Highly Efficient Electro-permeabilization of Mammalian Cells Using Micro-electroporation Chip

Young Shik Shin[*], Jung Kyung Kim[**], Sun Hee Lim[**], Young Kyung Lee[**], Keunchang Cho[**], Yongku Lee[*], Seok Chung[**], Chanil Chung[**], Dong-Chul Han[*] and Jun Keun Chang[**, ***]

[*] School of Mechanical & Aerospace Engineering, Seoul National University, Seoul, 151-742, Korea
[**] Digital Bio Technology Co., Seoul, 151-742, Korea
[***] School of Electrical Engineering and Computer Science, Seoul National University, Seoul, 151-742, Korea

ABSTRACT

Electroporation, is widely used method that can be directly applied to the field of gene therapy. However, very little is known about the basic mechanisms of DNA transfer and cell response to the electric pulse. As a more convenient and effective tool for the investigation on the mechanisms of electroporation and the enhancement of the functional efficiency, we developed a micro-electroporation chip with polydimethylsiloxane (PDMS). Owing to the transparency of PDMS, we could observe the process of Propidium Iodide (PI) uptake in real-time, which shows promise in visualization of gene activity in living cells. Furthermore, the design of the electroporation chip as a microchannel offers advantages in terms of efficiency in permeabilization of PI into SK-OV-3 cells. We also noticed the geometric effect on the degree of electroporation in microchannels with diverse channel width, which shows that the geometry can be another parameter to be considered for the electroporation when it is performed in microchannels.

Keywords: electroporation, PDMS, microchannel, bio-MEMS, propidium iodide

1 INTRODUCTION

Electroporation is widely used method to introduce macromolecules into living cells by applying electric pulses. Under the high electric field, cell membranes are transiently rendered to porous and they become permeable to otherwise impermeable foreign materials. Electro-permeabilization depends on several factors: pulse amplitude, pulse duration, the number of pulses and other experimental conditions. With those parameters, many research groups have performed various theoretical as well as experimental studies about the electroporation to understand the mechanism and to enhance the efficiency of the transfection.

As the theory about the mechanisms of electroporation and gene transfer has not been developed well, the visualization is another important issue on the electroporation. Golzio *et al.* investigated the DNA transfer process at the single-cell level by using fluorescence microscopy digitized imaging [1]. Deng *et al*. assessed the effect of intense submicrosecond electric pulses on cells by means of temporally resolved fluorescence and light microscopy [2].

In common electroporation processes, a cuvette which is consisted of two parallel electrode plates is used to contain the mixture of cell suspension and genes. By applying a high electric field across those two electrodes, it is possible to deliver genes into cells. Aluminum is commonly-used material for disposable cuvettes. In this case, however, the release of Al^{3+} from the aluminum electrode, which has adverse effects on cells, has been observed by several research groups. In case of using aluminum electrodes, different field strength is expected because of the significant voltage drop across the oxide layer on the electrode surface. Therefore, although it is more expensive, platinum or gold electrodes are more advantageous [3].

In this work, we designed a micro-electroporation chip which has a microchannel and investigated the characteristics of electroporation induced by that specific design. The microchip was fabricated with polydimethylsiloxane (PDMS) by using MEMS techniques. PDMS has a great potential as a fabrication material. It is easy to fabricate, inexpensive, transparent, and biocompatible. Owing to the transparency of PDMS, it is possible to observe the process of transfection in real-time. Furthermore, PDMS chips can be easily integrated with other systems because of many preceded works using PDMS such as PCR, CE, mixer and filter. To show the advanced functionality of the micro-electroporation chip, we observed Propidium Iodide (PI) uptake into SK-OV-3 cells by means of fluorescence and light microscopy. To check additional effects induced by miniaturization, we performed experiments in different conditions by changing the width of microchannel.

2 MATERIALS AND METHODS

2.1 Fabrication of electroporation chips

The micro-electroporation chip has a simple design which contains a microchannel and two reservoirs for inlet and outlet. The channel height is 20 μm and the length is 2 cm. We varies the channel width as 100, 200, 300, 400, and 500 μm. Figure 1 shows the fabricated micro-electroporation chip.

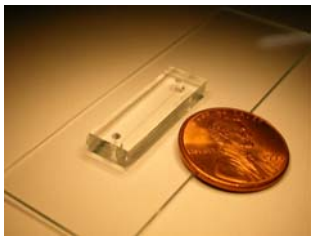

Figure 1. Fabricated micro-electroporation chip.

The micro-electroporation chip was fabricated with the replica molding method. The microchannel patterns were fabricated by photolithography using chrome photo mask. Figure 2 shows the fabrication process of the micro-electroporation chip schematically. Negative photoresist (SU-8, MicroChem, MA, USA) was spin-coated onto a silicon wafer to create a mold master of 20 μm thick structure. After soft-bake, the pattern of the mask was transferred to the SU-8 coated silicon wafer by mask aligner (MA-6, Karl Suss GmbH, Germany). Post-exposure bake, developing and hard baking of the exposed SU-8 patterns are followed by pouring the mixture of PDMS and curing agent on the pattern (Sylgard® 184, Dow Corning Co., USA). The curing condition was 120 °C for 30 min. The PDMS replica was bonded with a glass substrate to form a microchannel by 25 W oxygen plasma treatments.

2.2 Cell preparation

SK-OV-3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Sigma), penicillin (100 units/ml), streptomycin (100 μg/ml) and L-glutamine (4 mM) at 37 °C in a humidified 5% CO_2 incubator. Cells were dissociated from the 25 cm^2 tissue culture flask by using Trypsin-EDTA. The final cell suspension density was adjusted to 1×10^7 cells/ml.

Propidium Iodide (PI) was added to the cell media before applying an electric pulse. PI is a fluorescent marker that intercalates into nucleic acid molecules. When cell membrane becomes permeable, PI enters the cell and emits red fluorescence by binding to nucleic acids in the nucleus. Quantitative study is possible because the intensity of red fluorescence varies with the amount of PI bound to the nucleic acids. For the experiments, PI (1.0 mg/ml) was added to cell media as the ratio of 1:20 (v/v).

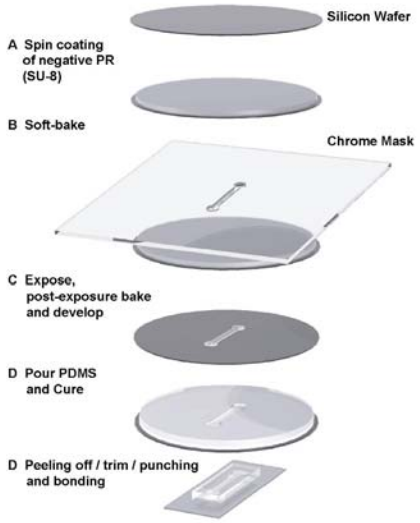

Figure 2. Schematic process for the fabrication of the micro-electroporation chip

2.3 Experimental setup

Experimental setup for the electroporation is consisted of a home made power supply, Pt electrodes and an electrode holder. As the electrode holder was mounted on a microscopy, we could observe the process of electro-permeabilization while applying the electric pulse. The power supply was connected to a computer via an analog output board (COMI-CP301, COMIZOA, Korea) and controlled with LabVIEW ver 6.1 (National Instruments, USA) program. To verify the performance of the micro-electroporation chip, a Square Wave Electroporation System (ECM® 830, BTX, USA) and a 2-mm gap cuvette with parallel-plate aluminum electrodes (BTX, USA) were used as a reference. To analyze the performance of two systems in the same electric field, we applied 200 V for cuvettes, and 2 kV for chips respectively. The electric field was 1 kV/cm and the pulse duration was 10 ms. We performed the experiments for five cases by changing the channel width: 100, 200, 300, 400, and 500 μm. Figure 3 shows the system setup for the experiment: (a) microchip and Pt electrode; (b) electrode holder on the microscopy.

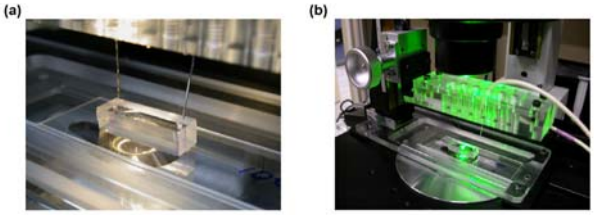

Figure 3. System setup: (a) microchip and electrode; (b) electrode holder

To observe the PI uptake, we used an inverted fluorescence microscopy (IX70, Olympus, USA) equipped with a 100 W Mercury lamp and 20×/0.4 NA objective. The excitation light was filtered by a 510~550 nm bandpass filter and induced fluorescence from the electroporated cells was filtered with a 590 nm longpass filter. 640 × 480 pixel images were acquired by a 12 bit cooled CCD camera (PCO, Kelheim, Germany) at 10 Hz frequency.

3 RESULTS AND DISCUSSION

3.1 Electrolysis effect while applying electric pulse

During the electroporation within a cuvette, a two-phase layer of liquid and gas was created because bubbles were generated electrochemically at the surface of electrodes. Figure 4 shows the bubble formation on the surface of an electrode right after applying an electric pulse. The generated bubbles rose very fast and caused complex liquid motion. This bubble movement, combined with electrophoresis during pulse, constituted non-homogeneous condition of bulk media as well as cells. Our hypothesis for the mechanism of the bubble formation is that oxide layer on the aluminum electrodes acts as a high register layer. Aluminum is the material that forms oxide layer (Al_2O_3) very easily by intervening oxidizable electrolyte. Pliquett *et al*. also showed the same hypothesis with us and explained it very minutely [4].

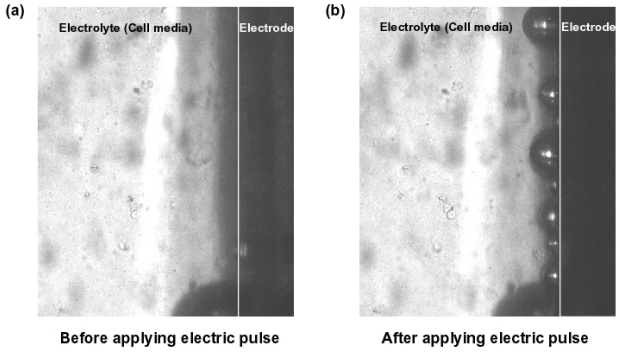

Figure 4. Bubble generation on the electrode of a cuvette: (a) before applying an electric pulse; (b) after applying an electric pulse. Applied voltage was 180 V and the pulse duration was 5 ms.

From the micro-electroporation chip, we couldn't observe bubble generation as well as complex movement. We may be able to explain this phenomenon in terms of the material. Pt electrodes that we use for the micro-electroporation experiments are very stable material. Therefore, it does not form oxide layer easily and even in case that oxide layer was formed, it can be removed with ease. As the motion of the bulk media within a cuvette is very intense, the stable condition of the media within the micro-electroporation chip can be advantageous especially for the case of investigating the mechanisms of electroporation.

3.2 Transfection rate and detection of electro-permeabilization process

The transfection rates of SK-OV-3 cells in a cuvette and a microchip were compared under the same experimental conditions: 1 kV/cm electric field and 10 ms pulse duration. In a cuvette based system, the transfection rate was 34.5 %. The transfection rate was calculated by counting the number of cells with PI uptake using an Improved Neubauer Hemocytometer (Superior, Germany). In a microchip, the permeabilization of PI was detected from almost every cell in the microchannel even though the same experimental condition was applied.

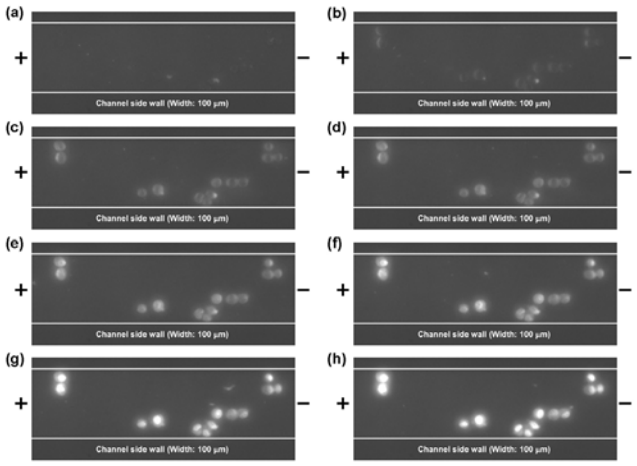

Figure 5. The PI inflow process within a 100 μm wide microchannel: before the pulsation (a) and after the pulsation (b) 1 ms; (c) 10 ms; (d) 5 s; (e) 10 s; (f) 15 s; (g) 25 s; (h) 35 s.

A localized inflow of PI in the mili-seconds was observed within microchannels after the pulse. Figure 5 shows the PI inflow process within a 100 μm wide microchannel. Right after the pulsation, the PI inflow was present only on the side facing the anode. This phenomenon shows that only the cell membrane on the left side was altered and the same observation was reported in the preceded work by Muriel Golzio *et al.* [5]. As time goes on, the fluorescence spreads into the whole cell interior (c-d) and after 10 s, the nucleus begins to emit fluorescence (e-h). This observation directly reflects the PI characteristics, binding to nucleic acids in the nucleus. The functionality of observing the real-time process in a single living cell is very advantageous because it can provide valuable information on the fundamental cellular processes.

3.3 The effect of channel width variation in the micro-electroporation chip

For microchips, the intensity of fluorescence by dye uptake was variable according to the channel width. As the channel width increases, the intensity of gray scale unit for cell area decreases even though the same electric pulses were applied. Figure 6 shows the representative microscopic images of cells for two different channels: 100 μm and 500 μm in width respectively. The electric field was 1 kV/cm and pulse duration was 10 ms. Pictures were taken at 30 s after the pulsation. It can be clearly noticed that PI uptake in a narrow microchannel (100 μm) is more than that in a wide microchannel (500 μm).

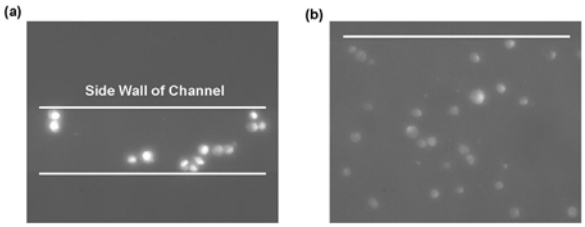

Figure 6. Fluorescence microscopic images of cells for two different channels: (a) 100 μm in width; (b) 500 μm in width. The images were taken at 30 s after the pulsation.

To compare the PI uptake for the five different cases of microchannels, images were acquired at 10 Hz and stored while performing the experiments. The image processing was performed for images at every 50 frames. The average intensity of gray scale unit for the background was subtracted from that for the cell area by using graphic software (Paint Shop Pro 7.0, Jasc Software, USA) and MATLAB program (MathWorks, Inc., USA). The comparative data for PI intensity are shown in figure 7. The effect of the channel width on PI uptake is obvious. As in a cuvette based system, the geometric parameters except for the electrode gap are not considered seriously, this unique phenomenon in a microchannel is noteworthy. Istávn P. Sugar et al. developed the low-voltage electroporator, which contains filter pores to increase local current density [6]. Owing to the large increase in current density in the filter pores, cells could be transfected by applying only 25 V. Their study was, however, based on local geometric changes for the reduction of current shunt pathways. So it can be differentiated from the phenomena that we observed in our microchips.

From this result, we could identify that we should consider the channel dimension when it comes to the electroporation in a microchannel. Further study is in progress to investigate the cause of the difference in a degree of electroporation within microchannels with various widths and the mechanism of it.

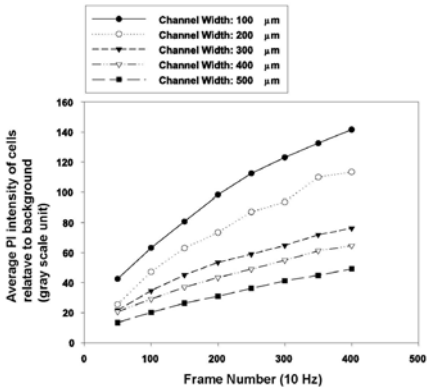

Figure 7. Average intensity of gray scale unit for cell area relative to the background for five different cases of microchannels. The relative intensity was calculated by image processing at every 50 frames. Images were acquired at 10 Hz.

4 CONCLUSIONS

We developed a micro device to perform electroporation in a microchannel. Owing to the advantageous material, PDMS, we could directly visualize the whole transfection process in real-time. In a microchannel, the bubble generation and complex motion of cell media as well as cells are not observed. This homogenious experimental condition led to the high transfection rate of PI into SK-OV-3 cells. The geometric effect on the degree of electroporation was identified through the experiments within microchannels with various channel width. This result reflects that the channel geometry can be another new parameter on the electropration when it is performed in a microchannel. Based on these experimental results, this new approach for the electroporation is expected to offer a lot of favorable possibilities for the investigation of the mechanism as well as the enhancement of efficiency.

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