Simulation of micro/nano electroporation for cell transfection

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Abstract. The 3D micro/nano electroporation for transfection has become a powerful biological cell research technique with the development of micro-nano manufacturing technology. The micro channels connected the cells with transfection reagents on the chip were important to the transmembrane potential, which directly influences the electroporation efficiency. In this study, a two-dimensional model for electroporation of cells was designed to address the effects of channels’ sizes and number on transmembrane potential. The simulation results indicated that the transmembrane potential increased with increasing size of channels’ entrances. Moreover, compared with single channel entrance, the transmembrane potential was higher when the cells located at multiple channels entrances. These results suggest that it IS required to develop higher micro manufacturing technology to create channels as we expected size.

1. Introduction
Electroporation is widely adopted in cell transfection that uses external electric field to create transient pores in the cell membrane, which contributes to the delivery of biomolecules into cells. When a high voltage is applied, the original hydrophobic pores occur. Afterward, these pores in the cell membrane change into from the transient hydrophilic pores, thereby increasing its electrical conductivity and membrane permeability. The formation of these hydrophilic pores depends mainly on the strength of the electric field [1].

The conventional bulk electroporation has several significant disadvantages: the entire cells need to be suspended in the buffer and have to be exposed to very high voltages, which will causes high fatality rate. To overcome these problems, the Espinaosa group [2] invented nanofountain probe electroporation device for a individual adherent cell transfection with high viability at lower voltage. Afterward, they utilized the porous carbonate membrane to develop a microfluidic device for high-throughput cell transfection [3]. However, the pores in the carbonate membrane were randomly distributed, which led to a non-uniform pore-cell contact. Chang group [4-6] developed a 3D nanochannel array chip for high-throughput cell electroporation with high uniformity and dosage control. The 3D nano electroporation silicon chip was a uniform and intense nanochannel array with a radius of 650 nm, which was made by projection photolithography and deep reactive ion etching technique. The experimental results indicated that the nanochannel electroporation chip showed a 20 fold improvement in uniformity and dosage control. However, limited by the microfabrication
technology and the material anisotropic, the etched channels on the chip could not present uniform morphology. The cross sections of channels most had trapezoid shape. Moreover, the cells during transfection experiment may locate at multiple channels’ entrances. These uncertain factors would result in different transmembrane potential difference and cause trouble in uniformity and dosage, even transfection validity.

To stress these issues, we performed infinite element analysis to study the effects of channels’ sizes and channels’ number on the transmembrane potential. The simulation results indicated that the bigger sizes resulted in higher transmembrane potential on condition that the cells were under safe potential difference. What’s more, the transmembrane potential was higher when the cells located at multiple channels entrances compared with single channel entrance.

2. Theory and simulation

2.1 Electroporation theory

After applying an electric field in the cell culture medium, ions move to the surface of the cell membrane. The accumulated ions create an electric field in the cell membrane. The potential difference between the inside and outside of the cell membrane is called the transmembrane potential, which is proportional to the external electric field and the diameter of the cell. For a spherical cell as shown in Figure 1(a), the transmembrane potential can be described by the Schwan equation:

\[ \text{TMP} = 1.5rE_0 \cos \theta \]  

(1)

where the \( r \) is the cell radius, \( E_0 \) is the applied electric field intensity, and \( \theta \) is the angle between the electric field direction and normal direction of the cell membrane surface point [7]. The transmembrane potential will increase when imposing to a long pulse voltage (ms) and the threshold transmembrane potential is in the range of 0.2~1 V [8]. Then the transient hydrophilic pores occur. Remove the pulse voltage, the cell membrane will be repair, there is no mechanical damage, a phenomenon called reversible electroporation. However, when a very high external electric field is applied in bulk electroporation, the most cells permanently deform and the cell membrane cannot be repaired, resulting in cell lysis. Unlike bulk electroporation, the micro/nano electroaporation shown in Figure 1(b) creates a localized electric field on the cell membrane area connected to micro/nano channel, resulting in pores of cell membrane at low voltages. So the cell viability can be improved greatly compared with bulk electroporation.

![Figure 1](image)

**Figure 1.** (a) Schematic of a spherical cell in the electric field; (b) Schematic of channels connected to a single cell at the top and transfection reagents at the bottom; (c) The corresponding circuit diagram of (b).

2.2 Modeling

To investigate the transmembrane potential at low voltage in micro/nano electroporation, we used Laplace equation \( \nabla (\sigma \nabla V) = 0 \) to calculate in COMSOL with appropriate boundary conditions and geometrical model. In the partial differential equation, \( \sigma \) is the electrical conductivity and \( V \) is the input electrical potential [9]. In the experiments, the input voltage was applied on the glass slide.
connected with transfection reagents and the other electrode was submerged into the cell culture buffer. To satisfy the boundary condition, we applied input voltage at the bottom boundary while the top boundary was electrically grounded. And the other boundaries were set electrically insulated. The cell was modeled as a circle of 10 μm in diameter enclosed by a 5nm layer. The relevant schematic is shown in Figure 2, where \( l \) is membrane channel length, \( h \) is the gap between the cell and the channel, \( r \) is the culture chamber diameter and \( w \) is the micro/nano channel width. In this simulation, the electrical conductivity of cell membrane, cytoplasm, and buffer were respectively set to \( 5 \times 10^{-7}, 0.2, 0.8 \) Sm\(^{-1}\) [10].

![Figure 2. The schematic of simulation model and relevant dimension parameters in simulation.](image)

3. Results and discussion

3.1 Effect of channels’ sizes on the transmembrane potential

Due to different manufacturing techniques and anisotropic materials, the etched channels were not absolutely straight. As shown in Figure 3, we performed electrostatic numerical simulation with channels of different sizes to analyze transmembrane potential. It was found that the potential dropped quickly in the micro/nano channels. Therefore, the cell viability was improved due to that only a small potential difference was located at a tiny area of the cell membrane. We predicted that the resistance in narrow channels was larger than that in wide channels. As predicted, the transmembrane potential drop increased with increasing width of channel entrances which side of channel the cells located at. Moreover, the transmembrane potential was lower than 1 V with a width of less than 0.5 μm at the input voltage of 10 V. The results suggested that it is required higher input voltage to achieve effective electroporation when the channel had a very small size.
Figure 3. Numerical simulation of electroporation of different channels’ sizes at input voltage of 10, 15, 20 V.

3.2 Effect of channels’ number on the transmembrane potential

It was not certain how many channels cells locate at in actual micro/nano electroporation experiments. So in order to identify if the number of channels the cells placed on could affect the transmembrane potential, we set respectively one, two, three channels in our simulations. The width of rectangle channel was 1 μm and the spacing distance between two adjacent channels was 2 μm in our simulation. We could see from Figure 4, the highest transmembrane difference all occurred at the nearest area from the channel. The resistance were influenced directly by the number of channels. The potential dropped slower with multiple channels. Therefore, the multiple channels could result in higher potential difference than single channel. Despite effective electroporation could be more likely happen at a higher transmembrane potential, it may cause trouble in dosage control. This was because the potential difference could influence the diffusion efficiency of transfection reagents.
Figure 4. Numerical simulation of electroporation of different channels’ number.

4. Conclusions

Toward the development of high-through micro/nano electroporation for transfections of the cells, we have emphasized the importance of channels’ sizes and number on microporous chip to effective electroporation. It was found that the size and spacing distance between two adjacent channels affect the transmembrane potential significantly. The results suggested that it is required higher input voltage to achieve effective electroporation when the channel had a very small size. Moreover, the more channels could result in higher potential difference. These findings suggest us we need more precise micro manufacturing techniques to create the channels on the chip as we designed.

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6. References

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