Simulation of molecular transport through an electroporated cell using COMSOL Multiphysics

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Abstract: Electroporation is a highly efficient transfection method which is used to inject molecules into cells by creating temporary pores in cell membranes using an electrical field. Electroporation has been successfully utilized in medicine in the processes of producing knockout mice, tumor treatments, gene therapy, and cell-based therapy. Electroporation is a simple and easy method to implement and transfect a large number of cells in a short time. However, there are major drawbacks in electroporation, such as substantial cell death caused by high voltage pulses and not having control over the molecular intake per pulse. In this work, we have used COMSOL software to investigate the molecular transport through pores.

Keywords: Electroporation, Electric Field, Diffusion

Introduction

Ability to transport molecules in a controllable manner through cell membranes is required in many biomedical applications such as gene transfection into cells, cancer chemotherapy, introduction of foreign proteins into cells, cell killing for sterilization and transdermal drug delivery, [1, 2]. For most of the applications, it is necessary to have the controlled transport of molecules into cells with high cell viability. However, only few studies have focused on developing these necessary capabilities. Electroporation is creation of pores in lipid bilayer membranes of living cells by application of a short electric pulse. These pores can be reversible or irreversible, depending on the conditions used for electroporation. Current electroporation techniques are not capable of transfecting cells with controllable manner without harming the cell viability. For example, studies have shown that the cell viability of electroporation using traditional macro-scale cuvettes is around 30-60%, [1].

To address this issue, here we propose a method that electroporate cells with high cell viability and controllable molecular transport. To implement the electroporation, we pattern the cells as single files in an array of interdigitated electrodes. Each electrode pair in the interdigitated electrode (IDE) is parallel to each other and produce electric fields equally in all cells in the pattern. Patterning is needed to equally expose all the cells to the external electric field and produce an equal induced membrane potential in cells. We will utilize dielectrophoretic forces (DEP) on cells to manipulate them in the electrode and produce cell patterns in a perfect line before electroporation so that almost all the cells will experience the same electric field. Once the cells are patterned, we will turn off the AC electric field and apply a small DC voltage pulse to the IDEs. The DC voltage pulses will induce membrane electric potentials on the cell membranes. The induced transmembrane potential controls the production of pores, size of the pores, and therefore this is the key towards developing a controlled molecular transport through pores. Furthermore, this ensures that the molecular uptake of each cell is approximately equal for all the cells. After the production of pores equally in all the cells, mechanisms for molecular transport through the pores needs to be fully understood. In this paper, we have developed a COMSOL software to simulate a potential mechanism.

Theory and Calculations

Molecular transport under electroporation is expected to occur in different times and mechanisms [1-3]. During a pulse, molecule transport can occur through pores, due to diffusion and electrophoretically driven transport. After a pulse, transport could be through pores, just by diffusion and with a small influence of transmembrane diffusion voltage [1-5]. Although it is shown that the pores can last seconds to minutes, it's found that most of the pores will be resealed within milliseconds after the electric field is turned-off [4]. Thus, most of the molecular uptake occurs during the period that the pulse is applied. If we can transport the desired number of molecules while the pulse is applied, we can ensure that our molecular injection has succeeded.

COMSOL software facilitates the modelling of chemical species transport such as molecular transport through pores. Depending on the initial concentration of the species, we can use, either

"Transport of diluted species" or "Transport of concentrated species". [6] In COMSOL, several molecular transport mechanisms such as "The molecular transport in an electric field", "The molecular transport in a porous media" and "The molecular transport due to diffusion" can be combined together. We have used, "Transport of diluted species" physics for our study. We then combined the Fick's law with molecular migration in porous media under an electric field to calculate the molecular transport.

$$R_i = \frac{\partial c_i}{\partial t} + \nabla.(-D_i.\nabla c_i - z_i.u_{m,i}.Fc_i.\nabla V) \qquad [6]$$

Where R_i is the reaction rate expression for the species, c_i is the concentration of the molecules in the solution, D_i is the diffusion coefficient, z_i is the charge number (dimensionless, but requires a plus or minus sign), $u_{m,i}$ is the mobility of molecules in an electric field, V is the electric potential in the cell membrane which is the addition of resting and induced potentials [6]. Then the induced membrane potential (**Vm**) is calculated using the following equation.

$$V_m = 1.5.E.a.\cos\theta \qquad [4]$$

Where V_m is the induced membrane potential, **E** is the applied electric field, **a** is the radius of the cell and θ is the polar angle of a position with respect to the electric field [4]. Typically, resting membrane potential is much smaller than the induced membrane potential and therefore often induced membrane potential do not include in calculations. In the calculation, V_m was taken as 1V, as it was mentioned as the minimum required induced transmembrane potential to open a pore [4, 5]. Then the required external Electric field (E) was calculated for $\theta = 0°$. The supply voltage was then calculated as 6.17V considering the electrode gap, which is 30µm.

Simulation of molecular transport through pore

Here we modeled the molecular transport through an electroporated cell that has an external diameter of 7 µm and a 5 nm thick cell membrane. The cell membrane consists of a pore of 20 nm radius (represent average area of all the pores) [10]. In the simulation this cell was placed between two parallel electrodes, molecules are suspended in the buffer that has conductivity of 2 S/m. To implement the simulation, first, the cell was drawn to the scale (Figure 1) in AutoCAD 2D software and the model was saved as a ".dxf" format. Then, the COMSOL software was opened and 2D space dimension was selected under the model wizard to continue the simulation. In order to model the molecular transport through an electric field, "The transport of diluted species" physics and "The electric current" physics both were selected in the simulation. The time domain analysis was selected to observe the molecular concentration variation over the time. Then the geometry unit was set to µm and the AutoCAD drawing of the cell was imported. The rest of the electrodes and the molecular suspension area were drawn using the COMSOL geometry addition feature (Figure 2.a). Then custom materials for each domain were assigned as in Table 1. Then the boundary conditions and the initial values of the each parameter were applied. The initial concentration of the cell exterior was set to 200 mol/cm^3 (200 mM) and the concentration of molecules of cytoplasm was set to 0M. In the simulation, two types of molecular

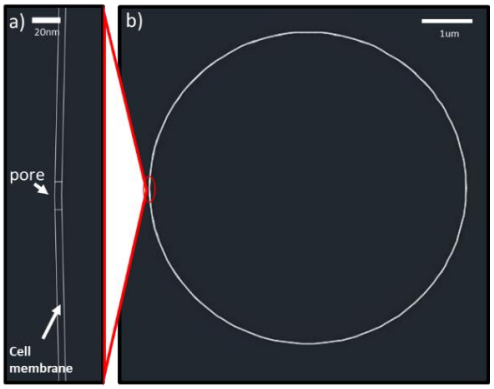

Figure 1. (a) Zoomed view of the pore, (b) Cell.

Domain	Material	Conductivity (S/m)	Relative Permittivity	Diffusion Coefficient (m^2s)
1,2 (Electrodes)	Gold	$43x10^6$	6.9	
3 (Cytoplasm)	Custom	0.95	87.7	$2.07x10^{-9}$
4 (Membrane)	Custom	$5x10^{-8}$	16.8	
5 (Buffer solution)	Custom	2	80	$2.02x10^{-20}$
6 (Aqueous pore)	Custom	1.3		$5x10^{-14}$

Table 1: Materials assigned for each domain

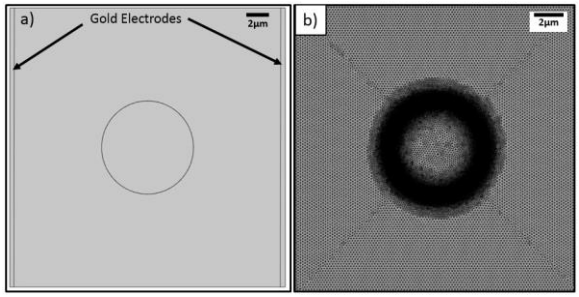

Figure 2. (a) Final geometry of the cell, and electrodes, (b) The geometry after meshing with the custom mesh size.

transport methods were defined depending on the materials used. To model the transport of molecules through the pore, "Migration in electric fields" and "The dispersion in porous media" were added as additional transport mechanisms, while the main transport mechanism is defined as diffusion. To model the molecular transport in extra to intracellular, only the "Migration in electric fields" was selected as the additional transport mechanism. Then a voltage of 6.17 V was assigned to one of the electrodes and the other electrode was defined as a ground. The outside boundary of the geometry was selected as an insulator boundary. In order to monitor the variables that are needed to be calculated, a boundary probe and two of domain probes were set under the "Definition" tab parameters. For example, "Electric potential" model input for the migration in electric field through the pore is defined by the value of the boundary electric potential probe, which is placed in the outer boundary of the pore.(Figure 1 a).

Then the geometry was meshed using a free triangular mesh with a custom mesh size. The minimum element of the custom mesh was set to 0.5 nm, as the minimum geometry size is 5 nm. (Figure 2.b). This mesh sizes were sufficient to produce the results with proper resolution. Then the time domain analysis was conducted with the time intervals of 10μs to analyze the concentration variation with time.

The COMSOL Multiphysics was used to calculate the electric field, the electric potential and the concentration of molecules. The electric field was observed in a space plot and the inbuilt parameter *ec.normE* was used to calculate the normalized electric field. The boundary and domain probes were also monitored separately for each parameter. Then the concentrations of the exterior solution and the interior of the cell were plotted in the same graph with time. The time for the saturation or the concentration equilibrium was measured for several initial exterior concentrations. Furthermore, to understand the concentration gradients and the molecular crowding near the electroporated pore, an animation was created by space plotting the concentration with time. [8,9]

Simulation Results

The simulation was configured to analyze the results from 0 -15 seconds. Results are indicated in the Figure 4. For example, if the desired level of molecular concentration in the cytoplasm is 50mM, electroporation time of 300 ms with 400 mM in the extracellular is needed (Figure 4.a). Similarly, if we use 200 mM as the initial concentration of the exterior solution, it will take about molecular transport time of 800 ms to get 50 mM in the cytoplasm. Then for a 100 mM exterior solution it will take 1.5 s. There, it can be seen that, the molecular uptake changes nonlinearly with the initial concentration of the extracellular solution. As we know, in an electroporation, a pulse will be applied in milliseconds of time period.

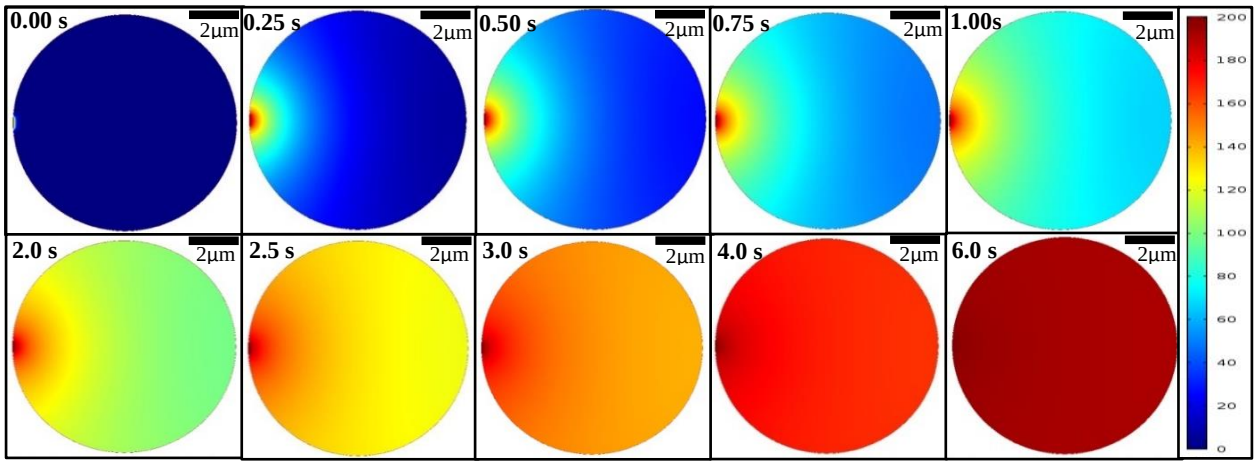

Figure 3: COMSOL simulation results of molecular concentration (mol/cm^3) variation inside the cell with the time. Initial outside concentration is 200 mol/cm^3.

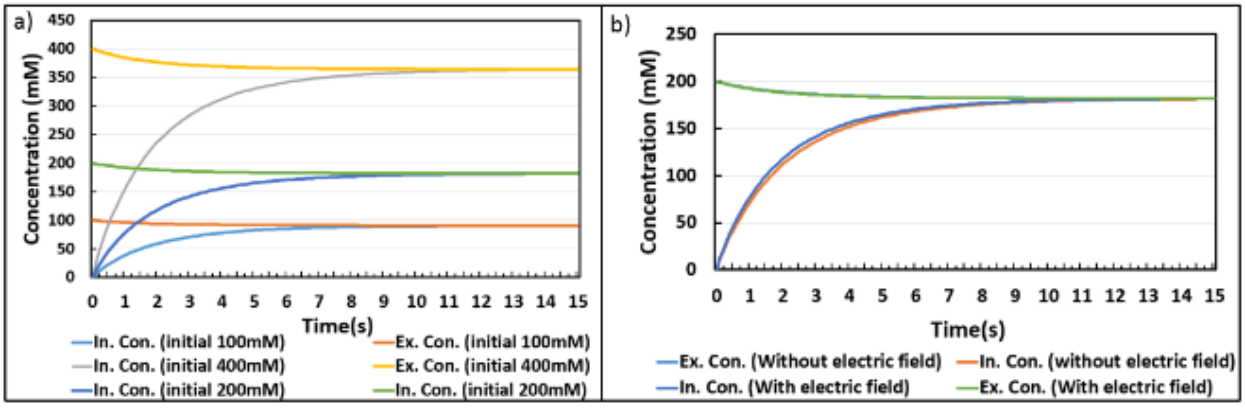

Figure 4: (a) Concentration variation inside (In.Con.) and outside (Ex.Con) of the cell with different initial extracellular molecular concentrations plot with time, (b) Concentration variation inside (In.Con.) and outside (Ex.Con.) of the cell with electric field applied and without applying electric field plot with time.

Therefore in this case we need a concentration above 400 mM for the exterior solution to successfully transfer molecules. If we know the width of the pulse applied, we can calculate and set the initial concentration of the exterior for the exact value and control the molecular uptake.

Then keeping the initial concentration as a constant, the voltage applied was changed in order to check the contribution from the electric field to the migration of molecules. For that the results were observed for two cases. Which are, "Without applying an electric field" and "With an application of an electric field of 2 kV/cm" (with 6.17 V applied to electrodes). Then the results were plotted in the same graph. (Figure 4.b). There we can see the graphs show a slight difference between the molecular uptake speeds in two cases. This is due to defining a low mobility value ($2x10^{-13}$ s.mol/kg) for the molecular solution in the simulation. This clearly shows why in some cases transport can occur in part or almost completely by diffusion and in some cases the transport caused by electroporation is predominantly electrically driven [3]. It depends on the type of molecule we are going to put in to the cell.

Conclusions

In this study, we have used COMSOL to analyze the molecular uptake in an electroporated cell. If the cells are patterned in a single file in between two parallel electrodes (as we suggest in our method), this simulation can be used to obtain a quantitative molecular transport to more than one cell.

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