Fabrication of 3D Nano-channel Electroporation Chip for High Throughput Cell Transfection

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Nano-Channel Electroporation (NEP) is a powerful technology for gene delivery and probing intracellular markers[1]. It has many advantages over commercial (bulk) electroporation including both cell safety and transfection uniformity. The unique cell-to-nano-channel environment allows for active delivery of bio-reagents into living cells, and the dosage is controlled by the external electric-field[2]. However, current NEP chip throughput is fundamentally limited due to its 2D configuration. Most clinical applications require a high throughput NEP chip equipped to handle thousands of cells while also offering excellent transfection uniformity at the single-cell level.

To meet such requirements, we designed and fabricated a silicon based 3D NEP chip (Fig.1 (A)) by using projection photo-lithography and deep reactive ion-etching (DRIE). A deep but uniform micro-trench array was etched with high aspect ratio in 5:1, by optimizing the parameters in Bosch Process (Fig.1 (B)). A nano-pore array (400 nm in diameter) was patterned on the other side by using projection photolithography, followed by DRIE for connecting the nano-trench to the bottom of the micro-trench. This completes the channel and creates a well-ordered nano-pore array(Fig.1 (C)). Nano-pore diameters were measured and confirm good uniformity of the array across the wafer (Fig.1 (D)).

Preliminary experiments were conducted to test the performance of 3D NEP for high throughput cell transfection. Fig.2 (A) shows the nano-pore array (inverted microscope used for imaging). Mouse embryonic stem cells (MEFs) were pre-stained with Hoechst and randomly seeded on the chip (Fig. 2(B)). Propidium iodide (PI dye) was then injected into cells using high voltage (150 V) electroporation. The results demonstrate that the 3D NEP platform achieved a high efficiency of intracellular delivery (Fig. 2(C)), as well as a high cell viability indicated by Calcein AM (Fig. 2 (D)). The 3D NEP can provide a real high throughput where > 50, 000 cells are transfected on a 1 cm^2 chip.

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[1] Boukany, P.E., *et al*. 2011, Nature Nanotechnology, 6, 747.
[2] Gao, K.L, *et al*. 2013, Small, *DOI: 10.1002/smll.201300116.*

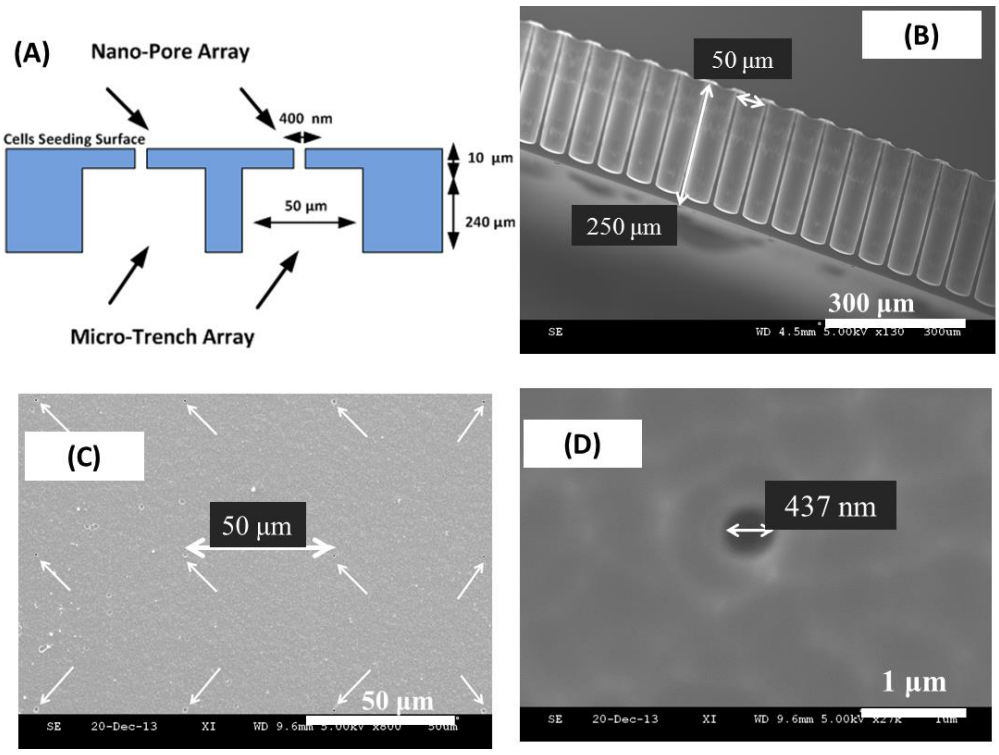

Figure 1: The 3D NEP chip schematic and micrographs: (A) The schematic of 3D NEP chip. Cells are seeded on the flat surface for electroporation; (B) the cross-section of micro-trench; (C) the arrangement of nano-pore array (top-view). The pore-pore distance is 50 µm. The pores are indicated by white arrows. (D) zoom-in image of a single nano-pore, with the size ~ 400 nm.

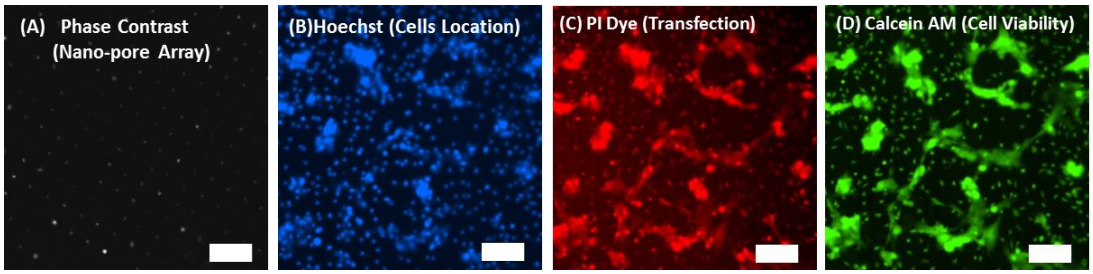

Figure 2: The 3D NEP chip for high throughput cell transfection: (A) The phase contrast show the nano-pore array, visualized by white spots array; (B) Cells (MEFs) were pre-stained with nuclei dye, Hoechst, for visualizing the cells location; (C) PI dye showed red fluorescence right after electroporation (150 V, 10 ms, 5 pulses). A high transfection efficiency was obtained by comparing (B) and (C); (D) Transfected cells were stained with Calcein AM after 2 hours. Calcein AM indicated a high cell viability after electroporation. Scale bar = 50 µm.