Stochastic Diffusion of Calcium Ions through a Nanopore in the Cell Membrane Created by Electroporation

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Abstract: In this work we simulated the diffusion of calcium ions through a nanopore created in the cell membrane by electroporation, in presence and absence of the external electric field responsible of the membrane permeabilization. First we solved the set of coupled differential equations that describe the process of ionic diffusion in a 2D nanopore model using the AC/DC and Transport of Diluted Species modules of COMSOL Multiphysics, and then we carried out a simulation of the stochastic molecular dynamics of the calcium ions in the nanopore using Matlab via LiveLink. Finally we compared the results obtained in both simulations. We found a difference of about one order of magnitude in the values of the ionic flux in the nanopore, while the qualitative description of the diffusion process evolution was similar in both cases.

Keywords: Electroporation, nanopore, calcium ions, stochastic diffusion, COMSOL-Matlab LiveLink.

1. Introduction

When a cell is under the influence of an strong enough external electric field hydrophilic nanopores will be created in the plasma membrane in a process known as electroporation [1][2]. There is transport of fluids and ions through these nanopores. The standard approach to study this phenomenon is the use of a set of differential equations that describe the diffusion, migration and convection of the ionic flux in the nanopore [3]; however, due to the nano-scale of the pores it is possible that the discrete nature of the ions and the randomness characteristic of their diffusive processes may affect the results obtained through the aforementioned deterministic equations [4]. During electroporation the study of calcium ions is of important because of their role in cellular signaling and in apoptosis or programmed cell death [5][6], therefore, the study of possible deviations in the behavior of calcium ions

transport through a nanopore from that predicted by deterministic differential equations is of special interest.

2. Mathematical model

2.1 Ionic diffusion

In a system of several ionic components a diffusion process will arise if at least one of them is not uniformly distributed. The diffusion processes caused by concentration gradients are classically described by Fick's laws.

$$J_{d,j} = -D_j \nabla c_j \tag{1}$$

$$\frac{\partial c_j}{\partial t} = D_j \nabla^2 c_j \tag{2}$$

Where $J_{d,j}$ is the ionic flux, and D_j and c_j are the diffusion and concentration coefficients of ion j, respectively. When an electrolyte, like the ones present in the extracellular and intracellular mediums, is under the effect of an external electric field, the ionic flux has an additional component due to the forced exerted by the electric potential upon the charged particles (migration flux). The total ionic flux is described by the Nernst-Planck equation [7].

$$J_j = -D_j \left(\nabla c_j + \frac{c_j z_j F}{RT} \nabla \Phi \right) \tag{3}$$

Where J_j is the total ionic flux, z_j is the valence of ion j, F is Faraday's constant, R is the ideal gas constant, T is the absolute temperature and Φ is the electric potential. Finally, the time change rate of the ionic concentration can be described by equation (4).

$$\frac{\partial c_j}{\partial t} = \nabla \cdot \left(D_j \nabla c_j + \frac{D_j z_j F}{RT} c_j \nabla \Phi_i \right) \tag{4}$$

The electric potential Φ is obtained solving equation (5)

$$\nabla\left(-\varepsilon\nabla\Phi(x,y,z,t)\right) = F\sum_j z_j c_j \qquad (5)$$

2.2 Brownian dynamics

Brownian dynamics allows the study of the movement of individual molecules in a solution. The effects of the solute-solvent interactions on the molecular movement are simplified assuming that the solute molecules diffuse with a mathematically ideal Brownian motion [4][8]. This approach replaces the deterministic molecular movements and is the stochastic source in the theory. Since this model considers individual molecules instead of bulk concentrations, Fick's second law is rewritten as a probability density function, thus, the position of molecule j at time t is given by the density $p_j(\boldsymbol{r}, t)$ that changes in time according to equation (6).

$$\frac{\partial p_j(\boldsymbol{r}, t)}{\partial t} = D_j \nabla^2 p_j(\vec{r}, t) \qquad (6)$$

The initial positions of the molecules are assumed as known and from there they diffuse for a given time interval. The solution of equation (6) with those initial conditions yields a probability density for the molecules displacement after each time interval with a Gaussian profile for each Cartesian coordinate.

$$p_j(\boldsymbol{r} + \varDelta\boldsymbol{r}, t + \varDelta t) = G_{s_j}(\varDelta x)G_{s_j}(\varDelta y)G_{s_j}(\varDelta z) \quad (7)$$

$$G_s(\varDelta x) = \frac{1}{s_j\sqrt{2\pi}}e^{\left(-\frac{(x-x_0{}^2)}{2s_j{}^2}\right)} \qquad (8)$$

$$s_j = \sqrt{2D_j\varDelta t} \qquad (9)$$

s_j is the standard deviation of the Gaussian and it's known as the root mean square displacement length [4][9].

3. Numerical simulation

3.1 Deterministic diffusion

The deterministic study of calcium ions diffusion through the nanopore in the cell membrane is based on the solution of equations (4) and (5) with an appropriate set of boundary conditions. To solve these coupled equations we used the AC/DC and the Transport of diluted species modules of COMSOL Multiphysics, following the methodology proposed in [3] for a bi-dimensional geometry. Besides the change in the problem's dimensionality in this case, we simulated a whole nanopore, neglected the effect of electrosmosis on the ionic transport, and set up the study of ionic concentrations as time dependant. We also neglect the effect of the Calcium ions concentration on the electric potential; reducing equation (5) to Laplace's equation. First we solved the problem in absence of the external electric field to obtain only the diffusion that arises from the concentration difference between the intracellular and extracellular mediums, and that accounts for the main transport mechanism once the electroporation pulses have ceased. Then we included the external electric field in the model to study the effects of ionic migration, coupling the AC/DC and the Transport of diluted species modules. In Table 1 we present the constants used in the simulation.

3.2 Stochastic diffusion

From the ionic concentrations listed in Table 1 we obtained the average number of calcium ions in the interest regions around the nanopore: about 430 ions in the extracellular medium and between 0 and 1 ion in the intracellular medium. The initial position of each ion was sampled from a Gaussian with a mean vector and a covariance matrix such that the large majority of ions would be located in the extracellular medium. If after the sampling there were ions present in forbidden regions, i.e. the interior of the cell membrane, these were relocated. To simulate the ions diffusion in absence of the external electric field the position of each ion was updated iteratively choosing random displacements with Gaussian distributions of the form given in (8) for coordinates x and y. In this case we used a time interval of $5 \times 10^{-12}\ s$ to update the position of each ion 1×10^5 times. To include the effects of the ionic migration in the diffusion process our first approach was to export the data of the electric field magnitude in the x and y coordinates from the simulation previously carried out in COMSOL Multiphysics to a text file and then loaded them into Matlab, afterwards we used the COMSOL-Matlab livelink instead, with no alteration in the final

behavior of the probabilistic distribution of the calcium ions in the nanopore. Based on the magnitude of the electric field in the x and y coordinates we obtained the electric force exerted on each calcium ion in each position, and superimposing it with the stochastic diffusive forces we modeled the ions dynamics. For this analysis we consider the ions as point charges. The time interval we used in this case was 2×10^{-12} and we updated the ions positions 500 times. The differences in the time intervals and the number of updates is due to the fact that the presence of the electric field accelerates considerably the ions transport through the nanopore and the phenomenon of interest can be observed in a shorter time span [3][10].

The version of the software used to solve the coupled differential equations was COMSOL Multiphysics 4.2. The model that describes the stochastic ions dynamic was solved in Matlab R2009a. Both simulations were carried out in a DELL Precision T3600 computer with a four Intel Xeon processor, 32 GB RAM and Nvidia Gforce 4600 2 MB.

Table 1. Constants and parameters used in the simulations [3].

| Parameter	Value
Nanopore radius	$5\ \mu m$
Intracellular medium radius	$50\ nm$
Extracellular medium radius	$50\ nm$
Relative permittivity	80
Surface charge density	$-0.0001\ \dfrac{C}{m^2}$
Temperature	$298\ K$
Faraday constant	$96485.3415\ \dfrac{C}{mol}$
Ideal gas constant	$8.314\ \dfrac{J}{K\ mol}$
Diffusion coefficient of calcium	$7.9 \times 10^{-10}\ \dfrac{m^2}{s}$
Intracellular calcium concentration	$0.000079\ mM$
Extracellular calcium concentration	$1.8\ mM$
Transmembrane potential	$1\ V$

4. Results and discussion

4.1 Results

A) Deterministic diffusion: We obtained graphics that describe the calcium ions diffusion, in both presence and absence of the external electric field (figures 1 and 2), we calculated the values of the ionic flux at the entrance of the nanopore in both cases for specific moments in time (Table 2), and finally we obtained the electric potential distribution and the electric field direction in the nanopore (figure 3).
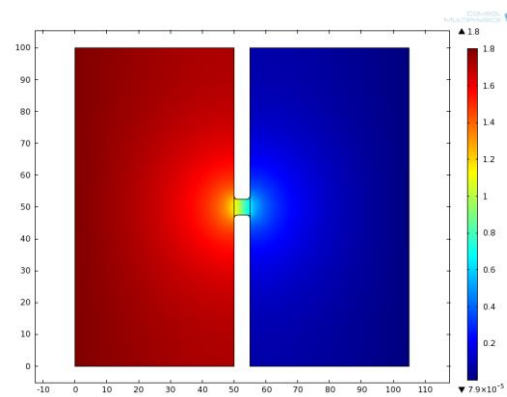
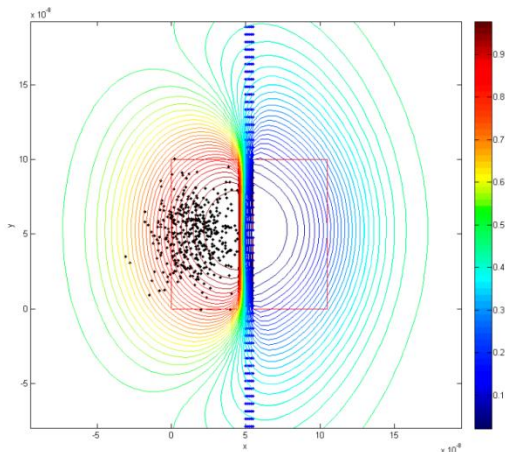**Figure 1.** Nanopore calcium diffusion in absence of the electric field.
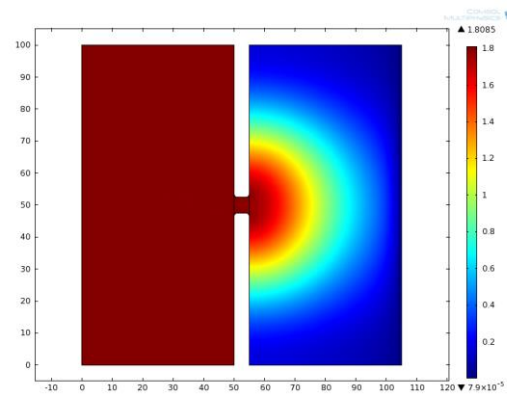
**Figura 2.** Nanopore calcium diffusion after the application of the external electric field.
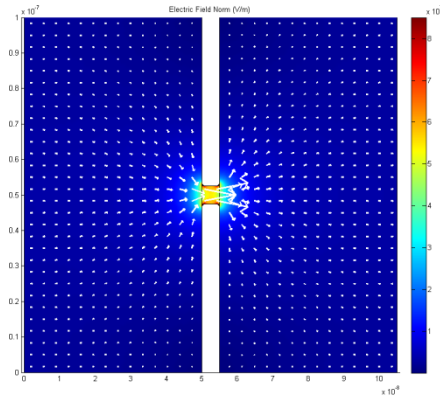
**Figura 3.** Electric potential distribution inside and around the nanopore.

B) Stochastic diffusion: We simulated the dynamics of ionic diffusion and computed the average time that took for 10% of the ions to go through the nanopore (Table 2). This limitation in the amount of ions considered was intended to minimize possible inaccuracies that could arise from the fact that physically there is a continuous flux of new ions towards the region of the extracellular medium surrounding the nanopore and that this ions aren't part of the model; Then we generated graphics with the probabilistic profiles of the calcium ions distribution before and after the diffusion took place (Figures 4-7). The above described procedure was first carried out without considering the electric field, and then taking it into account.

Figura 4. Initial probabilistic distribution of the calcium ions in absence of the electric field.
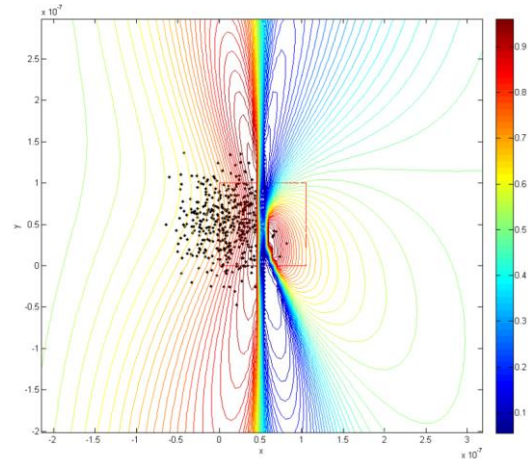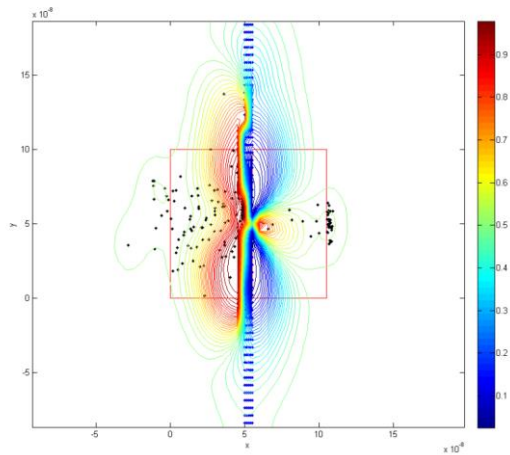

Figura 5. Final probabilistic distribution of the calcium ions after diffusion, in absence of the electric field.
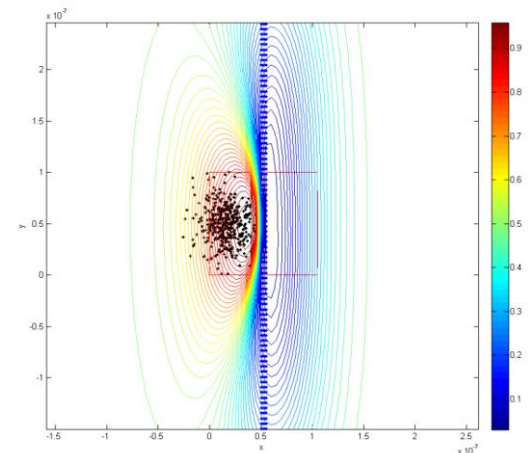
Figura 6. Probabilistic distribution of the calcium ions in the nanopore before the application of the external electric field.

Table 2. Ionic flux at the entrance of the nanopore.

Ionic Flux $\left(\frac{mol}{s}\right)$	Deterministic	Random
Diffusion (t = 0,438 µs)	$14,15 \times 10^{-12}$	$4,27 \times 10^{-13}$
Diffusion-migration (t = 0,157 ns)	$19,94 \times 10^{-16}$	$2,68 \times 10^{-17}$

Figura 7. Probabilistic distribution of the calcium ions in the nanopore after the application of the external electric field.

4.2 Discussion

The behavior of the observed diffusion processes, both through the solution of the coupled differential equations and the simulation of the model that describes the molecular dynamics of calcium ions, exhibits the same basic characteristics exposed in [3][10] and [11] for processes of this nature (figures 1,2 and 4-7). The most significant of which is the marked increase in the ionic flux through the nanopore when the effects of the electric field applied during electroporation are taking into account.

The variable we used to compare the results obtained by both approaches was precisely the ionic flux at the entrance of the nanopore. Due to the effects of the randomness of the diffusion processes on the ionic flux in the model that describes the molecular dynamics of calcium ions, the comparison was made using average values (Table 2). The most remarkable result we observed was a difference of about one order of magnitude in the values of the variable under study between both simulations. The lower values were always present when the stochastic behavior of the ionic movement was taken into account. This suggests that for low ionic concentration, like that of calcium, and for pores in the cell membrane in the range nanometers, the ions discrete nature and their stochastic movement can affect the values found from the solution of deterministic differential equations. However, it's important to emphasize that in the model of the molecular dynamics of calcium ions

we ignored the ions interactions among themselves and with the membrane [12], which has the potential to alter the obtained results.

5. Conclusions

In this paper we studied the diffusion of calcium ions through a nanopore in the cell membrane created by electroporation. This study was divided into two stages. In the first place we used a set of coupled differential equations to obtain the change in time of the calcium concentration, both in presence and absence of the external electric field. Then we simulated the molecular dynamics of the ions, taking into account the randomness of their diffusion processes, under the same conditions previously mentioned regarding the electric field. In both cases the transport of ions through the nanopore showed the general characteristics describe in the literature for this type of phenomenon, however the comparison between the results obtained for the ionic flux at the entrance of the nanopore in both approaches indicates that, for calcium ions, ignoring the stochasticity of their movement and their discrete nature could lead to underestimate the magnitude of this physical variable. Nonetheless the model of molecular dynamics we used in this work has some limitations that arise from theoretical assumptions, and that need to be addressed before a definite result can be reached.

6. References

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7. Acknowledgements