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A porous membrane-based culture substrate for localized *in situ* electroporation of adherent mammalian cells

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Abstract

We report herein the invention of and proof of function for a porous membrane-based electroporation device that can deliver molecules into spatially restricted and predefined areas of a cell monolayer. The device's cell culture substrate is a microporous alumina membrane (pore size 0.02 μm), with an underlying thin poly-(dimethylsiloxane) (PDMS) film that has one or more holes with diameters in the one-tenth millimeter range. When a transient electric field is generated between the device's two planar electrodes – one of which is placed above the cells and the other below the PDMS layer – the filed condenses only in the volume defined by hole in the PDMS film and therefore localized electroporation can occur. We demonstrate that Lucifer Yellow (LY) and plasmid DNA are selectively introduced into only those HeLa cells located above the holes. Using the device containing a PDMS film with multiple holes, a patterned array of LY-stained cells was resulted. Compared with the operation of and the results obtained from a conventional cuvette electroporation device, our device greatly decreases the necessary operating voltage, can be used with cells attached to a substrate, and increases the number of conditions that can be screened in a single experiment. Finally, since a PDMS film with different sized holes, produces different localized electric field strengths, it is possible to determine the optimum electroporetic conditions in a single experiment.

Keywords: Electroporation; Cell culture; PDMS

1. Introduction

Intracellular delivery of biological molecules such as DNA, proteins and drugs is an important biomedical technique with numerous applications. Depending on the experimental goal, different delivery methods based on biological (viral vector [1]), chemical (calcium phosphate precipitate [2], liposomale [3,4]), and physical [5] (electroporation, microinjection, sonoporation, and laser irradiation) techniques, are used. Certain mammalian cell-based *in vitro* bioassays require delivery of a molecule into cells that are attached to a substrate [6–9]. Additionally, controlled and localized molecular delivery into pre-determined regions of a cell monolayer is essential for cell–cell communication studies and for high-throughput analyses that involve cellular microarrays [10]. Ziauddin and Sabatini [11] developed the 'reverse transfection technique'. Their method involves incorporating a plasmid microarray into a cultivation substrate so that when cells first adhere to the substrate they are genetically modified by the underlying DNAs. Presently, such transfected-cell microarrays are the preferred platform for fundamental genomic studies [12–15]. The technique involves cationic lipid-enhanced, spontaneous cellular uptake of DNAs and transfection occurs during or shortly after cell adhesion. Conversely, electric field-induced temporal permeablization of cells, i.e. electroporation, can be used to control the timing of gene delivery [16–19]. For example, Yamauchi et al. [20,21] designed a plasmid DNA-loaded electrode array that, as the cellular substrate, can be manipulated to control spatial and temporal cell transfection. There are many reports concerning electroporation of cells attached directly to micropatterned electrode surfaces [22–28]. Unfortunately, cell damage often accompanies electroporation owing to direct contact between the cells and the electrode.

Recently, we designed and validated the function of a microsystem that can micropattern chemicals onto predetermined sections of a cellular monolayer or line pattern [29,30]. The system is a permeable filter membrane that is masked on

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its underside by a PDMS film stenciled with holes. Using cardiac myocytes attached to the membrane, we showed that when octanol, a gap junction inhibitor, was delivered to the underside of the membrane/PDMS film only the intercellular communications of cells that were not masked by the PDMS film, i.e. were located above the holes, were inhibited [30]. For the work reported herein, we converted this porous membrane-based local-dose system to one that uses localized electroporation by placing electrodes on both sides of the membrane. Since these electrodes are not in direct contact with the cultured cells, the usual problems associated with electrolysis of cells in direct contact with the electrode are eliminated [31,32]. The electric field geometrically condenses at the holes of the PDMS film, which, in turn, causes electroporation only for cells above the holes. When the underlying PDMS film has multiple holes, an array of modified cells can be produced. In addition, as the size of a hole controls the local electric field strength, a PDMS film with holes of different sizes can be used to efficiently screen for the best electroporation condition. Herein, we prove the preceding claims through experiments involving electroporation-assisted LY-uptake and DNA transfection of HeLa cells.

2. Experimental

2.1. Plasmids preparation

The pDsRed-C1 plasmid (Clontech) encoding for the enhanced red fluorescent protein (DsRed) was propagated in competent *Escherichia coli* (BL21(DE3)) cells. The plasmid was extracted and purified using the EndoFree Plasmid Maxi Kit protocols and chemicals (Qiagen). The pDsRed solution concentration was calculated from the solution's absorbance at 260 nm.

2.2. The design and construction of the porous membrane-based electroporation device

Fig. 1 includes illustrations of the device's architecture and a generalized experimental procedure. The cell culture substrate system is a commercially available, alumina membrane culture insert (161395, NUNC; pore size = 0.02 μm) with a thin polydimethylsiloxane (PDMS) film attached underneath. A brief description summarizing the construction of a PDMS film, whose pattern is copied from a 0.1 mm thick columnar photoresist pattern etched into a glass plate [29,30], follows. The PDMS polymer was prepared by first pouring the prepolymer (10:1 KE-106:CAT-RG; both of which were purchased from Shin-Etsu Chemical) over the glass template. Then a smooth glass plate was placed on top of the prepolymer and the two plates calmped together. Finally, the system was heated at 100 °C for 1 h to cure the polymer. The polymerized PDMS film was peeled off the template and placed underneath the culture insert with the adhesive nature of PDMS providing a tight seal [29].

To make the planar Pt electrodes, Ti was deposited onto a glass plate and then Pt was deposited on top of the Ti layer. The Pt-deposited plate was cut into pieces with areas of 2.5 cm^2(top electrode) or 6.0 cm^2(bottom electrode). One electrode was placed above and another below the membrane. The gap between the two electrodes was always 3 mm.

2.3. Cell culture

A HeLa cell line was used. The cells were donated by the Cell Resource Center for Biomedical Research (Tohoku University) and maintained in GIT medium (Wako Pure Chemicals) supplemented with 100 Units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Gibco), under a 5% CO_2 atmosphere at 37 °C. When cell densities reached ~80% confluency, cells were detached by a 0.25% trypsin/0.01% EDTA solution (Gibco) and then further propagated or used for experimentation.

Before culturing cells on the membrane-based substrate, it was first treated with O_2 plasma (60 W, 30 s). This treatment makes the polymer surface hydrophilic, which prevents bubble formation in a PDMS hole. Next, the substrate was immersed in a fibronectin solution (50 μg mL^{-1}) and incubated at room temperature for 30 min. After the substrate was rinsed with PBS, HeLa cells were seeded onto the substrate at a density of 1×10^5

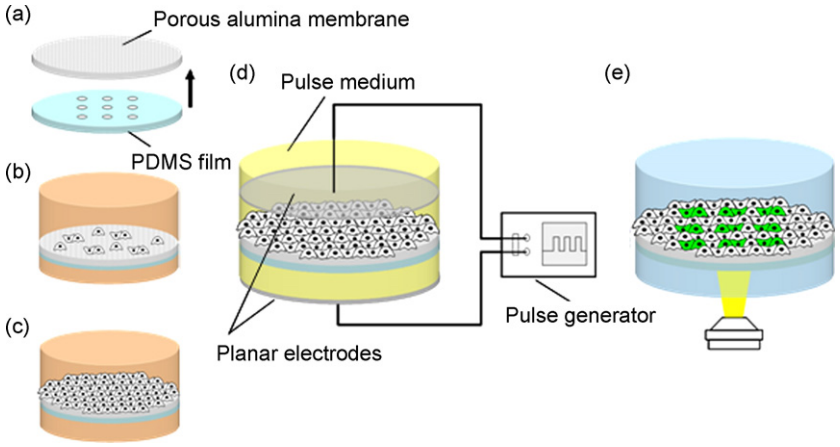

Fig. 1. Schematics diagramming the general electroporetic protocol (a) preparation of the membrane-based culture substrate, (b) cell seeding, (c) cell growth to confluency, (d) insertion of the electrodes and addition of pulse medium containing the molecular delivery into the chamber, and (e) microscopic imaging of the cellular sheet after electroporation.