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Microfluidic Device for Localized Electroporation

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Abstract

Electroporation is a common method of transfection due to its relatively low risk and high transfection efficiency. The most common method of electroporation is bulk electroporation which is easily performed on large quantities of cells yet results in variable levels of viability and transfection efficiency across the population. Localized electroporation is an alternative that can be administered on a similar scale but results in much more consistent with higher quality transfection and higher cell viability. This paper discusses the creation and use of a simple and cost-effective device using porous membrane for performing localized electroporation.

Keywords

lab-on-a-chip; localized electroporation; microfluidic; cell transfection

1 Introduction

Transfection is the transfer of nucleic acids into cells for gene therapy. Common methods for transfection include viral, nanoparticles, nanostructure penetration, and electroporation. Conventional carrier-mediated delivery methods using engineered viruses, lipids and conjugated nanoparticles are limited by sizes and types of molecules, and are often cell specific [2]. More importantly, they may introduce undesirable and unsafe mutagenesis of the cell, not to mention significant cytotoxicity [3]. Physical methods such as electroporation enables delivery of molecules into a cell by modulating cell membrane permeability, specifically by inducing transient and reversible nanopores in the cell membrane [4]. Electroporation is widely utilized due to safety concerns associated with viral transfection and a higher transfection efficiency than other non-viral methods [5]. Bulk electroporation executes a high voltage in a cuvette filled with suspended cells [6]. The process results in multitude of cell damage from the strong electrical field, leading to large amount of cell death [7]. Similar to carrier mediated delivery, current electroporation techniques are only applicable to a population of cells, where uniformity and precision in dosage are beyond control [8, 9]. Further, this process is only applicable to cells in suspension.

Localized electroporation is an alternative to bulk electroporation utilizing adherent cells on a perforated surface. The perforated surface allows the cells to adhere and grow naturally, thus improving the health of the cells and providing a better reference to cells as they adhere

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in the body [1, 10]. An electric field is generated between electrodes on each side of the perforated surface with electrical conduction only occurring through nanochannels. Cells will be porated only in the membrane areas exposed to the electrical field through the pores on the perforated surface. The reduction in surface area of the membrane exposed to the electrical field means lower voltages can be used, thus smaller and more uniform electric fields can be generated, and smaller pores are produced in the cell membrane, all resulting in increased viability and transfection efficiency [10, 11]. Moreover, the adherent state of the cells allows them to be repeatedly transfected with varying molecules and observed over a prolonged period.

This chapter discusses the creation and use of a simple and cost-effective lab-on-a-chip micro-device for localized electroporation. Utilizing a porous membrane with nanopores (size ranging from 50 nm to 200 nm), the device applies voltage to a small patch of the cell membrane to effectively reduce the exposed area and thus the amount of voltage required to induce pore formation on the cell membrane. Two layers of polydimethylsiloxane (PDMS) sandwiches the membrane in the middle; the top layer provides a cell culture chamber with media, while the bottom layer consists of a microchannel for the transport of genetic materials. This simple device consists of common, biocompatible materials such as polydimethylsiloxane (PDMS) and indium-tin oxide (ITO) and is small enough to fit inside of a petri dish for cell culturing. A conductive and transparent lower electrode consisting of a glass slide coated in ITO serves as the foundation of the chip. A layer of PDMS containing microfluidic channels for the material to be delivered is bonded to the conductive surface of the glass slide. The microfluidic channels protect the cells from fluid flow and the resulting shear stresses which can be damaging to the cells [12]. A perforated polycarbonate (PC) surface is placed above the PDMS followed by a second layer of PDMS which acts as the cell culture chamber. This chapter discusses delivery of green fluorescent protein (GFP) plasmid into HeLa or HT1080 cells but the same chip has also been used to transfect differentiated neural stem cells [1].

2 Materials

2.1 Micro-device Fabrication and Assembly

1. Indium-tin oxide (ITO) glass slide, MilliporeSigma, USA.
2. Wafer
3. Optical microscope
4. Polydimethylsiloxane (PDMS)
5. Oxygen plasma machine
6. Oven
7. Pluronic F-127 (poloxamer)
8. Phosphate buffered saline (PBS)
9. Polycarbonate (PC) membrane, AR Brown, USA (*see* Note 1).

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2.2 Cell Culture

1. HeLa cells (ATCC CCL-2), American Type Culture Collection, USA.
2. Dulbecco's Modified Eagle's medium (DMEM), MilliporeSigma, USA.
3. pH indicators: L-glutamine and phenol red
4. Fetal bovine serum (FBS), MilliporeSigma, USA.
5. 1X Penicillin-streptomycin, MilliporeSigma, USA.

2.3 Plasmid DNA, Stains, and Dye

1. 2 MDa green fluorescent protein (GFP) plasmid
2. Hoechst 33342 stain, Invitrogen, USA.
3. Propidium iodide (PI) stain, eBioscience, USA.
4. Calcein acetoxymethylester (AM) dye, Invitrogen, USA.

2.4 Electroporation

1. Function generator: DS345, Stanford Research Systems, USA (*see* Note 2).
2. Voltage amplifier: OPA445, Texas Instruments, USA.
3. Oscilloscope: 9384L, Teledyne LeCroy, USA.
4. Inverted microscope: Eclipse Ti-U, Nikon, Japan.
5. Charge-coupled device (CCD) Camera: Neo-sCMOS, Andor, Northern Ireland.

3 Method

The fabrication and assembly of the microfluidic platform are performed in the clean room. Briefly, the microfluidic channel of the bottom layer is fabricated by micromolding of PDMS. A silicon model is first fabricated by deep reactive ion etching (DRIE). The top layer consists of no patterns; thus it can be directly cured from PDMS. The PC membrane is bonded between the two PDMS layers with PDMS gel. The following methods are from an existing protocol [1].

3.1 Microchannel Fabrication by Micromolding

1. Contact lithography is used to transfer photomask patterns onto the photoresist layer of a silicon wafer.
2. The silicon wafer is spin coated with a uniform layer of 1.2 μm-thick photoresist and exposed to UV light in the mask and bond aligner before being placed in the developer.
3. The patterned photoresist is used as a mask for DRIE to produce 10 μm tall silicon micropost arrays.
4. The silicon mold is cleaned with oxygen plasma and coated with the FOTS solution at 65°C for 5 minutes to remove redundant surfactant.

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5. The silicon mold is cleaned with pure heptane at 100°C for 10 minutes to strengthen the release layer.

3.2 Device assembly

1. The bottom layer has a microchannel through which solution with target molecules is introduced. The height of the microchannel is about 50 μm. To make the bottom layer, PDMS solution with a ratio of 15:1 between base and curing agent is poured onto the mold fabricated in the previous step. In order to minimize the distance from microchannel to cells, the bottom layer needs to be very thin, typically 2–3 mm. Bake at 120^{oC} for 30 min.
2. The top layer is a piece of PDMS without any patterns. Therefore, the same PDMS solution is poured onto a clean silicon wafer. The top layer needs to be thick enough to hold cell culture solution (~ 6 mm). Bake at 120^{oC} for 30 min.
3. Gently peel off the PDMS layers from silicon wafers prepared in Step 1 & 2. Stack two layers together, but make sure the side without microchannel patterns faces to the top layer.
4. Cut the assembly into individual pieces following the microchannel patterns with a razor blade. After cutting, punch through the assembly with a 4 mm puncher to make the cell culture chamber. Make sure the hole is laid in the center of the microchannel. Separate two layers which are ready for final assembling described in the following steps.
5. The PC membrane has different roughness on two sides. The smooth side gives more light reflection, which can be easily identified with naked eyes. Place a piece of the PC membrane on a cutting pad with the smooth side facing up. Cut the membrane into circular pieces with a 6 mm puncher.
6. The top and bottom layers are placed under UV plasma for cleaning (*see* Note 3).
7. Spin coat a clean silicon wafer with PDMS solution with a ratio of 5:1 between base and curing agent under 3000 rpm for 30 seconds. Ink the side without microchannel of the bottom layer by bringing the PDMS layer in direct contact with the PDMS solution on the wafer. Place the bottom layer on side with the inked side facing up. Place a PC membrane piece on top of the bottom layer and make sure the center of the membrane aligns with the center of the chamber and the smooth side facing up.
8. Ink the top layer and bring the inked side in direct contact with the inked surface of the bottom layer, such that the PC membrane is sandwiched in between.
9. Gently press the assembly with a tweezer to make sure the contact is solid. Bake the final device at 65^{oC} for 1 hour.
10. Inlet and outlet are then introduced using a 2 mm and 1 mm punchers, respectively.

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3.3 Cell Culture and Plasmid DNA

1. Cells in suspension are placed in the cell culture chamber of the device.
2. The device is placed in an incubator at 37°C and 5% CO_2 for 24 hours (*see* Note 5).
3. Cells should adhere to the PC membrane after 24 hours. This can be easily verified by microscope imaging.

3.4 Electroporation

1. 10 μL of a 3:1 (V/V) solution of PBS and GFP plasmid in DMEM at the desired concentration was placed in the cell culture chamber (*see* Note 6).
2. The electrodes are connected to the function generator, voltage amplifier, and oscilloscope.
3. The resistance is measured to test the electrical connection (5–10 kΩ).
4. Bi-level electric pulses are applied for electroporation (*see* Note 7).

4 Notes

1. PC membrane pore size is an important parameter because it determines the size of the pore generated in the cell membrane and thus influences cell viability and cargo delivery. Smaller pores result in higher viability and dosage control yet limit cargo size. PC membrane pore size also affects the membrane channel resistance and therefore the voltage delivered to the cell membrane. The most effective pore size choices are 50 nm and 200 nm.
2. The function generator is used to produce the electric field for electroporation and therefore must be able to operate at the chosen frequency and voltages. A commercialized electroporation system from BioRad, Gene Pulser II, can also be used for this purpose.
3. Oxygen plasma cleaning surfaces makes them more hydrophilic, allowing them to bond to other hydrophilic surfaces. Oxygen plasma cleaning also reduces the conductivity of ITO coatings so exposure of the glass slides should be minimized [13].
4. These settings are recommended for culturing HeLa or HT1080 cells but may vary depending on the cell line used.
5. Concentrations of 0.01–1 μg μL^{-1} have been used for this experiment. Concentrations of 0.01 or 0.02 μg μL^{-1} are recommended because values closer to 1 μg μL^{-1} yielded a lower transfection efficiency [1].
6. The parameters for the electric pulses depend on the PC membrane selected (*see* Note 1). Generally, pulses are most effective when they consist of a short high voltage pulse alternated with a long low voltage pulse [14], but this was not performed with this device. The high voltage pulse must exceed 0.2–1 V *at the membrane* for transfection to occur [15]. For a PC membrane with pores that are

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2 μm in diameter and 24 μm in length, high voltage pulses of 10–20 V have been used and 7.7 V is the theoretical minimum [1].

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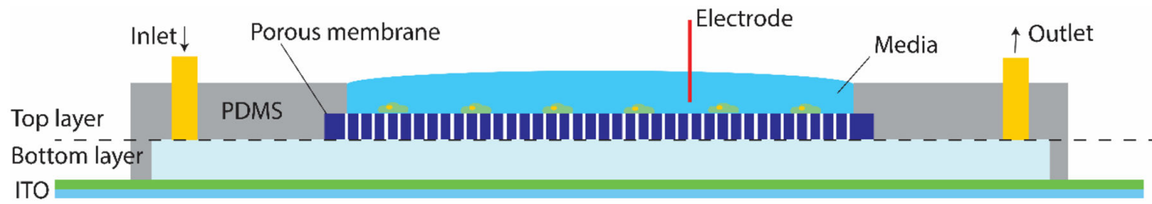

Fig. 1.
A schematic of the localized electroporation device. The device consists of three layers: the top PDMS layer, the bottom PDMS layer and the ITO glass layer. The PC membrane is sandwiched between the two PDMS layers. The top PDMS layer provides a cell culture chamber, where cells can grow on top of the PC membrane. The bottom layer is equipped with a microfluidic channel and an inlet and outlet to facilitate the transport of buffer solution containing the genetic materials to be transfected. The microfluidic channel is made of PDMS molding with a silicon mold. Reproduced from Ref. [1] with permission from The Royal Society of Chemistry.