A Novel Engineered Membrane to Measure Electroporation: Effect of Tethers and Bioelectronic Interface

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Abstract

This paper reports on the construction and predictive models for an engineered tethered membrane. A key feature of the engineered membrane is that it provides a controllable and physiologically relevant environment for the study of the electroporation process. The mixed self-assembled membrane is formed via a rapid solvent exchange technique. The membrane is tethered to the gold electrode and includes an ionic reservoir separating the membrane and gold surface. Above the membrane there is an electrolyte solution, and a counter gold electrode. A voltage is applied between the gold electrodes and the current measured. The current is dependent on the energy required to form aqueous pores and the conductance of each pore. Modelling the membrane as a dielectric and elastic continuum, we construct a free energy model for pores in the membrane. The free energy model is constructed using the Poisson-Nernst-Planck (PNP) system of equations with an activity coefficient to model the steric effects of ions in the electrolyte. The electrical energy required to form a pore is estimated using the PNP system of equations coupled to the Maxwell stress tensor. The tethers are modelled as Hookean spring anchors to the membrane surface. The conductance of pores in the membrane are estimated using the generalized PNP equations to account for the finite size of ions, multiple ionic species, and the Stern and diffuse layers present. The population and radii of pores in the membrane are estimated using asymptotic approximations to the Smoluchowski-Einstein equation that are dependent on the energy required to form the pores. Using an equivalent circuit model of the tethered membrane, the current is predicted using the estimated pore population, radii, and the conductance of the pores, and compared with experimentally measured current for different tethering densities.

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Introduction

Electroporation is a technique that causes the permeability of a biological membrane to increase in response to an applied electric field. The application of electrochemotherapy for antitumor treatment, protein insertion, gene and drug delivery are all of significant interest to the pharmaceutical industry (1). Over the past three decades the complex process of electroporation has been studied both experimentally and theoretically; however, the process is still poorly understood (1, 2). The electroporation process can be described as having two stages: the first is the nucleation and destruction of aqueous pores, and the second is the dynamics of formed pores. The nucleation of pores is currently an active area of research employing stochastic methods and molecular dynamics simulations (2, 3). The dynamics of created pores are governed by the free energy of the pores.

Experimental validation of theoretical models of electroporation is very difficult as the size of pores is on the nm scale and typical electroporation measurements are on the length scale of \( \mu m \). Since the dynamics of pore radii can not be measured, the validation of the models is carried out using conductance measurements of the membrane. The validation of the theoretical models using synthetic bilayer lipid membranes is useful but is not a good representation of physiological systems as effects
caused by the cytoskeletal network are not present. Using cells \textit{in vitro} provides a physiological system for validation; however, it is impossible to fully define the physiological environment which effects properties associated with electroporation. In this work, the novel engineering and predictive modelling of a tethered lipid membrane platform is presented for the study of electroporation in a physiologically relevant environment. The key feature of the engineered tethered membrane is that the experimentalist can select the desired density of tethers and membrane composition (4–7). Using the rapid solvent exchange technique to construct the membrane also reduces the defect density as compared with membranes constructed using the frequently used vesicle fusion technique allowing the engineered membrane to have a lifetime of several months (6, 8, 9).

A schematic of the \textit{engineered tethered bilayer lipid membrane} is given in Fig. 1. The engineered tethered membrane is composed of a self-assembled monolayer of mobile lipids, and a self-assembled monolayer of tethered and mobile lipids. The tethered lipids are anchored to the gold electrode via polyethylene glycol chains. Spacer molecules are used to ensure the tethers are evenly spread over the gold electrode. The intrinsic spacing between tethers and spacers is maintained by the benzyl disulfide moieties which bond the spacers and tethers to the electrode surface. The ratio of the total number of tethers to spacers and tether molecules in the engineered tethered membrane is given by the tethering density (i.e. a 10% tether density defines that for every 9 spacer molecules there is 1 tether molecule). In the special case of 100% tethering, the engineered tethered membrane is composed of a tethered archaeabacterial based monolayer with no spacer molecules. As experimentally illustrated in (8), it is not possible to construct a 0% tethered membrane as any formed membrane binds to the gold surface. As the electrolyte reservoir separating the membrane and electrode surface is required for the normal physiological function of the membrane, and noting that all prokaryotic and eukaryotic cell membranes contain cytoskeletal supports with a 1% to 10% tether density, the inability to construct a 0% tethered membrane does not inhibit the study of the electroporation process in a physiologically relevant environment. A time-dependent voltage potential is applied between the electrodes to induce a transmembrane potential of electrophysiological interest; this results in a current \( I(t) \) related to the charging of the double-layers and the nucleation and destruction of aqueous pores in the engineered tethered membrane.

To draw quantitative conclusions about the electroporation process using the engineered tethered membrane requires a predictive model. The first models of electroporation were based on using statistical physics of stochastic processes that are dependent on the energy required to form a pore. The most widely used model for the statistics of aqueous pores formed via electroporation is the Smoluchowski-Einstein equation derived from statistical mechanics (11–13). Several models governing the pore energy have been presented in the literature (14–21). The membrane is generally modelled as a dielectric and elastic continuum (14, 22–27). Aqueous pore formation involves the creation of a hydrophilic pore, generally assumed to be a water filled cylinder with a free energy given in terms of modified Bessel’s functions, which either spontaneously converts to a stable hydrophilic pore or spontaneously collapses (21, 28, 29). Although different pore shapes and corresponding pore energies can be considered for the hydrophobic pore, typically a toroidal pore is assumed (18, 20, 30). Note that the toroidal structure corresponds to the estimated hydrophilic pore shape obtained from molecular dynamics simulations (3, 31, 32). The classical free energy model for a hydrophobic aqueous pore \( W(r, V_m) \) in the membrane consists of four energy terms: the pore edge
energy $\gamma$, the membrane surface tension $\sigma$, the electrostatic interaction between lipid heads, and the transmembrane potential energy contribution $W_{es}(r, V_m)$. Several other contributions have been considered in the literature including effects from the cytoskeletal network (19), osmotic pressure contributions (33), electrocompressive forces (34), and bound peptides (35, 36). The classical pore energy typically used is given by (20):

$$W(r, V_m) = 2\pi\gamma r - \pi\sigma r^2 + (C_r^{-1}) + W_{es}(r, V_m) + W_m. \quad (1)$$

with the energy contribution from the mechanobiological properties of the cell included as $W_m$ (i.e. cytoskeleton). With the energy of a pore defined by Eq. 1, the key element that couples the experimental measurements with the predicted model is the aqueous pore conductance $G_p$ (29, 30, 37–39). Discussion of $G_p$, $W_{es}$, and $W_m$ is provided below.

Estimates of $G_p$ model the conduction of aqueous pores as originating from two contributions: one from the internal conductance of the pore which includes effects from steric hindrance, and the external “spreading conductance” (12, 38, 40, 41). Near the pore entrance significant nonlinear potential gradients are present which restrict the current flowing through the pore, this effect is denoted as the spreading conductance. Assuming an infinite electrolyte bath on both sides of the membrane and neglecting electrodiffusive effects, the spreading conductance for a pore of radius $r$ and electrolyte conductivity $\varsigma_e$ is given by $2\varsigma_e r$, computed from the Laplace equation for electrostatics (39, 41, 42). The total pore conductance, neglecting electrodiffusive effects, is given by (12, 38, 40):

$$G_p = \frac{2\pi r^2 \varsigma_c \varsigma_p}{\pi r \varsigma_p + 2h_m \varsigma_c}, \quad (2)$$

where $h_m$ is the membrane thickness and $\varsigma_p$ is the pore conductance which implicitly includes the steric effects of ions entering the pore. Recent models for computing $W_{es}$ utilize the Maxwell stress tensor with the electric field computed from the solution of the Laplace equation for electrostatics (18). As we show in this paper, including electrodiffusive effects is important when estimating the pore conductance $G_p$ and electrical energy required to form a pore $W_{es}$.

The mechanobiological properties of the membrane directly effect the electroporation process, as seen in (1). Neglecting the energy contribution from the cytoskeleton network, $W_m$, can introduce severe errors in the estimated energy (1). Experimental evidence shows that the cytoskeleton provides a degree of mechanical integrity to the cell membrane and is therefore a vital component of the pore energy (44–50). The linkages of the cytoskeleton to the membrane are analogous to “springs” and act to restrain the enlargement of aqueous pores. Modelling the mechanical properties of the membrane as an elastic continuum and assuming a permanent cytoskeletal network anchorage, the effect of these anchors are accounted for via the energy required to deform the Hookean springs—formally, the energy contribution can be modelled using $W_m = 0.5K_{cyto} r^2$ with $K_{cyto}$ denoting the spring constant of the cytoskeleton network (19). The accuracy of this model for the anchoring of a membrane have not been studied quantitatively as a result of the complexities associated with the experimental measurements (46–49).

In this paper we report on the construction and predictive models of the engineered tethered membrane that includes contributions from asymmetric electrolytes, multiple ionic species, and the Stern and diffuse electrical double layers present. The models are constructed by assuming the membrane behaves as a dielectric and elastic continuum. The electrodiffusion properties of the electrolyte are modelled using a Generalized Poisson-Nernst-Planck (GPNP) system of equations with a “Langmuir type” activation coefficient (51–53). To estimate $G_p$, we use the GPNP in a toroidal pore geometry. Based on the GPNP and Maxwell stress tensor we construct a model for the electrical energy required to form a pore $W_{es}$ that includes electrodiffusive effects. The energy required to deform the tethers is accounted for using the energy model $W_m = 0.5K_{cyto} r^2$. Modelling the engineered tethered membrane system as an equivalent circuit model, shown in Fig. 3, and using asymptotic approximations to the Smoluchowski-Einstein equation for electroporation, we construct a predictive model for the current $I(t)$ in Fig. 1 that is dependent on the energy to form a pore $W$, defined in Eq. 1. Numerical results and experimental measurements with different tethering densities and lipid compositions are used to validate the accuracy of the models and exemplify the application of the engineered tethered membrane to quantify the contribution the tethers and bioelectronic interface have on the electroporation process. Specifically, we estimate the electroporation parameters of the engineered tethered membrane in Fig. 1 for the membrane tether densities of 1% and 10% for the tethered DphPC lipid bilayer, and the 100% tethered DphPC monolayer membrane.

**Materials and Methods**

Novel features, construction, and predictive models of the engineered tethered membrane are provided in this section. The accuracy of the models are verified using experimental measurements of the engineered tethered membrane with different tethering densities.
The engineered tethered membrane structure is illustrated in Fig. 1. The membrane is supported by a 25mm × 75mm × 1mm polycarbonate slide onto which is patterned a 100 nm sputtered gold electrode array possessing six 0.7mm × 3mm active areas of membrane each of which is enclosed in a flow cell with a common gold return electrode. The formation of the tethered membrane is performed in two stages. The first stage anchors the inner layer of the membrane to the gold surface via benzyl disulphide groups. The inner layer components are introduced to the freshly deposited gold surface as ethanolic solutions of 370 µM benzyl disulphide concentrations in engineered ratios of tethers and spacers. For example the inner layer solution for the 10% tethering density is prepared by co-dissolving benzyl disulphide C20 diphytanylether bis-tetra-ethylene glycol and benzyl disulphide tetra-ethylene glycol in the ratio 1:10. The solution is exposed to the coating solution for 30 min and the electrode is then rinsed in ethanol and air dried for approximately two minutes. The coated slide is immediately assembled into a flow cell cartridge comprising six individual membranes with a common large area gold return electrode facing the membranes and separated by a 100 µm laminate defining the flow cell chamber height. The second stage of the membrane formation now occurs with the addition of 8 µL of 3 mM C20 diphanytanylether lipids comprising a 70:30 mole ratio of C20 diphytanylether glycerophosphatidylcholine: C20 diphtyanylether diglyceride being added to each of the flow chambers covering the membrane areas. The solution is incubated for two minutes at 20°C following which, 300 µL of phosphate buffered saline was flushed through each flow cell which forms the tethered bilayer. The quality of the bilayer is measured continuously using an SDx tethered membranes tethaPodTM swept frequency impedance reader operating at frequencies of 1000, 500, 200, 100, 40, 20, 10, 5, 2, 1, 0.5, 0.1 Hz and an excitation potential of 20 mV (SDx Tethered Membranes, Roseville, Sydney). The membrane was equilibrated for 30 min prior to the electroporation measurements. The electroporation measurements were performed using an eDAQ™ ER466 potentiostat (eDAQ, Doig, Denistone East) and a SDx tethered membrane tethaPlate™ adaptor to connect to the assembled electrode and cartridge. Individual triangular voltage ramps were applied from zero to 500 mV with a period of 2 ms. Current waveforms were recorded.

**Formation of the Engineered Tethered Membrane**

In this section we define the electrodiffusion model for the movement of ions in the engineered tethered membrane system illustrated in Fig. 1. The model provides the basis for computing the pore conductance \( G_p \), and the electrical energy of an aqueous pore \( W_{eq} \) in the membrane.

The ionic concentrations and electric potential dynamics in an electrolyte solution are classically modelled using the Poisson-Nernst-Planck (PNP) system of equations (54). A known weakness of the PNP model is that it does not suitably model the electrical double layers present at the membrane surface or at the electrode-electrolyte interface where Stern and diffuse charge layers are present (54). To overcome these limitations, (51) used a modified “Langmuir type” activity coefficient to account for the steric effects of ions in the electrolyte, termed the Generalized PNP (GPNP) model. The constructed GPNP model for electrodiffusion is given by (51):

\[
\frac{\partial c^i}{\partial t} = -\nabla \cdot (J^i),
\]

\[
J^i = -D^i \nabla c^i - F z^i q_i u_m^i c^i \nabla \phi
- D^i c^i \ln \left(1 - \sum_{i=1}^{N} N_A \alpha_i^3 c^i \right),
\]

\[
- \nabla \cdot (c \nabla \phi) = - \sum_{i} F z^i e^i .
\]

In Eq. 3a, \( J^i \) is the concentration flux, \( c^i \) is the concentration, \( \phi \) is the electrical potential, \( D^i \) is the diffusivity, \( N_A \) is Avagadro’s number, \( \alpha_i \) is the effective ion size, and \( u_m^i \) is the ionic mobility with \( i \) denoting the ionic species. In Eq. 3b, \( F \) is Faraday’s constant where the superscript defines the chemical species \( i \), \( q \) is the elementary charge, \( z^i \) is the charge valency, and \( \epsilon \) is the electrical permittivity. The electrodiffusive model Eq. 3 is able to account for asymmetric electrolytes, multiple ionic species, and the Stern and diffuse electrical double layers present at the surface of the electrodes and membrane. Note that for \( \sum_{i=1}^{N} N_A \alpha_i^3 c^i \ll 1 \), the steric effects are negligible in Eq. 3 and the standard PNP formulation can be used to model the electrolyte dynamics.

The membrane surface is assumed to be perfectly polarizable (i.e. blocking) such that the normal ionic flux vanishes at the surface of the membrane. There are no surface reactions present at the gold electrode to electrolyte interface; therefore, we have a no-flux boundary condition present at the gold surface. Formally these no-flux interface and boundary conditions
are given by
\[ n \cdot J^i = 0 \text{ in } \partial \Omega_m \cup \partial \Omega_e \cup \partial \Omega_{cc}, \] (4)
with \( n \) the normal vector pointing into the electrolyte solution and \( J^i \) defined below Eq. 3. The diffusion coefficient \( D^i \) in Eq. 3a is spatially dependent as the tethering reservoir has a lower diffusion than the bulk electrolyte solution with no tethers present. We define this condition as:
\[ D^i(x) = \begin{cases} D^i_r & \text{if } x \in \Omega_r, \\ D^i_w & \text{if } x \in \Omega_w, \end{cases} \] (5)
where \( \Omega \) defines the respective domain.

The dielectric permittivity in Eq. 3b is spatially dependent as defined below:
\[ \varepsilon(x) = \begin{cases} \varepsilon_w & \text{if } x \in \Omega_r \cup \Omega_w \\ \varepsilon_m & \text{if } x \in \Omega_m. \end{cases} \] (6)
To ensure the well-posedness of the Poisson equation Eq. 3b, the internal boundary conditions on the membrane to electrolyte interface are satisfied by the following (55):
\[ \phi_m - \phi_w = 0 \text{ in } \partial \Omega_m, \]
\[ \varepsilon_m \nabla \phi_m \cdot n - \varepsilon_w \nabla \phi_w \cdot n = 0 \text{ in } \partial \Omega_m. \] (7)

At the electrode surface a compact Stern layer exists with a capacitance per unit are given by \( C_s \). The Stern layer adjacent to the electrodes is modelled using the following boundary conditions
\[ C_s (\phi_e - \phi) + \varepsilon_w n \cdot \nabla \phi = 0 \text{ in } \partial \Omega_e, \]
\[ C_s (\phi_{ec} - \phi) + \varepsilon_w n \cdot \nabla \phi = 0 \text{ in } \partial \Omega_{ec}, \] (8)
with \( \phi_e \) and \( \phi_{ec} \) the prescribed potentials at the respective electrodes. The ambient boundary conditions of the axi-symmetric pore are given by:
\[ c^i = c^i_o \text{ in } \partial \Omega_w \cup \partial \Omega_r, \]
\[ n \cdot \nabla \phi = 0 \text{ in } \partial \Omega_{hm}, \] (9)
with \( c^i_o \) the initial concentration, refer to Fig. 2.

Using equations Eq. 3, with the material properties defined by Eq. 5 and 6, and the boundary conditions Eq. 4, 7, 8, and 9, all that the remains is to define the geometry of the aqueous pores in the engineered tethered membrane.

Initially, stable pores that form in the membrane are approximately cylindrical (56). As expansion occurs, the pores become toroidal in nature. In this paper we consider the toroidal pore structure illustrated in Fig. 2 to compute the conductance of a pore. The current travelling through the pore is computed using:
\[ I_p = F \sum_i J^i \int_0^r 2 \pi r dr, \quad G_p = \frac{I_p}{V_m}, \] (10)
where \( J^i \) and \( V_m \) are computed using Eq. 3 with the geometry given in Fig. 2.

To compare the numerical accuracy of the GPNP model with the previously developed model in (39), we assume electroneutrality (i.e. \( \sum z^i c^i = 0 \)) and no steric effects \( a_i = 0 \) (39). The governing equations of \( \phi_w \), the electrical potential in the electrolyte solution \( \Omega_w \), in the limit of electroneutrality and no steric effects, can be derived by substituting the time derivative of Eq. 3b into Eq. 3a for charge neutrality resulting in the elliptic equation:
\[ \nabla \cdot (\varsigma \nabla \phi + \nabla \kappa) = 0 \]
\[ \varsigma = \sum_{i=1}^N \frac{(qz^i)^2 D^i}{k_B T c^i}, \quad \kappa = \sum_{i=1}^N qz^i D^i c^i. \] (11)
with the parameters defined below Eq. 3. The boundary conditions of Eq. 11 at the electrode surfaces \( \partial \Omega_e \) and \( \partial \Omega_{cc} \), and at the ambient boundary \( \partial \Omega_w \), \( \partial \Omega_{hm} \), and \( \partial \Omega_r \) are given by Eq. 8 and 9 respectively, refer to Fig. 2. In the membrane domain \( \Omega_m \)
the electrostatic potential $\phi_m$ is governed by Laplace’s equation for electrostatics $\nabla \cdot (\varepsilon_m \nabla \phi) = 0$. The interface conditions between the domains $\Omega_w$ and $\Omega_m$ are given by:

$$n \cdot \nabla \phi_w = 0 \text{ in } \partial \Omega_m,$$

$$\phi_m = \phi_w \text{ in } \partial \Omega_m. \quad (12)$$

From the continuity of potential on $\partial \Omega_m$, Eq. 12, there exists a surface charge on the membrane given by $\rho_s = \varepsilon_m n \cdot \nabla \phi_m - \varepsilon_w n \cdot \nabla \phi_w$; therefore, the system of equations Eq. 11 with boundary conditions Eq. 12 implicitly includes the membrane surface charge $\rho_s$ (18, 39, 57).

We denote the governing equations Eq. 3a with $a_i = 0$ coupled with Eq. 11, the materials defined by Eq. 5 and 6 and the boundary conditions Eq. 4, 8, 9, and 12 as the Electroneutral Model (EM). Given the solution of the EM system of equations, the conductance $G_p$, Eq. 10, can be estimated assuming negligible steric effects and electroneutrality.

### Electrical Energy Required to Form a Pore

In this section we construct a model for the electrical energy of a pore, $W_{es}$, in the engineered tethered membrane by modelling the membrane as a dielectric continuum in an electrolyte solution. The computation of $W_{es}$ involves computing the force that causes a displacement of the pore structure. Consider the pore structure presented in Fig. 2. The pore boundary is assumed to only expand in the radial direction $r$. If we denote $F(r)$ as the total force acting on the pore boundary of radius $r$, then the electromechanical energy can be computed using (18, 43, 58):

$$W_{es}(r) = -\int_0^r F(r) dr. \quad (13)$$

Using the Maxwell stress tensor we show how $F(r)$ can be computed for the pore structures in Fig. 2.

The electric field induces a stress on the membrane surface. The total stress on the surface of the membrane can be computed using the Maxwell stress tensor $T$ given by (18, 43, 59–65):

$$T = \varepsilon \left(\frac{1}{2} |\nabla \phi|^2 I - \nabla \phi \otimes \nabla \phi\right), \quad (14)$$
where \( \varepsilon \) and \( \phi \) are defined below Eq. 3, \( I \) denotes the identity matrix, and \( \otimes \) is the dyadic product. The stress on the membrane from the electrolyte \( p_w \), and the stress on the electrolyte from the membrane \( p_m \) are given by:

\[
p_w = -T_w n = -\varepsilon_w \left( \frac{1}{2} |\nabla \phi_w|^2 I - \nabla \phi_w \otimes \nabla \phi_w \right)n, \\
p_m = T_m n + f \\
= -\varepsilon_m \left( \frac{1}{2} |\nabla \phi_m|^2 I - \nabla \phi_m \otimes \nabla \phi_m \right)n + f, \\
\]

where \( f \) is the stress induced from all other elastic properties of the membrane (66–68). Note that \( f \) denotes the electrical force density acting on the membrane surface.

The membrane is assumed to be at local mechanical equilibrium at the pore surface such that \( f = (T_w - T_m)n \). Therefore, to maintain local equilibrium the total force acting on the pore boundary \( F(r) \) is given by:

\[
F(r) = \int_S n \cdot (p_w - p_m) dS,
\]

with \( p_w \) and \( p_m \) defined in Eq. 15, and the surface \( S \) and normal vector \( n \) given in Fig. 2. Given \( \phi \) computed using Eq. 3, we can compute \( F(r) \) using Eq. 16. Substituting \( F(r) \) into Eq. 13 gives the electrical energy contribution to pore formation in the membrane. Note that \( F(r) \) includes electrical double layer and electrodiffusive effects caused by asymmetric electrolytes.

Note that for narrow cylindrical pores \( \partial \phi / \partial r \approx 0 \) and \( \partial \phi / \partial z \approx V_m / h_m \) on \( S \); substituting into Eq. 15 and 16 we see that \( F(r, V_m) \propto V_m^2 \) with \( V_m \) the transmembrane potential. The proportionality of \( F(r, V_m) \propto V_m^2 \), which also implies \( W_{\text{el}}(r, V_m) \propto V_m^2 \), is critical for the derivation of the electrical energy required to form a pore in (18, 28, 40). This allow the computation of \( F(r, V_m) \) to be conducted using a single instance of \( F(r) \) for a given transmembrane potential \( V_m \), which is used to estimate the proportionality constant.

For numerical comparison to previously published results (18), we estimate the pore energy \( W_{\text{es}} \), Eq. 13, assuming electroneutrality, negligible steric effects, and steady-state current (i.e. \( \nabla \phi = 0 \)). With these assumptions the electrical potential \( \phi \) is governed by Laplace’s equation \( \nabla \cdot (\varepsilon \nabla \phi) = 0 \) with \( \varepsilon \) defined by Eq. 6, and the interface and boundary conditions defined by Eq. 8, 9, and 12. We denote this as the Laplace Model (LM).

**Equivalent Circuit Electroporation Model of the Engineered Tethered Membrane**

The electrophysiological properties of the engineered tethered membrane are modelled using an equivalent circuit model (5, 8, 10, 69, 70). Note that for a sufficiently low applied potential (i.e. less then 50 mV) the effects of electroporation are negligible. This allows the circuit parameters to be estimated with a sinusoidal excitation potential with magnitude less then 50 mV.

The tethered membrane platform is composed of three distinct regions: the electrical double layers at the gold electrodes, the bulk electrolyte reservoir, and the tethered membrane. The bulk electrolyte solution is modelled as completely ohmic to maintain local equilibrium the total force acting on the pore boundary.

The equivalent circuit model of the tethered membrane platform is given in Fig. 3.

The governing equations of the tethered membrane system are given by:

\[
\frac{dV_m}{dt} = -\left( \frac{1}{C_m R_e} + \frac{G_m}{C_m} \right) V_m - \frac{1}{C_m R_e} V_{dt} + \frac{1}{C_m R_e} V_s, \\
\frac{dV_{dt}}{dt} = -\frac{1}{C_{dt} R_e} V_m - \frac{1}{C_{dt} R_e} V_{dt} + \frac{1}{C_{dt} R_e} V_s, \\
I = \frac{1}{R_e} (V_s - V_m - V_{dt}),
\]

where \( C_{dt} \) is the total capacitance of \( C_{tdt} \) and \( C_{bdt} \) in series. Given \( V_s(t) \), and the static circuit parameters \( C_{tdt}, C_{bdt}, R_e, C_m \), and the membrane conductance \( G_m \) can be estimated from the measured current \( I(t) \). However, for drive potentials below
approximately 50 mV, the membrane conductance is approximately constant and is assumed at its equilibrium value \( G_o \) such that
\[ G_m(t, V_m) \approx G_o. \]

How can we experimentally verify that the formed membrane does not contain significant defects? Possible membrane defects include patches with the gold electrode directly exposed to the bulk electrolyte, or with portions of bilayer sandwiched together. Using the measured current response resulting from an excitation potential below 50 mV, we can compute the mean squared error (MSE) between the predicted current from Eq. 17 and the experimentally measured current. If a significant MSE is obtained then the model of a homogeneous membrane Eq. 17 is not suitable and the membrane is concluded to contain inhomogeneities (i.e. defects). A major concern when performing electroporation experiments is the detection of the catastrophic voltage breakdown of the membrane causing separated areas of membrane to degrade. This effect can be detected by a high MSE and a significant increase in the estimated membrane conductance \( C_m \) resulting from the electrode surface capacitance coming into contact with the bulk electrolyte. Typical values for membrane capacitance and conductance are 0.5-1.3 \( \mu F/cm^2 \) and 0.5-2.0 \( \mu S \) for an intact 1%-100% tethered membrane with surface area 2.1 mm².

For transmembrane potentials above 50 mV, the TMP conductance \( G_m \) can be estimated by

\[
G_m = \sum_{i=1}^{N(t)} G_p(r_i),
\]

\[
dr_i \over dt = -D \over k_B T \partial W(r) \over \partial r_i \quad \text{for} \quad r_i \in \{1, 2, ..., [N(t)]\},
\]

\[
dN \over dt = \alpha e^{(V_m/V_{ep})^2} (1 - \frac{N}{N_o} e^{-q(r_m/r^*)^2}). \tag{18}
\]

In Eq. 18, \( \alpha \) is the pore creation rate coefficient, \( V_{ep} \) is the characteristic voltage of electroporation, \( N_o \) is the equilibrium pore density at \( V_m = 0 \), and \( q = (r_m/r^*)^2 \) is the squared ratio of the minimum energy radius \( r_m \) at \( V_m = 0 \) with \( r^* \) the minimum energy radius of hydrophilic pores (56, 71–73). The parameters \( \alpha, V_{ep}, N_o, \) and \( \gamma \) are estimated by fitting the measured current response \( I \) to the predicted current response from Eq. 17 and Eq. 18 given the drive voltage \( V_s \).

The derivation of Eq. 18 is based on making physiologically relevant approximations to the Smoluchowski-Einstein equation for electroporation. The Smoluchowski-Einstein equation governs the distribution of pores as a function of their radius \( r \) and time \( t \) (11–13). If we denote \( n(r, t) \) as the pore density distribution function, then the Smoluchowski-Einstein equation is given by:

\[
\partial n \over \partial t = D \over \partial r \left[ n \over k_B T \partial W \over \partial r + \partial n \over \partial r \right] + S(r), \tag{19}
\]

where \( D \) is the diffusion coefficient of pores, \( k_B \) is the Boltzmann constant, \( T \) is the temperature, \( W \) is the pore energy, and \( S(r) \) models the creation and destruction rate of pores. Making the physiologically relevant assumption that diffusion term in
Eq. 19 is negligible, and the characteristic time scale of \( W \) is longer then 0.1 \( \mu s \), then the process of electroporation can be modelled by Eq. 18. Note that Eq. 18 has been used by several authors for modelling DNA translocation into cells (56, 71–75).

**Numerical Methods**

The governing equations Eq. 3 with boundary conditions Eq. 4, and 7-9 are solved numerically with the commercially available finite element solver COMSOL 4.3a (Comsol Multiphysics, Burlington, MA). To solve the GPNP and PNP models the COMSOL modules *Transport of Diluted Species* and *Electrostatics* are utilized; and to solve the EM model the modules *Nernst-Planck* and *Electrostatics* are utilized. Eq.(10) is used to compute the pore conductance with the integration done in the region defined in Fig. 2. The simulation domain is meshed with approximately 270,000 triangular elements constructed using an advancing front meshing algorithm. The GPNP and PNP are numerically solved using the *multifrontal massively parallel sparse direct solver* (76) with a variable-order variable-step-size backward differential formula (77). The conductance is computed for a finite number of equally spaced radii between 0.5-10 nm with a step-size of 0.25 nm. The steady-state conductance \( G_{p} \), Eq. 10, is estimated when the percentage change in conductance between successive steps (i.e. \( |(G_{p}(t_{i+1}) - G_{p}(t_{i}))/G_{p}(t_{i})| \)) is less then 1%. The total force acting on the toroidal pore \( F(r) \), Eq. 16, is computed using the results from the conductance computation. Substituting \( F(r) \) into Eq. 13, the total electrical energy required to form the pore \( W_{es} \) is computed. The numerical estimate of \( I(t) \) is computed using Eq. 17 and 18 assuming there are a finite number of possible pore radii using the algorithm presented in (56, 71).

**Results and Discussion**

In this section we apply the electroporation model for the engineered tethered membrane. The predictive accuracy of the electroporation model is verified using experimental measurements of tethered DphPC membranes with a tethering density of 1%, 10%, and 100%.

**Numerically Predicted Aqueous Pore Conductance and Electrical Energy Required to form a Pore**

To estimate the conductance \( G_{p} \), Eq. 10, and the electrical energy required to form a pore \( W_{es} \), Eq. 13, we utilize the GPNP, Eq. 3, PNP, EM, and LM models defined in the Materials and Methods section.

In Fig. 4 the estimated pore conductance computed using the GPNP, PNP, and EM models is presented. As seen the pore conductance numerically predicted using the GPNP follows a \( G_{p} \propto r \) relationship. For membranes with sufficiently large electrolyte baths and pore radii (i.e. electrolyte bath is hundreds of nm thick and \( r > t_{m} \)), the pore conductance follows \( G_{p} \propto r (12, 38–40) \), in agreement with the spreading conductance derived from Laplace’s equation in (42, 78). Note that the effect \( G_{p} \propto r \) for \( r < t_{m} \) and \( h_{r} = 4 \) nm is only numerically predicted when the effects caused by asymmetric electrolytes, finite ion size, and Stern and diffuse layers are accounted for. In Fig. 4 A, the GPNP and PNP models produce differing conductance estimates as a result of the steric effects present. Recall that for \( \sum_{i=1}^{N} N_{A} \alpha_{i} c_{i} \ll 1 \) the steric effects are negligible and the estimated conductance using the GPNP and PNP models would be identical. As seen by comparing the estimated conductance \( G_{p} \) in Fig. 4 A, the assumption of electroneutrality causes a noticeable decrease in the computed conductance \( G_{p} \). As discussed in the Introduction the pore conductance \( G_{p} \) may be dominated by the spreading conductance, which follows a \( G_{p} \propto r \) proportionality, when the electrolyte solution is sufficiently geometrically constrained. From Fig. 2, the tethering reservoir is \( h_{r} = 4 \) nm, and from Fig. 4 A we see that \( G_{p} \propto r \); therefore, we conclude that the conductance of an aqueous pore in the engineered tethered membrane is dominated by the spreading conductance. As the diffusivity in the tethering reservoir, \( D_{r} \), decreases the pore conductance decreases, as seen in Fig. 4 B. This is expected as less ions can flow through the pore as a result of reduced ion mobility.

Fig. 5 compares the numerically estimated electrical energy required to form a pore computed using the GPNP, PNP, EM, and LM models as defined in the Materials and Methods section. For small pores below 1 nm all the models provide similar estimated for \( W_{es} \), as seen in Fig. 5 A. The PNP and EM models provide a significantly lower estimate of \( W_{es} \) compared to the GPNP and EM models for large pore radii above 4 nm. The discrepancy between the estimated \( W_{es} \) is a result of the assumption of negligible steric effects in the PNP model, and the assumption of negligible steric effects and electroneutrality in the EM model. Note that although the GPNP and LM models provide similar predictions of \( W_{es} \), the LM assumes negligible steric effects, electroneutrality, and steady-state current (i.e. \( \nabla c = 0 \)) which results in the estimated voltage distribution on the surface of the membrane to differ with the voltage distribution predicted from the GPNP. Qualitatively at the surface of the membrane the GPNP model has the interface condition Eq. 7 such that \( \varepsilon_{m} \nabla \phi_{m} \cdot n = \varepsilon_{w} \nabla \phi_{w} \cdot n \); however, the interface condition for the LM model Eq. 12 causes \( \varepsilon_{m} \nabla \phi_{m} \cdot n \neq \varepsilon_{w} \nabla \phi_{w} \cdot n \) on the surface. This results in the LM overestimating...
FIGURE 4: Numerically predicted pore conductance $G_p$, defined in Eq. 10. (A) provides the predicted pore conductance $G_p$ computed using the GPNP, PNP, and EM models. (B) is the predicted $G_p$ for different tethering reservoir diffusivities. The geometry of the pore is given in Fig. 2 with the parameters of the governing equations and boundary conditions provided in Table S1 in the Supporting Material.

the voltage potential when compared with the GPNP. From Eq. 15, the overestimated potential causes the computed $W_{es}$ to be larger when using the LM model as compared with the GPNP model. As discussed, the assumption of $W_{es}(r, V_m) \propto V_m^2$ is typically invoked to simplify the computation of $W_{es}(r, V_m)$ (18, 28, 40). From Fig. 5 B, we compute $W_{es}(r, V_m)$ explicitly for several transmembrane potentials and find that the proportionality follows a fractional power law. This illustrates the importance of including effects caused be electrodiffusion. As illustrated in Fig. 5 C, reducing the diffusion coefficient in the tethering reservoir $D_r$ causes a slight reduction in the estimated $W_{es}$. In comparing Fig. 5 B with Fig. 5 C, the main contribution to the change in $W_{es}$ results from a change in transmembrane potential.

FIGURE 5: Numerically predicted electrical energy $W_{es}$, Eq. 13, required to form an aqueous pore. (A) compares the predicted $W_{es}$ computed using the GPNP, PNP, EM, and LM models defined in the Materials and Methods section for the transmembrane potential of $V_m = 500$ mV. (B) presents estimates of $W_{es}$ computed using the GPNP for the transmembrane potentials listed. (C) provides estimates of $W_{es}$ computed using the GPNP for $V_m = 500$ mV for different tether reservoir diffusivities. The parameters of the governing equations and boundary conditions can be found in Table S1.

**Experimental Verification of Predictive Models**

In this section we use the numerically predicted pore conductance $G_p$, Eq. 10, and electrical energy $W_{es}$, Eq. 13, estimated using the GPNP model with the electroporation model given by Eq. 17 and 18 to predict the current response of the engineered
tethered membrane. The predicted current response is compared to experimentally measured data to validate the accuracy of the model. Note that all electroporation processes were reversible and did not cause permanent damage to the membrane.

From Eq. 17 and 18, if the drive potential $V_s$ is applied and the resulting current is $I_s$; then if the drive potential $-V_s$ is applied the resulting current must be $-I_s$ if only the process of electroporation is present. For all the tethering densities and membrane compositions tested this relation was observed in all experimental current measurements and therefore we concluded that the only process present is that of electroporation. The drive potential $V_e(t)$ used to produce the results in Fig. 6 and 7 is defined by a linearly increasing potential of 100 V/s for 5 ms proceeded by a linearly decreasing potential of -100 V/s for 5ms.

The experimental measurement and predicted voltages, pore radii, membrane resistance, and current are presented in Fig. 6 for the 10% tethered DphPC bilayer membrane. From Fig. 6 A, the experimentally measured and numerically predicted current are in excellent agreement. As seen in Fig. 6 B, the application of the voltage excitation immediately causes an increase in the double-layer voltage $V_{dl}$ as a result of the charge increase in the charge distribution at the electrode surface. The transmembrane potential $V_m$ simultaneously increases as a result of the excitation potential. The increase in $V_m$ results in the formation of pores. As seen in Fig. 6 C, a dramatic change in the resistance results after the application of the drive potential. In Fig. 6 D the maximum radius $r_{max}$ and mean radius $\bar{r}$ are provided to illustrate the spread in pore radii. As $V_m$ increases, pores are generated and expand according to Eq. 18. From Eq. 18, all pores diffuse to the minimum-energy pore radius given by $\partial W/\partial r = 0$ with an advection velocity proportional to $D/k_B T$. As seen in Fig. 6 D, generated pores rapidly expand to the minimum-energy pore radius as the spread between $r_{max}$ and $\bar{r}$ is negligible.

As seen from Fig.6 the predictive model is able to estimate the current response of the engineered tethered membrane; however an immediate question arises: how sensitive is the predicted current response to errors in the model parameters of Eq. 17 and Eq. 18? To reduce the number of free parameters we set $\gamma$, $\sigma$, $C$, $D$ to the experimentally measured values given in (13, 20, 29, 34, 56, 71). The estimated values of $G_o$, $C_m$, $C_{dl}$, and $R_e$ are provided in Table S2 of the Supporting Material and correspond to a homogeneous membrane with negligible defects, as discussed in the Materials and Methods section. Note that all errors in Table S2 are estimated by computing the range of values in good agreement with the experimentally measured current using Eq. 17 and Eq. 18. As seen, the electrolyte resistance $R_e$ has a negligible effect on the current response as the membrane conductance and capacitive charging dominate the current flow. Using a triangular drive potential allows the estimate of $C_m$, $R_m$, and $C_{dl}$ using specific sections of the measured current. The initial jump in current at the start of the triangular pulse is dominated by $C_m$. The current response proceeding the initial jump is dominated by $G_o$ with the slope...
proportional to the membrane conductance $G_m$. Note that any deviation from a linear current response in this region is a result of the electroporation process. The double layer capacitance $C_{dl}$ effect dominates the current response as the triangular drive potential decreases. By estimating each of the circuit parameters using specific regions of the current response allows the circuit parameters to be reliably estimated, as seen in Table S2. The pore creation rate Eq. 18 is not significantly effected by $\alpha$ and $N_o$, because $dN/dt$ is exponentially dependent on $V_m$, $V_{ep}$, and $q$. This dependence can be seen by referring to Table S2 where the estimated error of $\alpha$ and $N_o$ is significantly larger then $V_{ep}$ and $q$. The estimated values for $N_o$, $\alpha$, $V_{ep}$, $q$ are in excellent agreement with experimentally estimated values found in the literature (29, 74, 79).

Fig. 7 provides the experimental current measurement and the predicted current and membrane resistance $R_m = 1/G_m$ for the 1% and 10% DphPC bilayer, and the 100% DphPC monolayer membrane. In comparing the resulting current between the 1%, 10%, and 100% tethered case, Fig. 7A, we see that as the tethering density increases the effects of electroporation decreases. This is an expected result as the tethers provide structural support hindering the nucleation of pores reducing the equilibrium pore density $N_o$, and increasing the characteristic voltage of electroporation $V_{ep}$. As seen in Fig. 7B, the resistance begins to change at approximately 1 ms when the transmembrane potential reaches a sufficiently high to cause the nucleation of pores. The estimated spring constant $K_t$ for the 1%, 10%, and 100% tethering density are: 0 mN/m, 2±0.5 mN/m, and 20±4 mN/m. For the 1% tether density the spring constant is negligible as expected. For the 100% tether case pores can not expand as a result of the spring constant $K_t$, therefore the decrease in resistance is primarily a result of pore nucleation and destruction governed by Eq. 18. For the 100% tether density membrane, it may be the case that all pores in the membrane are hydrophilic as the tethers may prevent the transition from the hydrophilic to hydrophobic structure. If only hydrophilic pores are present, the membrane resistance would be dominated by the nucleation of pores and not the dynamics of the pores. Note that the molecular structure of the aqueous pores can not be reliably inferred using continuum theory models and would require the use of molecular dynamics or similar non-continuum models. Interestingly, for the 1% membrane structures the resistance begins to decrease at 9.2 ms, and for the 10% membrane at 9.4 ms after the initial application of the drive potential $V_s(t)$ defined at the beginning of this section. This is a result of the charge accumulation in the electrical double layers at the gold electrode surface, $V_{dl}$, discharging causing an increase in the magnitude of the transmembrane potential $V_m$. This illustrates the importance of including electrical double-layer effects when modelling gold electrodes. Note that when using Eq. 17 and Eq. 18 for estimating the effects of electroporation for rapidly changing drive potentials, the double-layer capacitance in Eq. 17 can become time dependent (51). In such cases the dynamics of the time dependent capacitance can be estimated using the GPNP model defined in Eq. 3 using the method outlined in (51) with the electroporation model developed in this paper. The thickness of the membrane can be estimated using $h_m = \varepsilon_m A_m/C_m$ with $A_m = 1.2 \text{ mm}^2$, the area of the membrane surface, and $\varepsilon_m$ and $C_m$ given in Table S1 and S2 in the Supporting Material. For the 1%, 10%, and 100% membranes we obtain a thickness of: 3.54 nm, 3.54 nm, 3.40 nm. These values are in excellent agreement with NMR measurement of similar DphPC based tethered membranes (8). As seen, the thickness of the tethered DphPC membrane is approximately constant between the 1% and 10% tether densities. The 100% DphPC monolayer is slightly thinner then the 1% and 10% DphPC bilayer membrane. The reduction in thickness between the 100%, and the 1% and 10% is a result of the combined effect of an increased tether density and the dibenzyl group that binds the phytanyl tails in the tethered DphPC monolayer.

![FIGURE 7: Experimentally measured and numerically predicted current $I(t)$ (A), and membrane resistance $R_m = 1/G_m$ (B) for the drive potential $V_s(t)$ defined at the beginning of this section. The tethering densities 1% and 10% correspond to the DphPC bilayer and the 100% corresponds to the DphPC monolayer. All predictions are computed using Eq. 17 and Eq. 18 with the parameters defined in Table S2.](image-url)

In Fig. 8 the experimentally measured and numerically predicted current $I(t)$ is provided for several different linearly increasing and decreasing drive potentials. As seen from Fig. 8A-D, we obtain excellent agreement between the experimentally measured and numerically predicted current. For small magnitude drive potentials one would expect the membrane...
Engineered Tethered Membrane

resistance to remain constant as the effects of electroporation, governed by Eq. 17 and Eq. 18, are negligible. Indeed from Fig. 8 A and D, we see that the electroporation effects are negligible for drive potentials from 50 to 80 V/s for the 1 ms rise, and 10-40 V/s for the 5 ms rise. The reason the 5 ms rise, Fig. 8 A and C, has larger relative electroporation effects as compared with the 1 ms rise, Fig. 8 B and D, is that the nucleation and dynamics of pore radii evolve for a longer period of time at a sufficiently high transmembrane potential. As expected, the magnitude of the current response for the 10% tethered membrane, Fig. 8 A and B, is less then the current response for the 10% tethered membrane, Fig. 8 C and D, as a result of the tethers hindering the nucleation and expansion of pores. The estimated electrical double-layer capacitance used to compute the current for the 10% membrane is $C_{dl} = 65$ nF, and that for the 1% tether density is $C_{dl} = 39$ nF. In reference to Table S3 of the Supporting Material, the expected value of $C_{dl} \in [118, 137]$ nF. Despite this minor discrepancy, the estimated current, using the model given in Eq. 17 and Eq. 18, is in excellent agreement with the experimentally measured current.

FIGURE 8: Experimentally measured and numerically predicted current response $I(t)$ for the 10% tethering density DphPC membrane, panels (A) and (B), and the 1% tether density DphPC membrane, panels (C) and (D). In panels (A) and (C), the drive potential $V_s(t)$ is defined by a 1 ms linearly increasing with a rise time of 50 to 500 V/s in steps of 50 V/s preceded by a linearly decreasing potential of -50 to -500 V/s in steps of -50 V/s for 1 ms. In panels (B) and (D), the drive potential $V_s(t)$ is defined by a 5 ms linearly increasing potential for 10 to 100 V/s in steps of 10 V/s for 5 ms. The numerical predictions are computed using Eq. 17 and Eq. 18 with the parameters defined in Table S3 of the Supporting Material.

Conclusion

The construction and predictive models for an engineered tethered membrane is presented in this paper. The self-assembled membrane can be setup with ease and provides a physiologically relevant environment for the study of electroporation. The analysis presented reveals several interesting features regarding how the Stern and diffuse double-layers, and tethers effect the electroporation process. Numerically we found that the electrical energy required to form a pore has the property $W_{es}(r, V_m) \propto V_m^2 V_m^2$ and that the pore conductance has the property $G_p \propto r$, where $V_m$ is the transmembrane potential and $r$ is the radius of the pore. As shown, the experimental results compare favourably with the results computed from the predictive models.

SUPPORTING MATERIAL

Tables S1, S2, and S3 are available at www.biophys.org/biophysj/supplemental/S0006-3495(XX)XXXXX-X.
References


### TABLE S1: Parameter Values for $G_p$ and $W_{es}$ Numerical Predictions

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<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
</tr>
</thead>
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<tr>
<td>$c_{Na}\big</td>
<td>_{t=0}$</td>
<td>Initial Na$^+$ concentration</td>
</tr>
<tr>
<td>$c_{K}\big</td>
<td>_{t=0}$</td>
<td>Initial K$^+$ concentration</td>
</tr>
<tr>
<td>$c_{Cl}\big</td>
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<td>Initial Cl$^-$ concentration</td>
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</tr>
<tr>
<td>$\alpha_{K}$</td>
<td>K$^+$ effective ion size</td>
<td>5 Å</td>
</tr>
<tr>
<td>$\alpha_{Cl}$</td>
<td>Cl$^-$ effective ion size</td>
<td>4 Å</td>
</tr>
<tr>
<td>$D_{Na}$</td>
<td>Na$^+$ electrolyte diffusion coefficient in $\Omega_w$</td>
<td>$1.33 \times 10^{-9}$ m$^2$/s</td>
</tr>
<tr>
<td>$D_{K}$</td>
<td>K$^+$ electrolyte diffusion coefficient in $\Omega_w$</td>
<td>$1.96 \times 10^{-9}$ m$^2$/s</td>
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<td>$D_{Cl}$</td>
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<td>$2.07 \times 10^{-9}$ m$^2$/s</td>
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<tr>
<td>$\varepsilon_w$</td>
<td>Electrolyte electrical permittivity</td>
<td>$7.083 \times 10^{-10}$ F/m</td>
</tr>
<tr>
<td>$\varepsilon_m$</td>
<td>Membrane electrical permittivity</td>
<td>$1.771 \times 10^{-11}$ F/m</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant</td>
<td>$9.6485 \times 10^4$ C/mol</td>
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<tr>
<td>$C_s$</td>
<td>Stern layer capacitance</td>
<td>1 pF</td>
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<td>$k_B$</td>
<td>Boltzmann constant</td>
<td>$1.3806488 \times 10^{-23}$ J/K</td>
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<td>$T$</td>
<td>Temperature</td>
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<td>Membrane thickness</td>
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<td>$h_e$</td>
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<td>Value</td>
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<tr>
<td>--------</td>
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<tr>
<td>$\gamma$</td>
<td>Edge energy</td>
<td>$1.8 \times 10^{-11} \text{ J/m}$</td>
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<tr>
<td>$\sigma$</td>
<td>Surface tension</td>
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<tr>
<td>$C$</td>
<td>Steric repulsion constant</td>
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<tr>
<td>$D$</td>
<td>Radial diffusion coefficient</td>
<td>$1 \times 10^{-14} \text{ m}^2/\text{s}$</td>
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<tr>
<td>$\alpha$</td>
<td>Creation rate coefficient</td>
<td>$1 \text{ Gs}^{-1} \left[10 \text{ Ms}^{-1} - 0.1 \text{ Ts}^{-1}\right]$</td>
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<tr>
<td>$q$</td>
<td>$q = (r_m/r_*)^2$ with the symbols defined below Eq. 17</td>
<td>$2.46 \pm 0.07$</td>
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### DphPC Membrane Tether Density:

<table>
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<th></th>
<th>1%</th>
<th>10%</th>
<th>100%</th>
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<tbody>
<tr>
<td>$G_0$ (Initial membrane conductance)</td>
<td>$1.67 \pm 0.3 \mu S$</td>
<td>$0.91 \pm 0.04 \mu S$</td>
<td>$0.43 \pm 0.03 \mu S$</td>
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<tr>
<td>$C_m$ (Membrane capacitance)</td>
<td>$10.5 \pm 0.8 \text{ nF}$</td>
<td>$10.5 \pm 0.7 \text{ nF}$</td>
<td>$11.0 \pm 0.2 \text{ nF}$</td>
</tr>
<tr>
<td>$R_e$ (Electrolyte resistance)</td>
<td>$3.5 \pm 1 \text{ k}\Omega$</td>
<td>$3.5 \pm 1 \text{ k}\Omega$</td>
<td>$5.0 \pm 2.0 \text{ k}\Omega$</td>
</tr>
<tr>
<td>$C_{dl}$ (Total electrode double-layer capacitance)</td>
<td>$136.3 \pm 6 \text{ nF}$</td>
<td>$136.3 \pm 8 \text{ nF}$</td>
<td>$118.2 \pm 8 \text{ nF}$</td>
</tr>
<tr>
<td>$V_{ep}$ (Characteristic voltage of electroporation)</td>
<td>$430 \pm 5 \text{ mV}$</td>
<td>$430 \pm 5 \text{ mV}$</td>
<td>$580 \pm 10 \text{ mV}$</td>
</tr>
<tr>
<td>$N_o$ (Equilibrium pore density)</td>
<td>$1068 [120-15k]$</td>
<td>$582 [90-10k]$</td>
<td>$275 [42-43k]$</td>
</tr>
<tr>
<td>$K_t$ (Spring constant)</td>
<td>$0 \text{ N/m}$</td>
<td>$2 \pm 0.5 \text{ mN/m}$</td>
<td>$20 \pm 5 \text{ mN/m}$</td>
</tr>
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</table>

### DphPC Membrane (Reservoir Double-Layer Effect) Tether Density:

<table>
<thead>
<tr>
<th></th>
<th>1%</th>
<th>10%</th>
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<tbody>
<tr>
<td>$G_0$ (Initial membrane conductance)</td>
<td>$1.00 \pm 0.1 \mu S$</td>
<td>$1.00 \pm 0.1 \mu S$</td>
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<tr>
<td>$C_m$ (Membrane capacitance)</td>
<td>$14.6 \pm 0.1 \text{ nF}$</td>
<td>$16.0 \pm 0.4 \text{ nF}$</td>
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<tr>
<td>$R_e$ (Electrolyte resistance)</td>
<td>$1.0 \pm 0.5 \text{ k}\Omega$</td>
<td>$1.0 \pm 0.5 \text{ k}\Omega$</td>
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<tr>
<td>$C_{dl}$ (Total electrode double-layer capacitance)</td>
<td>$65 \pm 3 \text{ nF}$</td>
<td>$39 \pm 2 \text{ nF}$</td>
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<tr>
<td>$V_{ep}$ (Characteristic voltage of electroporation)</td>
<td>$366 \pm 6 \text{ mV}$</td>
<td>$400 \pm 5 \text{ mV}$</td>
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<tr>
<td>$N_o$ (Equilibrium pore density)</td>
<td>$641 [100-2k]$</td>
<td>$641 [100-50k]$</td>
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<tr>
<td>$K_t$ (Spring constant)</td>
<td>$0 \text{ N/m}$</td>
<td>$2 \pm 0.5 \text{ mN/m}$</td>
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