A Simulation Study of the Electrode Profile on Electroporation Efficacy

Funian Xiao and Raji Sundararajan

***Abstract*— Electroporation has been used to treat tumors at research laboratories and clinical trials recently. However, the fundamental mechanism of the electroporation to the drug transport is still not clear even though it is proven that electroporation enhances drug and nanoparticle delivery efficacy. In this paper, we used Finite Element Analysis (FEA) method to choose the arrangement and the shape of the electrodes for single cell drug delivery The intensity and the distribution of the electrical field produce a variety of outcomes regarding pore formation in the cell membrane. The pore formation outcomes range from irreversible to reversible pores. Therefore, we propose to investigate and analyze the FEA models of the electrical fields of various electrodes in order to increase the cell viability during electroporation. The simulation results showed that the electrical field has impact on cell viability via its influence to the poration distribution in the cell membrane.**

***Index Terms*—Electroporation, Finite Element Analysis, Electric Field, Cell, Membrane.**

I. Introduction

Electrical pulses have been applied in many areas, such as food science [1], bio-thechnology [2], molecular biology medicine [3], and so on. Electroporation, an application of high intensity, low duration pulses, has been used in tumor treatments at research laboratories and clinical trials. Current applications of electroporation include Electrogenetransfection (EGT), gene transport through these pores into cells [4, 5], Electrochemotherapy (ECT), drug delivery into cells [6, 7], and Electrofusion (EF), membrane fusion in close-contact adjacent cells [8]. Electroporation increases irreversible pores under higher electric fields, thus causing cell to lose its cytoplasm and die. However, it is still widely used in food processing and bacterial decontamination even with unwanted effects. Another important application of Electroporation is to deliver nanoparticle into cellular cytoplasm without any damage to cell function.

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Various physical and biological factors can affect the electroporation efficacy. The most important factor is the electric field intensity. This factor may cause cells to reach unstable conditions, which may fail to close membrane pores, therefore causing cells to lose their viability. In addition, other physical and chemical parameters, such as cell interior conductivity and permittivity, membrane and its surrounding medium, and the intensity of the applied electric field., osmotic pressure [9, 10], ionic strength, and composition [9, 11], and temperature [12], can also change the electroporation efficacy after applying the electric field to the cell. Hence, understanding of the relation between these factors and electroporation performance is critical to electroporation applications.

In this paper, we studied the electric field's influence to cell membranes in order to improve cell viability during electroporation. A Finite Element Analysis (FEA) model was built to analyze the electric field distribution in two different single cell shapes by first selecting the electrode type around the cell, and then configuring the voltage of each of the electrodes. The FEA model was built from a combination of the cell modeling and the electrode modeling. The FEA result successfully helped us identify the best configuration after comparison of all cases. It is believed that the research in this paper will help understand the electroporation impact to the cell drug delivery theoretically.

II. Methods and Materials

A. Background

Certain types of structural rearrangements occur in the cell membrane when a cell is placed in a static electric field. The rearrangement arises because electric charges accumulate along the plasma membrane, thus causing the potential difference across the cell and the cell membrane to change from its initial value. The plasma membrane behaves as an osmotic membrane and some temporary channels form in the lipid bilayer when the potential difference increases to around 1V. The cell membrane consists of plenty of pores under electric field. These temporary channels increase the cell membrane permeability, which allow ionic, nanoparticles, DNA, and molecules to across the cell membrane. In addition, the electric field pushes ionic and molecular to pass through the temporary channels.

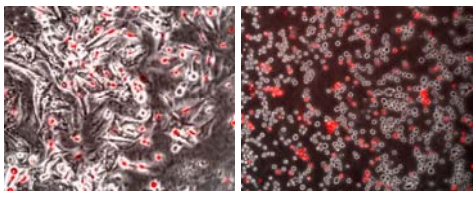
The nanoparticles are introduced to the cellular cytoplasm without any damage in cell function. The electric field will not pass through the membrane to the cytoplasm if the frequency

of the applied pulsed electric field is lower than the charging time of the plasma membrane. On the contrary, the electric field will pass through the membrane into the cytoplasm, therefore affecting the internal cell structure if the frequency of the applied pulsed electric field is sufficiently high enough. Pores will form in such membranes if the amplitude of the applied field is higher than the voltage threshold across the intracellular membranes.

The hypothesis of the charging mechanism to intracellular membranes is widely accepted although it is not completely clear to be understood. It is commonly acknowledged that various factors influence the electroporation efficacy. The most significant factors are cell interior conductivity and permittivity, membrane and its surrounding medium, and the strength of applied electric field. Inappropriate setting of these factors may bring cells to unstable conditions keeping membrane pores to continually open, thus causing cells to lose their viability. It is yet to be understood how the combinations of these factors can be improved to achieve better electroporation efficacy. Therefore, we decided to investigate the role of conductivity and permittivity regarding to the pore formation in cell membrane in particular. The FEA modeling is a step towards effective treatment planning, not only in clinical electro-chemotherapy, but also in other electroporation-based applications, such as gene eletrotransfer, electroporation drug delivery, and irreversible tumor ablation. The FEA model in this study includes two major parts, geometric model in section II.*B* and boundary condition specification in Section II.*C*.

B. Geometric Model

Two geometrical factors involved in the FEA model are, cell size and shape, and electrode geometry and its voltage. Cells have diverse shapes, which can be irregular when the cells attach to the cell culture flask (Fig. 1a). Cells fall from the flask bottom to suspend in media after trypsin usage (Fig. 1b). Cell shapes typically stabilize to circular (round) or elliptical after cells detach from the flask bottom. Hence, the FEA model employs round and elliptical cell shapes, as shown in Fig. 2. The diameter of outer cell membrane is 30μm and inner membrane is 20μm for round cell. The long axis of elliptical cell is 30μm and short axis is 10μm. Some cancer cells, such as MCF-7 cells, have diameter from 15μm to 30μm [13]. MATLAB was used for the study.



(a) (b)

Fig. 1. a) Adherent (cells attached to the surface) and b) Suspended neonatal rat cells

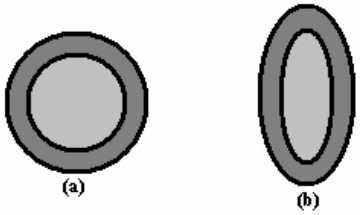


Fig. 2. Cells shapes (a) round and (b) elliptical

Electrodes have various profiles depending on their shapes and arrangements in practical usage. In this study, three different electrodes were selected: plate, four-needle, and six-needle; and two arrangements in needle electrodes: parallel, and hexagon. In particular, the four-needle electrode only has parallel arrangement. Fig. 3 shows the electrode shapes and arrangements studied in this project. The distance between the rightmost and the leftmost electrodes is 120μm. For instance, the distance between the left plate and the right plate is 120μm in the parallel plate electrode.

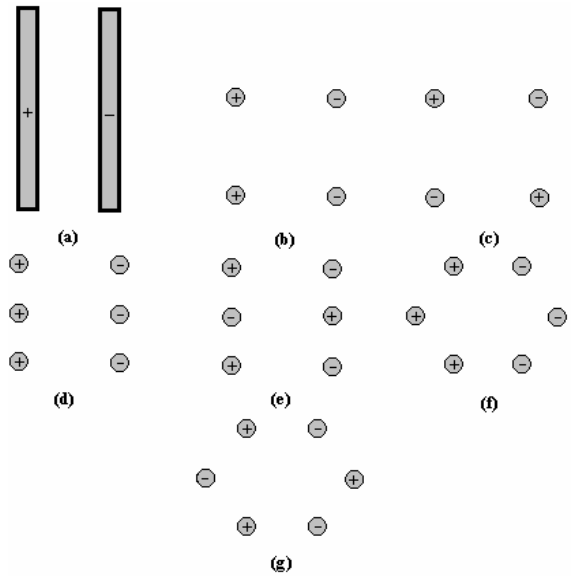


Fig. 3. Electrodes shapes and arrangements (a) Parallel plate electrode (b) Four-needle electrode with positive voltage on one side (c) Four-needle electrode with positive voltage at diagonal position (d) Parallel six-needle electrode with positive voltage on one side, (e) Parallel six-needle electrode with positive voltage at alternation (f) Six-hex-needle electrode with positive voltage on one side (g) Six-hex-needle electrode with positive voltage at alternation.

C. Boundary conditions

In electrostatics, the potential V is related to the electric field E by $E = -\nabla V$. The Poisson equation can be derived by combining this relationship with Maxwell's Equation, as illustrated below.

$$-\nabla \bullet (\varepsilon \nabla V) = \rho \tag{1}$$

where ε is the permittivity coefficient and ρ is the charge density. Two boundary conditions, Dirichlet and Neumann, are defined for V a scalar value, to solve this Poisson's equation. Dirichelet conditions are used to define the voltage of each electrode. The electrostatic potential V is specified on

the boundary under Dirichlet conditions. The voltages in electrodes generate an electric field of intensity, 200V/cm. Neumann conditions are used to define the media edge between two electrodes. The surface charge is specified on the boundary under Neumann conditions. Neumann condition can be represented as $\vec{n} \bullet (\varepsilon \nabla V)$, where $\vec{n}$ is the outward unit normal.

The cell conductivity and permittivity are also important factors in electric field simulation, as illustrated in Table 1.

TABLE I.
Cell conductivity and permittivity under different cell components

Cell component	Conductivity (S/m)	Permittivity (F/m)
Suspension Medium	5E-7	7.17E-10
Membrane	2E-1	5.81E-8
Cytoplasm	5E-7	7.17E-10

III. Results and Discussions

A. *Experimental Results*

A number of configurations of the cells types and the electrode profiles were chosen to study the electroporation effect based on the model described in Section II.A. Each electrode voltage was set up to generate a 200V/cm electric field. Fig. 4 shows the FEA result from the configuration that the round cell was placed with the parallel plate electrodes. Each electrode of the parallel plate electrode is placed beside the right and the left sides of the cell. The left electrode voltage was maintained at 2.4V, and the right electrode was at 0V. Another voltage configuration is that the left electrode was set up to 1.2V and the right electrode was set up to -1.2V. The result shows that the electric fields at both situations have the same distribution.

Fig. 5 shows the FEA result from the configuration that the round cell was placed in the four-needle electrode. These electrodes were placed at the four corners around the cell, as indicated in Fig. 5. The electric field intensity had the same distribution even at four different voltages from four electrodes. The round cell in the six-hex-needle electrode, as illustrated in Fig. 6, has a similar distribution of the electric field as that of the round cell in the four-needle electrode, except a very slightly difference electric field intensity. The electric field distribution of the six-hex-needle array is more uniform than that of the four-needle electrode array.

The elliptical cell was also studied under various electrode profiles with different voltage configurations. In addition, the cell orientation was also included in this study. Fig. 7 shows the FEA result for the configuration that the elliptical cell was placed across the parallel plate electrode profile with the ellipse's minor axis parallel to the electrode surface. The plate electrodes were placed on the right and the left sides of the cell. The electric field intensities at the left and the right side of the cell are much higher than the other parts of the cell.

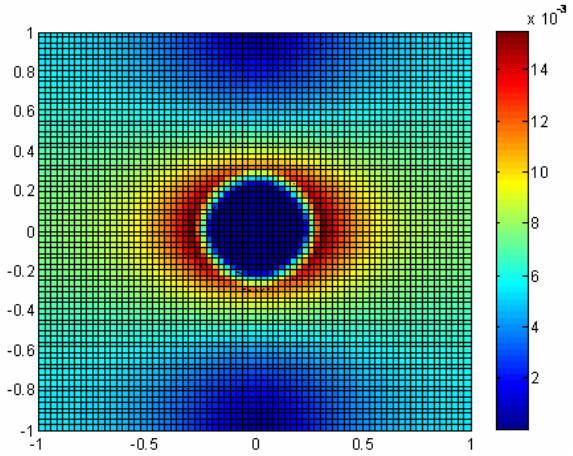


Fig. 4. The intensity distribution of the round cell in the parallel plate electrode

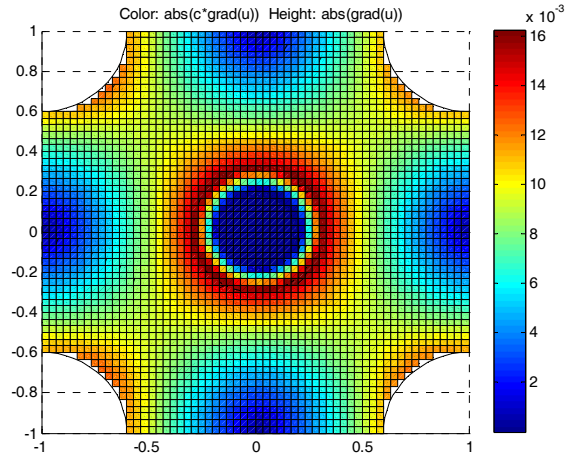


Fig. 5. The intensity distribution of the round cell in the four-needle electrode

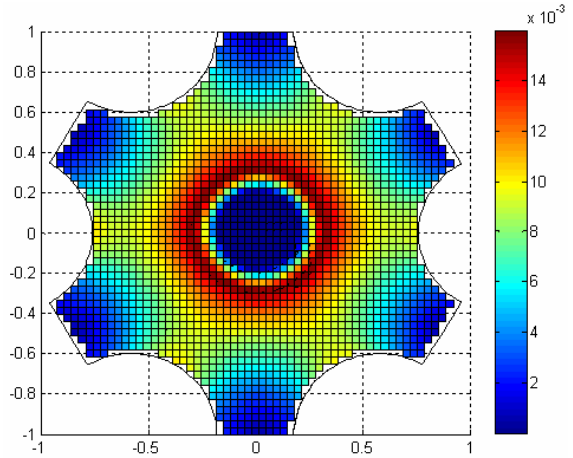


Fig. 6. The intensity distribution of the round cell in the six-hex-needle electrode

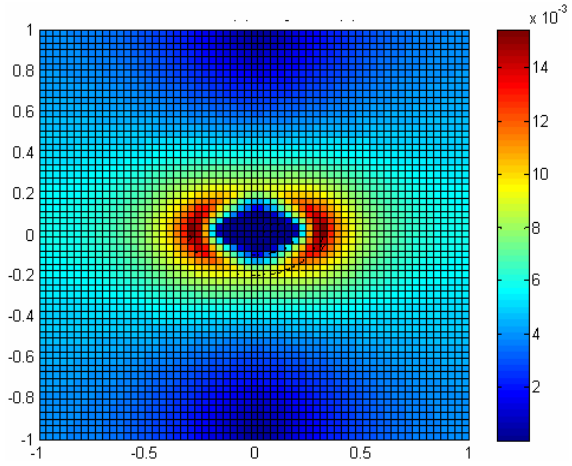


Fig. 7. The electric field distribution of the elliptical cell in the parallel plate electrode

Fig. 8 shows the FEA result from which the parallel plate electrode was kept at the same position, but the cell was rotated 90° at its center point. The FEA result demonstrates that the electric field distribution is much more uniform than the distribution presented in Fig. 7. Fig. 9 shows the FEA result from the elliptical cell placed under the four-needle electrode. The intensity of electric field along the minor axis of the cell is higher than the major axis of the cell. Fig. 10 shows the FEA result from the configuration that the elliptical cell was placed in the six-needle electrode with the cell's ellipse's long axis parallel to the right or the left three electrodes. The electric field intensity is nonhomogenous; it is slightly higher along the minor axis.

Fig. 11 shows the FEA result from the condition that the cell was rotated 90° at its center point with the same electrod configuration, and under the same electrode voltages as that in Fig. 10. The electric field intensity in Fig. 11 is the most non-uniform and it converges to the minor axis. Fig. 12 shows the FEA result from the configuration that the elliptical cell was placed under the six-hex-needle electrode configuration. The electric field is nonhomogenous with higher intensity at the short edge of cell.

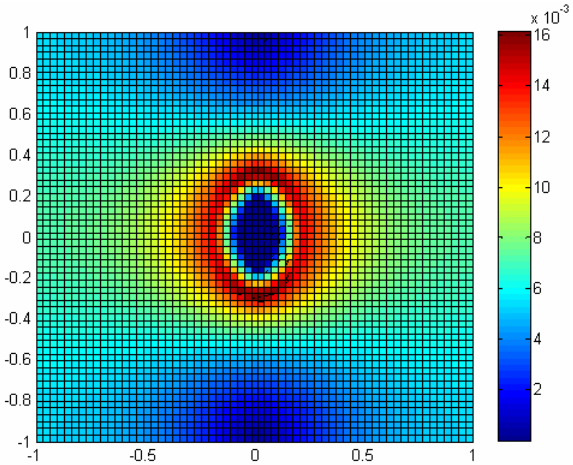


Fig. 8. The electric field distribution of the elliptical cell with 90º rotation in the parallel plate electrode

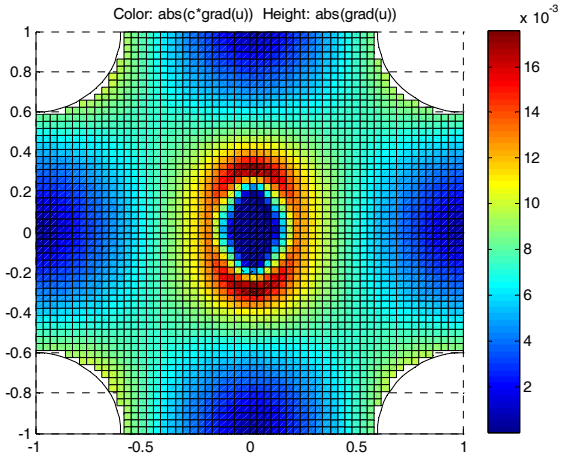


Fig. 9. The electric field distribution of the elliptical cell with 90º rotation in the four-needle electrode

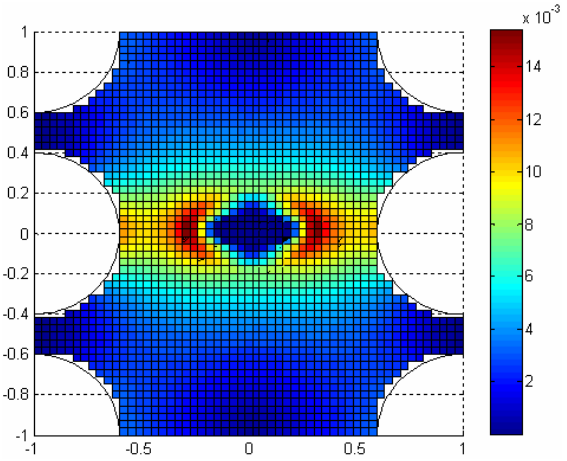


Fig. 10. The electric field distribution of the elliptical cell in the the parallel six-needle electrode

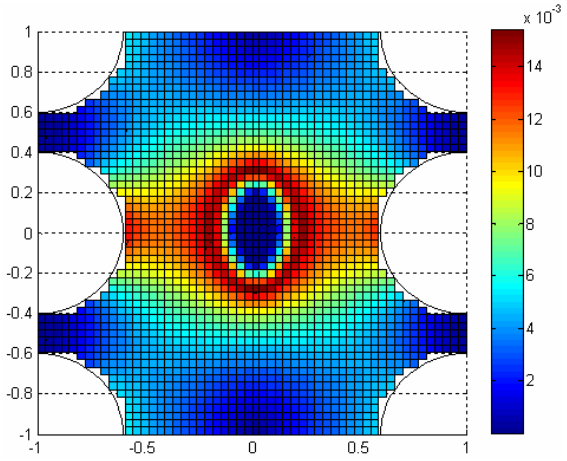


Fig. 11. The electric field distribution of the elliptical cell with 90º rotation in the parallel six-needle electrode

B. Discussion

There are several observations from the FEA simulation results presented in Section III.*A*. From these, it is seen that the electrode profiles, the cell shapes and the cell orientations are important factors that influence the electric field

distribution. The round cell tends to have a uniform distribution of the electric field intensity in the needle electrode configuration compared to the elliptical cell. In addition, the elliptical cell's orientation relative to the electrode influences the uniformly of the electric field distribution.

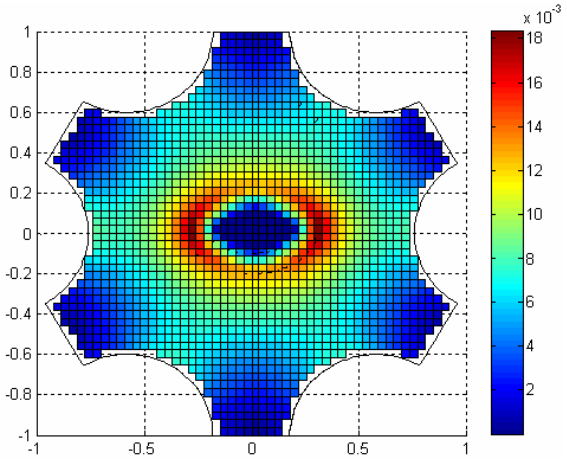


Fig. 12. The electric field distribution of the elliptical cell in the six-hex-needle electrode

The main reason could be that the electric field is the gradient of electric potential and is given by,

$$\vec{E}(x,y,z) = \hat{i}\frac{\partial V(x,y,z)}{\partial x} + \hat{j}\frac{\partial V(x,y,z)}{\partial y} + \hat{k}\frac{\partial V(x,y,z)}{\partial z}. \qquad (2)$$

The distance from the long edge of the elliptical cell to the electrode is different from the distance from the ellipse's minor axis to the electrode. Hence, the elliptical cell has more uniform distribution of the electric field intensity when its major axis is closer to the electrode. In contrast, the distribution of the electric field intensity in the elliptical cell is non-uniform when the ellipse's minor axis is close to the electrode. In addition, the electric field intensity distribution of the electrode is non-diagonal-symmetrical relative to the cell orientation. For instance, when the orientation of cell is fixed, the rotation of electrode cannot change the distribution of the electric field intensity. The electric field is completely diagonally symmetric from the four-needle electrode and the six-hex-needle electrode arrays for both types of cells. However, the electric field is only x and y axis symmetric from the plate electrode and the parallel six-needle electrode. Hence, the round cell cannot acquire uniform distribution of the electric filed intensity even though the cell has a symmetrical shape.

Ion accumulates in cell membrane uniformly when the electric field intensity has a uniform distribution in cell membrane. In addition, it also leads to a uniform reversible pore formation in the cell membrane, which improves the cell viability after the electroporation. When the electric field does not have a uniform distribution in cell membrane, some parts of the cell membrane under stronger electric field intensity tend to form irreversible pore, which damage the structure of the cell membrane, and increase the number of dead cells. Therefore, an appropriate electrode configuration can be selected to treat a tumor when the cell shape in the tumor is found under microscope before electroporation. In summary, a six-hex-needle electrode has better efficacy to a round cell, while a four-needle electrode is better for an elliptical cell. However, a four-needle electrode is recommended when a tumor has both shapes of cells.

One investigation still needs to be continued in the future. It was assumed that the cell shape is constant in this study. However, in reality, cell shape might change during electroporation, and the conductivity in the cell and the cell membrane also change due to electroporation. In the future, analysis of the transient FEA model can be explored for better understanding and better applications of the electroporation on cell drug delivery.

IV. Conclusions

In this paper, FEA model was built to simulate the distribution of the electric field intensity under various configurations of electrode types, cell shapes, cell orientation, and voltage settings. The purpose of this study is to better understand the result of the pore formation in the cell membrane, and the ion movement in the cells under the electric field, therefore to help improve the cell viability during electroporation. The FEA results showed correlation to the cell pore formation from these factors such as the electrode types, the cell shapes, and the cell orientations. A round cell tends to have a uniform pore formation in a needle electrode, while an elliptical cell has different pore formation depending on the cell orientation. Hence, an appropriate electrode has to be selected based on the cell shapes to treat the tumor before electrochemotherapy is applied. In the future, more effort will be focused on improving the current FEA model and verifying the FEA model using more real world examples.

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