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Cell electroporation chip using multiple electric field zones in a single channel

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We present cell electroporation chip using a single channel with multiple electric field, \(E\), zones. The present chip, where multiple \(E\) zones are generated by a pair of external electrodes across a stepwise single channel, provides the optimal \(E\) conditions for stable cell electroporation with high viability in a single experiment. The optimal \(E\) for both H23 and A549 cells was 0.4 kV/cm with the maximum percentage of the viable and electroporated cells of 51.4 \(\pm\) 3.0\% and 26.6 \(\pm\) 0.7\%, respectively. The present cell electroporation chip has potential for use in integrated cell chips to find the optimal \(E\) conditions for the electroporation study. © 2012 American Institute of Physics.

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Cell electroporation, the temporary increase of cell membrane permeability due to an applied electric field, is widely used in cell-based experiments for the intracellular transfer of bio-molecules such as genes,1–5 antibodies,6 proteins,7 Rb+ ions,8,9 and drugs.10 At the critical electric field, the cells were ruptured and killed due to permanent damage of the cell membrane; the critical electric field value is different for every cell type.11–13 Therefore, monitoring both the electroporation rate and the viability of the target cells at varying electric fields is required for stable electroporation with maximum viability. The conventional cuvette system, generally used for cell electroporation, requires high cost electroporation equipment (electroporator) as well as a complicated handling procedure.10 In addition, cuvette systems can easily cause harmful side effects on the cells due to electrochemical reactions.12 Recently, a number of microfluidic chips were developed to overcome the problems of conventional cuvette systems.10,13

The previous microfluidic chips can be classified into two groups according to the electroporation method used: continuous and discontinuous types. In the continuous type of chips, cells were injected into micropores14–17 or orifices,18–20 where the electric field was concentrated to electroporate the flowing cells. The continuous type of cell electroporation chips enables high throughput cell analysis since they perform the electroporation without settling cells in the chip. However, they cannot analyze the response of the cells simultaneously for multiple electric fields. In the discontinuous type of chips, cells were settled or cultured in the microfluidic channels or chambers, where a spatial electric field gradient was applied to the cells. Therefore, they can analyze the response of the cells against multiple electric fields at once. However, the previous discontinuous type of chips4,5 require complicated multiple parallel microchannels with a pair of electrodes4 or a one cell chamber with multiple electrodes5 to generate the multiple electric field levels. In the chip with multiple microchannels,4 since the microchannels share the same reservoir and are connected in parallel, there are problems of cell clogging and non-uniform cell seeding.

This paper proposes a simple method for cell electroporation and viability monitoring using a single channel and a single pair of electrodes with multiple electric field zones. The present chip generates multiple electric field zones by a pair of external electrodes across a stepwise single channel, thus finding the optimum electric field condition for stable cell electroporation with high viability in a single experiment.

The present cell electroporation chip (Fig. 1) has a single microchannel with varying widths between inlet and outlet ports. After seeding the cells in the microchannel, a pulse signal was applied for the electrodes located at the inlet and outlet ports, thus generating multiple electric field zones with different electric field values, \(E_i\), along the microchannel:

\[
E_i = \frac{V_o \rho}{R w_i h} \quad (i = 1, \ldots, n),
\]

where \(V_o\) is the pulse voltage, \(\rho\) is the resistivity of fluid, \(R\) is the total resistance, \(w_i\) is the width of the \(i\)th microchannel width, and \(h\) is the microchannel height. The electric field, \(E_i\), in the microchannel induces the transmembrane potential of the cells as follows:21

\[
\Delta \psi_i = 1.5 r E_i \cos \theta,
\]

where \(r\) is the cell radius and \(\theta\) is the angle between the field line and the normal to the point of interest in the membrane. It is known that the transmembrane potential from 0.4 V to 1 V temporarily increases the cell membrane permeability.10,16,22 The cell membrane permeability reversibly returns to the normal level after the elimination of the applied...
electric field. At a transmembrane potential over 1 V, the cell viability decreases due to the irreversible breakdown of the cell membrane. After generating the multiple electric fields, \( E_i \), in the single microchannel with varying widths, the cell electroporation rate and viability were measured to find the optimal electric field condition for stable electroporation and high viability.

The single stepwise microchannel is designed to have 5 different widths \((n = 5)\). The total channel length is 8.5 mm. The channel lengths, \( L_1-L_5 \), are designed to be 1100 \( \mu \)m. The channel height, \( h \), is 200 \( \mu \)m in order to generate a stable electric field regardless of the cell height of 20 \( \mu \)m. The channel widths, \( w_1-w_5 \), are designed to be 334, 286, 250, 222, and 200 \( \mu \)m, respectively, to generate the discrete electric fields of 0.3, 0.35, 0.4, 0.45, and 0.5 kV cm\(^{-1} \), respectively, which have widely been used in cell electroporation studies.  

The H23 and A549 non-small cell lung cancer (NSCLS) cell lines were used for the experimental study, which were obtained from American Type Culture Collection (ATCC). The H23 and A549 cells were cultured in complete media consisting of RPMI-1640 (Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco), and 1% (v/v) penicillin-streptomycin (Invitrogen). The cells were cultured in a humidified 37\(^\circ\)C and 5% CO\(_2\) incubator. The cells were washed and detached using 0.25% trypsin/EDTA (Invitrogen), which was neutralized with 10% RPMI-1640 medium (Gibco) and spun down with a centrifuge at 1500 rpm for 3 min and resuspended in fresh medium. After adjusting the cell concentration using a hemocytometer (Marienfeld), the cells were seeded in the fabricated chip using a micropipette (Eppendorf).

The cells were injected into the microchannel at the cell concentration of \( 3 \times 10^6 \) ml\(^{-1} \), and then incubated for 3 h in a 37\(^\circ\)C and 5% CO\(_2\) incubator to be attached on the fabricated chip. After the cell incubation, the microchannel was filled with the mixture of PBS (conductivity = 1.6 S m\(^{-1} \)) and 50 \( \mu \)g ml\(^{-1} \) propidium iodide (PI), and applied a single rectangular electric pulse of 30 ms width to the electrodes. If the cells are successfully electroporated, the PI molecules stain the cells red by penetrating through the permeable cell membrane. The mixture of PBS and 2.5 \( \mu \)M Calcein AM (Invitrogen) solution was injected into the microchannel for 30 min to stain the viable cells green. After washing out the solution by injecting PBS, the cells in the chip were observed using the fluorescent microscope. As a result, the electroporation rate was obtained from the number of cells stained with red PI out of the total cell numbers, as shown in the equation

\[
\text{Electroporation rate} = \frac{\text{Number of electroporated cells}}{\text{Number of total cells}} \times 100(\%)
\]

The viability of the cells also measured from the number of cells stained with green Calcein AM out of the total cells, as shown in the equation

\[
\text{Viability} = \frac{\text{Number of viable cells}}{\text{Number of total cells}} \times 100(\%)
\]

The percentage of the viable and electroporated cells was characterized by rating the number of cells stained with both red PI and green Calcein AM among the number of total cells, as shown in the equation

\[
\text{Percentage of viable and electroporated cells} = \frac{\text{Number of cells stained with both red PI and green Calcein AM}}{\text{Number of total cells}} \times 100(\%)
\]

Figures 2 and 3 show the red and green fluorescent images of the H23 and A549 cells, respectively, at the discrete electric fields of 0.3, 0.35, 0.4, 0.45, and 0.5 kV cm\(^{-1} \). By analyzing the number of stained cells out of the total cells in Figs. 2 and 3, the electroporation rate and viability of the H23 and A549 cells were characterized, as shown in Fig. 4. In Fig. 4, the electroporation rate of the H23 and A549 cells shows rapid increase at the different electric fields of 0.35 and
Based on Eq. (2), the electric fields of 0.35 and 0.4 kV cm$^{-1}$ are expected to induce cell transmembrane potentials of 0.44 and 0.45 V, respectively, which agree with the general breakdown potential of 0.4–1 V. By analyzing the cell images of Figs. 2 and 3, the percentage of the viable and electroporated cell number out of the total cell number was also characterized, as shown in Fig. 5. As a result, the H23 and A549 cells showed the maximum percentage of the viable and electroporated and cells out of the total cells, 51.4 ± 3.0% and 26.6% ± 0.7%, respectively, at the optimal electric field of 0.4 kV cm$^{-1}$. The different results of the percentage of the viable and electroporated cells can be attributed to size, shape, and membrane rigidity of the H23 and A549 cells. In addition to these, the expansion of the nuclei size in tumor cells is the important factor regarding its higher susceptibility to the electric field. Comparing the nuclei size of the two cell lines used in this study, the nuclei sizes of the H23 and A549 cells were 110.7 μm$^2$ and 216.9 μm$^2$, respectively.

This paper proposes the cell electroporation chip using a single microchannel with multiple electric field, $E$, zones between a pair of electrodes. The present cell electroporation chip was designed to generate multiple discrete $E$ values of 0.3, 0.35, 0.4, 0.45, and 0.5 kV cm$^{-1}$ across stepwise channel width. From the experimental study using H23 and A549 human lung cancer cells, the performance of the cell electroporation chip

![FIG. 3. Fluorescent images of the A549 cells in the microchannel: (a) Red fluorescent image of the electroporated cells stained with PI; (b) green fluorescent image of the viable cells stained with Calcein AM.](image)

![FIG. 4. Measured electroporation rate and viability. The error bar shows the standard deviation of the three repeated measurements of the cell electroporation rate and viability: (a) H23 cells at 5 different electric field zones in the microchannel; (b) A549 cells at 5 different electric field zones in the microchannel.](image)

![FIG. 5. Measured percentage of the viable and electroporated cells at 5 different electric field zones in the microchannel. The error bar shows the standard deviation of the three repeated measurements of the cell percentage: (a) H23 cells and (b) A549 cells.](image)
was characterized. The optimum electric field for H23 and A549 cells was identically measured as 0.4 kV cm\(^{-1}\), with the percentages of the viable and electroporated cells of being 51.4% ± 3.0% and 26.6% ± 0.7%, respectively. The cell electroporation chip has potential for use in integrated cell chips for the study of electroporation.

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