Electronic Supplementary Material (ESI) for Lab on a Chip.

A flexible microneedle array as low-voltage electroporation electrodes for *in vivo* DNA and siRNA delivery

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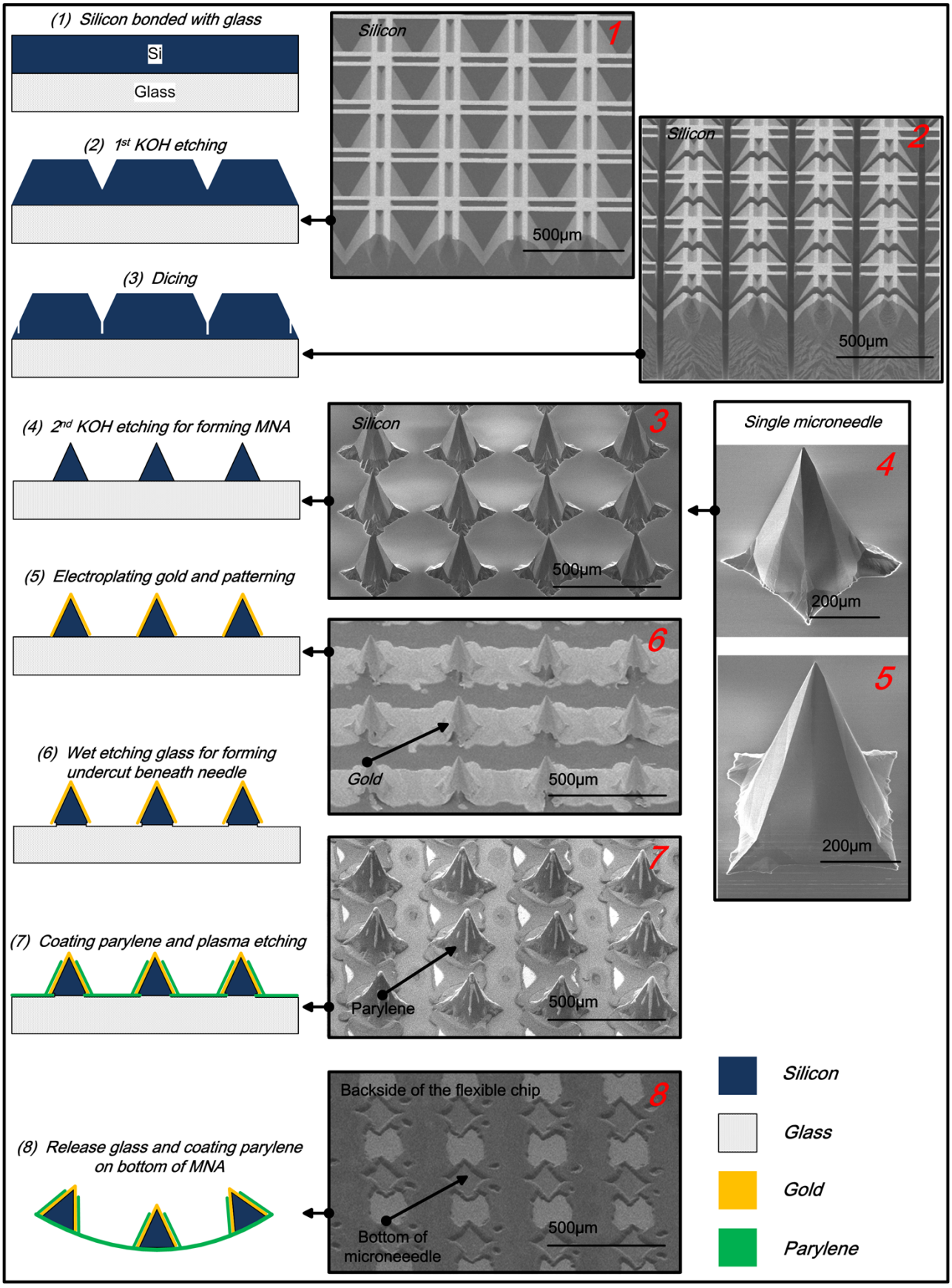
Supplementary Data S1

The fabrication of the flexible microneedle array electrode (MNAE)



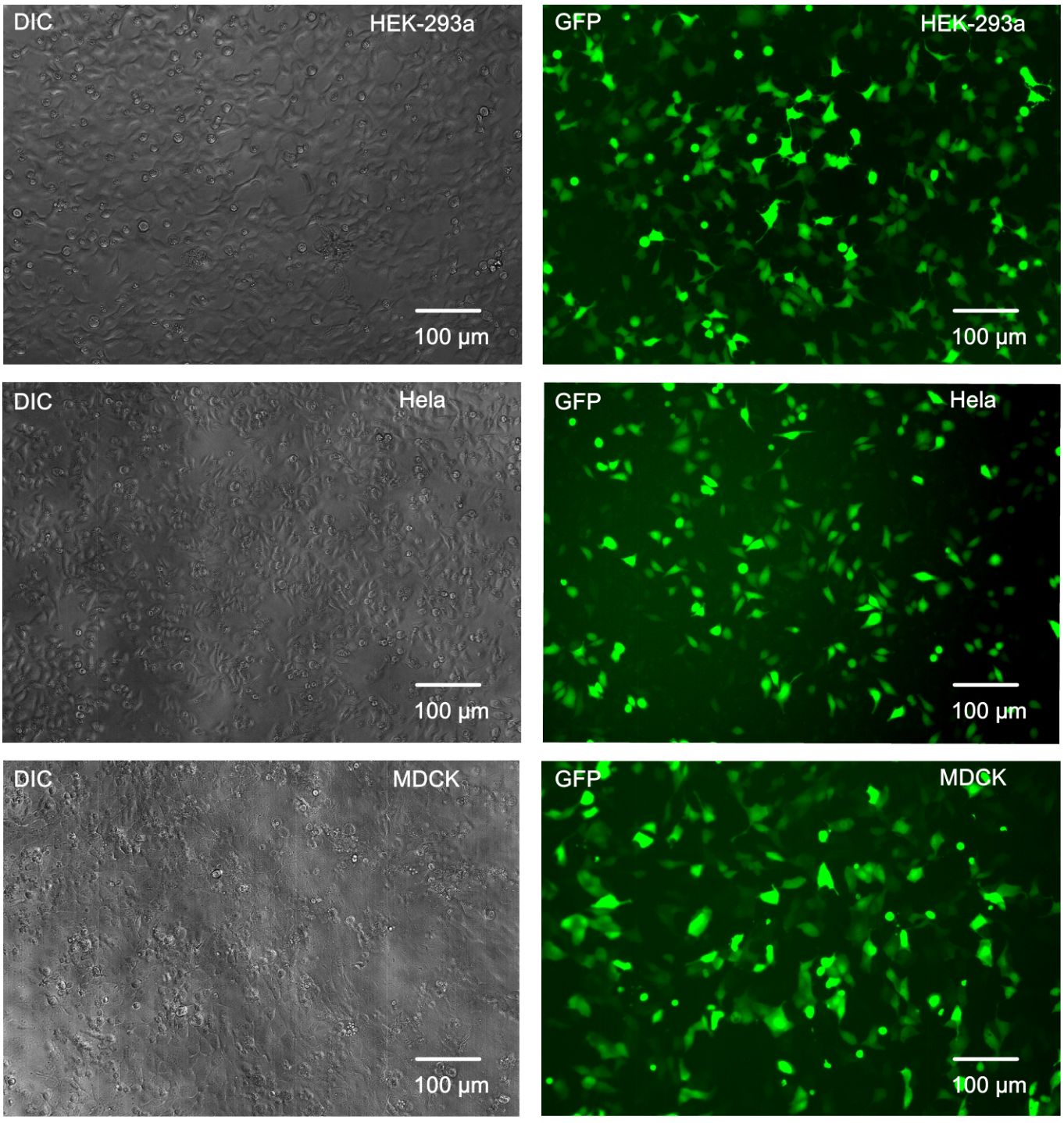
In the fabrication of MNAE chip, one challenge was to reduce the space between needles (to lower the electroporation voltage), while maintaining the needle height (to facilitate the skin insertion). According to the mechanism of the KOH wet etching, the width/depth ratio should be under a certain limit, and otherwise the etching would stop before forming the microneedle (as shown in the uppermost SEM figure 1). To solve this problem, we introduced a dicing process after the first wet etching to open a fresh side wall for the next wet etching (as shown in SEM figure 2).

In detail, the fabrication process started with bonding a 4-inch silicon wafer to a glass substrate, then two wet etching (with a dicing process between them) were used to form silicon microneedles (SEM figure 3). SEM figure 4 and 5 are the close-up views of a single silicon microneedle from different angles, showing the pyramid-like profile of the silicon microneedle. A gold layer was sputtered to coat the microneedles and patterned as electrodes (a chrome layer was used beneath the gold layer for the adhesion between silicon and gold. To withstand the strong electroporation current, electroplating was employed to thickening Au layer to 6 μm (SEM figure 6). An undercut beneath the microneedle was then formed by wet etching of glass to facilitate a good encapsulation of the microneedle root. BHF solution (buffered hydrofluoride acid, mass ratio HF:NH4F: DI water=3:6:10) was used to etch glass under 40 ℃. The etching depth was controlled by time. The etching rate was found to be 0.8 μm/min. It cost 10 minutes to etch glass for 8 μm. This depth is enough for depositing parylene layer to encapsulate the root of the microneedle. Using PDS2010 system (Specialty Coating System, USA), a parylene C film (8 μm) was coated on the top side of microneedles. The gold electrodes were then exposed by plasma etching of the parylene film (SEM figure 7). After releasing the flexible microneedles from the glass substrate,

another parylene layer (2 μm) was coated to encapsulate the bottom of microneedles, finally forming the MNAE. SEM figure 8 is the backside of released MNAE.

Supplementary Data S2

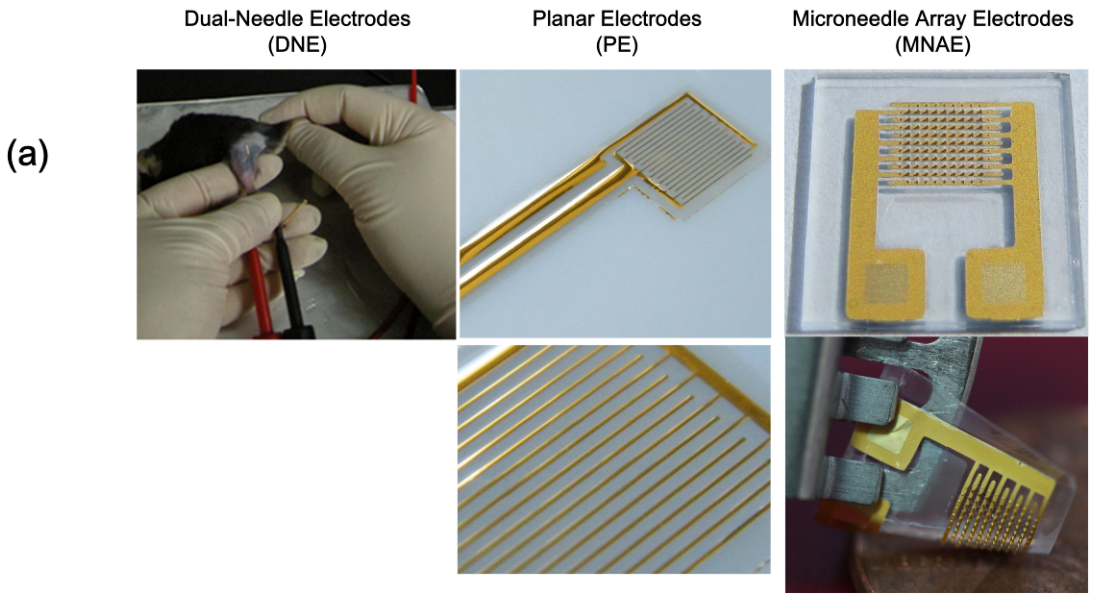
In vitro electroporation of plasmid DNA by MNAE

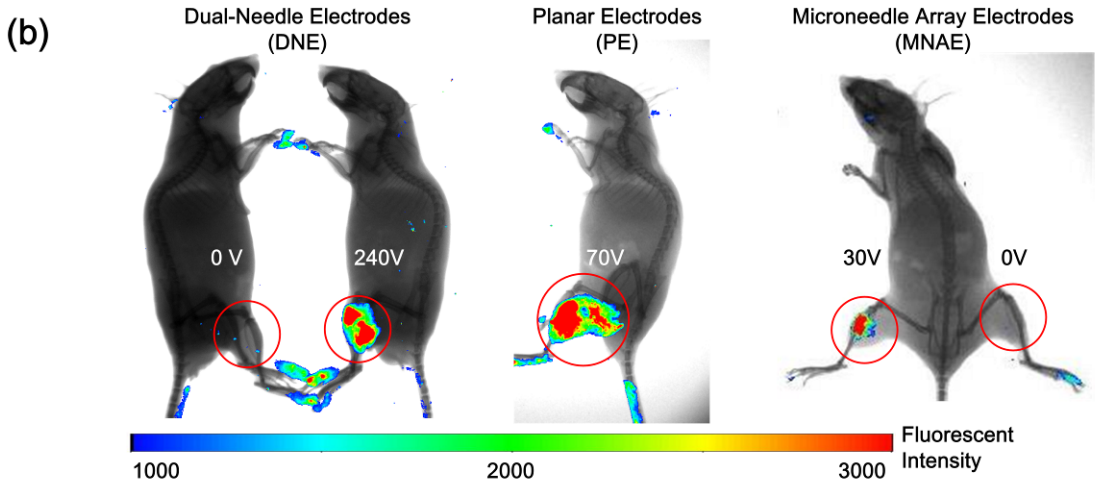


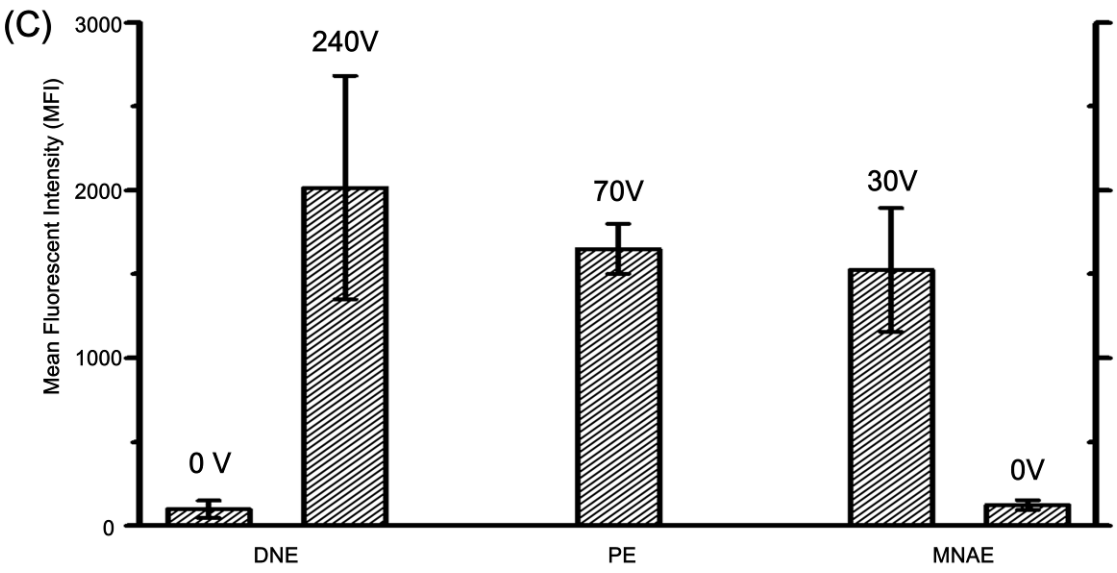
Cell lines HEK-293a (Human Embryonic Kidney 293 Cells), Hela and MDCK (a cocker spaniel-derived kidney cell) were used to characterize the efficacy of *in vitro* cell transfection of green fluorescent protein (pEGFP-C3). Cells were cultured in a 75 cm^2 flask for 24 hours, and then were harvested and re-suspended in electroporation buffer[1]. After adding pEGFP-C3, 20 μl mixture was dispensed on to MNAE surface and three electrical pulses (0.1 ms pulse duration, 2 s pulse interval) were applied right away to perform electroporation. Then the mixture was transferred to a 96-well plate filled with enough cell culture medium. After 24 hours incubation, the transfection efficiency was determined by comparing the number of fluorescence cells with total cell number. As shown in Figure S2, the transfection efficiencies of supernatant HEK-293, Hela and MDCK, are about 85%, 80% and 65%, respectively. It is worthwhile to note that differing from the easy-to-transfect cells, MDCK cell is a hard-to-transfect one. The efficient transfection of MDCK better proves the capability of MNAE chip.

Supplementary Data S3

The comparison test of DNA transfection efficiency on microneedle array electrodes (MNAE), dual-needle electrodes (DNE) and flexible planar electrodes (PE)







(a) Three kinds of electrodes for *in vivo* electroporation.

The conventional dual-needle electrodes (DNE) required a deep tissue penetration to generate proper electric filed in tissue for *in vivo* electroporation. While the flexible planar electrodes (PE) and the flexible microneedle array electrodes (MNAE) were capable of adapting the tissue profile and generating the proper electric field by simple patching on the living tissue.

(b) The DNA transfection of DNE, PE and MNAE.

To electroporate mouse myocytes with DNE, PE and MNAE, the mice were firstly anesthetized, injected with hyaluronidase and RFP plasmid sequentially. The reagents, injection method and reaction time were the same with the protocol described in section 2.3. For DNE, twenty minutes after RFP plasmid injection, two identical needles were inserted into mouse muscle. The diameter and the length of the needle are 0.8mm and 7cm respectively. Since it's difficult to maintain two needles parallelly placed by manual operation, typical spacing between two needles was 0.8-1cm, while the biggest spacing was about 1.5cm. It's observed that that RFP expression area was divided into two separate parts around the two electroporation needles, which proved that electric field was not even for the traditional needle-based electroporation system. For PE and MNAE, the electrodes were patched on the tissue surface. Different pulse voltages, 70 V (for PE) and 30 V (for MNAE) were used.

(c) The quantification of RFP fluorescence

24 hours after electroporation, the RFP fluorescent images were quantitatively analysed. The results revealed that the introduction of MNAE significantly reduced the applied voltage from 240 V (DNE) or 70 V (PE) to 30 V, yet the similar mean fluorescent intensities still remained.

References

1. H. Huang, Z. Wei, Y. Huang, D. Zhao, L. Zheng, T. Cai, M. Wu, W. Wang, X. Ding, Z. Zhou, Q. Du, Z. Li and Z. Liang, *Lab on a chip*, 2011, 11, 163-172.