Dielectrophoresis-assisted 3D nanochannel electroporation for high-throughput cell transfection with dosage control

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Precise transfection of large cell populations by non-viral methods is critical for many frontier biomedical applications such as gene therapy, regenerative medicine and cancer vaccine. However, this is not achievable by any of the existing methods. We reported a unique electro-transfection technology, nanochannel electroporation (NEP)[1], which can precisely control the dosage when delivering exotic molecules into living cells with negligible cell damage. However, the previously reported 2D NEP device can only handle a small number of cells making it undesirable or infeasible for many clinical applications. Recently we demonstrated a 3D microchannel electroporation (MEP)[2] capable of handling a large number of cells for transfection with little observable cell damage, but with poor dosage control. Herein, we report a novel 3D NEP system for precise, benign, and high-throughput (from 10,000 up to million) cell transfection (Figure 1(A)).

A Si-based nanochannel array chip was fabricated using projection photolithography and deep reactive ion-etch (a modified Bosch Process), as shown in Figure 1(B). 650 nm nanochannel arrays were adopted for electro-transfecting cells in the Z-direction. Due to the fact the NEP cannot occur if the cell is far from the nanochannel, dielectrophoresis (pDEP) was developed for precisely localizing individual cells in the proximity of nanochannels, as shown in Figure 1(C). By optimizing the working voltage and frequency of pDEP, high efficiency cell-array trapping was achieved (Figure 1D), a critical factor for achieving efficient cell electroporation. When compared to conventional bulk electroporation (BEP), the NEP chip shows a 10-20 fold improvement in dosage control and uniformity, while still maintaining high cell viability (>90%) even in "difficult to transfect" cells such as cardiac cells. The applied voltage is also investigated by numerical simulation to discern optimal electroporation conditions and account for any variable cell positioning after seating. Clinical testing was carried out, using the 3D DEP-NEP system for genetically engineering difficult-to-transfect natural killer (NK) cells with plasmids encoding for the chimeric antigen receptor (CAR); a model of high relevance for adoptive immunotherapy applications.[3] The system offered significantly higher CAR transfection efficiencies (>70%) compared to conventional bulk electroporation (<30%), as shown in Figure 2. The 3D DEP-NEP system provides an innovative and medically valuable platform with uniform and reliable cellular transfection, allowing for a steady supply of healthy, engineered cells.

[1] Boukany, P.E., *et al*. 2011, Nature Nanotechnology, 6, 747.
[2] Chang, L., *et al*. 2014, Small, DOI: 10.1002/smll.201402564
[3] Porter, D.L., *et al*. 2011, New England Journal of Medicine, 365, 723.

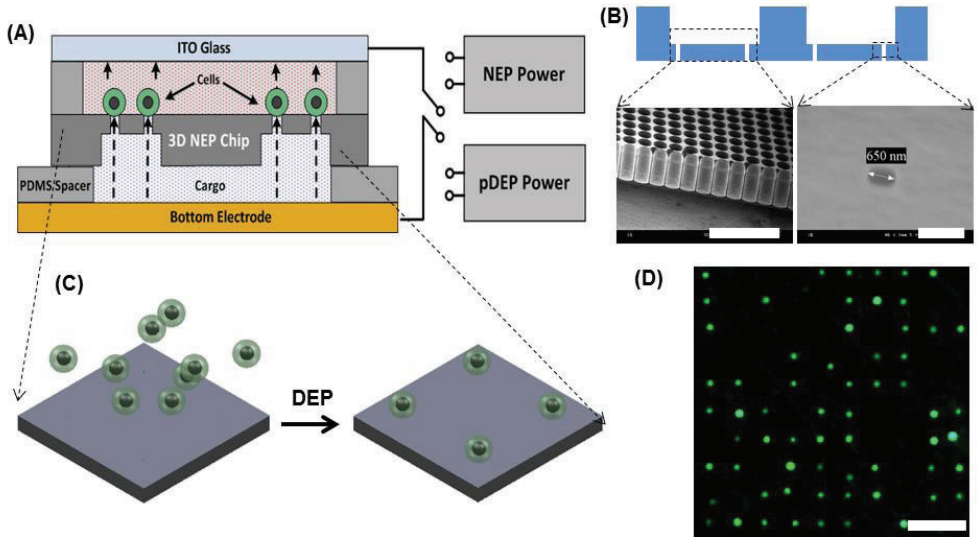

Figure 1: Fabrication and assembly of DEP - 3D NEP System: (A) The schematic of DEP - NEP system; (B) NEP chip schematic with scanning electron micrographs showing microchannel (left, scale bar = 500 μm) connecting nanochannels (right, scale bar = 1 μm); (C) Positive DEP (pDEP) is turned on for positioning the randomly loaded cell specific to nanochannel array, which leading to (D) a well-ordered cell array (NK-92 cell, stained with Calcein AM) on the chip.

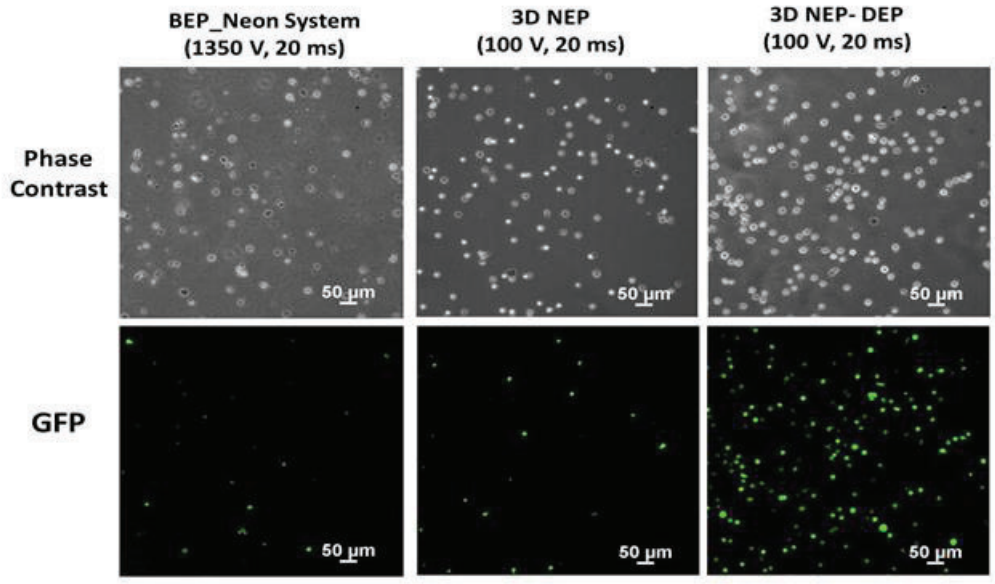

Figure 2: The DEP- NEP system for high-throughput NK cells engineering with chimeric antigen receptors (CAR) plasmid. Fluorescence and phase contrast images demonstrated DEP-NEP system showed significantly higher efficiency than BEP for transfecting NK cells, a difficult-to-transfect cell.