Phase-Field Model of Electroporation

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Abstract

In this work, a model and simulation method to study the dynamics of pore formation and annihilation in a lipid membrane under an applied electric field has been developed. A continuum-level diffusive interface model (phase-field) method is applied to model the evolution of the pore in a lipid membrane patch. The numerical method and results are presented.

1 Introduction

Vesicles have biomedical applications as vectors for drug and gene delivery. Manufactured vesicles can be injected with drug molecules, or other particles such as DNA, which can in theory be delivered directly to a particular region. An ability to release the interior of the drug in a controlled environment is crucial for any application. One possible approach is so called "electroporation". Under the action of high potential difference across the biological membrane, they lose their barriers functions (electric breakdown). It is generally accepted now that electric breakdown is based on the formation of pores in the lipid areas of the membrane. It is also accepted that due to extra surface tension produced by potential difference cause the formation of these pores.

In this paper the diffusive interface approach (phase-field) is adopted in order to simulate the electroporation of a simple model system based on planar lipid bilayers. It is believed the study of planar system paves the way to study more complex systems such as lipid vesicles and biological cells.

2 Theoretical Background

2.1 Transient Pores Model

The initial observation of pores on membranes did not involve the electrical behaviour. The possibility of spontaneous poration initially suggested by two groups and later on studied more carefully [7, 8]. The assumption is that during the constant thermal fluctuation of lipid molecules hydrophobic pores are spontaneously formed in the lipid matrix (Figure 1a).

Exceeding to the critical radius, a reorientation of lipids occur and converts the pore into hydrophilic pores (Figure 1b) with the head groups forming the pore walls.

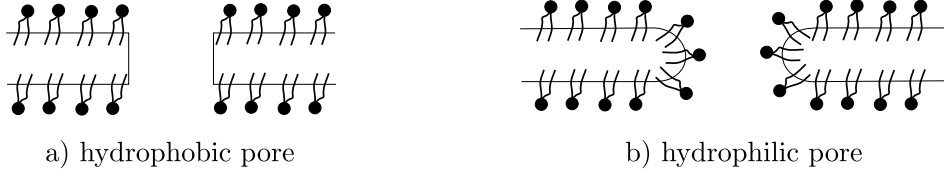a) hydrophobic pore   b) hydrophilic pore

Figure 1: Types of pores in lipid membrane

The life time of the hydrophobic pores is in the order of the lipid fluctuations. Therefore, it is assumed that they are only intermediate stages in the formation of hydrophilic pores which are more stable and larger in size [6]. In this paper the intermediate stage of hydrophobic pores is neglected.

The mechanism of hydrophilic pores formation/annihilation is described by two competitive deriving force: the energy per length along the pore edges γ, we call it line tension, and the membrane tension or the energy per area of the membrane of a flat pore-free membrane σ_0 where it is simply called the surface tension. The energy of transient pore ΔW_p is usually given for a single pore with radius of R:

$$\Delta W_p(R) = 2\,\pi\,\gamma\,R - \sigma_0\,\pi\,R^2 \tag{1}$$

One can simply generalize this for pore region instead of one pore and rewrite the free energy equation in the form of two integrals. The first integral is the interracial contribution due to line tension γ acting on the pore edges $\partial\Gamma$, and the second integral is the surface tension contribution to the porated area Γ_p of the lipid membrane:

$$\Delta W_p = \oint_{\partial\Gamma} \gamma\,ds - \int_{\Gamma_p} \sigma_0\,dA \tag{2}$$

Surface tension σ_0 is responsible for appearance of transient pores in vesicles and lipid membranes. The surface tension for the membrane in relaxed state is very small. However, due to many reasons such as thermal fluctuations or increased hydrodynamic pressure inside the vesicle the membrane eventually becomes tense and pore formation occurs. After the pore formation and releasing the surface tension, pores will eventually reseal, the deriving force here is the line tension γ. In Figure 2 the opening and closing deriving forces are shown.

2.2 Electroporation Model

Many theories describing the electroporation process have surfaced in recent (and not so recent) years [9, 10, 1]. Electroporation is a dynamic phenomenon that depends on the local transmembrane voltage U_m of the lipid part of the membrane. The induced voltage over the lipid membrane creates an extra surface tension σ_e over the membrane in addition to the membrane tension σ_0 that already exists on the membrane. The overall surface tension then is:

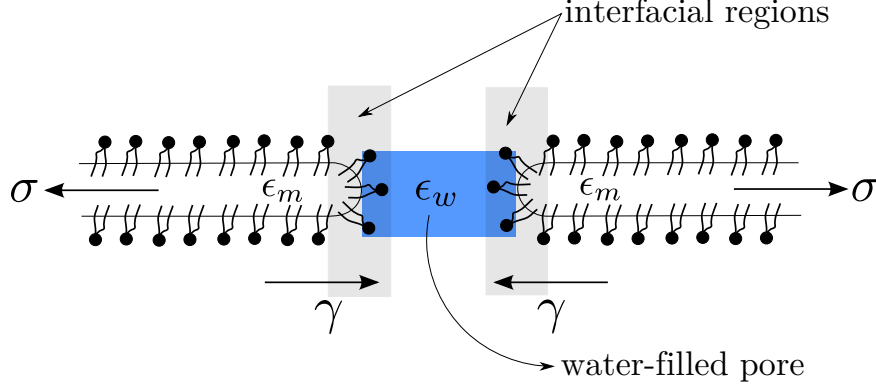$$\sigma = \sigma_0 + \sigma_e \tag{3}$$

Figure 2: Schematic of one water-filled pore in a lipid membrane

Pore regions can be treated as having an energy associated with the change of its specific capacitance, C_{LW}, as lipid is replaced by water (a water-filled capacitor) shown in Figure (2). In the presence of a transmembrane electric field, the free energy of pore formation should be:

$$\Delta W_p = \oint_{\partial \Gamma} \gamma \, ds - \int_{\Gamma_p} \sigma_0 \, dA - \int_{\Gamma_p} \sigma_e \, dA \tag{4}$$

The last term is an additional surface tension due to electric potential induced over the membrane surface. Electric surface tension defined as [9]:

$$\sigma_e = \frac{1}{2} C_{LW} \bar{U}_m^2 \tag{5}$$

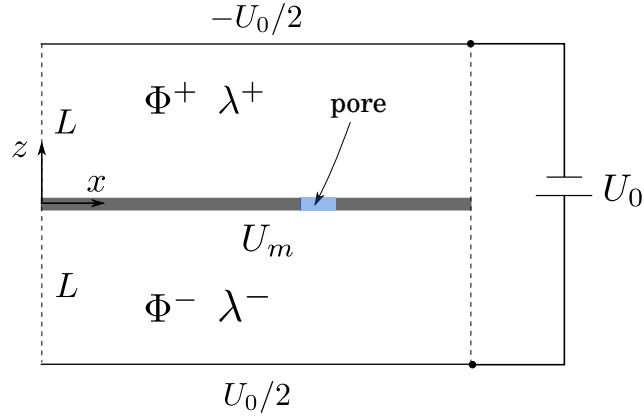Here $\bar{U}_m$ is the spatially averaged transmembrane voltage,

Figure 3: A lipid membrane placed in a uniform electric field.

The membrane voltage U_m can be obtained from solving electric field equation of the system:

$$\nabla^2 \Phi^{\pm} = 0 \tag{6}$$

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where $\mathbf{E}^{\pm} = -\nabla\Phi^{\pm}$ is the electric field. At the electrodes, the potential satisfies the boundary conditions $\Phi(x, y, \pm L, t) = \mp U_0/2$, conservation of normal currents requires

$$C_m \frac{d\bar{U}_m}{dt} + G_m \bar{U}_m + I_p = \lambda^{\pm} \mathbf{n} \cdot \mathbf{E}^{\pm} \tag{7}$$

where $U_m(t) = \Phi^+(x, y, 0, t) - \Phi^-(x, y, 0, t)$ is the membrane voltage and $\mathbf{n}$ is the unit normal vector. G_m is the conductance of the lipid membrane and I_p is the current density of pore regions:

$$I_p = \frac{\bar{U}_m}{R_p} \tag{8}$$

where $R_p = h/\lambda A_p$ is the pore resistance with A_p being the area of the pore region and λ is the conductivity of the solution filling the pore and h is the thickness of the membrane.

At $t = 0$, when the field is applied, the potential is assumed to be continuous, $\bar{U}_m(t = 0) = 0$. These system of equation can be solved numerically. where $\lambda^{\pm}$ is the interior and exterior electrolyte (i.e. water) conductivity. This solution is the result of solving the electric field system of equation and the leaky dielectric assumption applied on the lipid membrane.

The change of the pore's specific capacitance as water displaces lipid to form a pore is simply

$$C_{LW} = \left(\frac{\epsilon_w}{\epsilon_m} - 1 \right) C_m \tag{9}$$

Here $\epsilon_w = K_w \epsilon_0$ is the permittivity of water and $\epsilon_m = K_m \epsilon_0$ is the permittivity of lipid membrane, and C_m is the constant capacitance per area of a pore free membrane, i.e $C_m = \epsilon_m/h$ where h is the thickness of the membrane. Typically $K_w = 80$ and $K_m = 2$, so when the potential $\bar{U}$ increases ΔW decreases which means pore nucleation is more desirable.

3 Phase-field Formulation

We are adopting the first order phase transition model. This model implies that broken membrane states (pore phase) have lower free energy than intact membrane states (lipid phase). This concept first introduced on the study of stability of soap films [3, 2]. This approach assumes at the presence of electric field the membrane breakdown occurs because it is energetically more desirable i.e. transitioning to more stable phase.
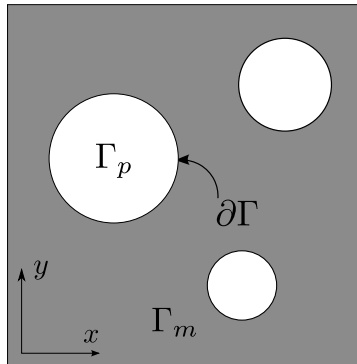

Figure 4: Schematic of the whole domain $\Gamma = \Gamma_m \cup \partial\Gamma \cup \Gamma_p$. Γ_m indicates lipid membrane phase, Γ_p is the pore phase, and $\partial\Gamma$ is the interface

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The phase-field function ϕ is defined for two states $\phi = 1$ for lipid membrane (grey regions in Figure 4) and $\phi = 0$ for pores (voids in Figure 4), and the phase interfaces are replaced by thin layer across which ϕ changes its value between 0 to 1. The line tension energy in Equation 4 can be replaced with Ginzburg-Landau energy form [5, 4]. Therefore,

$$\Delta W_p[\phi] = \int_\Gamma \bar{\gamma} \left(\frac{\varepsilon}{2} |\nabla \phi|^2 + \frac{1}{\varepsilon} g(\phi) \right) - \int_\Gamma \sigma \left(1 - H(\phi) \right) c_0 \tag{10}$$

where $g(\phi) = \frac{1}{4}\phi^2 (1 - \phi)^2$ is a double-well potential function and ε a small length scale. The coefficient $\bar{\gamma}$ is related to the line tension coefficient γ by

$$\bar{\gamma} = \frac{3}{4} \gamma \tag{11}$$

The function $H(\phi)$ is the Heaviside or the step function. The smooth Heaviside function $H(\phi) \approx \frac{1}{2} + \frac{1}{2} \tanh \left(k \left(\phi - \frac{1}{2} \right) \right)$ were chosen in order to utilize the better separation behaviour of two phases where larger k corresponds to a sharper transition at $\phi = 1/2$. The coefficient c_0 is the area correction coefficient in order to keep the area constant

$$c_0 = \frac{\int_\Gamma (1 - \phi)}{\int_\Gamma (1 - H(\phi))} \tag{12}$$

The Allen-Cahn type dynamic formulation can be obtained from the energy functional in Equation 10:

$$\frac{\partial \phi}{\partial t} = -M \frac{\delta \Delta W_p}{\delta \phi} + \eta(\mathbf{x}, t)$$
$$= -M \left(-\bar{\gamma} \varepsilon \nabla^2 \phi + \frac{\bar{\gamma}}{\varepsilon} \frac{dg}{d\phi} + \sigma \delta(\phi) c_0 \right) + \eta(\mathbf{x}, t) \tag{13}$$

where $g'(\phi) = \frac{1}{2} \left(\phi - 3\phi^2 + 2\phi^3 \right)$ and also the Dirac's delta function $\delta(\phi) = dH(\phi)/d\phi$ is the derivative of Heaviside function. M is related to the time scale for atomic rearrangement from the disordered phase to ordered one called the mobility coefficient where can be related to the diffusion coefficient D and temperature T by the Einstein relation $D = M k_B T$, and the coefficient $k_B = 4.11 \times 10^{-12}$ J is called is the Boltzmann's constant, and $\eta(\mathbf{x}, t)$ is the stochastic noise due to thermal fluctuations and satisfies the relation:

$$\langle \eta(\mathbf{x}, t) \, \eta(\bar{\mathbf{x}}, \bar{t}) \rangle = 2M k_B T \, \delta(\mathbf{x} - \bar{\mathbf{x}}) \, \delta(t - \bar{t}) \tag{14}$$

4 Numerical Method

We introduce the following non-dimensional variables:

$$\mathbf{x}' = \frac{\mathbf{x}}{l_c}, \quad t' = \frac{t}{\tau}$$

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where l_c is a characteristic length of the membrane and $\tau = l_c^2/D$ is the characteristic time associated with diffusion of the lipid molecules. Where D is the diffusion coefficient of lipid membrane and $D = M\,k_B T$. Also the dimensionless surface and line tension can be acquired:

$$\gamma' = \frac{\gamma\,l_c}{k_B T}, \quad \sigma' = \frac{\sigma\,l_c^2}{k_B T}$$

The non-dimensionalized and discretized form of Equation 13 is going to be:

$$\frac{\phi_{i,\,j}^{n+1} - \phi_{i,\,j}^{n}}{\Delta t'} = \gamma'\,\varepsilon'\bar{\nabla}^2 \phi_{i,\,j}^{n} - \frac{\gamma'}{\varepsilon'}\,(\phi_{i,\,j}^{n}) - \sigma'\,\delta(\phi_{i,\,j}^{n})\,c_0 + \eta'(\mathbf{x}',t') \tag{15}$$
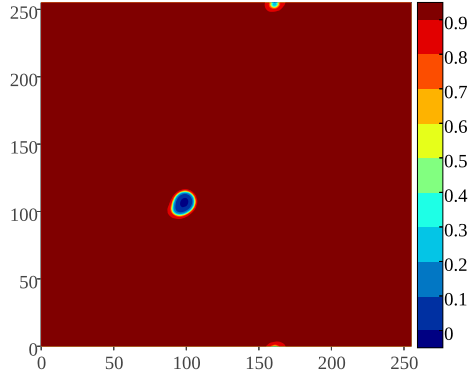
The form of discrete Laplacian operator $\bar{\nabla}^2$ is used where it introduced first by Oono and Puri:

$$\begin{aligned}
\bar{\nabla}^2 \phi_{i,\,j}^{n} &= \frac{\phi_{i+1,\,j} + \phi_{i-1,\,j} + \phi_{i,\,j+1} + \phi_{i,\,j-1}}{2\,\Delta x'^2} \\
&+ \frac{\phi_{i+1,\,j+1} + \phi_{i-1,\,j+1} + \phi_{i+1,\,j-1} + \phi_{i-1,\,j-1}}{4\,\Delta x'^2} \\
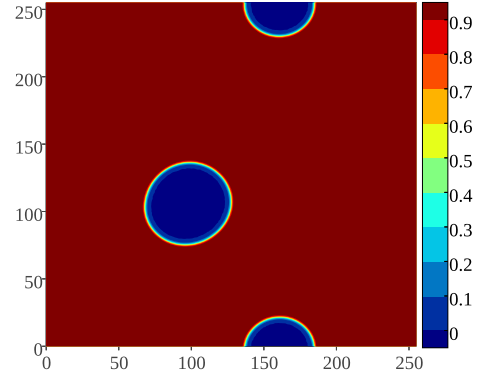&- \frac{3\,\phi_{i,\,j}}{\Delta x'^2}
\end{aligned} \tag{16}$$

Take the lipid molecules the line tension γ is reported to be between $10\,\mathrm{pN}$ to $30\,\mathrm{pN}$. We choose the characteristic length $l_c = k_B T/\gamma$. This gives $l_c = 6.4111 \times 10^{-11}$ m.
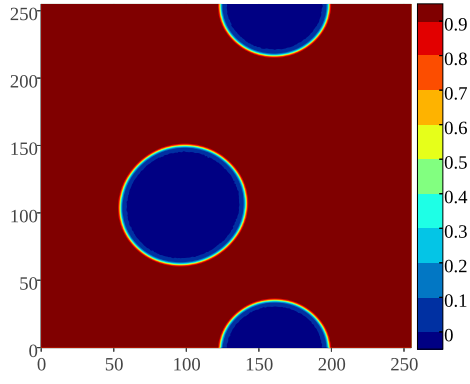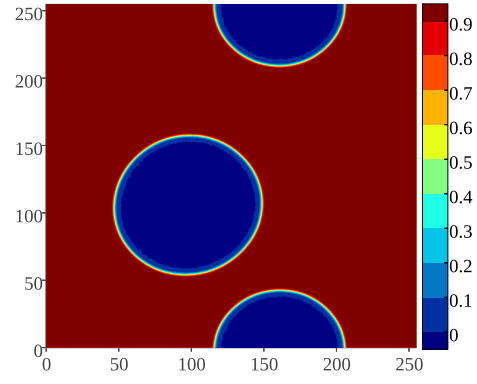
5   Results
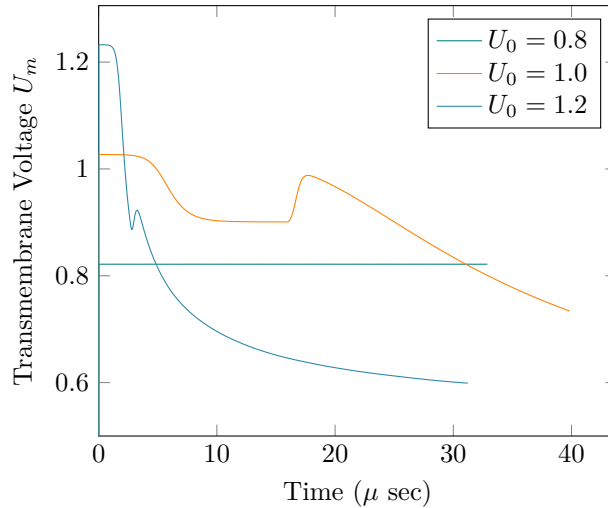
(a) $t = 10000\,\Delta t'$

(b) $t = 15000\,\Delta t'$

(c) $t = 20000\,\Delta t'$

(d) $t = 25000\,\Delta t'$

Figure 5: Initiation of the pore and evolution during time ($U_0 = 1.0$ v)

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