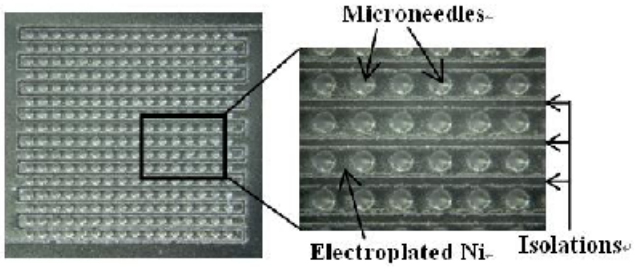

Figure 3. *Photomicrograph of microneedle electrode device, showing an interdigitated pattern of electrically isolated rows of microneedles.*

needles), in which the height of each needles is 400 μm, the base diameter is 100 μm, and the pitch between microneedles is 250 μm.

These microneedle arrays were then coated with metal and laser-etched to provide electrical functionality, as shown in Figure 3. Rows of needles were electrically isolated from each other so that alternating rows provided alternating electrical polarity. Each array was interfaced with an electroporation power source using two wires connected to the microneedle array.

To determine if microneedle electrode arrays could be inserted into skin without breaking, several insertion tests were performed on human subjects. PMMA microneedle arrays with only a thin metal seed layer (30 nm Ti and 300 nm Cu) were not strong enough to penetrate human skin. To enhance the mechanical strength, a 25-μm thick layer of Ni was electroplated on top of a pre-patterned seed layer, and insertion tests were performed. After removing the microneedle electrode array, the skin was stained and then observed by microscopy. As shown in Figure 4, the skin was stained in the pattern of the microneedle electrode array, indicating that the microneedle electrodes pierced into the skin. Subsequent microscopic examination of the arrays showed that microneedle electrode tips were not damaged, even after multiple insertions.

To determine if microneedle arrays were electrically functional and capable of electroporating cells, microneedle electrode arrays were immersed in a suspension of bovine red blood cells. Electrical pulses over a range of voltages were applied to cause electroporation. Using hemoglobin release as a marker of electroporation,

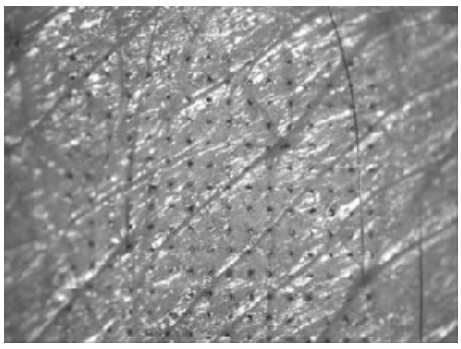

Figure 4. *Photomicrograph of human skin in vivo after piercing with microneedle electrodes and staining with blue dye.*

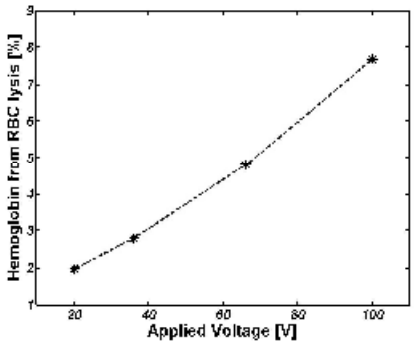

Figure 5. *Hemoglobin released from red blood cells after electroporation using the microneedle electrode array.*

microneedle electrodes caused between 2 – 8% of hemoglobin to be released, as shown in Figure 5. Although the procedure requires additional optimization, these results demonstrate that the microneedle electrode arrays were electrically active and capable of causing electroporation.

CONCLUSION

This study demonstrated the fabrication of microneedle arrays made of polymer, coated with a metal layer, and etched to act as alternating electrodes suitable for electroporation of epidermal cells. Experiments in human subjects established that microneedle electrode arrays are strong enough to insert into skin. Additional experiments with red blood cells showed that these microneedle electrodes are electrically active and capable of electroporating cells.

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