ABSTRACT

In recent years, more studies focused on single cell electroporation. However, not only the position cannot be confined in the specific region of the cells, but also the employed electrodes are not transparent, causing difficulty on optical observation. Here, we demonstrated localized single cell membrane electroporation (LSCMEP), which an efficient to deliver drugs into single cell by selective and localized way from millions of cells. The ITO microelectrodes fabricated by wet etching as well as FIB technique, which successfully deployed 1µm gap between two microelectrodes. Due to submicron level gap, electric field can more intense in a narrow region. The diffusion mechanism with PI dye intensity variation has been analyzed by imaging software. In our approach, we have successfully achieved 0.99 µm electroporation regions on the cell membrane to inject PI dye into the cell with high cell viability by trypan blue test. We demonstrated the cell self-recovery process from the injected PI dye intensity variation. Our localized single cell membrane electroporation technique, not only generates well-controlled nano-pore allowing rapid recovery of cell membrane, but also provides a clear optical path potentially monitoring drug/DNA delivered into single cell level even in single molecule level.

KEYWORDS

Localized single cell electroporation, ITO micro-electrodes, FIB technique, transparent chip

INTRODUCTION

When a certain strong electric field pulses applied across a cell and tissue, then it’s have ability to rearrangement of their structure causes the permeabilization of the cell membrane named in early 1980’s “electroporation” [1-2]. In the past decades, high electric field pulses were applied to the whole cells between two large electrodes which resulted in permeabilizing the membrane of millions of cells simultaneously without reversibility [3]. In recent years, more studies focused on single cell electroporation on chip. For single cell electroporation the electric field parameter can be controlled better to avoid the cell death. For this electroporation, the electric field applied locally surrounding the single cell whereas, in bulk electroporation a homogeneous electric field applied to suspension of millions of cells together. The success rate like surviving cells for single cell electroporation is far better compare with bulk electroporation. This technique is faster and easy to perform with less toxicity and technical difficulty for application of wider tissue and cell types. However to allow selective manipulation of single organelles with in a cell, the electrode size must be reduce to nano-scale level. Thus the localized single cell membrane electroporation (LSCMEP) concept has come in last few years [4]. By this technique selective manipulation organelles and biochemical effect can be analyzed of the individual cell and this techniques more advance compare to single cell electroporation. The cell rapture and cell death can be minimized because of electric field concentrated in more localized region of the cell membrane surface compare to single cell electroporation.

EXPERIMENT

Fig. 1(a) demonstrate our experimental setup for localized single cell membrane electroporation (LSCMEP) technique, in where ITO electrodes fabricated on top of the glass (130 µm) substrate. The distance between two electrodes is 1µm. Fig. 1(b) shows an intense electric field in between two microelectrodes (Results simulated by Comsol Multiphysics).
Fig. 1. (a) Experimental setup for electroporation. (b) The Comsol Multiphysics simulation of electric-field result for 1μm gap Localized single cell electroporation chip. The electric field raises $0.7 \times 10^7$ V/m in between two electrodes.

During simulation, we consider ITO resistivity is $2.16 \times 10^{-3}$ Ω cm and top of the ITO electrodes, we consider SiO$_2$ layer with conductivity $10^{-13}$ S/m. Due to high resistance, we have applied 8V potential difference to achieve electric field $0.7 \times 10^7$ V/m from this simulation result. For our experiment, single cell distributed on top of the chip surface and those cell present in between two micro-electrodes, we applied voltages for our localized single cell membrane electroporation experiment. Fig. 2(a) demonstrates the fabrication process, where 100nm ITO film was deposited by RF sputter onto the cover glass and patterned the electrodes by wet chemical etching technique. After that we deposited 300 nm SiO$_2$ layer by PECVD process and final pattern of ITO electrodes have done by FIB technique, where, we make 1μm gap between two micro-electrodes. Fig. 2(b) shows the scanning electron microscope (SEM) image of ITO micro-electrodes with 1μm gap, while Fig. 2(c) illustrates the optical microscope image of the patterned ITO micro-electrodes.

After fabrication of our chip, Hela cells were incubated on top of the chip surface. As result, cells are distributed randomly throughout the chip surface. From randomly distributed cells, we chose such of cell, those are distributed in between two micro-electrodes for our electroporation experiment.

Figure 2. (a) Fabrication process of ITO micro-electrode based chip (b) Scanning electron microscope (SEM) image of ITO micro-electrodes with 1μm gap between two electrodes (c) Optical image of the patterned ITO micro-electrodes.

Figure 3.(a) 8Vpp 20 ms pulse, the pore is open and the cell self-recover to seal the pore(triangular shape green curve) (b) After two pulse (one pulse at zero second and another pulse at 300 second) ,the survival fluorescence images of single HeLa cell in different time scale.
PI dye was introduced into the incubation buffer to indicate the perforation status of the cells. Initially PI dye was not visible when it was not entering inside the cell. Due to application of voltage (1-2V, cell membrane threshold voltage), cell membrane can permeabilize molecules to enter inside the cell. If PI dye enters into the cell, it illustrated a red color surrounding the electrodes gap (single cell was top of the two micro-electrodes). In Fig. 3(a) shows the experimental results, where shorter pulse (8Vpp 20 ms, we applied higher voltage, because of ITO resistivity was very high) was applied into the cell, as a result, intensity kept increasing (triangular shape green curve) until saturation at 200 seconds in the 1st cycle. Then the second pulse was applied afterward (at 300 sec.) and showed a further raising of the intensity and it’s re-saturated after another 200 seconds. The signal saturation suggested a reseal of the holes on the cell surface to stop, continuous entering of the PI dye into the single cell. The diamond shape red curve, suggested PI dye continually entering into HeLa cell, which indicated, after application of pulse, hole is open up but it is not close again. So Dye can enter continuously and intensity can increase linearly. As a result, we can conclude that cell is died. Fig. 3(b) illustrates the fluorescence image of the survival cell at different times.

![Graph showing PI dye diffusion and fluorescence image](image)

Fig.4. (a) shows the PI dye diffusion inside the cell membrane with different time (b) the effective diffusion length versus the diffusion time (t^{1/2}), a linear relationship which indicates the diffusion model inside the cell membrane is followed by 2D diffusion model.

To analyze the PI dye intensity inside the single cell, raw data were fitted by image pro plus software. Fig. 4(a) shows that, the data were captured from the different times respectively for PI dye diffusing into the single cell. The intensity was normalized with the highest value (350sec) that indicated localized region from the electroporation region. We consider normalized intensity from middle of the cell surface. As results, intensity is higher for each seconds in middle of the cell surface (zero position). In Fig 4(b), shows that the PI dye follows the diffusion mechanism, because of the effective diffusion length is a linear relationship with square root of diffusion time (t^{1/2}) indicating PI dye is followed by the 2D diffusion model inside the cell membrane. Using this diffusion model, we achieved 0.99µm electroporation region (for micro sec. pulse) by our localized electroporation experiment.

REFERENCES

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