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Cell electroporation with a three-dimensional microelectrode array on a printed circuit board

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Electroporation is a commonly used approach to rapidly introduce exogenous molecules into cells without permanent damage. Compared to classical electroporation protocols, microchip-based electroporation approaches have the advantages of high transfection efficiency and low consumption, but they also commonly rely on costly and tedious microfabrication technology. Hence, it is desirable to develop a novel, more affordable, and effective approach to facilitate cell electroporation. In this study, we utilized a standard printed circuit board (PCB) technology to fabricate a chip with an interdigitated array of electrodes for electroporation of suspended cells. The electrodes (thickness ~ 35 μm) fabricated by PCB technology are much thicker than the two-dimensional (2D) planar electrodes (thickness < 1 μm) fabricated by conventional microfabrication techniques and possess a smooth corner edge. Numerical simulations showed that the three-dimensional (3D) electrodes fabricated by PCB technology can provide a more uniformly distributed electric field compared to 2D planar electrodes, which is beneficial for reducing the electrolysis of water and improving cell transfection efficiency. The chip constructed here is composed of 18 individually addressable wells for high throughput cell electroporation. HeLa, MCF7, COS7, Jurkat, and 3T3-L1 cells were efficiently transfected with the pEGFP-N1 plasmid using individually optimal electroporation parameters. This work provides a novel method for convenient and rapid cell transfection and thus holds promise for use as a low-cost disposable device in biomedical research.

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1. Introduction

Being able to introduce various impermeable molecules such as DNA, RNA, and proteins into living cells in vivo or in vitro is important for many cell biological experiments. Among the numerous established methods for biomolecule delivery (i.e., viral vectors [1], liposome fusion [2], calcium phosphate co-precipitation [3], and gene guns [4]), electroporation is a non-toxic way to introduce exogenous molecules into cells by transiently and reversibly permeabilizing the cell membrane with electrical pulses, yielding a high transfection rate [5]. Therefore, electroporation has been applied for many biomedical approaches, including gene transfer [6], protein introduction [7], drug delivery [8], and other specialized applications [9–11].

Currently, microchip-based electroporation methods are attracting great interest because they consume small amount of cells and biomolecules, as well as improve cell viability by reducing cell damage induced by high voltage, compared to conventional cuvette-type electroporation systems. Additionally, unlike traditional bulk electroporation, microchips enable the high throughout electroporation of various molecules and cell types, providing an ideal tool for gene function screening [12–18]. These chips with the microelectrode array (MEA) on the bottom are easily acceptable for potential compatibility with cell culture plates. Lin et al. utilized an MEA chip for electroporation in 2004 [13] and then integrated electrophoresis with the electroporation process to enhance gene transfection efficacy in 2007 [15]. Yamauchi et al. [14] and Jain and Muthuswamy [16,17] constructed chips with individually addressable square electrode arrays for site-specific transfection of target cells, which made parameter optimization more convenient than traditional bulk transfer methods. Recently, Huang et al. [18] reported an electroporation chip with an angular formatted MEA that is compatible with standard multi-well plates for the transfer of plasmid DNA and synthetic siRNA into various types of cells.

Although the usefulness of MEA-based electroporation chips for cell transfection was presented in the abovementioned studies, the practicability of these electroporation chips has not been comprehensively accepted by biologists. A major reason is that the MEA chips rely on microfabrication processes that involve lithography, metal sputtering, metal lift-off or etching, and dielectric deposition, which are costly, time consuming, and require specialized equipment. In contrast, as a mature technology in electronics industry, printed circuit board (PCB) technology can quickly achieve mass cost-effective lab-on-a-chip (LOC)

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devices. Currently, PCB technology has a resolution of tens of micrometers, which is sufficient for many LOC applications [12,19]. In addition, the biocompatibility of materials used in PCB manufacturing processes has been demonstrated for multiple cell types [20,21], indicating the potential for PCB-based chips in development of biological platforms. Indeed, PCB-based chips for dielectrophoresis have already been used in cell manipulation [22], positioning [23], noncontact electroporation [24], and impedance measurement [25].

In this study, we demonstrate a simple and economical approach for electroporation of suspended cells using interdigitated electrodes fabricated on a PCB. In a previous study [24], the border areas of the electrodes with rectangular cross-section on the PCB were covered with a photosensitive epoxy layer and therefore the electrodes functioned as the common planar electrodes. Here we used naked gold-coated electrodes fabricated by standard PCB technology to generate electric filed for cell electroporation, which were found to be beneficial for improving electroporation efficiency. The scanning electron microscopy (SEM) images showed that the gold-coated electrodes fabricated by PCB technology present a three-dimensional (3D) profile with smooth corner. Numerical simulations demonstrated that the electric field distribution among 3D electrodes was more uniform than that among two-dimensional (2D) electrodes. Moreover, in 3D electrodes, the electrolysis of water was less obvious, and transfection efficiency was higher compared to 2D electrode controls. Our PCB-based electroporation chip consists of 18 individually addressable wells, facilitating the optimization of the electric pulse parameters for electroporation. Using this chip, we successfully transferred the pEGFP-N1 plasmid into several cell types with high transfection rates.

2. Experimental section

2.1. Materials and reagents

Pyrex glass wafers were purchased from Corning Inc. (New York, NY). Polydimethylsiloxane (PDMS, Sylgard 184) was supplied by Dow Corning, Inc. (Midland, MI). Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 media, fetal bovine serum, trypsin/EDTA solution, penicillin, and streptomycin were purchased from Gibco BRL Inc. (Grand Island, NY). All other reagents were supplied by Sigma-Aldrich (St. Louis, MO). The hypoosmolar electroporation buffer was composed of 25 mM KCl, 0.3 mM KH_2PO_4, 0.85 mM K_2HPO_4, and 90 mM myo-inositol. The pH value of this medium was adjusted to 7.4 at 25 °C.

2.2. Chip design and fabrication

The electrodes were designed using commercial electric circuit layout software (Altium Designer Winter 09, Altium Corp.) and fabricated using standard PCB technology. The PCB had 1-mm-thick FR-4 material as the insulating substrate and a 40-μm-thick metal layer on the surface as the electrodes. The interdigitated electrodes used for electroporation were 150 μm in width, and the inter-electrode distance was 250 μm. To avoid the toxicity of copper, the naked electrodes on the substrate were coated with gold. With this treatment, the electroporation electrodes were biocompatible, and the corner edge of the electrodes became smooth.

After the PCB was produced, the electroporation chip was encapsulated as shown in Fig. 1. Machined polymethyl methacrylate (PMMA) cavities (6 mm in diameter, 8 mm in height) were assembled on the electroporation area of the PCB to provide an environment for cell suspension. PCB and PMMA cavities were glued with polydimethylsiloxane (PDMS) pre-polymer and incubated over night at 37 °C to bind tightly. Dustproof PMMA lids covered the chip until use.

To compare the performance of 3D electrodes fabricated by PCB technology, a glass chip with a similar MEA pattern was produced by standard microfabrication technology [26,27], which consisted of a conductive layer of Ti/Au (30 nm/200 nm) and an insulating layer of Si_3N_4/SiO_2 (400 nm/200 nm). Reactive ion etching was used to remove the insulation layer on the electrodes and bonding pads. The PDMS cavities (6 mm in diameter, 8 mm in height) were covalently bonded with glass substrate using oxygen plasma treatment (FEMTO, Diener Plasma-Surface-Technology, Ebhausen, Germany) as a medium container. The MEA glass chip and an encapsulated device are shown in Fig. S1.

The geometry of the electrodes was assessed via scanning electron microscopy (SEM). Samples were mounted on an aluminum stage,

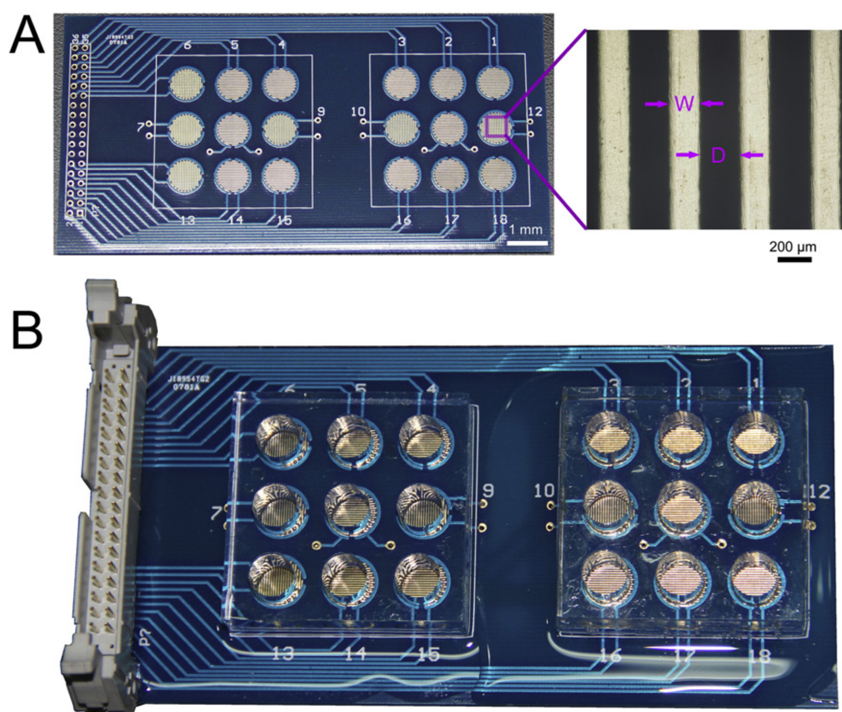

Fig. 1. Encapsulation of the PCB-based electroporation chip. (A) The PCB layer consists of an interdigitated microelectrode array. The width (W) and distance (D) of the electrodes are 150 and 250 μm, respectively. (B) The encapsulated chip with PMMA layers possesses 18 individually addressable wells for cell electroporation in parallel.

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