A high-throughput nano-electroporation platform for large-scale manipulation and transfection of adult mouse cardiomyocytes

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Electroporation is one of the most commonly-used physical techniques for gene/drug delivery. Compared to chemical methods, electroporation can bypass the endocytosis, thus overcoming the immune-responses from the cells. Commercial bulk electroporation systems (BEP) have been widely used *in vitro* and *in vivo*. However, these BEP designs usually result in serious cell damage and low transfection efficiency. Moreover, the applications of BEP in gene transfection have great limitations in certain cell types that are susceptible to electric fields. For instance, adult mammalian cardiomyocytes, whose major functions (beating for hearts) could be easily affected by external electric shocks, are usually led to apoptosis in BEP. Safe and efficient electroporation systems for gene delivery into adult cardiomyocytes *in vitro* are currently lacking[1]. Nanoscale electroporation (NEP) systems are recently reported for on-chip transfection of single cell with unique advantages[2]. However, most of them require sophisticated cell manipulation techniques, while compromising on cell numbers and working time[3].

In this work, we report a high-throughput nanochannel electroporation technique for on-chip manipulation and transfection of large-scale single cells. Our platform, based on a 3D nanochannel array chip, enables massive-parallel delivery of functional cargo into cellular array on the chip. A novel 'dewetting' method is applied to trap cells on 3D NEP chip for electroporation at high-throughput (Fig.1a). Micro-cap arrays are patterned on 3D NEP chip (Fig.1b) for localizing cells to the top of nanochannels. Cells trapped on the chip are delivered with cargos on a 3D NEP) platform (Fig.1c & d). By dewetting, tight-contact between cells and corresponding nanochannels is achieved (Fig.1e). Such a device can achieve electrophoresis of charged cargo in nanochannels, thus capable of controlling the dosage of molecules delivered into living cells (Fig.1f).

This 3D NEP platform is applied to transfection of adult mouse cardiomyocytes. In a sharp contrast to a commercial BEP system, our device has demonstrated safe and deterministic gene transfection at high-throughput. Fig.2a shows the primary cardiomyocytes delivered with GFP plasmids by 3D NEP started expressing GFP fluorescence within nuclei as ea rly as 6 hr after treatment. Fig.2b indicates that the metabolism of cardiomyocytes in vitro is much faster than commonly-seen cell types which start GFP expression ~ 12 hr after delivery (Fig.2b). Our platform has further demonstrated precise dosage control in delivery of biomolecules into adult mouse cardiomyocytes, by either working voltage (Fig.2c) or pulse lengths (Fig.2d). The unique performance of 3D NEP, assisted with the 'dewetting' method, provides a clinical-valuable platform for gene transfection of mass cells that cannot be accessed by reported electroporation devices. Compared to previous technologies, such a method is simple and quick and therefore it would be more applicable to clinical use.

[1] Klauke, N. *et al.* 2003, Biophysical Journal, 85, 1766.
[2] Boukany, P.E., *et al*. 2011, Nature Nanotechnology, 6, 747.
[3] Kang, W. *et al.* 2013, Nano Letters, 13, 2448.

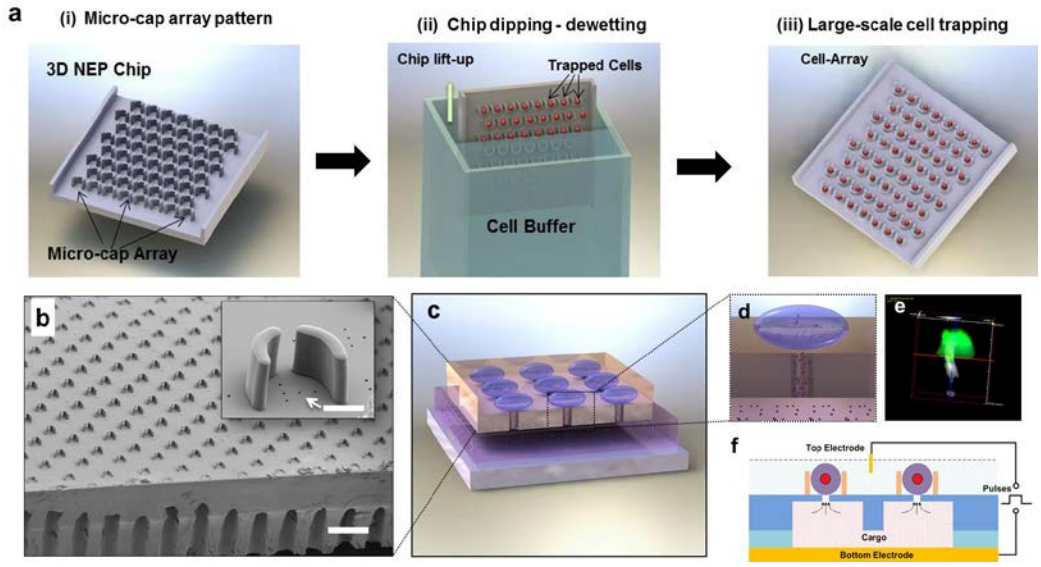

Figure 1:'Dewetting' technique for on-chip cellular array trapping and high-throughput gene transfection on 3D NEP platform: (a) The schematic of dewetting procedure; (b) Patterning micro-cap array on 3D NEP chip for cell trapping. scale bar: 100μm, Inset 5μm; (c) The schematic of 3D NEP platforms with large-scale cells trapped on the chip; (d) gene delivery into localized cell through nanochannel; (e) confocal microscopy image shows cellular-nanochannel contact; (f) the setup and working mechanism of 3D NEP platform.

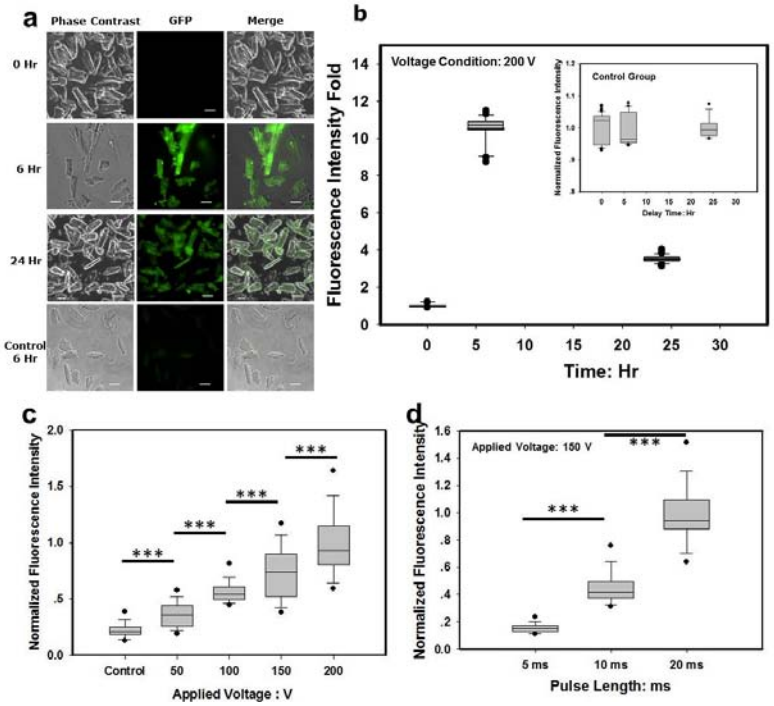

Figure 2: Precise and safe transfection of adult cardiomyocytes at high-throughput. (a) Time elapse GFP fluorescence expression after GFP plasmids delivery by 3D NEP. (b) The GFP fluorescence intensities as a function of elapsed time after NEP. Precise gene transfection with dosage control can be achieved by applied voltage and pulse length.