Modeling Cell and Tissue Electroporation

by

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Submitted to the Department of Electrical Engineering and Computer Science
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To my wonderful parents, Todd and Karen,
for supporting me in all of my endeavors.
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Abstract

Large, pulsed electric fields are becoming an increasingly important tool in drug delivery, gene delivery, and apoptosis induction. Nonetheless, much remains unknown about the fundamental mechanisms by which large electric fields interact with cells and tissue, in part because many critical features of the cell and tissue responses occur on time and length scales that are difficult to assess experimentally. Therefore, sophisticated models are needed to further understanding of the basic mechanisms of interaction.

Electroporation, in which transient, aqueous pores form in lipid bilayers, is one fundamental mechanism by which large electric fields may alter biological systems. Here cell and tissue electroporation models are presented that are based on the asymptotic model of electroporation and the new mesh transport network method (MTNM), which utilizes equivalent circuit networks to simulate nonlinear, coupled transport phenomena. The cell system simulations show that small magnitude (0.1 MV/m), long duration (100 µs) pulses result in conventional electroporation, in which pores form in only the plasma membrane, while large magnitude (10 MV/m), short duration (10 ns) pulses result in supra-electroporation, in which pores form in the plasma membrane and organelle membranes. The organelle membrane electroporation may be a primary mechanism by which large magnitude, short duration pulses lead to complex, experimentally observed responses, including apoptosis. The tissue system simulations show that dynamic spatial shifts in the electric field accompany electroporation. For certain pulses, the shifting electric field can lead to quite spatially extensive tissue electroporation.

The models presented here offer new insights into the dynamic electrical responses of cells and tissue to pulses of widely varying strength and duration and will contribute to the development of new therapies and biotechnologies based on electroporation.

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Chapter 1

Introduction

Electroporation is a phenomenon in which transient aqueous pores form in lipid bilayers subjected to large electric fields, thereby allowing transport between previously isolated compartments, such as a cell’s intracellular and extracellular spaces [1]. Electroporation can broadly be categorized as conventional electroporation, in which long duration, small magnitude pulses (e.g. 100 µs, 0.1 MV/m) cause electroporation of the cell plasma membrane only, and supra-electroporation, in which short duration, large magnitude pulses (e.g. 10 ns, 10 MV/m) cause significant electroporation of the cell plasma membrane and the organelle membranes. Conventional electroporation has been utilized extensively in research laboratories for efficient in vitro loading of cells with drugs and genes [2–12] and, more recently, supra-electroporation has been shown to result in diverse cellular responses, including apoptosis [13–24].

Electroporation is gaining increased importance because of its potential clinical applications. Following an initial demonstration by Okino et al. [25], Mir and colleagues pioneered electrochemotherapy [26–28], in which tumors are electroporated to facilitate uptake of highly toxic chemotherapy agents that do not normally pass through the plasma membrane in significant amounts. Several clinical trials in Europe have produced promising results [29]. If the recently discovered capability of short duration, large magnitude
electric pulses alone (no drug) to induce apoptosis can be confirmed in vivo, they will provide a novel method of treating cancer without drugs or their associated deleterious systemic effects. Electroporation may also find clinical usage as a non-viral method of gene delivery [30,31].

Despite the potential clinical applications of electroporation, much remains unknown about the basic molecular mechanisms of electroporation and other biophysical mechanisms coupling large electric fields to changes in cell biochemistry. New insights are being gleaned from supra-electroporation experiments, molecular dynamics simulations, and sophisticated spatially distributed continuum models. Theoretical and computational approaches have become increasingly important with the discovery of supra-electroporation because the perturbation of intracellular structures opens the door to many new candidate mechanisms for coupling between electric fields and biological events and the very short time scales and length scales of supra-electroporation pulses make experimental investigations difficult.

The objective of this thesis project was the development of robust, mechanistic models of cell and tissue electroporation in response to electric fields of essentially any strength and duration. The cell and tissue models presented here are based on the asymptotic model of electroporation [32] and the newly developed mesh transport network method (MTNM), a sophisticated, flexible framework for modeling nonlinear, coupled transport phenomena. The cell model characterizes the electrical response at the cell and organelle levels and provides a more accurate and comprehensive investigation of the cell response to pulsed electric fields than has been put forth previously. The multiscale tissue model determines macroscopic transport properties from spatially distributed single cell models and thereby is able to simulate the complex electrical dynamics of tissue electroporation that have been beyond the reach of previous models. The flexibility of the MTNM will allow future models to incorporate even greater sophistication, simulating pore expansion
and molecular transport.

This thesis begins with a detailed description of the mesh transport network method (MTNM), a robust, new method for modeling nonlinear, coupled transport phenomena using equivalent circuits (Chp. 2). The cell and tissue systems modeled using the MTNM are then described (Chp. 3), and the responses of the cell and tissue systems to pulses of widely varying strength and duration are examined (Chps. 4 and 5). The thesis closes with a comparison between the simulation results and the results of other experimental and theoretical investigations and a discussion of future extensions to the models (Chp. 6).
The Mesh Transport Network Method (MTNM) is an intuitive finite volume method (FVM) for modeling transport. The method uses a ground-up approach in which local constitutive relations are established between adjacent finite volumes and cast as equivalent circuit networks. The model is completed by enforcing conservation within each finite volume, which is done automatically in circuit-space by Kirchhoff’s Current Law (KCL).

The strength of the MTNM is its focus on defining local transport, which may be quite complex due to nonlinear or coupled transport mechanisms, rather than global transport equations. One need only define the transport equations between each pair of adjacent finite volumes in terms of quantities that are defined in those finite volumes. The transport may be simple diffusive or electrical transport. Or it may be the coupling of the two, electrodiffusion, or a highly nonlinear phenomenon such as electroporation. As will be shown, models of each of these transport phenomena may be created with the MTNM. Other types of transport phenomena, such as thermal transport, may be modeled similarly.

This chapter begins by considering transport in 1D (Sec. 2.1). The constitutive and
conservation relations for electrical, diffusive, and electrodiffusive transport are developed and used to find equivalent circuits for these transport mechanisms. The 1D concepts are then generalized for 2D transport (Sec. 2.2). Finally, the chapter closes with discussions of extension to 3D (Sec. 2.3), continuity conditions (Sec. 2.4), guidelines for translation to equivalent circuits (Sec. 2.5), and the computer simulation of circuit networks (Sec. 2.6).

2.1 One-dimensional Transport

Figure 2-1 shows a 1D system with concentrations $\gamma_j$ and electric potentials $\phi_j$ at positions $x_j$ and cross-sectional area $A$. The system is divided into Voronoi cells (VCs), each of which is associated with a single node. The VC is the collection of all points closer to a particular node than to any other node. Therefore, the VC interfaces lie halfway between the nodes:

\[
x_{j-1} + \frac{1}{2} (\Delta x)_{j-1,j} = x_j - \frac{1}{2} (\Delta x)_{j-1,j}
\]

and

\[
x_j + \frac{1}{2} (\Delta x)_{j,j+1} = x_{j+1} - \frac{1}{2} (\Delta x)_{j,j+1}.
\]

Here $(\Delta x)_{j-1,j}$ and $(\Delta x)_{j,j+1}$ are the distances between nodes $j - 1$ and $j$ and nodes $j$ and $j + 1$, respectively: $(\Delta x)_{j-1,j} = x_j - x_{j-1}$ and $(\Delta x)_{j,j+1} = x_{j+1} - x_j$. The nodes are not assumed to have uniform spacing. Initially diffusive and electrical transport will be assumed to be independent.

An equivalent circuit of the 1D system will be derived by first developing the constitutive relations that govern transport in Sec. 2.1.1 and then applying conservation relations in Sec. 2.1.2.

2.1.1 Constitutive relations

Many simple transport phenomena are described by Fick’s Law, which states that the flux of a quantity is proportional to the gradient of the quantity. Simple thermal, diffusive, and steady-state electrical transport obey Fick’s Law. In the 1D system,

\[
\vec{J}_D = -D \frac{\partial \gamma}{\partial x} \hat{x}, \quad \vec{J}_E = -\sigma \frac{\partial \phi}{\partial x} \hat{x},
\]

(2.1)
Figure 2-1: 1D transport system. The concentration $\gamma$ and electric potential $\phi$ vary in the $x$-direction, and the system has cross-sectional area $A$. The system is discretized into Voronoi cells (VCs), each enclosing the region of the system closer to that VC node than to any other. VC $j$ has length $(\Delta x)_j$ and the distances between the $j-1$ and $j$ nodes and $j$ and $j+1$ nodes are $(\Delta x)_{j-1,j}$ and $(\Delta x)_{j,j+1}$.

where $\vec{J}_D$ and $\vec{J}_E$ are the diffusive and electrical fluxes and $D$ and $\sigma$ are the diffusivity and conductivity of the medium. If we make the more general assumption that the system is not at steady-state and the transport medium is a dielectric, the electrical flux is not completely described by Fick’s Law; a second term must be added to account for the electrical displacement flux. Thus, the equations become

$$
\vec{J}_D = -D \frac{\partial \gamma}{\partial x} \hat{x}, \quad \vec{J}_E = -\left( \sigma \frac{\partial \phi}{\partial x} + \epsilon \frac{\partial}{\partial t} \left( \frac{\partial \phi}{\partial x} \right) \right) \hat{x},
$$

where $\epsilon$ is the permittivity of the medium.

The spatial derivatives may be evaluated at the interfaces of the VCs using simple, first-order approximations:

$$
\left. \frac{\partial \gamma}{\partial x} \right|_{\text{VC interface}} \approx \frac{\Delta \gamma}{\Delta x}, \quad \left. \frac{\partial \phi}{\partial x} \right|_{\text{VC interface}} \approx \frac{\Delta \phi}{\Delta x}.
$$
The fluxes at VC interfaces are then

\[ \vec{J}_{D}^{i-1,j} = -D \frac{(\Delta \gamma)_{j-1,j}}{(\Delta x)_{j-1,j}} \hat{x} \]

\[ \vec{J}_{E}^{i-1,j} = -\left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} + \epsilon \frac{d}{dt} \left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) \right) \hat{x} \]  \hspace{1cm} (2.4)

\[ \vec{J}_{D}^{i,j+1} = -D \frac{(\Delta \gamma)_{j,j+1}}{(\Delta x)_{j,j+1}} \hat{x} \]

\[ \vec{J}_{E}^{i,j+1} = -\left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} + \epsilon \frac{d}{dt} \left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} \right) \right) \hat{x} \]  \hspace{1cm} (2.5)

Multiplying the fluxes by the cross-sectional area \( A \) of the system yields the total currents:

\[ i_{D}^{i-1,j} = -DA \frac{(\Delta \gamma)_{j-1,j}}{(\Delta x)_{j-1,j}} \]

\[ i_{E}^{i-1,j} = -\sigma A \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} - \epsilon A \frac{d}{dt} \left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) \]  \hspace{1cm} (2.6)

\[ i_{D}^{i,j+1} = -DA \frac{(\Delta \gamma)_{j,j+1}}{(\Delta x)_{j,j+1}} \]

\[ i_{E}^{i,j+1} = -\sigma A \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} - \epsilon A \frac{d}{dt} \left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} \right) . \]  \hspace{1cm} (2.7)

Here currents in the +\( x \) direction (to the right in Fig. 2-1) are defined to be positive.

The \( \Delta x \) are fixed (not functions of time), and therefore

\[ \frac{d}{dt} \left( \frac{\Delta \phi}{\Delta x} \right) = \frac{1}{\Delta x} \frac{d}{dt} (\Delta \phi) . \]  \hspace{1cm} (2.8)

The terms in the currents may be regrouped as the products of time-independent and time-dependent quantities:

\[
\begin{align*}
\begin{cases}
\dot{i}_{D}^{i-1,j} = - \left( \frac{DA}{(\Delta x)_{j-1,j}} \right) (\Delta \gamma)_{j-1,j} \\
\dot{i}_{E}^{i-1,j} = - \left( \frac{\sigma A}{(\Delta x)_{j-1,j}} \right) (\Delta \phi)_{j-1,j} - \left( \frac{\epsilon A}{(\Delta x)_{j-1,j}} \right) \frac{d}{dt} (\Delta \phi)_{j-1,j} \\
\dot{i}_{D}^{i,j+1} = - \left( \frac{DA}{(\Delta x)_{j,j+1}} \right) (\Delta \gamma)_{j,j+1} \\
\dot{i}_{E}^{i,j+1} = - \left( \frac{\sigma A}{(\Delta x)_{j,j+1}} \right) (\Delta \phi)_{j,j+1} - \left( \frac{\epsilon A}{(\Delta x)_{j,j+1}} \right) \frac{d}{dt} (\Delta \phi)_{j,j+1} .
\end{cases}
\end{align*}
\]  \hspace{1cm} (2.9)
2.1 One-dimensional Transport

Defining parameters for the time-independent quantities

\[
R_{D}^{j-1,j} \equiv \frac{(\Delta x)_{j-1,j}}{DA} \quad R_{E}^{j-1,j} \equiv \frac{(\Delta x)_{j-1,j}}{\sigma A} \quad C_{E}^{j-1,j} \equiv \frac{\epsilon A}{(\Delta x)_{j-1,j}} \quad (2.11)
\]

\[
R_{D}^{j,j+1} \equiv \frac{(\Delta x)_{j,j+1}}{DA} \quad R_{E}^{j,j+1} \equiv \frac{(\Delta x)_{j,j+1}}{\sigma A} \quad C_{E}^{j,j+1} \equiv \frac{\epsilon A}{(\Delta x)_{j,j+1}} \quad (2.12)
\]

The currents may be expressed

\[
i_{D}^{j-1,j} = -\frac{(\Delta \gamma)_{j-1,j}}{R_{D}^{j-1,j}} \quad \quad i_{E}^{j-1,j} = -\frac{(\Delta \phi)_{j-1,j}}{R_{E}^{j-1,j}} - C_{E}^{j-1,j} \frac{\partial}{\partial t} (\Delta \phi)_{j-1,j} \quad (2.13)
\]

\[
i_{D}^{j,j+1} = -\frac{(\Delta \gamma)_{j,j+1}}{R_{D}^{j,j+1}} \quad \quad i_{E}^{j,j+1} = -\frac{(\Delta \phi)_{j,j+1}}{R_{E}^{j,j+1}} - C_{E}^{j,j+1} \frac{\partial}{\partial t} (\Delta \phi)_{j,j+1} \quad (2.14)
\]

The spatial dimensions have all been absorbed into the \( R \) and \( C \) parameters. The diffusive current is proportional to the concentration difference between two adjacent nodes and the electric current has a component proportional to the potential difference between two adjacent nodes and a component proportional to the time rate of change of the potential difference between two adjacent nodes.

2.1.2 Conservation relations

The constitutive relations (Eqs. 2.13 and 2.14) express the instantaneous diffusive and electrical currents at the interfaces of the VCs in terms of the concentrations and electrical potentials of the VCs. To complete the model of the 1D system, the effect of the instantaneous currents on the concentrations and electrical potentials of the VCs must be determined by applying conservation principles to establish conservation relations.

General statements of conservation of mass and charge for diffusive and electrical trans-
port are
\[ \frac{d}{dt} \int_V \gamma \, dV + \oint_S \vec{J}_D \cdot \hat{n} \, dS = 0 \]
\[ \frac{d}{dt} \int_V \rho \, dV + \oint_S \vec{J}_{E_o} \cdot \hat{n} \, dS = 0. \]  
(2.15)

Here \( V \) is an arbitrary volume, \( S \) is the surface enclosing \( V \), \( \hat{n} \) is an outward-pointing unit normal vector, \( \gamma \) is concentration, \( \rho \) is free charge density, \( \vec{J}_D \) is diffusive flux, and \( \vec{J}_{E_o} \) is the conductive, or ohmic, component of the electrical flux. These are simply statements that the total amount of a quantity within an arbitrary volume changes in time as a function of how much of the quantity flows in/out of the surface of the volume. Note that only the conductive contribution to the electrical flux is present in the statement of conservation of charge because a displacement flux does not contribute to translational charge movement. An additional conservation relation will be needed for the displacement flux contribution.

In many electrical systems,
\[ \int_V \rho \, dV \approx 0 \]  
(2.16)

because any net charge in \( V \) decays with a time constant \( \tau_{\text{ch, rel.}} = \frac{\epsilon}{\sigma} \). In the electrolyte of the systems of interest here, \( \epsilon \approx 10^{-9} \text{F/m} \) and \( \sigma \approx 1 \text{S/m} \) so \( \tau_{\text{ch, rel.}} = \frac{\epsilon}{\sigma} \approx 10^{-9} \text{s} \), which is on the short end of the time scales of interest here. Moreover, there is no net charge to begin with in the systems of interest here. Thus, there is no net charge within \( V \), and the time rate of change of charge within \( V \) is zero. Therefore, the conservation relations become
\[ \frac{d}{dt} \int_V \gamma \, dV + \oint_S \vec{J}_D \cdot \hat{n} \, dS = 0 \]
\[ \oint_S \vec{J}_{E_o} \cdot \hat{n} \, dS = 0. \]  
(2.17)

For the discretized 1D system, the conservation relations for \( VC \) \( j \) may be expressed
\[ V_j \frac{d\gamma_j}{dt} + A \left( J_{D,j}^{j+1} - J_{D,j}^{j-1} \right) = 0 \]
\[ A \left( J_{E_o,j}^{j+1} - J_{E_o,j}^{j-1} \right) = 0. \]  
(2.18)
2.1 One-dimensional Transport

Multiplying the fluxes $J$ by the cross-sectional area yields the currents $i$:

$$V_j \frac{d\gamma_j}{dt} + i^{j+1}_D - i^{j-1}_D = 0$$

$$i^{j+1}_E - i^{j-1}_E = 0. \quad (2.19)$$

Rearranging,

$$i^{j-1}_D - i^{j+1}_D = V_j \frac{d\gamma_j}{dt}$$

$$i^{j-1}_E - i^{j+1}_E = 0. \quad (2.20)$$

Finally, defining

$$C^j_D \equiv V_j,$$

the conservation relations become

$$i^{j-1}_D - i^{j+1}_D = C^j_D \frac{d\gamma_j}{dt}$$

$$i^{j-1}_E - i^{j+1}_E = 0. \quad (2.22)$$

A conservation relation is also needed for the electrical displacement flux. Gauss’s Law states

$$\int_S \epsilon \vec{E} \cdot \hat{n} dS = \int_V \rho dV. \quad (2.23)$$

But, from Eq. 2.16,

$$\int_V \rho dV \approx 0. \quad (2.24)$$

Therefore,

$$\int_S \epsilon \vec{E} \cdot \hat{n} dS = 0. \quad (2.25)$$

Taking the time derivative,

$$\frac{d}{dt} \int_S \epsilon \vec{E} \cdot \hat{n} dS = 0. \quad (2.26)$$
For the discretized 1D system, the conservation equation for VC \( j \) may be expressed

\[
\epsilon A \frac{d}{dt} \left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} - \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) = 0. \quad (2.27)
\]

The two terms are the displacement current contributions \(-i^{j+1,j}_{Ed} \) and \( i^{j-1,j}_{Ed} \) in Eqs. 2.7 and 2.6. Therefore,

\[
i^{j-1,j}_{Ed} - i^{j,j+1}_{Ed} = 0. \quad (2.28)
\]

Combining Eqs. 2.22 and 2.28, the set of conservation relations is complete:

\[
i^{j-1,j}_{D} - i^{j,j+1}_{D} = C^{j}_{D} \frac{d\gamma_{j}}{dt} \quad \left( i^{j-1,j}_{Eo} + i^{j-1,j}_{Ed} \right) - \left( i^{j,j+1}_{Eo} + i^{j,j+1}_{Ed} \right) = 0 \quad (2.29)
\]

For the diffusive system, the conservation relation states that the time rate of change of the concentration in VC \( j \) is equal to the net flow of solute into the VC divided by the volume of VC, and for the electrical system, the conservation relation states that the total electrical current (conductive and displacement) flowing into VC \( j \) is zero.

Substituting the currents (Eqs. 2.13 and 2.14) into Eq. 2.30 yields the complete system of equations characterizing the diffusive and electrical transport systems. For the diffusive system,

\[
-\frac{(\Delta \gamma)_{j-1,j}}{R^{j-1,j}_{D}} + \frac{(\Delta \gamma)_{j,j+1}}{R^{j,j+1}_{D}} = C^{j}_{D} \frac{d\gamma_{j}}{dt} \quad (2.31)
\]

for each node \( j \). And for the electrical system,

\[
\left( \frac{(\Delta \phi)_{j-1,j}}{R^{j-1,j}_{E}} + C^{j-1,j}_{E} \frac{\partial}{\partial t} (\Delta \phi)_{j-1,j} \right) - \left( \frac{(\Delta \phi)_{j,j+1}}{R^{j,j+1}_{E}} + C^{j,j+1}_{E} \frac{d}{dt} (\Delta \phi)_{j,j+1} \right) = 0 \quad (2.32)
\]

for each node \( j \).
2.1 One-dimensional Transport

(a) Diffusive transport equivalent circuit

(b) Electrical transport equivalent circuit

Figure 2-2: 1D diffusive and electrical transport equivalent circuits. (a) Diffusive transport equivalent circuit. Solute is transported by gradient-driven currents through the resistors and may accumulate in the small volumes associated the nodes by charging the capacitors to ground. (b) Electrical transport equivalent circuit. Charge is transported by gradient-driven currents through the resistors and by displacement currents through the capacitors.

2.1.3 Equivalent circuits

Transport in the circuits in Fig. 2-2 is equivalent to the transport in the 1D diffusive and electrical transport system. We can check this by applying Kirchhoff’s Current Law (KCL) at node $j$ in each circuit.

In the diffusive circuit (Fig. 2-2a), KCL specifies that the sum of the currents flowing into node $j$ must equal zero:

$$i_{D,j-1} - i_{D,j} + i_{D,j+1} = 0.$$  \hspace{1cm} (2.33)
Rearranging,

\[ i^{j-1,j}_D - i^{j+1,j}_D = i^j_D. \]  \hspace{1cm} (2.34)

Defining the currents in terms of node voltages, resistances, and capacitances,

\[ \frac{\gamma_{j-1} - \gamma_j}{R^{j-1,j}_D} - \frac{\gamma_j - \gamma_{j+1}}{R^{j+1,j}_D} = C^j_D \frac{d\gamma_j}{dt}. \]  \hspace{1cm} (2.35)

Defining \( \Delta\gamma \) as previously,

\[ -\left(\frac{\Delta\gamma}{R^{j-1,j}_D}\right) + \left(\frac{\Delta\gamma}{R^{j+1,j}_D}\right) = C^j_D \frac{d\gamma_j}{dt}. \]  \hspace{1cm} (2.36)

This is identical to the governing equation derived for 1D transport (Eq. 2.31).

In the electrical circuit (Fig. 2-2b), KCL specifies that the sum of the currents flowing into node \( j \) must equal zero:

\[ (i^{j-1,j}_{E_0} + i^{j-1,j}_{E_d}) - (i^{j+1,j}_{E_0} - i^{j+1,j}_{E_d}) = 0. \]  \hspace{1cm} (2.37)

Here the currents are defined as traveling to the right. This equation is identical to that found in applying conservation to the simple 1D system (Eq. 2.29). Defining the currents in terms of node voltages, resistances, and capacitances,

\[ \left(\frac{\phi_{j-1} - \phi_j}{R^{j-1,j}_E} + C^{j-1,j}_E \frac{d}{dt} (\phi_{j-1} - \phi_j)\right) - \left(\frac{\phi_j - \phi_{j+1}}{R^{j+1,j}_E} + C^{j+1,j}_E \frac{d}{dt} (\phi_j - \phi_{j+1})\right) = 0. \]  \hspace{1cm} (2.38)

Defining \( \Delta\phi \) as previously and multiplying by \(-1\),

\[ \left(\frac{(\Delta\phi)_{j-1,j}}{R^{j-1,j}_E} + C^{j-1,j}_E \frac{\partial}{\partial t} (\Delta\phi)_{j-1,j}\right) - \left(\frac{(\Delta\phi)_{j,j+1}}{R^{j+1,j}_E} + C^{j+1,j}_E \frac{d}{dt} (\Delta\phi)_{j,j+1}\right) = 0. \]  \hspace{1cm} (2.39)

This is identical to the governing equation derived for 1D transport (Eq. 2.32).
2.1 One-dimensional Transport

2.1.4 Electrodiffusion

A strength of the MTNM is the ease with which it can be used to model coupled transport. Electrodiffusion will be used as an important and illustrative example. In this type of transport, diffusive transport is coupled to the electrical transport. The solute is charged and therefore its transport has an electrical drift component in addition to a diffusive component. Assume that the solute is not a primary charge carrier and that the effect of the solute movement on the electric field is negligible, i.e. the coupling only goes one way. Because the electrical transport is not influenced by the diffusive transport, the governing equations for $\phi$ and the equivalent circuit are unchanged from Secs. 2.1.1–2.1.3 and Fig. 2-2b.

The charged solute transport, on the other hand, will be influenced by the electrical transport. As in the initial 1D example, the key to building the model is understanding the transport that occurs at the VC interfaces. The electrical drift components $\vec{J}_{ED_e}^j$ of the solute flux at the interfaces of VC $j$ are simply

$$
\vec{J}_{ED_e}^{j-1,j} = \mu z \gamma_{j-1,j} \vec{E}_{j-1,j} \\
\vec{J}_{ED_e}^{j,j+1} = \mu z \gamma_{j,j+1} \vec{E}_{j,j+1}
$$

where $\mu$ and $z$ are the mobility and valence of the solute. The VC interfaces were defined such that they were halfway between nodes. Therefore, the concentrations at the interfaces may be approximated as the averages of the concentrations of the VCs associated with the interfaces:

$$
\gamma_{j-1,j} = \frac{\gamma_{j-1} + \gamma_j}{2} \\
\gamma_{j,j+1} = \frac{\gamma_j + \gamma_{j+1}}{2}.
$$

The electric fields at the interfaces are

$$
\vec{E}_{j-1,j} = -\frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \hat{x} \\
\vec{E}_{j,j+1} = -\frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} \hat{x}.
$$
The electrical drift components of electrodiffusion at the interfaces are then

\[ \vec{J}_{ED}^{j-1,j} = -\mu z \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \left( \frac{\Delta \phi_{j-1,j}}{\Delta x_{j-1,j}} \right) \hat{x} \]  \hspace{1cm} (2.43)

\[ \vec{J}_{ED}^{j,j+1} = -\mu z \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) \left( \frac{\Delta \phi_{j,j+1}}{\Delta x_{j,j+1}} \right) \hat{x} \]  \hspace{1cm} (2.44)

The diffusive components of electrodiffusion \( \vec{J}_{ED_D} \) are unchanged from the original, uncoupled system (Eqs. 2.4 and 2.5):

\[ \vec{J}_{ED_D}^{j-1,j} = -D \frac{(\Delta \gamma)_{j-1,j}}{\Delta x_{j-1,j}} \hat{x} \]  \hspace{1cm}  (2.46)

\[ \vec{J}_{ED_D}^{j,j+1} = -D \frac{(\Delta \gamma)_{j,j+1}}{\Delta x_{j,j+1}} \hat{x}. \]  \hspace{1cm}  (2.47)

The total electrodiffusive fluxes \( \vec{J}_{ED} \) at the VC interfaces are the sums of the diffusive and drift components:

\[ \vec{J}_{ED}^{j-1,j} = -\left( D \frac{\Delta \gamma_{j-1,j}}{\Delta x_{j-1,j}} + \mu z \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \left( \frac{\Delta \phi_{j-1,j}}{\Delta x_{j-1,j}} \right) \right) \hat{x} \]  \hspace{1cm} (2.46)

\[ \vec{J}_{ED}^{j,j+1} = -\left( D \frac{\Delta \gamma_{j,j+1}}{\Delta x_{j,j+1}} + \mu z \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) \left( \frac{\Delta \phi_{j,j+1}}{\Delta x_{j,j+1}} \right) \right) \hat{x}. \]  \hspace{1cm} (2.47)

The electrodiffusive currents \( i_{ED} \) at the interfaces are found by multiplying the electrodiffusive fluxes by the cross-sectional area \( A \):

\[ i_{ED}^{j-1,j} = -DA \left( \frac{\Delta \gamma_{j-1,j}}{\Delta x_{j-1,j}} \right) \hat{x} - \mu z A \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \left( \frac{\Delta \phi_{j-1,j}}{\Delta x_{j-1,j}} \right) \]  \hspace{1cm} (2.48)

\[ i_{ED}^{j,j+1} = -DA \left( \frac{\Delta \gamma_{j,j+1}}{\Delta x_{j,j+1}} \right) \hat{x} - \mu z A \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) \left( \frac{\Delta \phi_{j,j+1}}{\Delta x_{j,j+1}} \right). \]  \hspace{1cm} (2.49)

Here currents in the \( +x \) direction (to the right in Fig. 2-1) are defined to be positive. Defining

\[ R_{D}^{j-1,j} \equiv \frac{\Delta x_{j-1,j}}{DA} \]  \hspace{1cm} \[ R_{D}^{j,j+1} \equiv \frac{\Delta x_{j,j+1}}{DA}, \]  \hspace{1cm} (2.50)
and substituting into the current equation,

\[ i_{ED}^{j-1,j} = -\frac{(\Delta \gamma)_{j-1,j}}{R_{D_{j-1,j}}} - \mu z A \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) \]  

\[ i_{ED}^{j,j+1} = -\frac{(\Delta \gamma)_{j,j+1}}{R_{D_{j,j+1}}} - \mu z A \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) \left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} \right). \]  

(2.51) \hspace{1cm} (2.52)

Note that the \( R_D \) defined here are unchanged from the original diffusion example because the diffusive contribution to transport is unchanged.

The conservation relation (Eq. 2.30) requires

\[ i_{ED}^{j-1,j} - i_{ED}^{j,j+1} = V_j \frac{d\gamma_j}{dt}. \]  

(2.53)

Defining

\[ C_D^j \equiv V_j, \]  

(2.54)

and conservation relation becomes

\[ i_{ED}^{j-1,j} - i_{ED}^{j,j+1} = C_D^j \frac{d\gamma_j}{dt}. \]  

(2.55)

Note that the \( C_D^j \) here is unchanged from the original diffusion example because the volume of the VC is unchanged. Substituting for the currents, the complete system of equations characterizing the electrodiffusive transport system is

\[ - \left( \frac{(\Delta \gamma)_{j-1,j}}{R_{D_{j-1,j}}} + \mu z A \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) \right) \]

\[ + \left( \frac{(\Delta \gamma)_{j,j+1}}{R_{D_{j,j+1}}} + \mu z A \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) \left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} \right) \right) = C_D^j \frac{d\gamma_j}{dt}. \]  

(2.56)

for each node \( j \).
Figure 2-3: 1D electrodiffusive transport equivalent circuit. (a) Electrical transport equivalent circuit. Charge is transported by gradient-driven currents through the resistors and by displacement currents through the capacitors. (b) Electrodiffusive transport. Solute is transported by gradient-driven currents through the resistors and by electrical drift current sources that are functions of the node voltages in (a). Solute may accumulate in the small volumes associated with the nodes by charging the capacitors to ground.

Figure 2-3 shows the electrical and electrodiffusive transport equivalent circuits. The nonlinear current sources $i_{ED}$ in the electrodiffusive transport equivalent circuit are coupled to the electric potentials $\phi$ in the electrical transport equivalent circuit. Note that the electrodiffusive circuit is quite similar to the diffusive circuit (Fig. 2-2a). The only difference is the addition of current sources driving electrical drift transport.
2.1 One-dimensional Transport

2.1.5 Electrodiffusion formulation validation

The claim has been made that characterizing quantities and transport at the interfaces of the VCs is the key to developing transport relations. In the case of electrodiffusion, the concentrations at the interfaces of the VCs were assumed to be the averages of VC concentrations and electric fields at the interfaces of the VCs were assumed to be the differences in electric potentials divided by the differences in positions of the VC nodes. If these assumptions are valid, then in the limit $\Delta x \to 0$, the derived discretized governing equation for electrodiffusion (Eq. 2.56) should approach the continuous differential equation governing electrodiffusion.

The conservation relation (Eq. 2.18) specifies

$$V_j \frac{d\gamma_j}{dt} = A \left( J_{\text{ED}}^{j-1,j} - J_{\text{ED}}^{j,j+1} \right). \tag{2.57}$$

Dividing by $A$ and substituting $\frac{V_i}{A} = (\Delta x)_j$, where $(\Delta x)_j$ is the length of VC $j$,

$$(\Delta x)_j \frac{d\gamma_j}{dt} = J_{\text{ED}}^{j-1,j} - J_{\text{ED}}^{j,j+1}. \tag{2.58}$$

Substituting for the fluxes $J_{\text{ED}}$ (Eqs. 2.46 and 2.47),

$$\begin{align*}
(\Delta x)_j \frac{d\gamma_j}{dt} &= -\left( D \frac{(\Delta \gamma)_{j-1,j}}{(\Delta x)_{j-1,j}} + \mu z \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) \right) \\
&\quad + \left( D \frac{(\Delta \gamma)_{j,j+1}}{(\Delta x)_{j,j+1}} + \mu z \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) \left( \frac{(\Delta \phi)_{j,j+1}}{(\Delta x)_{j,j+1}} \right) \right). \tag{2.59}
\end{align*}$$
Collecting terms of similar form and dividing by \((\Delta x)_j\),

\[
\frac{d\gamma_j}{dt} = D \frac{\gamma_{j+1} - \gamma_j}{(\Delta x)_j} + \mu z \left( \gamma_{j+1} - \gamma_j \right) \frac{\Delta \phi_{j+1}}{2(\Delta x)_j} - \gamma_{j-1} \frac{\Delta \phi_{j-1}}{2(\Delta x)_j} + \mu z \gamma_j \frac{\Delta \phi_{j+1}}{(\Delta x)_{j+1}} - \mu z \gamma_{j-1} \frac{\Delta \phi_{j-1}}{(\Delta x)_{j-1}},
\]

(2.60)

Further simplification yields

\[
\frac{d\gamma_j}{dt} = D \frac{\gamma_{j+1} - \gamma_j}{(\Delta x)_j} + \mu z \frac{\Delta \phi_{j+1}}{2(\Delta x)_j} + \mu z \gamma_j \frac{\Delta \phi_{j+1}}{2(\Delta x)_j} + \mu z \gamma_j \frac{\Delta \phi_{j+1}}{(\Delta x)_{j+1}} - \mu z \gamma_{j-1} \frac{\Delta \phi_{j-1}}{(\Delta x)_{j-1}},
\]

(2.61)

In the limit as the \(\Delta x \to 0\), the first term becomes the diffusivity times the second spatial derivative of concentration with respect to \(x\), the second term becomes the product of the mobility, valence, and first spatial derivatives of concentration and electric potential with respect to \(x\), and the last term becomes the product of mobility, valence, concentration, and the second spatial derivative of electric potential with respect to \(x\):

\[
\frac{\partial \gamma}{\partial t} = D \frac{\partial^2 \gamma}{\partial x^2} + \mu z \frac{\partial \gamma}{\partial x} \frac{\partial \phi}{\partial x} + \mu z \gamma \frac{\partial^2 \phi}{\partial x^2}.
\]

(2.62)

This is in fact the electrodiffusion equation [33], validating the approach taken in Sec. 2.1.4. If \(\mu, z, \text{ or } \frac{\partial \phi}{\partial x}\) is zero, the equation becomes the diffusion equation:

\[
\frac{\partial \gamma}{\partial t} = D \frac{\partial^2 \gamma}{\partial x^2}.
\]

(2.63)
2.1.6 Strong electrodiffusive coupling

Electrical transport was assumed in Sec. 2.1.4 to be independent of the solute concentration (weak electrodiffusive coupling) because the solute was assumed to be a minor charge carrier. The methods developed may be extended to make electrical transport a function of solute concentration, as would be the case in a model of ion transport in an electrolyte. In this case, local conductivity is a function position:

\[ \sigma(x) = \sum_{k=1}^{N} F |z_k| \mu_k \gamma_k(x) \]  

(2.64)

where \( N \) is the number of ion species, \( F \) is Faraday’s constant, and \( z_k, \mu_k, \) and \( \gamma_k(x) \) are the valence, mobility, and concentration of species \( k \). A separate electrodiffusion equivalent circuit of the type shown in Fig. 2-3b is created for each ion species with the resistors in the electrical equivalent circuit (Fig. 2-3a) replaced by current sources

\[ \dot{I}^{j-1,j} = -A \left( \frac{(\Delta \phi)_{j-1,j}}{(\Delta x)_{j-1,j}} \right) \sum_{k=1}^{N} F |z_k| \mu_k \left( \frac{\gamma_{j-1} + \gamma_j}{2} \right) \]  

(2.65)

\[ \dot{I}^{j,j+1} = -A \left( \frac{(\Delta x)_{j,j+1}}{(\Delta x)_{j,j+1}} \right) \sum_{k=1}^{N} F |z_k| \mu_k \left( \frac{\gamma_j + \gamma_{j+1}}{2} \right) . \]  

(2.66)

2.2 Two-dimensional Transport

The concepts developed for the 1D transport system are readily generalized to 2D and 3D. As in the 1D case, the focus of the method is on creating constitutive relations for the transport between each pair of adjacent VCs and then applying conservation. In 2D and 3D, unlike in 1D, the transport in the physical system is not in general normal to the imposed VC interfaces. However, this may easily be dealt with in systems with isotropic transport properties, as will be assumed in the following derivation.

This section will focus on electrical transport in 2D. Other forms of transport may easily
be generalized in a similar manner.

2.2.1 Constitutive relations

The constitutive relations for transport between adjacent VCs in 2D are of the same form as those for 1D (Eqs. 2.6 and 2.7). However, determining the equivalents of $A$ and $\Delta x$ requires further examination.

Figure 2-4a shows a typical triangular mesh and its associated VCs, which are polygons enclosing all points closer to the node associated with the VC than to any other node. The sides of the VC bisect the triangle edges at right angles. Figure 2-4b shows a pair of adjacent VCs $j$ and $k$ and the triangle edge connecting their nodes. The VC interface has length $w_{j,k}$, the triangle edge has length $l_{j,k}$, and the system has depth $d$. The area of the VC interface is $A_{j,k} = w_{j,k}d$. There is an electric field $\vec{E}_{j,k}$, electric flux $\vec{J}_{E}^{j,k}$, and unit normal vector $\hat{n}_{j,k}$ at the VC interface.

The electric flux $\vec{J}_{E}^{j,k}$ may be expressed as the sum of conductive and displacement flux contributions:

$$\vec{J}_{E}^{j,k} = \vec{J}_{E_o}^{j,k} + \vec{J}_{E_d}^{j,k}. \tag{2.67}$$

Here,

$$\vec{J}_{E_o}^{j,k} = \sigma \vec{E}_{j,k} \quad \text{and} \quad \vec{J}_{E_d}^{j,k} = \epsilon \frac{\partial \vec{E}}{\partial t}. \tag{2.68}$$

The electrical fluxes and electric field can be expressed as the sums of components normal and parallel to the VC interface:

$$\vec{J}_{E_o}^{j,k} = \vec{J}_{E_{o\perp}}^{j,k} + \vec{J}_{E_{o\parallel}}^{j,k} \quad \text{and} \quad \vec{J}_{E_d}^{j,k} = \vec{J}_{E_{d\perp}}^{j,k} + \vec{J}_{E_{d\parallel}}^{j,k} \quad \vec{E} = \vec{E}_{\perp}^{j,k} + \vec{E}_{\parallel}^{j,k}. \tag{2.69}$$

It is clear from Fig. 2-4b that the components parallel to the VC interface do not con-
Figure 2-4: 2D transport system. (a) Triangular mesh and Voronoi cells (VCs). A portion of a 2D system is discretized into a set of VCs (blue) associated with the nodes connected by triangulation (black). (b) Adjacent Voronoi cells. The VCs have depth $d$ and an interface of length $w_{j,k}$, and the distance between the VC nodes is $l_{j,k}$. The VCs have electric potentials $\phi_j$ and $\phi_k$ and, at the VC interface, there is an electric field $\vec{E}$ and flux $\vec{J}$, which can be broken into components normal ($\vec{E}_\perp$ and $\vec{J}_\perp$) and parallel ($\vec{E}_\parallel$ and $\vec{J}_\parallel$) to the interface.

The currents flowing from VC $j$ to VC $k$ are the product of the interface area $A_{j,k} = w_{j,k}d$ and the dot product of the fluxes with the outward-point unit normal vector:

$$i_{E_\parallel}^{j,k} = w_{j,k}d \vec{E}_\parallel \cdot \hat{n}_{j,k} \quad \quad i_{E_\perp}^{j,k} = w_{j,k}d \vec{E}_\perp \cdot \hat{n}_{j,k}. \quad \quad \quad \quad \quad (2.72)$$
Substituting Eq. 2.71, the currents become

\[
\begin{align*}
    i_{Eo}^{j,k} &= w_{j,k} d J_{Eo,\perp}^{j,k} \cdot \hat{n}_{j,k} \\
    i_{Ed}^{j,k} &= w_{j,k} d J_{Ed,\perp}^{j,k} \cdot \hat{n}_{j,k}.
\end{align*}
\]

The components of the fluxes normal to the VC interface may be expressed in terms of the normal component of the electric field at the VC interface:

\[
\begin{align*}
    \vec{J}_{Eo,\perp}^{j,k} &= \sigma \vec{E}_{j,k}^{\perp} \\
    \vec{J}_{Ed,\perp}^{j,k} &= \epsilon \frac{\partial}{\partial t} \vec{E}_{j,k}^{\perp}.
\end{align*}
\]

Substituting for the fluxes, the currents become

\[
\begin{align*}
    i_{Eo}^{j,k} &= \sigma w_{j,k} d \vec{E}_{j,k}^{\perp} \cdot \hat{n}_{j,k} \\
    i_{Ed}^{j,k} &= \epsilon w_{j,k} d \frac{\partial}{\partial t} \vec{E}_{j,k}^{\perp} \cdot \hat{n}_{j,k}.
\end{align*}
\]

A first-order approximation to the normal component of the electric field is

\[
E_{j,k}^{\perp} \approx \frac{\Delta \phi_{j,k}}{l_{j,k}},
\]

where \((\Delta \phi)_{j,k} = \phi_k - \phi_j\) and \(l_{j,k}\) is the distance between nodes \(j\) and \(k\). Substituting for the perpendicular electric field component, the currents become

\[
\begin{align*}
    i_{Eo}^{j,k} &= -\sigma w_{j,k} d \frac{(\Delta \phi)_{j,k}}{l_{j,k}} \\
    i_{Ed}^{j,k} &= -\epsilon w_{j,k} d \frac{d}{dt} \frac{(\Delta \phi)_{j,k}}{l_{j,k}}.
\end{align*}
\]

The terms in the currents may be regrouped as the products of time-independent and time-dependent quantities:

\[
\begin{align*}
    i_{Eo}^{j,k} &= -\left( \frac{\sigma w_{j,k} d}{l_{j,k}} \right) (\Delta \phi)_{j,k} \\
    i_{Ed}^{j,k} &= -\left( \frac{\epsilon w_{j,k} d}{l_{j,k}} \right) \frac{d}{dt} (\Delta \phi)_{j,k}.
\end{align*}
\]

Defining

\[
R_E^{j,k} = \frac{l_{j,k}}{\sigma w_{j,k} d} \quad \text{and} \quad C_E^{j,k} = \frac{\epsilon w_{j,k} d}{l_{j,k}},
\]

\[
(2.79)
\]
the currents may be expressed as

\[
\begin{align*}
    i_{E_0}^{j,k} &= -R_E^{j,k} (\Delta \phi)_{j,k}, \\
    i_{Ed}^{j,k} &= -C_E^{j,k} \frac{d}{dt} (\Delta \phi)_{j,k}.
\end{align*}
\]

(2.80)

Thus, the total current flowing from VC \( j \) to VC \( k \) is

\[
\begin{align*}
    i_E^{j,k} &= -R_E^{j,k} (\Delta \phi)_{j,k} - C_E^{j,k} \frac{d}{dt} (\Delta \phi)_{j,k}.
\end{align*}
\]

(2.81)

This constitutive relation is identical to that derived for the 1D system (Eqs. 2.13 and 2.14). The only difference is in the spatial parameters defining \( R_E \) and \( C_E \).

### 2.2.2 Conservation relations

The conservation theory developed in Sec. 2.1.2 may be applied in similar form to 2D transport. Conservation requires

\[
\begin{align*}
    \oint_S \vec{J}_{E_0} \cdot \hat{n} dS &= 0, \\
    \frac{d}{dt} \oint_S \vec{E} \cdot \hat{n} dS &= 0.
\end{align*}
\]

(2.82)

In the discretized 2D system these relations become

\[
\begin{align*}
    \sum_{k=1}^{N_j} (w_{j,k}d) J_{E_0}^{j,k} &= 0, \\
    \sum_{k=1}^{N_j} \left( \frac{\epsilon w_{j,k}d}{l_{j,k}} \right) \frac{d}{dt} (\Delta \phi)_{j,k} &= 0.
\end{align*}
\]

(2.83)

where \( N_j \) is the number of VCs adjacent to VC \( j \). Simplifying,

\[
\begin{align*}
    \sum_{k=1}^{N_j} i_{E_0}^{j,k} &= 0, \\
    \sum_{k=1}^{N_j} i_{Ed}^{j,k} &= 0.
\end{align*}
\]

(2.84)

Summing the conductive and displacement current contributions, the total current leaving VC \( j \) must also equal zero:

\[
\sum_{k=1}^{N_j} i_E^{j,k} = 0.
\]

(2.85)
Substituting the constitutive relation (Eq. 2.81) yields the system of equations governing transport:

$$\sum_{k=1}^{N_j} \left( R_{E}^{j,k} (\Delta \phi)_{j,k} + C_{E}^{j,k} \frac{d}{dt} (\Delta \phi)_{j,k} \right) = 0 \quad (2.86)$$

for each VC $j$.

Note the similarities between the 2D and 1D systems.

### 2.2.3 Electrodiffusion

The 2D analysis was limited to electrical transport for compactness. Applying concepts from the 1D electrodiffusive system and the translation of the electrical system from 1D to 2D, one easily arrives at the 2D electrodiffusive and simple diffusive transport relations.

The constitutive relations are

$$i_{ED}^{j,k} = -Dw_{j,k}d \frac{(\Delta \gamma)_{j,k}}{l_{j,k}} - \mu zw_{j,k}d \left( \frac{\gamma_j + \gamma_k}{2} \right) \left( \frac{(\Delta \phi)_{j,k}}{l_{j,k}} \right). \quad (2.87)$$

Defining

$$R_{D}^{j,k} \equiv \frac{l_{j,k}}{Dw_{j,k}d} \quad \quad i_{ED}^{j,k} \equiv -\mu zw_{j,k}d \left( \frac{\gamma_j + \gamma_k}{2} \right) \left( \frac{(\Delta \phi)_{j,k}}{l_{j,k}} \right) \quad (2.88)$$

The constitutive relations become

$$i_{ED}^{j,k} = -\frac{(\Delta \gamma)_{j,k}}{R_{D}^{j,k}} + i_{ED}^{j,k} \quad (2.89)$$
The general conservation relation for diffusion (Eq. 2.15) states
\[
\frac{d}{dt} \int_V \gamma dV + \oint_S \mathbf{J}_D \cdot \hat{n} dS = 0.
\] (2.90)

For the discretized 2D system, the conservation relation becomes
\[
V_j \frac{d\gamma_j}{dt} = - \sum_{k=1}^{N_j} i_{j,k}^{ED}.
\] (2.91)

Here \( V_j \) is the volume VC \( j \). \( V_j \) is the product of the VC depth and the cross-sectional areas of the VC. This cross-sectional area is evaluated as the sum of the area of the \( N_j \) triangles formed by placing line segments between the node and VC vertices, each of which has base \( w_{j,k} \) and altitude \( \frac{1}{2}l_{j,k} \) and therefore area \( \left( \frac{1}{2} \right) w_{j,k} \left( \frac{1}{2}l_{j,k} \right) \). Thus,
\[
V_j = \sum_{k=1}^{N_j} \frac{l_{j,k}w_{j,k}d}{4}
\] (2.92)

Defining
\[
C_j^D = V_j
\] (2.93)

and substituting for \( i_{j,k}^{ED} \), the 2D electrodiffusion governing equation is
\[
C_j^D \frac{d\gamma_j}{dt} = \sum_{k=1}^{N_j} \left( \frac{\Delta \gamma_{j,k}}{R_{j,k}^D} - i_{j,k}^{ED} \right)
\] (2.94)

for each VC \( j \). Setting \( i_{j,k}^{ED} \) equal to zero results in the 2D simple diffusion governing equation:
\[
C_j^D \frac{d\gamma_j}{dt} = \sum_{k=1}^{N_j} \frac{\Delta \gamma_{j,k}}{R_{j,k}^D}.
\] (2.95)
2.2.4 Equivalent circuits

The equivalent circuits for 2D electrical, diffusive, and electrodiffusive transport are shown in Figs. 2-5, 2-6, and 2-7. The equivalent circuit representations of 2D transport are very similar to those of 1D transport because the constitutive and conservation relations take the same form in 2D as in 1D. The primary difference is in the number of adjacent nodes to which nodes are connected. In the 1D systems, each VC (excluding those on a boundary) was adjacent to two other VCs, and therefore the nodes in the 1D transport equivalent circuit are connected to two other nodes. In the 2D transport systems, each VC (excluding those on a boundary) was adjacent to approximately six (in a triangularly meshed system) adjacent VCs, and therefore the nodes in the 2D transport equivalent circuit are connected to approximately six other nodes.

Applying KCL to node $j$ to the equivalent circuits results in the same equations as those derived (Eqs. 2.86, 2.94, and 2.95).

2.3 Three-dimensional Transport

Extending the MTNM methods developed here to 3D is conceptually straightforward. In 1D, only one spatial variable was used to determine the constitutive relations; the distance between nodes $\Delta x$ varied but the cross-sectional area $A$ was fixed. In 2D, there were two spatial variables used to determine the constitutive relations; the distance between nodes $l$ and VC interface length $w$ varied but the depth $d$ was fixed. It follows that in 3D, three spatial variables will be used to determine the constitutive relations.

In 3D, a system is discretized using tetrahedrons, just as in 2D a system is discretized using triangles. The VCs are polyhedrons rather than prisms of polygonal cross-section, as in 2D, and the VC interfaces are polygons rather than rectangles, as in 2D. The constitutive and conservation relations may be developed in the same manner as in 1D and 2D.
While applying the MTNM to 3D is conceptually straightforward, applying the method in practice is significantly more difficult than applying the MTNM in 1D or 2D. The challenge is in the technical details of implementation, such as specifying the system geometry and generating 3D tetrahedral meshes. As such, 3D models will not be used in this thesis. Moreover, the current state of the art is such that more sophisticated, biologically realistic 2D models are of more use than very simple 3D models. Eventually, however, developing 3D models will be necessary for truly compelling results.
Figure 2-6: 2D diffusive transport equivalent circuit. The general layout is the same as that of Fig. 2-2a. However, the VCs in 2D systems have more adjacent VCs than VCs in 1D systems, and therefore the nodes in the 2D equivalent circuit have more connections. Solute is transported by gradient-driven currents through the resistors and may accumulate in the small volumes associated with the nodes by charging the capacitors to ground.

2.4 Continuity Conditions

A requirement of the MTNM is that the triangular edges of the mesh must lie along changes in material (e.g. electrolyte-membrane interface). For electrical transport, continuity conditions require that the tangential electric field ($\vec{E}_\perp$ in Fig. 2-4b) and electric potential be constant across a boundary. The methods as derived are consistent with this requirement. The tangential fluxes are calculated separately for each side of the triangular edge using the transport parameters for the material on each side. Thus, in
2.4 Continuity Conditions

Figure 2-7: 2D electrodiffusive transport equivalent circuit. The general layout is the same as that of Fig. 2-3b. However, the VCs in 2D systems have more adjacent VCs than VCs in 1D systems, and therefore the nodes in the 2D equivalent circuit have more connections. Solute is transported by gradient-driven currents through the resistors and may accumulate in the small volumes associated with the nodes by charging the capacitors to ground.

In diffusive transport, steady-state concentrations can be discontinuous at material interfaces if the solute has differing solubilities in the two materials. The concentrations in the two materials are related at the interface by their partition coefficient. Thus, nodes at the interface must be designated as representing the concentration of one material or the other. Fluxes at the boundary in the other material are then determined by scaling
the concentration by the partition coefficient. This requires a slight departure from the simple resistor-capacitor circuit shown in Fig. 2-6 at the interfaces but can easily be implemented using more flexible circuit elements.

2.5 Translation to Equivalent Circuits

2.5.1 Comparison of electrical and diffusive transport

Electrical and diffusive transport are quite different, and it is worthwhile to examine how their differing transport properties translate into different equivalent circuits. There are two fundamental differences between electrical and diffusive transport and their translations into circuits. Electrical systems exhibit a displacement current in dielectric materials, but there is no equivalent in diffusive transport. Consequently, nodes in the equivalent circuit of the electrical system are connected by resistors (gradient-driven current) and capacitors (displacement current) in parallel. Nodes in the equivalent circuit of the diffusive system are connected only by resistors (gradient-driven current). The other fundamental difference between electrical and diffusive transport is the assumption in electrical transport that the rate of change of charge in an arbitrary volume is zero, an assumption that cannot be made for diffusion because of its much slower transport time scales. Consequently, the nodes in the diffusive equivalent circuit are connected to ground through capacitors (the volumes associated with the nodes), allowing solute accumulation in the volumes associated with the nodes. The electrical transport equivalent circuit does not have capacitors to ground because accumulation is assumed not to occur.

While it is natural to think of electrical systems using circuits, it is somewhat less natural to think of diffusive systems using circuits. Nonetheless, the underlying principles (e.g. potential differences drive transport) are similar and allow the derivation of equivalent quantities (Table 2.1) and equivalent relations for translating electrical and diffusive systems into equivalent circuits (Table 2.2).
2.5 Translation to Equivalent Circuits

<table>
<thead>
<tr>
<th>Electrical Transport</th>
<th>( \Leftrightarrow )</th>
<th>Diffusive Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantity</strong></td>
<td><strong>Symbol</strong></td>
<td><strong>Unit</strong></td>
</tr>
<tr>
<td>Charge</td>
<td>( q )</td>
<td>( C )</td>
</tr>
<tr>
<td>Current</td>
<td>( i_E )</td>
<td>( A = \frac{C}{l} )</td>
</tr>
<tr>
<td>Potential</td>
<td>( \phi )</td>
<td>( V = \frac{1}{C} )</td>
</tr>
<tr>
<td>Conductivity</td>
<td>( \sigma )</td>
<td>( \frac{C^2 \text{m}}{\text{J} \text{s}} )</td>
</tr>
<tr>
<td>Conductance</td>
<td>( G_E )</td>
<td>( \frac{C^2}{\text{J} \text{s}} )</td>
</tr>
<tr>
<td>Resistance</td>
<td>( R_E )</td>
<td>( \frac{\text{J}}{\text{C}^2 \text{s}} )</td>
</tr>
<tr>
<td>Capacitance</td>
<td>( C )</td>
<td>( \frac{C}{V} = \frac{C^2}{l} )</td>
</tr>
<tr>
<td>Time Constant</td>
<td>( \tau_E )</td>
<td>( \text{s} )</td>
</tr>
</tbody>
</table>

Table 2.1: Analogous electrical and diffusive transport quantities.

\[
\begin{align*}
G_E &= \sigma \frac{A}{l} \\
R_E &= \frac{1}{\sigma A} \\
i_E &= G_E \Delta \phi \\
C &= \epsilon \frac{A}{l} \\
qu &= C \Delta \phi \\
\tau_E &= R_E C
\end{align*}
\]

\[
\begin{align*}
G_D &= D \frac{A}{l} \\
R_D &= \frac{1}{D A} \\
i_D &= G_D \Delta \gamma \\
V &= Al \\
n &= V \gamma \\
\tau_D &= R_D V
\end{align*}
\]

Table 2.2: Analogous electrical and diffusive transport relations.
2.5.2 General rules for translation to equivalent circuits

The electrical, diffusive, and electrodiffusive transport examples give a sample of the types of transport systems that can be modeled by the MTNM and how to go about creating such models. The following provide some general guidelines for the translation of transport phenomena into equivalent circuits:

- **VC ↔ circuit node.** The physical system is discretized into VCs, each of which is associated with a node in the equivalent circuit. The discretization should be chosen to have sufficient fineness that quantities can be assumed to vary linearly between VCs. Then gradients evaluated at VC interfaces can be approximated as the change in the quantity between the VCs divided by the distance between the VC nodes. Additionally, quantities at VC interfaces can be approximated as the average of the quantities of the VCs.

- **Resistors provide gradient-driven flow.** The current through a resistor is proportional to the voltage drop across the resistor. Assuming a quantity varies linearly from one VC to the next, the gradient-driven flow between the VCs is proportional to the differences in the quantity between the VCs. Analogously, the current between nodes connected by resistors is proportional to the differences in the node voltages. Therefore, gradient driven flow in a physical system can be modeled as a circuit network with resistors between nodes whose values are determined by time-invariant material properties and the chosen discretization of the physical domain.

- **Capacitors provide time-dependent flow and integration.** The current through a capacitor is proportional to the time rate of change of the voltage drop across the capacitor. Therefore, flow that is proportional to the time rate of change of the gradient of a quantity (e.g. displacement flux) can be modeled as capacitors between circuit nodes. Additionally, capacitors to ground integrate the net current flowing into their circuit nodes. In the general transport case in which the amount
of a quantity flowing into a VC does not necessarily equal the amount of the quantity flowing out of the node (i.e. the quantity can accumulate), a capacitor from the circuit node associated with the VC to ground allows this accumulation.

- **Nonlinear current sources provide nonlinear flow.** When resistors and capacitors do not suffice, current flow between nodes can be represented by nonlinear current sources that are functions of voltages and currents elsewhere in the circuit.

- **KCL applies conservation principles.** KCL requires that the sum of the currents flowing into a circuit node equals the sum of the currents flowing out of the circuit node. In some modes of transport (e.g. electrical) or under other special conditions (e.g. steady-state) the amount of a quantity flowing into a small volume does equal the amount of a quantity flowing in, and therefore the associated nodes in an equivalent circuit are connected to each other but not to ground. In other modes of transport (e.g. diffusive, electrodiffusive, thermal), the amount of a quantity flowing into a small volume does not equal the amount of a quantity flowing out. Rather, the difference between the currents is related to the time rate of change of the quantity in the volume. Therefore, for the net current flowing into the associated circuit node, an additional current from ground must be added to account for the accumulation of the quantity. This is accomplished by adding a capacitor to ground, which integrates the net current flowing into the node from other nodes. When the sum of the currents flowing from adjacent nodes is positive, a current equal to this sum flows onto the capacitor, charging it and increasing the node voltage. Alternatively, when the sum of the currents flowing from adjacent nodes is negative, a current equal to this sum (in absolute value) flows out of the capacitor, discharging it and decreasing the node voltage.
2.6 MTNM Transport Simulation

Abstracting transport in physical systems using equivalent circuits is a powerful conceptual tool in thinking about how a quantity moves from place to place. This is not, however, the only reason to use this abstraction. Robust computer software exists for simulating circuit networks. Thus, the numerical difficulties one ordinarily encounters in simulating nonlinear transport are handled by the circuit simulation software, which has powerful numerical routines for simulating nonlinear devices, thereby decoupling the numerical problem of simulating transport from the problem of understanding the transport mechanisms and setting up a model that adequately characterizes the transport processes.

In this thesis, simulations were performed by Berkeley SPICE 3f5, a robust, open-source circuit simulator. MATLAB 7 was used to determine the circuit elements based on the transport parameters of the physical system and the geometry of the discretization, as described above. Having determined the values of all of the resistors, capacitors, etc., the circuit networks were written as a circuit netlists readable by SPICE (one line per circuit element, each detailing its node connections and transport parameters). SPICE loaded the files, simulated the circuits, and created a binary output files. The output files were then loaded into MATLAB and the results were analyzed.
Chapter 3

Modeling Cell and Tissue

Electroporation Using the MTNM

The mesh transport network method (MTNM) is ideal for modeling the responses of cells and tissue to pulsed electric fields. This chapter describes how the fundamentals of the MTNM developed in Chp. 2 can be extended and applied to modeling these systems.

The methods presented here are an extension and generalization of the transport lattice method (TLM), which was developed by Gowrishankar and Weaver [34] to model the responses of cells and tissue (at the scale of cells) to applied electric fields. The TLM uses Cartesian grids rather than meshes to discretize systems. While the method is robust and relatively accurate, the use of structured grids, rather than unstructured meshes as in the MTNM, causes artifacts and inaccuracies at the membranes because grids cannot resolve the fine details of membranes without using a prohibitively large number of nodes. Thus, the TLM simulation results exhibit “staircasing” effects [35,36]. Additionally, the fixed grid sizes of TLM models result in suboptimal node allocations that are computationally inefficient.

MTNM models avoid these problems because the edges of the mesh triangles follow the
contours of the structures in the domain (e.g. membranes), and the variable element sizing allows the resolution of tiny structures without requiring an unnecessarily fine mesh elsewhere (e.g. in the electrolyte far from the membrane). Consequently, the MTNM has proved highly accurate in simulations that can be compared with analytical expressions, such as the transmembrane potential on a passive dielectric shell in a uniform electric field [35].

While the MTNM cell model is in some sense a refinement of the TLM cell models, the MTNM tissue model is completely new. The MTNM tissue model is a multiscale model that uses simple cell models throughout the domain to capture the cell responses to the electric field at the microscopic level and then extrapolates the macroscopic tissue transport properties and resultant response from the cell responses. Because the macroscopic tissue response determines the local electric fields experienced by the cells at the microscopic level, the macroscopic electrical behavior directly influences the cell responses. Thus, there is constant feedback between the cell and tissue scales that allows for the examination of the dynamics of the tissue response that cannot be captured by passive tissue models.

This chapter begins by describing the geometry (Sec. 3.1) and meshing (Sec. 3.2) of the cell and tissue systems. The asymptotic model of electroporation (Sec. 3.3) and its implementation in the cell equivalent circuit model (Sec. 3.4) are then discussed. The description of the calculation of the electric field on a mesh (Sec. 3.5) and description of the simple cell impedance model (Sec. 3.6) then lead into the implementation of the tissue equivalent circuit (Sec. 3.7) and the tissue system electrical parameter fitting (Sec. 3.8). Finally, the chapter closes with a discussion of the electroquasistatic approximation (Sec. 3.9) and a brief description of the software and hardware used for MTNM simulations (Sec. 3.10).
3.1 System Geometry

3.1.1 Cell system

The cell system comprises a circular cell membrane (CM) enclosing one circular large organelle membrane (LOM) and one circular small organelle membrane (SOM) and a pair of planar electrodes (Fig. 3-1). The membranes have thickness $d_m = 5\, \text{nm}$ and radii $r_{CM} = 10\, \mu\text{m}$, $r_{LOM} = 3\, \mu\text{m}$, and $r_{SOM} = 0.5\, \mu\text{m}$. The electric field, $E_{app}$, is applied by planar electrodes at $y = 50\, \mu\text{m}$ (anode) and $y = -50\, \mu\text{m}$ (cathode). Here $E_{app}$ is the voltage applied between the electrode, $V_{app}$, divided by the distance between the electrodes. The bounding box for the system is $50\, \mu\text{m} \times 50\, \mu\text{m}$, with the cell centered. The bounding box was made much larger than the cell so that boundary effects would be negligible. The LOM and SOM centers are offset from the CM center by $(-2\, \mu\text{m}, 2\, \mu\text{m})$ and $(4\, \mu\text{m}, -4\, \mu\text{m})$, respectively.

3.1.2 Tissue system

Many different tissue systems were created, each comprising two circular electrodes in a large tissue region. Most of the simulations use the primary tissue system with electrode radius $r_e = 0.25\, \text{mm}$ and electrode spacing $L_e = 10\, \text{mm}$ (Fig. 3-2), but systems were also created in which the $r_e$ were varied from $0.1\, \text{mm}$ to $2.5\, \text{mm}$ with $L_e = 10\, \text{mm}$ or in which $L_e$ were varied from $2.5\, \text{mm}$ to $40\, \text{mm}$ with $r_e = 0.25\, \text{mm}$ (Fig. 3-3). Simulations of these alternate systems illustrate the effects that the electrode configuration have on tissue electroporation. In all simulations, the nominal applied electric field, $E_{app}$, is equal to the voltage difference between the electrodes, $V_{app}$, divided by the electrode spacing, $L_e$.

The tissue systems have symmetry about $y = 0$. Thus, a no-flux boundary was placed at $y = 0$ for each system and only the region $y \geq 0$ was actually meshed and simulated. This allowed the simulations to have higher node densities without any loss of information. Therefore, a $200\, \text{mm} \times 100\, \text{mm}$ region of tissue was simulated to represent the complete
Figure 3-1: Cell system. (a) The cell is centered in a 50 $\mu$m x 50 $\mu$m region. The upper (anode) and lower (cathode) boundaries are planar electrodes. (b) The radii of the cell membrane (CM), large organelle membrane (LOM), and small organelle membrane (SOM) are $r_{CM} = 10 \mu m$, $r_{LOM} = 3 \mu m$, and $r_{SOM} = 0.5 \mu m$. 
Figure 3-2: Tissue system. (a) The tissue system contains two circular electrodes in a 200 mm × 200 mm tissue region. (b) The electrodes have radii $r_e$ and separation $L_e$ (edge-to-edge). In the primary tissue simulations, $r_e = 0.25$ mm and $L_e = 10$ mm.
response of a 200 mm × 200 mm region of tissue.

The large bounding box was chosen to ensure that artifacts did not arise from the boundaries being too close to the electrodes and electroporating tissue, particularly in cases of pulses with large magnitude and long duration. Moreover, the computational expense of increasing the size of the bounding box is quite small because the triangles in the mesh grow linearly in size with distance from the electrodes.

### 3.2 Mesh Generation

The mesh generation algorithm used to allocate nodes in the cell and tissue systems is based on one developed by Persson and Strang [37]. The advantage of this algorithm is
that it is simple and easily modifiable, which cannot be said of most other “black box” meshing programs, such as that provided with the MATLAB PDE Toolbox.

The meshing algorithm treats the triangle edges and nodes analogously to struts and joints in a truss structure. The triangle edges are determined from the node positions using the Delaunay triangulation algorithm. The edge length function, $h_n(d_n, s_n)$, determines the desired edge length as a function of distance from the closest membrane (or electrode), $d_n$, and the distance between the nodes at the closest membrane (or electrode) location, $s_n$. The force between the nodes, $f_n(l_n, l_{n0})$, is then calculated as a function of the desired length, $l_{n0}$, and the actual length, $l_n$. The function

$$f_n(l_n, l_{n0}) = \begin{cases} 1 - \left(\frac{l_n}{l_{n0}}\right)^2 & \text{if } l_n < l_{n0}, \\ 0 & \text{if } l_n \geq l_{n0}. \end{cases}$$

(3.1)

was found to produce good results and used here.

The net forces acting on the nodes determine their displacements. The newly-displaced nodes are then re-triangulated and the forces between them recalculated. The loop continues until the displacements of the nodes at each step become small, indicating that the mesh has reached steady-state.

The MTNM requires that edges lie along changes in material (Sec. 2.4). An edge between an extracellular node and intracellular node, for example, is not permitted. Rather, the connections between the spaces must go through intermediate nodes, in this case extracellular node $\rightarrow$ extracellular-membrane interface node $\rightarrow$ membrane-intracellular interface node $\rightarrow$ intracellular node. Similarly, in the tissue model, nodes in the tissue can only be directly connected to nodes on the surface of the electrodes and not to nodes in the interior of the electrodes. The mesh boundary requirement is imposed during mesh gen-
eration by enforcing a buffer zone around the material interfaces that moving nodes may not enter. Nodes that are displaced into the buffer region are automatically pushed out of the buffer in the normal direction. The buffer region thickness on each side of the interface is approximately the same as the fixed node spacing on the interface. Any potential edge crossing an interface would be about twice as long as the alternate potential edge connecting the interface nodes, which is highly unfavorable in the triangulation algorithm, and therefore, the triangulation algorithm never creates edges that cross the interface.

During the generation of the cell system mesh, nodes are fixed along the centers of the membranes in the cell system. Following mesh generation, these nodes are replaced by nodes on the intracellular and extracellular boundaries of the membrane. During the generation of the tissue system mesh, nodes are fixed along the electrode boundaries.

Each triangulation generated has an associated set of Voronoi cells (VCs), as described in Sec. 2.2. The VCs contain the regions of physical space represented by circuit nodes in computational space. The transport between circuit nodes in computational space are equal to the transport between VCs in physical space. The local material properties and geometry of the triangles and VCs determine the transport parameters of the mesh as described previously.

### 3.2.1 Cell system mesh

Figure 3-4 shows the mesh and associated VCs generated for the cell system at four scales. The mesh has 14930 nodes, 29806 triangles, 44691 edges, and 80 nm membrane node spacing. The CM, LOM, and SOM have 786, 236, and 40 membrane node pairs, respectively. The current implementation of the meshing algorithm requires that the node spacing for all membranes be equal. In this system, an adequate description of the SOM requires that the membrane node spacing be very fine. Consequently, the number
of nodes on the CM is excessive, resulting in many more total membrane nodes and total system nodes than would be required if the membrane node spacings were independent. For future models of cells with more complicated internal structure the meshing algorithm will be modified to allow node spacings to be independently specified for each membrane.

### 3.2.2 Tissue system mesh

Figure 3-5 shows the mesh and associated VCs generated for the primary tissue system at three scales. The tissue mesh has 2713 nodes, 5228 triangles, 7940 edges, and 65.2 µm electrode node spacing. The electrodes (in the half-system simulated) have 13 surface nodes.

The meshes for the alternate electrode configuration systems (Fig. 3-3) are not shown, but they are similar to the mesh of the primary tissue system (Fig. 3-2).

### 3.2.3 Mesh quality

The quality factor, $Q$, of a triangular mesh element is commonly evaluated as the ratio of twice the radius of the largest inscribed circle to the radius of the smallest circumscribed circle [37]. $Q = 1$ for an equilateral triangle and $Q = 0$ for three collinear points. For reference, a right isosceles triangle has $Q = 0.838$. Figure 3-6 shows the triangle quality histograms for the cell and tissue systems.

In the cell system, 86.1% of the triangles have $Q > 0.90$ and 91.9% of the triangles have $Q > 0.80$, and in the tissue system, 97.6% of the triangles have $Q > 0.90$ and 99.8% of the triangles have $Q > 0.80$.

The cell system mesh has a collection of triangles with $Q \approx 0.12$ that are associated with
Modeling Cell and Tissue Electroporation Using the MTNM

(a) Mesh
3.2 Mesh Generation

(b) Voronoi cells

Figure 3-4: Cell system mesh and Voronoi cells. The cell system (a) mesh and (b) Voronoi cells are shown at four scales. The mesh has 14930 nodes, 29806 triangles, 44691 edges, and 80 nm membrane node spacing.
Figure 3-5: Tissue system mesh and Voronoi cells. The tissue system (a) mesh and (b) Voronoi cells are shown at three scales. The mesh has 2713 nodes, 5228 triangles, 7940 edges, and 65.2 µm electrode node spacing.
the membrane triangles (Fig. 3-6a). These triangles are very acute because the node spacing is much greater than the membrane thickness, which results in a low $Q$. The results of simulations are not adversely affected, however. The VC segments that intersect the hypotenuses of the membrane triangles are extremely short relative to the lengths of the hypotenuses. Consequently, these edges contribute negligibly to transmembrane transport. In fact, the results of cell system simulations in which these segments were left out were indistinguishable from simulations in which they were present.

### 3.3 Electroporation Model

#### 3.3.1 Asymptotic model of electroporation

The dynamics of electroporation are described by the Smoluchowski equation:

$$\frac{\partial n}{\partial t} = D_p \frac{\partial}{\partial r} \left( \frac{\partial n}{\partial r} + \frac{n}{kT} \frac{\partial W_{HI}}{\partial r} \right) + S(r), \quad (3.2)$$

where $n(r,t)$ is the pore density distribution function, $D_p$ is the diffusion coefficient in pore radius space, $k$ is the Boltzmann constant, $T$ is the absolute temperature, $W_{HI}$ is the...
3.3 Electroporation Model

formation energy for a hydrophilic pore, and $S(r)$ is the pore source term that describes the transition between hydrophobic and hydrophilic pores [32, 38, 39].

Neu and Krassowska developed an asymptotic model of electroporation that reduces the Smoluchowski equation to the ordinary differential equation [32]

$$\frac{dN(t)}{dt} = \alpha e^{q(V_m)} \left(1 - \frac{N(t)}{N_{\text{eq}}(V_m)}\right),$$  \hspace{1cm} (3.3)

where $N$ is the local pore density

$$N(t) \equiv \int_0^\infty n(r, t) \, dr,$$  \hspace{1cm} (3.4)

$\alpha$ is the pore creation rate coefficient, $V_m$ is the transmembrane potential, $V_{ep}$ is the characteristic voltage of electroporation, and $N_{\text{eq}}(V_m)$ is the equilibrium pore density at $V_m$:

$$N_{\text{eq}}(V_m) = N_0 e^{q(V_m)^2}.$$  \hspace{1cm} (3.5)

Here $N_0$ is the equilibrium pore density for $V_m = 0$ V and $q$ is the electroporation coefficient $q = \left(\frac{r_m}{r_*}\right)^2$, where $r_m$ is the most energetically favorable hydrophilic pore size at $V_m = 0$ V and $r_*$ is the pore radius above which the hydrophilic pore conformation is more energetically favorable than the hydrophobic pore conformation. Table 3.1 lists the electroporation parameters used in the cell and tissue electroporation models.

The primary simplification of the asymptotic electroporation model in its original form is that pores are assumed not to expand. This assumption is quite reasonable for strong fields of short duration but less so for intermediate to small fields of long duration. Two competing processes, pore creation and pore expansion, contribute to increased membrane conductance and associated maintenance of a transmembrane potential of $\sim 1$ V [38, 40]. Pore creation proceeds much more rapidly (insofar as it increases mem-
brane conductance) than pore expansion. As such, pore creation dominates pore expansion when the applied electric field is very large while pore expansion is at least commensurate with pore creation when the applied electric field is smaller [41]. Despite the details of the pore population being a function of the applied field, the electrical predictions of the model are quite robust. That is, whether pore creation or expansion dominates, the processes proceed toward a state in which the transmembrane potential drops from its peak to $\sim 1$ V. Pore expansion will become more important in future models that simulate molecular uptake, which, unlike the electrical consequences of applied fields, depends strongly on the details of the pore population.

### 3.3.2 Pore conductance

A pore with conductivity $\sigma_p$, radius $r_m$, and thickness $d_m$ has conductance

$$G_p(V_m) = \sigma_p \frac{\pi r_m^2}{d_m} \left( \frac{e^{\nu_m} - 1}{w_0 e^{w_0 \eta \nu_m + \eta \rho_{m}} - w_0 e^{w_0 \eta \nu_m + \eta \rho_{m}}} \right), \quad (3.6)$$

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>$1 \times 10^9 \text{ m}^{-2} \text{s}^{-1}$</td>
<td>pore creation rate coefficient</td>
</tr>
<tr>
<td>$V_{ep}$</td>
<td>0.258 V</td>
<td>characteristic voltage of electroporation</td>
</tr>
<tr>
<td>$q$</td>
<td>2.46</td>
<td>electroporation coefficient</td>
</tr>
<tr>
<td>$N_0$</td>
<td>$1.5 \times 10^9 \text{ m}^{-2}$</td>
<td>equilibrium pore density at $V_m = 0$ V</td>
</tr>
<tr>
<td>$r_m$</td>
<td>0.80 nm</td>
<td>minimum energy pore radius at $V_m = 0$ V</td>
</tr>
<tr>
<td>$\sigma_p$</td>
<td>1.2 S m$^{-1}$</td>
<td>conductivity in pore</td>
</tr>
<tr>
<td>$d_m$</td>
<td>5 nm</td>
<td>membrane thickness</td>
</tr>
<tr>
<td>$w_0$</td>
<td>2.65</td>
<td>pore energy barrier</td>
</tr>
<tr>
<td>$\eta$</td>
<td>0.15</td>
<td>pore relative entrance length</td>
</tr>
<tr>
<td>$T$</td>
<td>300 K</td>
<td>temperature</td>
</tr>
</tbody>
</table>

Table 3.1: Electroporation parameters (from DeBruin and Krassowska [38]).
where \( w_0 \) is the energy barrier inside a pore, \( \eta \) is the relative entrance length of a pore, and \( \nu_m \) is the dimensionless transmembrane potential \( \nu_m \equiv \frac{V_m q_e}{kT} \) [42]. Here \( q_e \) is the charge of a monovalent ion. Thus, the current \( i_p \) through pores in a small region of membrane with area \( A_m \) and pore density \( N \) is

\[
i_p(t) = G_p(V_m) N(t) A_m V_m(t).
\]

(3.7)

The following sections describe how this local current through pores is combined with the other electrical properties of the cell and tissue systems to create the complete MTNM models of these systems.

### 3.4 Cell Equivalent Circuit

Figure 3-7 shows the circuit components and their expressions for each pair of adjacent nodes \( j \) and \( k \) in the cell system equivalent circuit. Most of the nodes lie within electrolyte, and the transport between these nodes is simply described by the electrolyte resistance, \( R_{el}^{j,k} \), and capacitance, \( C_{el}^{j,k} \) associated with the transport between the Voronoi cells corresponding to the circuit nodes. \( R_{el}^{j,k} \) and \( C_{el}^{j,k} \) are calculated as described in Chp. 2, and are determined by the electrolyte conductivity, \( \sigma_{el} \), and permittivity, \( \epsilon_{el} \), and the distance, \( l_{j,k} \), between nodes \( j \) and \( k \) and the width, \( w_{j,k} \) and depth, \( d \), of their shared VC interface.

The equivalent subcircuit describing transmembrane electrical transport is somewhat more complicated than the subcircuit describing transport in the electrolyte because of the highly nonlinear change in membrane conductance with electroporation (Fig. 3-7). The passive membrane resistance, \( R_{m}^{j,k} \), and capacitance, \( C_{m}^{j,k} \) have conductivity, \( \sigma_m \), and permittivity, \( \epsilon_m \), and the same length parameters as the electrolyte, \( l_{j,k} \), \( w_{j,k} \), and \( d \). In this case, \( l_{j,k} = d_m \). The current through pores, \( i_{p}^{j,k}(t) \), is determined by the conductance
Figure 3-7: Cell system equivalent circuit. An electrolyte or membrane subcircuit is placed between each pair of adjacent nodes in the cell system equivalent circuit. The electrical transport is determined by the local mesh geometry, passive electrical properties, and, across the membrane, by the pore density and conductance. Each membrane subcircuit has an associated pore density subcircuit that is used to calculate the total current through pores.
per pore,\( G_{p}^{j,k}(V_{m}^{j,k}(t)) \), pore density, \( N_{j,k}(t) \), transmembrane potential, \( V_{m}^{j,k}(t) \), and local membrane area,\( w_{j,k} \). \( G_{p}^{j,k}(V_{m}^{j,k}(t)) \) is simply calculated by Eq. 3.6, but \( N_{j,k}(t) \) must be calculated by solving Eq. 3.3. This integration is performed by a small subcircuit with a capacitor, \( C_{N}^{j,k} \), and a current source, \( i_{N}^{j,k}(t) \) that is a function of \( N_{j,k}(t) \) and \( V_{m}^{j,k}(t) \) (Fig. 3-7). The constitutive relation for the capacitor relates its voltage, \( N_{j,k}(t) \), to its current, \( i_{N}^{j,k} \) by

\[
\frac{dN_{j,k}(t)}{dt} = \frac{i_{N}^{j,k}(t)}{C_{N}^{j,k}},
\]

where the expressions for \( C_{N}^{j,k} \) and \( i_{N}^{j,k}(t) \) are as shown in Fig. 3-7. This is the differential equation governing pore creation in Eq. 3.3. Therefore the subcircuit solves Eq. 3.3:

\[
N_{j,k}(t) = \frac{1}{C_{N}^{j,k}} \int_{t_0}^{t} i_{N}^{j,k}(\tau) \, d\tau + N_{j,k}(t_0)
\]

\[
= \alpha \int_{t_0}^{t} e^{\left(\frac{V_{j,k}(\tau)}{V_{ep}}\right)^2} \left(1 - \frac{N_{j,k}(\tau)}{N_0} e^{-q\left(\frac{V_{j,k}(\tau)}{V_{ep}}\right)^2}\right) d\tau + N_{j,k}(t_0).
\]

The initial condition at time \( t_0 \) is satisfied by placing the initial pore density, \( N_{j,k}(t_0) \), on \( C_{N}^{j,k} \) at the start of the simulation:

\[
N_{j,k}(t_0) = N_{0,k}^{j,k} e^{q\left(\frac{V_{j,k}^{\text{rest}}}{V_{ep}}\right)^2},
\]

where \( V_{j,k}^{\text{rest}} \) is the resting potential of the membrane between nodes \( j \) and \( k \).
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_{el}$</td>
<td>1.2 S m$^{-1}$</td>
<td>electrolyte conductivity</td>
</tr>
<tr>
<td>$\sigma_m$</td>
<td>$9.5 \times 10^{-9}$ S m$^{-1}$</td>
<td>membrane conductivity</td>
</tr>
<tr>
<td>$\epsilon_{el}$</td>
<td>$80\epsilon_0 = 7.08 \times 10^{-10}$ F m$^{-1}$</td>
<td>electrolyte permittivity</td>
</tr>
<tr>
<td>$\epsilon_m$</td>
<td>$5\epsilon_0 = 4.43 \times 10^{-11}$ F m$^{-1}$</td>
<td>membrane permittivity</td>
</tr>
<tr>
<td>$V_{rest_{CM}}$</td>
<td>$-86$ mV</td>
<td>cell resting potential</td>
</tr>
<tr>
<td>$V_{rest_{LOM}}$</td>
<td>0 mV</td>
<td>large organelle resting potential</td>
</tr>
<tr>
<td>$V_{rest_{SOM}}$</td>
<td>$-174$ mV</td>
<td>small organelle resting potential</td>
</tr>
</tbody>
</table>

Table 3.2: Cell system electrical parameters ($\sigma$ and $\epsilon$ from Ref. [39]).

used here, in which the current source $I_{rest}^{j,k} = V_{rest}^{j,k} \left( \frac{1}{R_{m}^{j,k}} + G_{p}^{j,k} (V_{rest}^{j,k}) N_{eq}^{j,k} (V_{rest}^{j,k}) w_{j,k} d \right)$ was placed in parallel with the total membrane resistance, was found to result in faster simulation times.

Table 3.2 lists all of the electrical parameters used in the cell system model.

### 3.5 Electric Field Calculation on Mesh

The multiscale nature of the tissue system model requires that the electric field magnitude be calculated during the simulation. This field is then used to determine the voltage drop across the cell models throughout the domain. The electric field magnitude is not needed in the cell system model and is not calculated.

The electric field is calculated at each node in the tissue system using a linear least-squares method. As such, the mesh is expected to be sufficiently fine to consider the electric potential to vary linearly near each node.

Calculation of the electric field in two dimensions requires that the potential be known
3.5 Electric Field Calculation on Mesh

at three positions. These positions are the node at which the field is being calculated and the adjacent nodes that do not lie strictly inside an electrode, where the electric field is zero. The electric field calculation is always well-determined because the minimum number of adjacent nodes is two, which can occur in a corner of the domain. More generally, the calculation is over-determined and the electric field is calculated by a linear least-squares method for which there are more than the minimum of three potentials needed to define the field.

Consider node $j$ with $M_j$ adjacent nodes $k_1, k_2, \ldots, k_{M_j}$. The positions and potentials of the adjacent nodes $k_i$ relative to the central node $j$ are related to the gradient in potential by

$$
\begin{bmatrix}
(\Delta x)_{j, k_1} & (\Delta y)_{j, k_1} \\
\vdots & \vdots \\
(\Delta x)_{j, k_{M_j}} & (\Delta y)_{j, k_{M_j}}
\end{bmatrix}
\begin{bmatrix}
\frac{\partial \phi}{\partial x} \\
\frac{\partial \phi}{\partial y}
\end{bmatrix}_j =
\begin{bmatrix}
(\Delta \phi)_{j, k_1} \\
\vdots \\
(\Delta \phi)_{j, k_{M_j}}
\end{bmatrix},
$$

(3.13)

where $(\Delta x)_{j, k_i} \equiv x_{k_i} - x_j$, $(\Delta y)_{j, k_i} \equiv y_{k_i} - y_j$, and $(\Delta \phi)_{j, k_i} \equiv \phi_{k_i} - \phi_j$.

Rewriting Eq. 3.13 compactly,

$$
P_j g_j = v_j.
$$

(3.14)

The linear least-squares solution is found by multiplying by $P_j^T$ from the left and solving for $g_j$ [43]:

$$
P_j^T P_j g_j = P_j^T v_j
$$

(3.15)

$$
g_j = (P_j^T P_j)^{-1} P_j^T v_j
$$

(3.16)

$$
g_j = S_j v_j,
$$

(3.17)

where $S_j \equiv (P_j^T P_j)^{-1} P_j^T$ is a $2 \times M_j$ array that scales the $(\Delta \phi)_{j, k_i}$ to yield $\left(\frac{\partial \phi}{\partial x}\right)_j$ and
Here \( s_{x,j,k_1}, \ldots, s_{x,j,k_{M_j}} \) scale the relative potentials to determine the \( x \)-component of the electric field and \( s_{y,j,k_1}, \ldots, s_{y,j,k_{M_j}} \) scale the relative potentials to determine the \( y \)-component of the electrical field \( \vec{E}_j = -\hat{x} \left( \frac{\partial \phi}{\partial x} \right)_j - \hat{y} \left( \frac{\partial \phi}{\partial y} \right)_j \).

For the purposes of the model, only the magnitude of the electric field, \( E_j = |\vec{E}_j| \), is required, though the \( x \) and \( y \) components must be calculated to determine the magnitude. Thus,

\[
E_j = \sqrt{(S_{x,j} \cdot v_j)^2 + (S_{y,j} \cdot v_j)^2},
\]

where \( S_{x,j} \) is the \( x \) row of \( S_j \), \( S_{y,j} \) is the \( y \) row of \( S_j \), and the “\( \cdot \)” operator represents a scalar product.

## 3.6 Tissue Model Cell Unit

Because the scale of the system is several orders of magnitude larger than the scale of the cell, individual cells cannot be resolved by the mesh. Assuming cells have an average size of approximately 20 \( \mu \m \times 20 \mu \m \), the 200 \( \m \times 100 \m \) domain used for the simulations would contain \( \sim 10^8 \) cells! Clearly, the features of individual cells cannot by captured by the mesh. However, representative single cell models can be placed at each node in the domain and the bulk transport properties of the tissue can be extrapolated from the single cell models. Thus, the changes to single cells throughout the domain can be captured.

The single cell models distributed throughout the domain must be relatively simple for
Figure 3-8: Tissue model cell unit. The cell unit comprises extracellular, membrane, and intracellular regions in series and parallel shunt region. The spatial and electrical parameters of the regions are labeled.

the simulation time to be reasonable and for the local electrical properties of the tissue to be extracted from them, but the model must capture the essential features of cell and tissue electroporation to be of value. From an electrical standpoint, tissue may be thought of as an organized system of membrane and electrolyte, and the fundamental unit of tissue, the cell, may be considered a region of electrolyte enclosed by membrane and surrounded by electrolyte. Consequently, the most reasonable simple cell model is a cubic region of electrolyte surrounded by a membrane surrounded by yet more electrolyte. This model is shown Fig. 3-8.

The membrane and each region of electrolyte has an associated conductivity $\sigma$ and permittivity $\epsilon$. Additionally, each electrolyte region has a tortuosity $\nu$ to account for the
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Value</th>
<th>Description</th>
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<tr>
<td>$l_u$</td>
<td>21.7 µm</td>
<td>tissue unit length</td>
</tr>
<tr>
<td>$l_c$</td>
<td>20 µm</td>
<td>cell length</td>
</tr>
<tr>
<td>$w_u$</td>
<td>21.7 µm</td>
<td>tissue unit width</td>
</tr>
<tr>
<td>$w_c$</td>
<td>20 µm</td>
<td>cell width</td>
</tr>
<tr>
<td>$d_m$</td>
<td>5 nm</td>
<td>membrane thickness</td>
</tr>
<tr>
<td>$d$</td>
<td>1 m</td>
<td>system depth</td>
</tr>
<tr>
<td>$\sigma_e$</td>
<td>1.2 S m$^{-1}$</td>
<td>extracellular conductivity</td>
</tr>
<tr>
<td>$\sigma_i$</td>
<td>1.2 S m$^{-1}$</td>
<td>intracellular conductivity</td>
</tr>
<tr>
<td>$\sigma_s$</td>
<td>1.2 S m$^{-1}$</td>
<td>shunt conductivity</td>
</tr>
<tr>
<td>$\sigma_m$</td>
<td>9.5 × 10$^{-9}$ S m$^{-1}$</td>
<td>membrane conductivity</td>
</tr>
<tr>
<td>$\epsilon_e$</td>
<td>$80\epsilon_0 = 7.08 \times 10^{-10}$ F m$^{-1}$</td>
<td>extracellular permittivity</td>
</tr>
<tr>
<td>$\epsilon_i$</td>
<td>$80\epsilon_0 = 7.08 \times 10^{-10}$ F m$^{-1}$</td>
<td>intracellular permittivity</td>
</tr>
<tr>
<td>$\epsilon_s$</td>
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</tr>
<tr>
<td>$\nu_e$</td>
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</tr>
<tr>
<td>$\nu_i$</td>
<td>17.3</td>
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<tr>
<td>$\nu_s$</td>
<td>8.64</td>
<td>shunt tortuosity</td>
</tr>
</tbody>
</table>

Table 3.3: Tissue system spatial and electrical parameters ($\sigma$ and $\epsilon$ from Ref. [39]).

The structural complexity of tissue not captured by the cell model (see Sec. 3.8). These quantities are shown in Fig. 3-8 with subscripts $m$ for membrane, $i$ for intracellular, $e$ for extracellular (non-shunt), and $s$ for shunt. The relative sizes of the intracellular and extracellular spaces were chosen such that 15% of the total volume was extracellular and 85% was intracellular. The cell length, $l_c$, and width, $w_c$, were set at 20 µm, and the cell unit length, $l_u$, and width, $w_u$, were therefore set to 21.7 µm. The arbitrary system depth was set to 1 m. The values of the electrical and spatial parameters are listed in Tab. 3.3.

The impedance of tissue is essentially the same as that of a cell unit (cell plus elec-
trolleyte) within the tissue. Consider the region of tissue between two nodes. The tissue is assumed to be uniform and the cell unit is assumed to reasonably represent impedance on a microscopic scale. Thus, the tissue is just a grid of the cell units described above. Moreover, the impedance of a tissue region with the same relative dimensions as the cell unit is identical to the impedance of the cell unit. The impedance of a tissue region of different relative dimensions from the cell unit is the same as that of the cell unit scaled to account for the differing relative dimensions.

Consider a few simple cases. Assume that a voltage $V_u$ is placed across the cell unit and results in a current $I_u$. Then, the impedance of the cell unit is $Z_u = \frac{V_u}{I_u}$. Now, imagine that this cell is actually surrounded by other cells and the cells are in a uniform electric field. Then, the voltage drop across the cell and a neighbor in series with it is $2V_u$, but the current through the cell units, $I_u$, is unchanged because the voltage drop per cell unit is unchanged. Thus, the impedance of the two cells in series is $2Z_u$. By similar reasoning, the impedance of two cell units in parallel is $\frac{1}{2}Z_u$. A $2 \times 2$ grid of cell units would have impedance $Z_u$, as would a grid of $100 \times 100$ cell units.

Now consider a rectangular tissue region of arbitrary dimensions $l_t \times w_t$ filled with cell units with fixed dimensions $l_u \times w_u$ (both have the same depth $d$). There are $\frac{l_t}{l_u}$ rows of cells and $\frac{w_t}{w_u}$ columns of cells, so the impedance of the tissue is $\left(\frac{l_t}{l_u}/\frac{w_t}{w_u}\right)Z_u$. Using the values in Tab. 3.3, $l_u = w_u$, and therefore the impedance of a region of tissue with dimensions $l_t \times w_t$ is $\frac{l_t}{w_t}Z_u$, where $Z_u$ is the impedance of a single cell unit in that region.

While the cell model used is a rectangular prism, the prism has no particular orientation relative to the system (e.g. electrodes). Rather, the prism is always oriented with respect to the field. That is, the voltage drop across the cell unit is a function of the electric field magnitude but not the electric field direction. Thus, the model implicitly assumes that the tissue conductance is independent of direction, which would not be the case for
muscle, for example, and the increased tissue conductivity associated with electroporation is a scalar (independent of electric field direction), not a tensor (dependent on electric field direction). In reality, there likely is some dependence of the electroporation and impedance on direction. Accounting for this dependence would require multiple cell models per node, each with a different permanent orientation relative to the system. The conductance in a particular direction would then be determined by weighting the models based on their orientation relative to the electric field.

### 3.7 Tissue Equivalent Circuit

Figure 3-9 shows the circuit components and their expressions for each pair of adjacent nodes $j$ and $k$ in the tissue system equivalent circuit. The circuit can be divided into the subcircuits that describe the microscopic tissue response (Fig. 3-9a) and the subcircuits that describe the macroscopic tissue response (Fig. 3-9b). The macroscopic subcircuits determine the transport between the spatially distributed nodes in the system, while the microscopic subcircuits determine the macroscopic transport properties.

Each node in the tissue system has a simple cell model associated with it. This simple cell model, or cell unit, is shown in Fig. 3-8 and has the equivalent circuit shown in Fig. 3-9a. The passive components of the circuit are calculated as functions of the conductivity $\sigma$, permittivity $\epsilon$, tortuosity $\nu$, and geometry of the region with which they are associated. These electrical and spatial parameters are listed in Tab. 3.3. Note that only one membrane is explicitly represented in the circuit (Fig. 3-9a). Rather than use two membranes, one membrane was used with the resistance doubled, capacitance halved, and current through pores halved. It is not true in general that sets of parallel components placed in series can be combined in this manner, but it is true in the special case of the parallel components being identical. Here, of course, the electrical properties of the two membranes are assumed identical.
3.7 Tissue Equivalent Circuit

The current through pores, \( i_p^j(t) \), is determined by the conductance per pore, \( G_p^j(V_m^j(t)) \), local pore density, \( N_j(t) \), transmembrane potential, \( V_m^j(t) \), and local membrane area, \( w_c d \). \( G_p^j(V_m^j(t)) \) is simply calculated by Eq. 3.6, but \( N_j(t) \) must be calculated by integrating Eq. 3.3. This integration is performed by a small subcircuit with a capacitor, \( C_j^N \), and a current source, \( i_N^j(t) \) that is a function of \( N_j(t) \) and \( V_m^j(t) \) (Fig. 3-9a), as described further in Sec. 3.4. Note that the quantities here are associated only with node \( j \), in contrast to the quantities in the cell circuit, which were associated with adjacent nodes \( j \) and \( k \).

The voltage across the simple cell, \( V_u^j(t) \), is a function of the local electric field magnitude, \( E_j(t) \). \( E_j \) couples the microscopic electrical behavior to the macroscopic electrical behavior. Because the electric field magnitude and tissue properties are assumed approximately uniform in any small region of tissue, the voltage across a single cell unit in the small region is \( V_u^j(t) = E_j(t) l_u \), where \( l_u \) is the length of the single cell unit. \( E_j(t) \) is calculated by scaling the relative potentials of the adjacent nodes \( k_1, k_2, \ldots, k_{M_j} \) by the constants \( s_{x,j,k_1}, s_{x,j,k_2}, \ldots, s_{x,j,k_{M_j}} \) and \( s_{y,j,k_1}, s_{y,j,k_2}, \ldots, s_{y,j,k_{M_j}} \) and then evaluating the square root of the squares of the \( x \)-and \( y \)-components of the electric field, as described in Sec. 3.5.

The primary electrical nodes throughout the system are connected by the circuit shown in Fig. 3-9b. This circuit is nearly identical to the cell unit circuit shown in Fig. 3-9a because the tissue impedance is electrically equivalent to the impedance of the cell unit, as discussed in Sec. 3.6. The circuit resistors are scaled by \( \frac{L_{j,k}}{w_{j,k}} \) and the capacitors by \( \frac{w_{j,k}}{L_{j,k}} \) to account for the tissue’s relative dimensions being different from the cell unit’s.

The macroscopic model does not have an explicit electroporation model. Rather the information about electroporation and membrane conductance are provided by the mi-
(a) Microscopic equivalent circuit

\[ V_{u}^{j}(t) = E_{j}(t) l_{u} \]

\[ R_{e} = \left( \frac{\nu_{e}}{\sigma_{e}} \right) \left( \frac{l_{u} - l_{c}}{w_{c} d} \right) \]

\[ C_{e} = \epsilon_{e} \left( \frac{w_{c} d}{l_{u} - l_{c}} \right) \]

\[ R_{m} = \left( \frac{2}{\sigma_{m}} \right) \left( \frac{d_{m}}{w_{c} d} \right) \quad R_{s} = \left( \frac{\nu_{s}}{\sigma_{s}} \right) \left( \frac{l_{u}}{(w_{u} - w_{c}) d} \right) \]

\[ C_{m} = \left( \frac{\epsilon_{m}}{2} \right) \left( \frac{w_{c} d}{d_{m}} \right) \quad C_{s} = \epsilon_{s} \left( \frac{(w_{u} - w_{c}) d}{l_{u}} \right) \]

\[ i_{p}^{j}(t) = G_{p}^{j}(V_{m}^{j}(t)) N_{j}(t) V_{m}^{j}(t) w_{c} d \]

\[ R_{i} = \left( \frac{\nu_{i}}{\sigma_{i}} \right) \left( \frac{l_{c}}{w_{c} d} \right) \]

\[ C_{i} = \epsilon_{i} \left( \frac{w_{c} d}{l_{c}} \right) \]

\[ C_{N} = \frac{1}{\alpha} \]

\[ i_{N}^{j}(t) = e^{\left( \frac{V_{m}^{j}(t)}{V_{cp}} \right)^{2}} \left( 1 - \frac{N_{j}(t)}{N_{0}} e^{-q \left( \frac{V_{m}^{j}(t)}{V_{cp}} \right)^{2}} \right) \]

\[ E_{j}(t) = \sqrt{\left( \sum_{i=1}^{M_{j}} s_{x,j,k_{i}} (\Delta \phi(t))_{j,k_{i}} \right)^{2} + \left( \sum_{i=1}^{M_{j}} s_{y,j,k_{i}} (\Delta \phi(t))_{j,k_{i}} \right)^{2}} \]
Figure 3-9: Tissue system equivalent circuit. (a) Microscopic equivalent circuit. The cell unit subcircuit associated with each node determines the local pore density and pore conductance. The voltage across the cell unit is calculated from the local electric field magnitude, which is determined from the macroscopic circuit. (b) Macroscopic equivalent circuit. The electrical transport in the tissue unit subcircuit is determined by the mesh geometry, passive electrical properties, and current through pores, which is calculated from the pore density and pore conductance of the microscopic circuit.
crosscopic model. The membrane conductance for transport between nodes $j$ and $k$ is taken to be the average membrane conductance of the cell unit models at $j$ and $k$. That is,

$$G_{p}^{j,k}(t) = \frac{1}{2} \left( G_{p}^{j} \left( V_{m}^{j}(t) \right) + G_{p}^{k} \left( V_{m}^{k}(t) \right) \right). \quad (3.20)$$

This is analogous to the approximation described in Chp. 2 in which the concentration at the interface of two VCs was taken to be the average of the concentrations at the nodes associated with the VCs.

The key to the tissue model is the manner in which the microscopic and macroscopic models are coupled. The macroscopic model determines the electric field, which in turn determines the electric field magnitude and voltage drop across each microscopic cell model. The changes in membrane conductance that accompany electroporation of the microscopic cell model then determine the tissue impedance at the macroscopic scale. The changes in tissue impedance alter the electric field, which in turn changes the electrical environments of the microscopic cell modes, et cetera. This coupling allows the dynamic characteristics of tissue electroporation to be captured.

### 3.8 Tuning Cell Unit Parameters

The effective conductivity of the intracellular and extracellular spaces is significantly less than the conductivity of electrolyte because of the many structures (organelles, cytoskeletal fibers, extracellular matrix, etc.) in and around cells. Such structures cannot be directly modeled by the simple cell model used here. Thus, the effect of the spatial complexity of real tissue is described by the tortuosities of these spaces, which scale the conductivities in the calculations of resistances in the circuit. These tortuosities represent free parameters. As such, they were selected to make the impedance of the tissue model as close as possible to the impedances of real tissues.
Figure 3-10: Tissue impedance. The passive tissue impedance of the tissue model is compared with experimental measurements of the impedances of several tissues over a broad range of frequencies. The model follows the major trends of the real tissue impedances.

Figure 3-10 shows the impedance of common tissues and the impedance of the cell unit. The tissue in this model is not meant to represent any particular tissue. Rather, it is meant to capture the main electrical properties of generic tissue. Therefore, the tortuosities were chosen such that the model impedance followed the trends of the experimentally measured tissue impedances [44]. With extracellular, intracellular, and shunt tortuosities \( \nu_e = 8.64 \), \( \nu_i = 17.3 \), and \( \nu_s = 8.64 \), the model closely reproduces the passive impedance of tissues over a wide range of frequencies (Fig. 3-10).

The tissue impedance has two frequency breakpoints (Fig. 3-10). The first occurs at \( \sim 10 \) kHz. As the frequency is increased beyond \( \sim 10 \) kHz, the electric field begins to penetrate the membrane, decreasing the total impedance. The second breakpoint occurs at \( \sim 10 \) MHz, beyond which displacement currents dominate conduction currents, and
the impedance decreases with the increasing frequency.

### 3.9 Electroquasistatic Approximation Justification

The models fundamentally assume that the signal propagation times in the system are sufficiently short as to be insignificant. That is, the electroquasistatic (EQS) approximation is assumed reasonable. Given the short time scales of the applied pulses and relatively large size of the systems relative to the wavelength of light, this assumption requires justification, particularly for the tissue system. Given the much smaller scale of the cell system compared to the tissue system, EQS is considered reasonable for the cell system for pulses of similar, or even moderately higher, frequency content to those shown appropriate in the tissue system.

The EQS approximation is valid when propagation effects may be ignored:

\[
\frac{2\pi f L_{\text{char}}}{c \sqrt{\epsilon_{\text{el}}}} \ll 1, \tag{3.21}
\]

where \( f \) is frequency, \( L_{\text{char}} \) is the characteristic length of this system, and \( \frac{c}{\sqrt{\epsilon_{\text{el}}}} \) is the speed of light in electrolyte with dielectric constant \( \epsilon_{\text{el}} \) [33]. Here \( L_{\text{char}} \) the electrode spacing \( L_e \), which is 10 mm. Solving Eq. 3.21 for \( f \),

\[
f \ll \frac{\epsilon}{2\pi L_e} \approx 500 \text{ MHz}. \tag{3.22}
\]

Therefore, a reasonable cutoff frequency is \( f_{\text{co}} = 100 \text{ MHz} \).

The validity of the EQS approximation can be gauged by examining pulses and determining the impact of removing frequencies that exceed the cutoff frequency. Here two pulses are considered. The first is a 30 ns pulse with 10 ns rise- and fall-times, and the second is a 1 \( \mu \)s pulse with 10 ns rise- and fall-times (Fig. 3-11). Each pulse was converted
to the frequency domain using the Fast Fourier Transform (FFT), the frequency content greater than 100 MHz was removed, and the pulse was then converted back to the time domain using the Inverse Fast Fourier Transform (IFFT). Both filtered pulses closely resemble the unfiltered pulses, demonstrating that the high frequency content (> $f_{co}$) is of limited importance and the EQS approximation is reasonable.
3.10 Circuit Generation and Simulation

MATLAB 7 and Berkeley SPICE 3f5 were the primary software programs used to generate and simulate the cell and tissue systems. MATLAB generated the meshes and determined all of circuit element values based on the electrical and electroporation parameters and the mesh geometry. MATLAB output large circuit netlists, which are text files that list each circuit element and its parameters and connections. The netlists were then loaded by Berkeley SPICE and the circuits contained within were simulated. SPICE then created a binary output file containing all of the circuit node voltages and currents through dependent sources. These SPICE output files were loaded by MATLAB and all of the important variables were extracted, analyzed, and plotted.

The SPICE simulations were run on a MOSIX cluster with 12 Linux nodes, each with dual 2.66 GHz Intel Xeon processors and 4 GB of RAM. Parallel processing was not used. Rather, the simulation jobs were submitted to the cluster and the jobs were moved to individual processors as they became available.

The simulation times varied widely. In general, circuits with electroporation models and large applied electric fields took the longest (2–3 hours), but the majority of the simulations took less than an hour. ~600 simulations were run for this thesis. The total runtime on the cluster for these simulations was ~25 hours, which is remarkably fast and testament to the power of the methods used.
Chapter 4

Cell Electroporation

Cell electroporation has long been used as a laboratory tool for loading cells with various molecules, including DNA [1]. Nonetheless, much has remained unknown about the basic mechanisms of electroporation [45]. Recently, there has been a renewed interest in electroporation research because of the observation that short duration, large magnitude pulses can electroporate the membranes of the cell interior, opening the possibility for electro-manipulation of the cell interior and new biotechnological and therapeutic applications of electroporation [13–24]. Because electroporation experiments generally only examine secondary effects of electroporation, such as the transmembrane transport of fluorescent dyes, biologically realistic models of cell electroporation play an important role in explaining the basic mechanisms that lead to the observed secondary effects. Furthermore, simulations contribute to an understanding of the complex electrical responses of cells to short duration, large magnitude electric pulses and suggest possible mechanisms by which the intracellular fields could interact with the cell organelles to lead to desirable, or undesirable, biological responses [46].

This chapter presents the results of simulations of the electrical response of the cell system (Chp. 3) to a variety of applied electric pulses. The chapter begins with a brief comparison of conventional electroporation and supra-electroporation (Sec. 4.1). The
electrical response of a cell to a conventional electroporation pulse is then examined in more detail (Sec. 4.2), and then the rest of the chapter (Sec. 4.3) focuses on the electrical responses of the cell to supra-electroporation pulses. The discussion of each type of electroporation begins with a series of spatial contour plots of electric potential and pore density to provide a general sense of the response to each pulse. The responses are then examined more quantitatively with plots of transmembrane potential and pore density versus angle at selected time points and temporal plots of transmembrane potential and pore density at the cell and organelle poles. For supra-electroporation, summary data is presented for the responses at the cell and organelle poles to 10 ns pulses with a wide range of pulse strengths, and the importance of the pulse rise-time is discussed.

4.1 Conventional vs. Supra-electroporation

Electroporation can be categorized as conventional electroporation, in which there is substantial electroporation of only the cell plasma membrane, and supra-electroporation, in which there is substantial electroporation of the cell plasma membrane and the intracellular organelle membranes. Conventional electroporation occurs in response to long duration (microseconds–milliseconds), relatively small applied electric fields ($< 0.1 \text{ MV}_m$) while supra-electroporation occurs in response to very short duration (sub-microsecond), relatively strong applied electric fields ($> 1 \text{ MV}_m$).

Figure 4-1 shows the disparate responses of a single cell with two idealized organelles to conventional electroporation and supra-electroporation pulses. The conventional electroporation pulse is a 100 $\mu$s, 0.1 $\text{MV}_m$ pulse with 1 $\mu$s rise- and fall-times and the supra-electroporation pulse is a 60 ns, 10 $\text{MV}_m$ pulse with 10 ns rise- and fall-times.
4.1 Conventional vs. Supra-electroporation

4.1.1 Conventional electroporation

Upon application of the conventional electroporation pulse (Fig. 4-1a), the cell membrane (CM) slowly charges (∼0.1–1 µs) (Fig. 4-1a, 0.5 µs). The electric field is initially largely excluded from the cell interior because the CM resistance is much greater than the electrolyte resistance. Essentially the entire drop in potential between the extracellular space and the intracellular space occurs across the CM if it is unaltered.

By the end of the rise-time (Fig. 4-1a, 1 µs), the polar regions of the cell plasma membrane (CM) charge beyond the threshold for reversible electrical breakdown (REB) (∼1 V), and pores form, as indicated by the regions of white overlaying the equipotential lines. The increased membrane conductance that accompanies the electroporation allows the electric field to penetrate the cell interior, but the electric field magnitude inside the cell, as evidenced by the density of equipotential lines, remains significantly smaller than the electric field magnitude outside the cell.

The spatial extent of electroporation of the polar regions of the CM increases during the rest of the pulse, and the electric field magnitude inside the cell increases slightly (Fig. 4-1a, 10 µs, 99 µs). The intracellular electric field magnitude does not, however, become large enough to electroporate the membranes of the organelles.

Following the pulse, the CM discharges within 1 µs (Fig. 4-1c). The transmembrane potential is largest at the cell poles, where the membrane is most charged. The increased CM conductance temporarily prevents the reestablishment of the cell resting potential. As the pore density exponentially decays with an assumed time constant (pore lifetime) of 1.5 s, the membrane conductance returns to its original value and the cell resting potential is reestablished (not shown; time scale too long).
During pulse

<table>
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<th>$\phi$ (V)</th>
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<td>10 µs</td>
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<tr>
<td>99 µs</td>
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<td>50 ns</td>
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(a) $0.1 \text{ MV/m}$

(b) $10 \text{ MV/m}$
4.1 Conventional vs. Supra-electroporation

Figure 4-1: Conventional vs. supra-electroporation. (a) and (c) show the electric potential and pore distribution resulting from a conventional 100 µs, 0.1 MV/m pulse with 1 µs rise- and fall-times (a) during and (c) after the pulse. (b) and (d) show the electric potential and pore distribution resulting from a 60 ns, 10 MV/m pulse with 10 ns rise- and fall-times (b) during and (d) after the pulse. Pore density is indicated by the white line thickness (\(10^{14}\), \(10^{15}\), \(10^{16}\) pores/m²).
4.1.2 Supra-electroporation

Upon application of the supra-electroporation pulse (Fig. 4-1b), the electric field is essentially uniform throughout the domain because the high frequency components of the pulse cause voltage division between the membrane and electrolyte to be largely determined by the dielectric, rather than conductive, properties of the membrane. Consequently, the transmembrane potentials of all regions of the cell and organelle membranes other than the equatorial regions rapidly increase and exceed the threshold for REB (~1 V) and electroporate (Fig. 4-1b, 5 ns).

Most of the electroporation that results from this particular supra-electroporation pulse occurs during the pulse rise-time (Fig. 4-1b, 10 ns). The pore density and transmembrane potential do not change significantly during the remainder of the pulse. The high membrane conductance state that accompanies the extensive electroporation causes the membranes to remain “electrically invisible” during the remainder of the pulse (i.e. the equipotential lines appear unperturbed by the presence of the cell), even as the conductive membrane properties become more significant (Fig. 4-1b, 50 ns).

The end of the pulse, like the beginning of the pulse, contains high frequency components. These high frequency components are of opposite sign from those at beginning of the applied pulse, and the accompanying voltage division again becomes significant. The voltage division is largely determined by the membrane dielectric properties and results in an initially rapid discharge of the membrane, particularly near the membrane poles, which are normal to the electric field. Additionally, there is a switch in the sign of the potential of the electrolyte within the cell and organelles that persists briefly because the vast majority of the total applied voltage falls across the electrolyte. The transmembrane potential remains elevated, particularly near the un-electroporated regions near the equatorial regions of the membranes, where the lesser membrane conductance impedes rapid dissipation of transmembrane potential (Fig. 4-1d, 0 ns). This complex discharge
4.2 Conventional Electroporation

is so short and so small (the potentials are much smaller than during the pulse) that it is unlikely to have any significant biochemical effect on the cell, but the phenomenon is interesting and results in striking electrical potential patterns.

Following the pulse, the cell and organelle membranes discharge within tens of nanoseconds (Fig. 4-1d, 10 ns). The equatorial regions of the membranes are slowest to discharge because of the relatively low conductance of these regions relative to the electroporated regions. The increased membrane conductances temporarily prevent the reestablishment of the cell and organelle resting potentials. As the pore density exponentially decays with a time constant of 1.5 s, the conductances of the membranes return to their original values and the cell resting potentials are reestablished (not shown; time scale too long).

4.2 Conventional Electroporation

A number of models have been developed for conventional electroporation [38, 41, 47, 48]. As such, conventional electroporation is not the primary focus of this chapter. However, it is worthwhile to present some conventional electroporation results to facilitate comparison with previous models and with the supra-electroporation results. The conventional electroporation results are qualitatively very similar to those of DeBruin and Krassowska [38, 47], though they simulated longer duration, lower magnitude applied fields on a larger cell.

4.2.1 Angular response

Figure 4-2 shows the transmembrane potential and pore density on the cell membrane (CM) and organelle membranes (LOM, SOM) as functions of angle at a number of time points. The results are the counterparts to the spatial results shown in Fig. 4-1a, c.

Upon application of the electric pulse, the membranes begin to charge. During the ini-
Figure 4-2: Angular response: 100 µs, 0.1 MV/m pulse. (a), (c), and (e) transmembrane potential and (b), (d), and (f) pore density of the cell membrane (CM) and organelle membranes (LOM, SOM). Times shown (from blue to red) are 0.5, 0.85, 0.93, 1.5, and 99 µs during the pulse (solid lines) and 0 ns, 10 ns, and 1 µs after the pulse (dashed lines).
tial charging phase, the transmembrane potential with respect to the polar angle with respect to the \( x \)-axis, \( V_m(\Theta) \), is sinusoidal and increases in amplitude with time for each membrane. After \( \sim 0.75 \mu s \), the \( V_m \) at the poles of CM begin to exceed the threshold for REB (\( \sim 1 \text{ V} \)) (Fig. 4-2a). The anodic pole reaches its peak \( V_m \) of 1.37 V at 0.84 \( \mu s \) and the cathodic pole reaches its peak \( V_m \) of 1.38 V slightly later at 0.93 \( \mu s \).¹ The difference is a consequence of the cell resting potential, which produces an electric field in the same direction as the applied electric field at the anodic pole but in the opposite direction at the cathodic pole. The rates of pore creation peak as \( V_m \) peak (Fig. 4-2b), and the pore densities, \( N \), of the polar regions of the CM increase dramatically, thereby increasing the conductances of these membrane regions and driving the \( V_m \) toward \( \sim 1 \text{ V} \).

\( V_m \) and \( N \) peak first at the poles of the CM and later farther toward the equator of the CM (Fig. 4-2a). \( V_m(\Theta) \) of the un-electroporated regions (those further from the poles) continues to conform to a sinusoidal profile of increasing amplitude. As the active site of electroporation moves out from the poles, the \( V_m \) of the electroporating regions diverge from the sinusoid and decreases toward \( \sim 1 \text{ V} \). Thus, \( V_m(\Theta) \) has the appearance of a sinusoid with flattened peaks (Fig. 4-2a). The CM regions more than \( \sim 50^\circ \) from the poles do not charge sufficiently to electroporate significantly (Fig. 4-2b).

Neither the LOM nor the SOM electroporate because the intracellular electric field magnitude remains too small to charge them much beyond their resting potentials, even after electroporation of the CM (Figs. 4-1a, 4-2c–f). Additionally, the LOM and SOM require larger electric field magnitudes to electroporate than the CM because of their smaller sizes.

¹To facilitate comparison between the anodic and cathodic membrane poles, the transmembrane potential, \( V_m \), generally refers to the electric potential on the anodic side of the membrane minus the electric potential on the cathodic side, rather than the traditional definition of the electric potential on the interior side of membrane minus the electric potential on on the exterior side of the membrane. More explicitly, at the anodic pole \( V_m \) refers to the exterior potential minus the interior potential, and at the cathodic pole \( V_m \) refers to the interior potential minus the exterior potential.
By the end of the pulse, the $V_m$ at the poles of the CM decrease to 1.11 V and the $N$ at the poles of the CM reach $2.1 \times 10^{14}$ pores/m$^2$ (Fig. 4-2a, b). The $V_m$ at the poles of the LOM peak at 0.22 V (anodic) and $-0.21$ V (cathodic) and the $N$ at the poles also remain at their basal equilibrium values for the duration of the pulse (Fig. 4-2c, d). The $V_m$ at the poles of the SOM peak at 0.21 V (anodic) and $-0.14$ V (cathodic) and the $N$ at the poles remain at their basal equilibrium values for the duration of the pulse (Fig. 4-2e, f). Note that the basal equilibrium pore density of the SOM exceeds that of the LOM because the SOM has a 174 mV resting potential while the LOM has no resting potential. The angles at which the SOM $V_m(\Theta)$ peaks occur shift noticeably during the pulse (Fig. 4-2e) because the intracellular electric field does not point directly in the $-y$-direction. Rather, it also has a component in the $-x$-direction (Fig. 4-1a, 99 $\mu$s). There is also a shift in the angles at which the LOM $V_m(\Theta)$ peak (Fig. 4-2c), but the effect is less pronounced.

Following the pulse, the membranes quickly discharge (Fig. 4-2). The CM $V_m(\Theta)$ remains at 0 V until the CM pores reseal and the resting potential can be reestablished. $N(\Theta)$ decreases with a 1.5 s time constant. The LOM and SOM quickly discharge to and remain at their resting potentials (0 mV and 174 mV) because they are not electroporated.

### 4.2.2 Temporal response

Figure 4-3 shows the temporal response of the transmembrane potential and pore density at the poles of the cell membrane and organelle membranes. Two time scales are shown for each to give a sense of the changes both over the duration the entire pulse and during the early phase of the pulse.

For the CM, the polar $V_m$ increase approximately linearly for the first 0.84 $\mu$s (anodic) and 0.93 $\mu$s (cathodic) to reach 1.37 V (anodic pole) and 1.38 V (cathodic) (Fig. 4-3a). The polar $V_m$ then decrease slightly with the onset of electroporation (Fig. 4-3b) briefly
before assuming steady \( V_m \) of \( \sim 1.3 \, \text{V} \) for the remainder of the 1 \( \mu \text{s} \) pulse rise-time. The polar \( V_m \) then slowly decrease to 1.11 \( \text{V} \) at the end of the pulse, and the polar \( N \) increase slightly to \( 2.1 \times 10^{14} \text{ pores/m}^2 \).

The organelle polar \( V_m \) and \( N \) change little during the pulse (Fig. 4-3). The polar \( V_m \) of the organelles remain essentially unchanged from their resting potentials until the end of the pulse rise-time when the electroporation of the CM increases the intracellular electric
field. Nonetheless, the polar $V_m$ of the LOM and SOM only change by fractions of a volt by the end of the pulse and their polar $N$ remain at their basal equilibrium values.

After the pulse, the polar $V_m$ quickly approach 0 V for the CM because the electroporated membrane cannot sustain a resting potential. The polar $V_m$ approach the resting potentials for the LOM and SOM because they are not electroporated (Fig. 4-3).

4.3 Supra-electroporation

Supra-electroporation has become an area of considerable interest in the electroporation research community in recent years because it will potentially allow the electro-manipulation of the cell interior for technologically and clinically useful applications, such as apoptosis induction [13–24]. The defining feature of supra-electroporation, as the name implies, is the extensive electroporation of both the cell membrane and organelle membranes. For pulses of sufficient magnitude, simulations show that nearly all cell and organelle membranes become highly electroporated, largely independent of their geometry.

4.3.1 Membrane-electrolyte voltage division

Supra-electroporation requires that special consideration be given to the frequency content of the applied pulses and how it affects the electrical responses of the membranes. For the long pulses used in conventional electroporation ($\sim 10 \mu s$), the frequency content is on the low end of the spectrum, and the voltage division between electrolyte and membrane is determined by the conductive properties of the materials. For short pulses, the frequency content is shifted toward the high end of the spectrum, and the dielectric properties the materials become significant.
A simple model of the membrane-electrolyte voltage division illustrates the importance of the pulse frequency content. The total electrolyte and membrane impedance may be represented by a parallel resistor-capacitor pair for the electrolyte placed in series with a parallel resistor-capacitor pair for the membrane, as shown in Fig. 4-4. The electrolyte has conductivity $\sigma_{\text{el}}$ and permittivity $\varepsilon_{\text{el}}$. Thus,

$$R_{\text{el}} = \frac{l_{\text{el}}}{\sigma_{\text{el}}A} \quad C_{\text{el}} = \frac{\varepsilon_{\text{el}}A}{l_{\text{el}}}, \quad (4.1)$$

where $l_{\text{el}}$ is the length of the electrolyte and $A$ is the cross-sectional area of the 1D model. The membrane has an effective conductivity that is a function of pore density $\sigma_{\text{m}}(N)$ and permittivity $\varepsilon_{\text{m}}$. Thus,

$$R_{\text{m}}(N) = \frac{l_{\text{m}}}{\sigma_{\text{m}}(N)A} \quad C_{\text{m}} = \frac{\varepsilon_{\text{m}}A}{l_{\text{m}}}, \quad (4.2)$$

where $l_{\text{m}}$ is the thickness of the membrane. Let $l_{\text{el}} = 50 \, \mu\text{m}$ and $l_{\text{m}} = d_{\text{m}} = 5 \, \text{nm}$ to make the system as similar as possible to the cell system. $\sigma_{\text{m}}(N)$ is calculated as the sum of...
the membrane conductivity and total pore conductivity:

\[
\sigma_{m_{\text{eff}}}(N) = \sigma_m + l_m G_p(V_m) N.
\] (4.3)

For the purpose of estimating \(G_p(V_m)\), \(V_m\) is assumed to be 1 V, the approximate transmembrane potential after electroporation. The membrane resistance is

\[
R_m(N) = \frac{l_m}{(\sigma_m + l_m G_p(V_m) N) A}. \quad (4.4)
\]

The magnitude of the voltage drop across the membrane relative to the applied voltage is

\[
\left| \frac{V_m(j\omega)}{V_{\text{app}}(j\omega)} \right| = \left| \frac{Z_m(N, j\omega)}{Z_m(N, j\omega) + Z_{\text{el}}(j\omega)} \right|, \quad (4.5)
\]

where

\[
Z_m(N, j\omega) = \frac{R_m(N)}{1 + j\omega R_m(N) C_m} \quad Z_{\text{el}}(j\omega) = \frac{R_{\text{el}}}{1 + j\omega R_{\text{el}} C_{\text{el}}}
\] (4.6)

and \(\omega = 2\pi f\) is the angular frequency.

Figure 4-5 shows the membrane voltage division as a function of frequency for a number of pore densities. At low frequencies (<~10^5 Hz), essentially the entire applied voltage drops across the membrane for low pore densities (<~10^{13} \text{ pores/m}^2). However, as the pore density is increased beyond ~10^{13} \text{ pores/m}^2, the bulk of the voltage drop shifts to the electrolyte.

At higher frequencies (>~10^5 Hz), most of the applied voltage drops across the electrolyte, even when the pore density is low, and the voltage division becomes less sensitive to low pore densities. At 100 MHz, for example, only ~0.005 of the applied voltage drops across the membrane for low pore densities, and increasing the pore density does not significantly alter the voltage division until it surpasses ~10^{16} \text{ pores/m}^2. At extremely
4.3 Supra-electroporation

Figure 4-5: Membrane-electrolyte voltage division for different pore densities. The voltage division between the membrane and electrolyte in the simple model shown in Fig. 4-4 is a function of frequency and pore density.

While this model of voltage division is quite simple, it illustrates why very short duration, high magnitude pulses cause supra-electroporation while long duration, low magnitude pulses do not. At high frequencies, the great majority of the voltage drop occurs over the electrolyte. As such, the applied voltage must be very large so that the small fraction of the total applied voltage that does drop across the membrane is large enough to cause REB (\( \sim 1 \text{ V} \)). Upon application of a large pulse, the transmembrane potential increases rapidly and exceeds the threshold for REB (\( \sim 1 \text{ V} \)). The pore creation rate then skyrockets, and because the voltage division is relatively unaffected by low pore densities, pore creation continues until very high pore densities are reached that are sufficient to signifi-
cantly alter the voltage division and decrease the transmembrane potential and end pore creation. Additionally, because the membrane impedance decreases dramatically at high frequencies, the intracellular electric field experienced by organelle membranes is of the same magnitude as that experienced by the cell membrane, and all regions of membrane electroporate for sufficiently large pulses.

Figure 4-5 is a powerful tool for demonstrating the role of pulse frequency content in determining the cell response to supra-electroporation pulses, and it will be referenced throughout the rest of the chapter.

4.3.2 Spatial response

Figures 4-6 and 4-7 show the responses of the cell system with two organelles to 60 ns and 10 ns pulses of 0.1, 2, and $10 \text{MV}_m$ magnitudes. The 60 ns pulses have 10 ns rise- and fall-times, and the 10 ns pulses have 1.5 ns rise- and fall-times.

60 ns pulses

The 60 ns, $0.1 \text{MV}_m$ pulse (Fig. 4-6a, d) has the same magnitude as the conventional electroporation pulse shown in Fig. 4-1a, c, but the duration and rise- and fall-times are greatly reduced. The high frequencies associated with the short rise-time cause the transmembrane potential to be largely determined by the dielectric, rather than conductive, properties of the membrane (Fig. 4-5). Consequently, the electric field is relatively uniform throughout the domain, including within cell and organelles. If this field could be maintained for a longer duration, it would likely lead to some organellar membrane electroporation, but the transmembrane potential is increasingly determined by the conductive properties of the membrane during the pulse plateau, and the intracellular field magnitude decreases. As conductive membrane properties increasingly determine the membrane impedance, the low conductance of the membrane begins to exclude the electric field from the cell interior (Fig. 4-1a, 50 ns). The cell membrane (CM) would elec-
troporate, as in the response to the conventional electroporation pulse, but the duration of the pulse is simply too short.

Following the 60 ns, 0.1 MV/m pulse, the cell and organelle membranes rapidly discharge (Fig. 4-6d). The end of the pulse is essentially equivalent to applying a second pulse to the system oriented in the opposite direction to the first. The voltage drop of the high frequency components of this second pulse then primarily falls across the electrolyte rather than the membrane. Therefore, the anodic side of the intracellular space becomes relatively negative and the cathodic side of the intracellular space becomes relatively positive. Within 1 µs, the cell returns to its original state (Fig. 4-6d, 1 µs). Because the membrane integrity is maintained during the pulse, the resting potentials of the CM and SOM are also maintained.

During the initial phase of the 60 ns, 2 MV/m pulse (Fig. 4-6b), the electric field looks similar to that in response to the 0.1 MV/m pulse. However, for the 2 MV/m pulse the magnitude of the electric field that penetrates the cell interior is sufficient to electroporate the LOM. The electroporation begins with CM because of its greater size and first occurs on the anodic pole of the cell because of the resting potential (86 mV), which creates an electric field in the same direction as the applied field. Electroporation of the cathodic pole commences soon after the electroporation of the anodic pole, and soon thereafter, the LOM electroporation begins. Because the LOM lacks a resting potential and is sufficiently distanced from other structures in the cell, the electroporation of both poles starts at the same time and proceeds to the same extent. Though the intracellular field is large enough to electroporate the LOM, it is not large enough to electroporate the SOM. The intracellular field does not decrease toward the end of the 2 MV/m pulse as it did toward the end of the 0.1 MV/m pulse because the greatly increased conductance of the CM allows the field to be maintained even as conductive membrane properties determine the membrane-electrolyte voltage division (Fig. 4-6b).
During pulse

<table>
<thead>
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<th>Time</th>
<th>( \phi ) (V)</th>
<th>Time</th>
<th>( \phi ) (V)</th>
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<th>( \phi ) (V)</th>
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<td></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>

(a) \(0.1 \text{ MV m}^{-1}\) (b) \(2 \text{ MV m}^{-1}\) (c) \(10 \text{ MV m}^{-1}\)
4.3 Supra-electroporation

After pulse

<table>
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<tr>
<th>Time</th>
<th>(d) 0.1 MV/m φ (V)</th>
<th>(e) 2 MV/m φ (V)</th>
<th>(f) 10 MV/m φ (V)</th>
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<tr>
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<td><img src="image9.png" alt="Image" /></td>
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</table>

**Figure 4-6:** Spatial response: 60 ns pulses. Electric potential and pore distribution resulting from 0.1, 2, and 10 MV/m pulses with 10 ns rise- and fall-times (a)–(c) during and (d)–(f) after the pulses. Pore density is indicated by the white line thickness (1: 10^{14}, 1: 10^{15}, 1: 10^{16} pores/m^2).
The electric potential in and around the cell exhibits complex patterns as the membranes discharge (Fig. 4-6e). The highest electric potentials exist near un-electroporated equators of the membranes, which are charged during the pulse (except at the very equator) but not beyond the threshold for REB. The negatively-oriented voltage division during the pulse fall-time causes a small but sharp drop in transmembrane potential at the poles, but because the electric field associated with the high frequency components is predominantly in the $y$-direction, this does not significantly alter the transmembrane potential in the equatorial regions of membrane, which are nearly parallel to the field. Within 1 µs the extracellular and intracellular regions reach 0 V; the electroporated CM cannot maintain a resting potential. The SOM, which was not electroporated, maintains its original resting potential of 174 mV (Fig. 4-6e).

The 60 ns, 10 MV/m pulse shown in Fig. 4-6c, f is the same as the supra-electroporation pulse shown in Fig. 4-1b, d that was previously described (Sec. 4.1.2). The magnitude of the electric field is large enough during the initial instant of the pulse to electroporate essentially all membrane regions that are not oriented parallel to the electric field. The tremendously increased conductance of the membranes allows the field to continue to penetrate the cell and its organelles as the conductive membrane properties become more significant in determining the membrane-electrolyte voltage division (Fig. 4-6c).

The membranes discharge very quickly after the pulse because of their highly elevated conductances (Fig. 4-6f). The extracellular, intracellular, and intraorganellar spaces all reach 0 V within 20 ns. Because all of the membranes are highly electroporated, none of the membrane-enclosed regions is able to maintain a resting potential until the pores reseal. This may have dramatic effects on the cell biochemistry and signaling, particularly if the pores require seconds to reseal, as assumed here.
4.3 Supra-electroporation

10 ns pulses

The cell responses to 10 ns pulses of 0.1, 2, and 10 MV/m magnitudes (Fig. 4-7) are qualitatively very similar to the responses to 60 ns pulses of the same magnitudes (Fig. 4-6). As such, only a few points will be made.

The 10 ns, 0.1 MV/m pulse (Fig. 4-7a, d), like the 60 ns, 0.1 MV/m pulse (Fig. 4-6a, d), is simply too short and too weak to electroporate any of the membranes, and cell returns to its original state soon after the end of the pulse (Fig. 4-7d).

The 10 ns, 2 MV/m pulse (Fig. 4-7b, e) electroporates the CM and LOM at the end of the pulse plateau, but the spatial extent of the electroporation is not quite as great as that in response to the 60 ns, 2 MV/m pulse (Fig. 4-6b, e) because of its shorter duration. The post-pulse discharge is similar, but the regions of highest potential (in magnitude) are located farther from the equator because the spatial extent of electroporation is less extensive. The regions of highest potential (in magnitude) are at the closest un-electroporated regions to the poles, where the transmembrane potential is large at the end of the pulse but the unaltered membrane conductance slows post-pulse discharge.

The 10 ns, 10 MV/m pulse (Fig. 4-7c, f) electroporates nearly all of the cell and organelle membrane area within nanoseconds. The extent of the electroporation is similar to that in response to the 60 ns, 10 MV/m pulse, and the post-pulse discharge is also quite similar (Fig. 4-6c, f).

4.3.3 Angular response

Figures 4-8 and 4-9 show the transmembrane potential and pore density of the cell membrane and organelle membranes as functions of angle at several time points for 10 ns pulses of 2 MV/m and 10 MV/m magnitudes. The results are the counterpart to the spatial
## During pulse

<table>
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<tr>
<td>3.6 ns</td>
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</tr>
<tr>
<td>8.5 ns</td>
<td><img src="image10.png" alt="Image" /></td>
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</tbody>
</table>
### Figure 4-7: Spatial response: 10 ns pulses. Electric potential and pore distribution resulting from 0.1, 2, and 10 MV/m pulses with 1.5 ns rise- and fall-times (a)–(c) during and (d)–(f) after the pulses. Pore density is indicated by the white line thickness (1: $10^{14}$, 2: $10^{15}$, 3: $10^{16}$ pores/m$^2$).
results shown in Fig. 4-7.

10 ns, $2 \text{MV}_m$ pulse

The anodic pole of the CM is the first region of membrane to electroporate. The $V_m$ reaches a peak value of 1.59 V on the anodic side at 6.0 ns, and the $V_m$ reaches a peak value of 1.59 V on the cathodic side soon thereafter at 6.6 ns (Fig. 4-8a). This asymmetry, which is caused by the hyperpolarization of the anodic pole due to the cell resting potential, causes the $N$ of the anodic pole to briefly exceed the $N$ of the cathodic pole (Fig. 4-8b). The poles of the CM rapidly electroporate because of the highly elevated $V_m$. The dramatic increase in the conductance of the membrane poles then drives $V_m$ at the poles toward $\sim 1$ V. As the $V_m$ is decreasing at the poles, it is still increasing across the adjacent regions of membrane that have not yet electroporated. After $V_m$ peaks in these regions, they too are driven toward $\sim 1$ V. At all times, $V_m(\Theta)$ of the un-electroporated regions of membrane conform to sinusoidal curves that increases in amplitude with time, and regions near the poles of the CM that have electroporated dip toward $\sim 1$ V (Fig. 4-8a). As such, the peak $V_m(\Theta)$ begin at the poles but then move out toward the equator as the pulse progresses. While the $N$ of the anodic and cathodic poles of the CM are essentially equal at the end of the pulse ($8.6 \times 10^{15}$ pores m$^{-2}$), a slightly greater fraction of the membrane area on the anodic side of the cell electroporates because of the cell resting potential (Fig. 4-8b).

Following the pulse, the CM quickly discharges (Fig. 4-8a). The electroporated regions discharge fastest because of the increased membrane conductance of these regions.

The progression of the LOM response (Fig. 4-8c, d) is similar to that of the CM. The primary difference is that the LOM lacks a resting potential and therefore $V_m(\Theta)$ and $N(\Theta)$ are identical (in magnitude) at both poles of the LOM at all times. At the poles,
Figure 4-8: Angular response: 10 ns, 2 MV/m pulse. (a), (c), and (e) transmembrane potential and (b), (d), and (f) pore density of the cell membrane (CM) and organelle membranes (LOM, SOM). Times shown (from blue to red) are 1.5, 6, 6.7, 7.3, and 8.5 ns during the pulse (solid lines) and 0 ns, 10 ns, and 1 µs after the pulse (dashed lines).
the $V_m$ peak at 1.58 V at 6.8 ns, and the $N$ reach $7.9 \times 10^{15}$ pores/m² by the end of the pulse (Fig. 4-8c). The $N$ is almost as large at the LOM poles as at the CM poles, but a lesser fraction of the LOM area is electroporated by the end of the pulse (Fig. 4-8d).

The SOM does not electroporate as extensively as the CM and LOM (Fig. 4-8f). The electric field required to cause electroporation is proportional to radius, and therefore, while the field is strong enough to extensively electroporate the CM and LOM, it is not strong enough to extensively electroporate the SOM in 10 ns. $V_m(\Theta)$ assumes a sinusoidal profile that is shifted by the SOM resting potential (Fig. 4-8e). The $V_m$ is still increasing at the end of pulse plateau and reaches 1.43 V (anodic) and 1.08 V (cathodic) at the poles. The anodic pole begins electroporating late in the pulse and $N$ reaches $1.35 \times 10^{13}$ pores/m² (Fig. 4-8f), but the pulse is not long enough for $N$ to reach the magnitude seen in the CM and LOM. The cathodic pole of the SOM does not electroporate at all because of the large resting potential, which causes the magnitude of the $V_m$ at the cathodic pole to lag behind that on the anodic pole. Without significant electroporation, $V_m(\Theta)$ maintains a sinusoidal profile with increasing amplitude during and decreasing amplitude after the pulse. (Fig. 4-8e).

10 ns, 10 MV/m pulse

The responses of the cell and organelle membranes to the 10 MV/m pulse are somewhat similar to their responses to the 2 MV/m pulse but are more extreme. Almost all of the membrane area electroporates. In fact, the membrane poles electroporate during the 1.5 ns rise-time, which leads to more complex $V_m(\Theta)$ profiles (Fig. 4-9) than those seen for the 10 ns, 2 MV/m pulse (Fig. 4-8).

The initially sinusoidal profiles of $V_m(\Theta)$ and the anode-cathode asymmetries produced by the membrane resting potentials are still present in the cell response to the 10 MV/m pulse (Fig. 4-9). However, in response to the 10 MV/m pulse, the polar regions of the membrane
Figure 4-9: Angular response: 10 ns, $10 \frac{MV}{m}$ pulse. (a), (c), and (e) transmembrane potential and (b), (d), and (f) pore density of the cell membrane (CM) and organelle membranes (LOM, SOM). Times shown (from blue to red) are 0.75, 1.4, 1.5, 1.8, and 8.5 ns during the pulse (solid lines) and 0 ns, 10 ns, and 1 µs after the pulse (dashed lines).
electroporate during the pulse rise-time, which leads to more elevated pore densities. When $V_m$ peaks during the rise-time of a short duration, large magnitude pulse, the high frequency components cause the dielectric properties of the membrane to be a significant determinant of the membrane-electrolyte voltage division (Fig. 4-5). Consequently, the electroporation of the membrane, which increases the conductance of the membrane but is not assumed to alter its permittivity, does not decrease the transmembrane potential to the same extent as it would with lower frequency-components and decreased influence of the membrane dielectric properties. As such, a higher membrane conductance state is reached in driving $V_m$ toward $\sim 1$ V, thereby ending pore creation. Therefore, $V_m$ and $N$ reach higher values than they tend to during a pulse plateau. This rise-time effect, as it will be called hereafter, is manifest in Fig. 4-9 by the relatively sharp transitions in $V_m(\Theta)$ and $N(\Theta)$ at the interfaces between regions of membrane that do and do not electroporate during the rise-time. This is apparent in the $V_m(\Theta)$ profiles by looking at the peaks of $V_m(\Theta)$ at the end of the 1.5 ns rise-time and noting that they align exactly with the sharp transitions in $V_m(\Theta)$ that are apparent at the next time point. The $V_m$ of the regions of membrane subject to the rise-time effect actually decrease below 1 V during the pulse plateau when conductive membrane properties become significant. This effect is clear in Fig. 4-5 by examining the shift in voltage division along lines of constant pore density as the frequency decreases. The rise-time effect occurs on every pole except the SOM cathode, where the resting potential prevents electroporation on such a short time scale, which causes asymmetry between the anodic and cathodic sides of the SOM. There is also asymmetry between the poles of the CM, which exhibit the rise-time effect to differing degrees (Fig. 4-9a, b).

Outside the polar regions, the progression of $V_m(\Theta)$ and $N(\Theta)$ are similar to those shown for less extreme pulses. The $V_m(\Theta)$ maintain sinusoidal profiles until they exceed $\sim 1$ V, at which point pore creation limits the peak $V_m$ and eventually drives the $V_m$ toward $\sim 1$ V (Fig. 4-9a, c, e).
4.3 Supra-electroporation

All membrane poles, except the SOM cathodic pole, reach peak $V_m$ of $\sim 1.67$ V between 1.40 ns and 1.48 ns. By the end of the pulse plateau these $V_m$ drop to $\sim 0.88$ V and the $N$ reach $\sim 5.7 \times 10^{16}$ pores/m$^2$. The SOM cathodic pole reaches a peak $V_m$ of 1.65 V at 1.79 ns, and by the end of the pulse plateau, the $V_m$ drops to 1.00 V and $N$ reaches $4.5 \times 10^{16}$ pores/m$^2$. Because of the rise-time effect, the peak $V_m$ and $N$ of the cathodic pole of the SOM are slightly less than those of the other membrane poles, and the polar $V_m$ does decrease as significantly during the pulse plateau. The $N(\Theta)$ profiles are very broad for all three membranes, with nearly all regions electroporating except the narrow bands near the membrane equators (Fig. 4-9b, d, f).

Following the pulse, the $V_m$ quickly fall to 0 V for all of the membranes, with the $V_m$ falling fastest in the highly electroporated polar regions (Fig. 4-9a, c, e).

4.3.4 Temporal response

Figures 4-10 and 4-11 show the temporal responses at the poles of the cell membrane and organelle membranes to 10 ns pulses of 2 MV/m and 10 MV/m magnitudes. Two time scales are shown for the 10 MV/m to give a sense of the changes both over the duration the entire pulse and during the early phase of the pulse.

10 ns, 2 MV/m pulse

The polar $V_m$ rapidly increase during the 1.5 ns rise-time and then continue to rise during the pulse plateau at slightly decreased rates (Fig. 4-10a). Initially, when the frequency content is highest, all three membranes charge with fairly similar rates, but the rates begin to differ more substantially during the pulse plateau with the poles reaching peak $V_m$ in order of decreasing membrane radius. This is consistent with the charging rates being less dependent on membrane radius at high frequencies (during the rise-time) and
more dependent on membrane radius at lower frequencies (during the plateau) [35].

The CM and LOM poles exceed the threshold for REB after $\sim 5$ ns and begin to electroporate (Fig. 4-10b). The $V_m$ of the anodic pole of the CM peaks at 1.59 V at 6.0 ns, and the $V_m$ of the cathodic pole peaks soon thereafter at 1.59 V at 6.6 ns. The $V_m$ of both poles of the LOM peak at 1.58 V at 6.8 ns. After peaking, the $V_m$ begin to decrease toward $\sim 1$ V. The $V_m$ of the SOM poles increase throughout the pulse and are still increasing at the end of the pulse plateau. By that time, the $V_m$ reaches 1.43 V at the anodic pole and 1.08 V at the cathodic pole, and $N$ reaches $1.4 \times 10^{13} \text{ pores m}^{-2}$ at the anodic pole while remaining at the basal equilibrium value at the un-electroporated cathodic pole. At the anodic and cathodic CM poles, the $V_m$ drop to 1.21 V and 1.27 V and the $N$ reach $8.6 \times 10^{15} \text{ pores m}^{-2}$. At both LOM poles, the $V_m$ drop to 1.27 V and the $N$ reach $7.9 \times 10^{15} \text{ pores m}^{-2}$ (Fig. 4-10).

During the fall-time and after the pulse, the polar $V_m$ of the CM and LOM decrease and quickly reach 0 V (Fig. 4-10a). The $V_m$ fall more quickly during the fall-time than follow-
4.3 Supra-electroporation

Figure 4-11: Temporal response at poles: 10 ns, 10 MV/m pulse. (a) Transmembrane potential and (b) pore density at the anodic (solid lines) and cathodic (dashed lines) poles of the cell membrane (CM) and organelle membranes (LOM, SOM). The two time scales show the evolution for the complete pulse (left) and the early phase of the pulse (right).

The applied 10 MV/m electric field is sufficiently large to drive all of the V_m of the cell and organelle membrane poles, other than the SOM cathodic pole, past the threshold for
REB and electroporate them during the 1.5 ns pulse rise-time (Fig. 4-11). Note that the charging rates are largely independent of the membrane radii because of the high frequency content of the rise-time (Fig. 4-11a). The polar \( V_m \) peaks first at the CM anodic pole at 1.40 ns, and then is closely followed by both poles of the LOM at 1.47 ns, the SOM anodic pole at 1.48 ns, and the CM cathodic pole at 1.49 ns. The SOM cathodic pole \( V_m \) peaks somewhat later at 1.79 ns, during the pulse plateau. All poles reach a peak \( V_m \) of 1.67 V except the SOM anodic pole, which reaches a slightly lesser 1.65 V. Accordingly, all of the poles reach \( N \) of \( \sim 5.7 \times 10^{16} \frac{\text{pores}}{\text{m}^2} \) except the SOM cathodic pole, which reaches a slightly lesser \( 4.5 \times 10^{16} \frac{\text{pores}}{\text{m}^2} \). Essentially all pore creation starts after \( \sim 1 \) ns and occurs within a \( \sim 0.1 \) ns window of time containing the time of peak \( V_m \) (Fig. 4-11b).

The rapid rises of the polar \( V_m \) are accompanied by rapid electroporation (Fig. 4-11b), which drives down the polar \( V_m \) (Fig. 4-11a). Because of the rise-time effect, the increase in the membrane conductance that accompanies electroporation is sufficient to drive the \( V_m \) to 0.88 V for all of the poles except the SOM cathodic pole, which reaches a larger plateau \( V_m \) of 1.00 V.

During the fall-time and following the pulse, the polar \( V_m \) of the membranes decrease and quickly reach 0 V (Fig. 4-11a). The polar \( V_m \) fall much more quickly during the fall-time than following the fall-time because of the negatively-oriented dielectric voltage division that is the counterpart to the quick rise in \( V_m \) during the pulse rise-time. The \( N \) decay exponentially with a 1.5 s time constant (not shown; time scale too long).

### 4.3.5 Pulse magnitude

Figure 4-12 shows the peak polar \( V_m \) and \( N \) for 10 ns pulses of varying magnitude. For \( E_{\text{app}} < 1 \frac{\text{MV}}{\text{m}} \), none of the membrane poles significantly electroporates. As \( E_{\text{app}} \) is increased beyond \( 1 \frac{\text{MV}}{\text{m}} \), the polar \( V_m \) begin to reach values in excess of 1.5 V and substantially electroporate. The CM and LOM poles electroporate for \( E_{\text{app}} \geq 1.5 \frac{\text{MV}}{\text{m}} \).
and the SOM poles electroporate for $E_{\text{app}} > 2.5 \text{ MV}_m$. For the CM and SOM, which have resting potentials, the anodic poles electroporate at slightly lower $E_{\text{app}}$ than the cathodic poles. The rise-time effect is manifest by the small but sharp increase in $N$ at $\sim 9 \text{ MV}_m$ (Fig. 4-12b).

### 4.3.6 Pulse rise-time

The pulse rise-time is a critical parameter in determining the cell response to supra-electroporation pulses, as the 10 ns, 10 $\text{ MV}_m$ pulse demonstrates. The high frequency content of the pulse rise-time causes dielectric properties of the membrane to be a significant determinant of its impedance and the membrane-electrolyte voltage division. Consequently, the voltage division between the electrolyte and membrane is less sensitive to changes in membrane conductance during the pulse rise-time than when the frequency content is lower during the pulse plateau (Fig. 4-5). Moreover, the transmembrane potential and pore creation rate reach higher values and lead to higher pore density when pore creation peaks during the rise-time. For pulses with longer rise-times, the transmembrane potential and pore creation rate remain elevated after the peaking, and they
can even peak one or more additional times, leading to elevated pore densities.

When pore creation peaks during the pulse plateau, the transmembrane potential and pore creation rate peak and then decrease monotonically. However, when pore creation peaks during the early phase of a long rise-time, the transmembrane potential and pore creation rate peak but the increased membrane conductance that results from the burst of electroporation is not sufficient to sustain a monotonic decrease in the transmembrane potential and pore creation rate as the applied voltage continues to increase. Rather, provided the rise-time is long enough, they will peak one or more additional times.

Figure 4-13 illustrates the rise-time effect for three pulse rise-times, pulse strength combinations chosen such that they all deliver the same energy \( (E_{app}^2 \cdot \text{rise} = \text{constant}) \) as the rise-time of the 10 ns, 10 MV m pulse. Here the pulses are sawtooth-shaped; the pulse duration is equal to the pulse rise-time.

The first pulse is a 10 ns, 3.84 MV m sawtooth pulse (Fig. 4-13a, b). The progression of the polar \( V_m \) and \( N \) are similar to those during the rise-time of the 10 ns, 10 MV m pulse with 1.5 ns rise-time (Fig. 4-11). The \( V_m \) of all of the membrane poles, except the SOM cathodic pole, peak by 9 ns and then decrease toward \( \sim 1.2 \) V at the end of the pulse. Had the pulse been longer, the \( V_m \) would inevitably had begun to increase again.

The second pulse is a 100 ns, 1.22 MV m sawtooth pulse (Fig. 4-13c, d). The CM polar \( V_m \) peak at 1.53 V at \( \sim 46 \) ns, decrease to \( \sim 1.2 \) V by \( \sim 62 \) ns and then increase to \( \sim 1.46 \) V before dropping slightly to 1.43 V by the end of the 100 ns pulse. The LOM polar \( V_m \) peak at 1.52 V at 55 ns, drop to 1.31 V by 68 ns, and then increase to 1.45 V before dropping slightly to 1.43 V by the end of the pulse. The SOM polar \( V_m \) increase monotonically throughout the pulse, which is not long enough for the damped oscillatory behavior of the CM and LOM polar \( V_m \) to arise.
Figure 4-13: Temporal response at poles: sawtooth, equi-energy pulses. Transmembrane potential and pore density at the anodic (solid lines) and cathodic (dashed lines) poles of the cell membrane (CM) and organelle membranes (LOM, SOM) for (a), (b) 10 ns, 3.84 MV/m; (c), (d) 100 ns, 1.22 MV/m; and (e), (f) 1000 ns, 0.387 MV/m sawtooth pulses.
The third pulse is a 1000 ns, 0.387 MV \text{m}^2 \text{sawtooth pulse} (Fig. 4-13e, f). The CM polar $V_m$ peak at 1.43 V at $\sim 0.33 \mu s$, decrease to a minimum of 1.29 V at $\sim 0.39 \text{ns}$, and exhibit damped oscillation approaching 1.36 V. During this plateau of elevated $V_m$, the CM polar $N$ increases linearly from $3.5 \times 10^{14} \text{ pores/m}^2$ to $8.7 \times 10^{14} \text{ pores/m}^2$. The LOM polar $V_m$ follow a similar, if delayed, trend, initially peaking at 1.4 V at $\sim 0.88 \mu s$, then exhibit damped oscillation approaching 1.36 V.

The rise-time effect stems from two sources. First, the rise-time is associated with high frequency components that cause the dielectric properties of the membrane to become significant, making the membrane impedance relatively insensitive to changes in membrane conductance. Thus, the peak $V_m$ and $N$ reach greater values before $V_m$ is driven toward $\sim 1 \text{ V}$ and pore creation ceases.

The second source of the rise-time effect is the inability of the increased membrane conductance that accompanies electroporation to sustainably drive down $V_m$ as the applied voltage continues to increase. After the initial peak in $V_m$ and initial burst of pore creation, the $V_m$ and pore creation rate decrease. But if the applied voltage continues to increase, at some point $V_m$ must again begin to increase, and a second, albeit lower, peak in $V_m$ and burst in pore creation ensue. The $V_m$ and the pore creation rate continue to oscillate as they approach a steady $V_m$ and associated constant pore creation rate that linearly increase the membrane conductance at a rate that prevents further swings in voltage. This oscillatory approach to constant $V_m$ is shown in Fig. 4-13c, e. This effect is also demonstrated by the conventional electroporation pulse in Fig. 4-3a. The CM polar $V_m$ settle to a constant $V_m$ at the end of the rise-time, but as soon as the pulse plateau begins, the frequency components shift, and the polar $V_m$ begins to decrease.

Fig. 4-13 shows the $V_m$ and $N$ at the poles. In a cell, these quantities vary not only as
functions of time but also of space. Consequently, one region of membrane may electroporate during the pulse rise-time while a nearby region of membrane electroporates after the rise-time. This leads to the abrupt spatial changes in $V_m(\Theta)$ and $N(\Theta)$ seen in Fig. 4-9.

Figures 4-14 and 4-15 show the peak polar $V_m$ and $N$ as they vary with the rise-time and applied electric field. In Fig. 4-14, sawtooth pulses are applied and $t_{rise}$ and $E_{app}$ are set such that all pulses deliver the same energy ($E_{app}^2 t_{rise} = \text{constant}$) as the rise-time of the 10 ns, 10 MV m pulse. In Fig. 4-15, $t_{rise}$ is fixed at 1.5 ns and $E_{app}$ is varied from 0.1 to 30 MV m.

For the equi-energy set of sawtooth pulses (Fig. 4-14), the long $t_{rise}$ pulses do not cause electroporation because the associated $E_{app}$ do not charge the membranes beyond the threshold for REB. As $t_{rise}$ decreases (and $E_{app}$ increases), the peak polar $V_m$ increase and the threshold for REB is exceeded, first for the CM poles, and then with progressively shorter $t_{rise}$ (larger $E_{app}$), the LOM and SOM poles. For $t_{rise} < 20 \text{ ns}$ ($E_{app} > 2.24 \text{ MV m}$),
both poles of the CM, LOM, and SOM electroporate. Further decreases in $t_{\text{rise}}$ (increases in $E_{\text{app}}$) cause higher peak $V_m$ and $N$ at all of the poles, except the SOM cathodic pole, as dielectric properties increasing affect the membrane-electrolyte voltage division and render the transmembrane potential less sensitive to changes in its conductance. Interestingly, as $t_{\text{rise}}$ decreases below $\sim 10$ ns, the peak $V_m$ and $N$ actually decrease at the cathodic pole of the SOM (Fig. 4-14) because the dielectric properties of the electrolyte become significant, decreasing the fraction of the total voltage applied voltage falling across membrane. Consequently, the anodic membrane pole simply does not have time to overcome the resting potential and significantly exceed the threshold for REB. The charging time constant is dependent on the membrane radius, though it does become less dependent at high frequencies.

An interesting feature of the set of sawtooth pulses is the very clear separation of the pulse rise-time, electric-field magnitude combinations at which electroporation occurs for the membranes (Fig. 4-14). More than an order of magnitude in rise-time separates the electroporation of the CM from the LOM and the LOM from the SOM. On the contrary, very small changes in $E_{\text{app}}$ separate the electroporation of the membranes for the 10 ns pulses (Fig. 4-12), and very short times separate the electroporation of the membranes in response to 10 ns pulses of $2 \text{ MV}_m$ and $10 \text{ MV}_m$ magnitudes (Figs. 4-10, 4-11). Whether this selectivity is unique to sawtooth pulses of the energy examined here or whether it is a more robust effect is not clear, but the ability to selectively electroporate organelles based on size with a fair degree of precision would certainly be useful in the electro-manipulation of the cells.

For the 1.5 ns rise-time set of sawtooth pulses of varying magnitude (Fig. 4-15), pulses with $E_{\text{app}}$ less than $\sim 8 \text{ MV}_m$ do not significantly electroporate any of the membranes poles. As $E_{\text{app}}$ is increased beyond $8 \text{ MV}_m$, the peak $V_m$ of the poles max out at $\sim 1.67$ V and the CM, LOM, and SOM poles electroporate. All of the membrane poles extensively
electroporate by $12 \text{MV}_m$. Figure 4-12 shows the peak polar $V_m$ and $N$ vs. $E_{app}$ at the end of the pulse plateau (8.5 ns) for 10 ns pulses with 1.5 ns rise- and fall-times. The pulses that electroporate extensively during the rise-time (Fig. 4-15,b) do not further electroporate during the pulse plateau (Fig. 4-12). Additionally, just as there are notches in Fig. 4-9 where the membranes switch from electroporating during the pulse plateau to pulse rise-time to during the plateau, there are slight notches Fig. 4-12 present where the membranes switch from electroporating during the pulse rise-time to during the plateau.
Chapter 5

Tissue Electroporation

Electroporation has traditionally been used as a laboratory tool for cell loading rather than as a clinical tool for drug delivery, in part because the mechanisms and effects of electroporation on single cells, let alone tissue, are still poorly understood. Electroporation of widely-spaced single cells in vitro provides researchers a simpler, more uniform, more accessible system for studying electroporation, and in vitro electroporation has therefore remained the primary experimental system. Some general features of tissue electroporation can be inferred based on the responses of single cells to similar pulses because tissue really is just a large collection of single cells held together by extracellular matrix. However, this only works to a point because it assumes that the electrical environment of a single cell in tissue is known, which in general it is not because of the tremendous spatial heterogeneity in the electrical response of tissue. This heterogeneity is not just a function of the simple variation in the electric field with position but also a function of the changing tissue impedance with time. That is, one cannot generally consider the electrical response of a single cell at a single position in the tissue based on a prediction of the electric field seen by the cell in passive tissue because the electric field at each position is a function of the changing impedance of the surrounding tissue.

The results presented here explore the dynamic electrical tissue response to a variety of
electric pulses using the multiscale model described in Chp. 3. The tissue electrical properties closely resemble those of actual tissue (Fig. 3-10), and the membrane impedance, and therefore the tissue impedance, is based on the established asymptotic model of electroporation. Thus, the model combines the experimentally measured macroscopic properties of tissue with knowledge of membrane electroporation gained from cell electroporation experiments to create a complete model that respects the tissue electrical properties at both the macroscopic and microscopic scales, thereby allowing a more sophisticated theoretical examination of tissue electroporation than previous models.

This chapter begins with an in-depth examination of tissue responses to four pulse strength-duration combinations (Sec. 5.1): 100 ms, $0.01 \frac{\text{MV}}{\text{m}}$; 1 ms, $0.1 \frac{\text{MV}}{\text{m}}$; 10 µs, $1 \frac{\text{MV}}{\text{m}}$; and 100 ns, $10 \frac{\text{MV}}{\text{m}}$. The total tissue current and impedance, which are experimentally measurable quantities, are then assessed (Sec. 5.2). The summary results of the tissue response to pulses of a wide range of strengths and durations are then presented (Sec. 5.3). Finally, the effects of electrode size and spacing on the tissue responses to applied electric fields are described (Sec. 5.4).

5.1 Responses to Pulses of Varying Strength and Duration

The response of a cell at any given location in the tissue domain will be reasonably similar to that of a cell in the previous chapter exposed to the same electric field. However, the electric field to which a given cell in the tissue will be exposed cannot be determined a priori because the field at a particular location will depend not only on what voltage is applied between the electrodes, but also the electrical response of the rest of the tissue. The electroporation of the region near the electrode, for example, decreases the impedance of that region, and a greater proportion of the total voltage drop shifts to un-electroporated regions. The effect of the spatial variation in the electric field was
also seen in the cell model, in which the electric field experienced by the organelles was determined in part by the response of the cell membrane to the applied field. The degree to which the time-varying voltage division is important will depend on the pulse strength and duration.

Some of the same features noted in the cell response to applied electric fields are also seen in the tissue response. The rise-time effect (Sec. 4.3.6), in which transmembrane potential and pore creation are sustained at a particularly high level when peaking during the pulse rise-time, occurs in response to large applied fields, particularly near the electrodes, where the electric field magnitude is largest. The applied pulse frequency content is an important determinant of the tissue response to applied fields, just as it was an important determinant of the cell response (Sec. 4.3.1). When the frequency content is high, the membrane impedance is determined primarily by its dielectric properties, and making it relatively insensitive to changes in membrane conductance by electroporation until the pore densities become particularly high (Fig. 4-5).

### 5.1.1 Tissue voltage division

Just as there is voltage division between the membrane and the electrolyte, which is dependent on electric field frequency and pore density, in the tissue system there is also voltage division on the macroscopic scale between adjacent regions of tissue, which is also dependent on electric field frequency and pore density. That is, the electroporation of one region of tissue decreases its impedance, and therefore the fraction of the applied voltage that drops across it also decreases. Consequently, the fraction of the applied voltage that drops across an adjacent region, perhaps not yet significantly electroporated, increases. This leads to a dynamic electrical behavior in response to certain pulses.

Before exploring the tissue response in depth, it is worthwhile to examine in a general
sense how electroporation affects the impedance of the tissue with the assumption that displacement currents are negligible. This assumption clearly is not valid on short time scales, but aids in developing basic concepts. Moreover, the shifting voltage division is of the greatest importance on the longer time scales of conventional electroporation pulses, for which this assumption is reasonable.

The total resistance of the cell unit, \( R_u \), is the parallel combination of the cell resistance, \( R_c \), and the shunt resistance, \( R_s \). \( R_c = 2R_m + R_e + R_i \), where \( R_m \) is the resistance of each membrane (there are two), \( R_e \) is the resistance of the extracellular space (non-shunt), and \( R_i \) is the resistance of the intracellular space. Because \( R_s \), \( R_e \), and \( R_i \) are fixed, \( R_s \) can be expressed in terms of \( R_e \) and \( R_i \) as \( R_s = \beta (R_e + R_i) \). With the parameters in this model, \( \beta = 6.146 \). \( R_u \) may be expressed in terms of the other resistances:

\[
R_u = \frac{R_s}{1 + \frac{R_s}{R_c}} = \frac{\beta (R_e + R_i)}{1 + \frac{\beta (R_e + R_i)}{2R_m + R_e + R_i}}
\]  

(5.1)

The voltage drop across the cell unit is \( V_u \) and the total voltage drop across both membranes is \( 2V_m \). Therefore, the total voltage drop across the intracellular and extracellular electrolyte regions is \( V_u - 2V_m \). Because voltage divides across series resistors in proportion to their resistances, the fraction in the denominator of Eq. 5.1 may be recast in terms of voltages:

\[
R_u = \frac{\beta (R_e + R_i)}{1 + \frac{\beta (V_u - 2V_m)}{V_u}} = \frac{\beta (R_e + R_i)}{1 + \beta \left(1 - \frac{2V_m}{V_u}\right)}
\]  

(5.2)

Let \( R_u^0 \) equal \( R_u \) prior to electroporation. Before electroporation, \( R_m \gg R_e + R_i \) and therefore \( 2V_m \approx V_u \) and the denominator of the \( R_u^0 \) expression is one, and \( R_u^0 = \beta (R_e + R_i) = R_s \). Thus, prior to electroporation, the high resistance of the transcellular pathway renders it inaccessible to current flow. Consequently, all current flows through the shunt pathway, and the total tissue unit resistance is simply that of the shunt pathway.
5.1 Responses to Pulses of Varying Strength and Duration

The ratio $R_u/R_0$ provides a useful metric for the effect that electroporation has in lowering the cell unit resistance:

$$\frac{R_u}{R_0} = \frac{\frac{\beta(R_e+R_i)}{1+\beta\left(1-\frac{2V_m}{V_u}\right)}}{\frac{1}{1+\beta\left(1-\frac{2V_m}{V_u}\right)}}. \quad (5.3)$$

$V_u$ may be expressed in terms of the electric field magnitude, $E$, and the length of the cell unit, $l_u$, as $V_u = El_u$. Therefore,

$$\frac{R_u}{R_0} = \frac{1}{1 + \beta\left(1 - \frac{2V_m}{El_u}\right)}. \quad (5.4)$$

Prior to electroporation, $R_u/R_0 = 1$. Electroporation causes $V_m$ to decrease such that $V_m \approx 1\, V$, independent of $E$. Thus, after electroporation $R_u/R_0$ is purely a function of $E$. $R_u/R_0$ quickly asymptotes to $\frac{1}{1+\beta}$ as $E$ is increased. In fact, increasing $E$ from $92.2\, kV/m$, at which $El_u = 2V_m = 2\, V$ and $R_u/R_0 = 1$, to $110\, kV/m$ decreases $R_u/R_0$ to 0.5.

The shifting voltage division that accompanies electroporation is most significant in altering the tissue response in regions where $E \approx 10^5\, V/m$. Electric fields of this magnitude are just large enough to cause electroporation. Moreover, the tissue resistance is very sensitive to fields of this magnitude, and fields that exceed $92.2\, kV/m$, even by a small margin, can significantly decrease the resistance of the region by electroporation. The decreased resistance shifts a greater proportion of the total voltage drop to adjacent regions, increasing $E$ in these regions. If $E$ was not initially large enough to electroporate these adjacent regions, the shift in the field may be enough to increase $E$ enough to cause electroporation.

For larger electric field magnitudes (e.g. $10^6\, V/m$), the tissue resistance is relatively insensitive to the electric field magnitude. Therefore, in tissue regions where the electric field is large, there will not be much spatial variation in the tissue impedance with the variation
in the electric field magnitude. Moreover, the largest electric fields are applied with the shortest duration pulses, and for such short pulses, the frequency content is high and the contribution of the membrane conductance to the total tissue impedance, with or without electroporation, is minimal. On the contrary, in regions where $E \approx 10^5 \frac{V}{m}$, the spatial variation in the tissue resistance will vary considerably with the variation in the electric field magnitude, and therefore, in these regions, the electroporation of tissue will significantly impact the electroporation of neighboring tissue.

5.1.2 100 ms, $0.01 \frac{MV}{m}$ pulse

A range of applied electric field strengths and durations have been applied to tissue experimentally [29]. Many have used conventional electroporation pulses of long duration, particularly for transfecting cells. This section examines just such a pulse. A 100 ms, $0.01 \frac{MV}{m}$ pulse with 10 $\mu$s rise- and fall-time is applied. Here the nominal applied electric field, $E_{app}$, is defined to be the applied voltage, $V_{app}$, divided by the electrode spacing, $L_e$. It should be emphasized that this is exactly the type of pulse for which the asymptotic model of electroporation, on which this tissue model is based, is least appropriate. For low magnitude pulses of such long duration, the energetically favorable increase in membrane conductance is not dominated by the rapid creation of many minimally sized pores, but rather by the expansion of the few pores created or already present [41]. This model does not account for such pore expansion. However, the overall electrical result of electroporation, whether dominated by pore creation or expansion, is the decrease of the transmembrane potential toward $\sim 1$ V, and thus the electrical responses predicted by this model are generally quite robust. It is the size and number of pores that is less well-defined, at least for low magnitude, long duration pulses.

In response to the $0.01 \frac{MV}{m}$ pulse, the electric field magnitude throughout most of the tissue is too small to charge the transmembrane potentials to anywhere near the threshold for reversible electrical breakdown (REB) ($\sim 1$ V). As such, little will be noted about the
tissue response to this pulse.

Spatial response

Figure 5-1 shows the spatial tissue response to a 100 ms, 0.01 MV pulse with 10 µs rise- and fall-times. The electric potential, $\phi$, and electric field magnitude, $E$, remain essentially unchanged during the pulse (Fig. 5-1a, b). The transmembrane potentials, $V_m$, throughout the domain charge to their maximum, final values, all significantly less than 1 V, early in the pulse (Fig. 5-1c). Because $V_m$ are so small, only the regions very near the electrodes, where $E$ and $V_m$ are largest, electroporate, and even there the pore densities, $N$, increase by only about an order of magnitude (Fig. 5-1d) over the duration of the pulse. It is because so few pores form that the tissue impedance remains unchanged and $E$ remains constant.

After the pulse, $\phi$, $E$, and $V_m$ rapidly approach zero (within microseconds) (Fig. 5-1e–g). The pore densities decay to their basal value with a time constant of 1.5 s.

Spatial response along electrode centerline

Figure 5-2 shows the tissue response to a 100 ms, 0.01 MV pulse along the electrode centerline ($y = 0$ mm). These results show the tissue response at the same time points as in the spatial results of Fig. 5-1.

$\phi$ and $E$ remain essentially unchanged during the pulse (Fig. 5-2a, b). The $E$ at the inner surfaces of the electrodes are 51.0 kV/m, while the $E$ at the tissue center is an order of magnitude smaller at 5.12 kV/m. The $V_m$, which is proportional to $E$ on long time scales with negligible electroporation, are 0.552 V on the inner surfaces of the electrodes and 0.0555 V at the tissue center (Fig. 5-2c). There is very limited electroporation near the electrodes. $N$ increases by only an order of magnitude over the equilibrium pore density (Fig. 5-2d). The $N$ on the outer surfaces of the electrodes are slightly smaller than on the
During pulse

(a) Electric potential $\phi$ (V)

(b) Electric field magnitude $E_{\text{m}}$ ($V/m$)

(c) Transmembrane potential $V_{\text{m}}$ (V)

(d) Pore density $N$ (pores/mm²)

(e) Tissue Electroporation

<table>
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<tr>
<th>Time</th>
<th>0.01 ms</th>
<th>0.1 ms</th>
<th>1 ms</th>
<th>10 ms</th>
<th>99.99 ms</th>
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<tr>
<td>$\phi$ (V)</td>
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<td>50</td>
<td>100</td>
<td>150</td>
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<td>$E_{\text{m}}$ ($V/m$)</td>
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<td>$10^4$</td>
<td>$10^5$</td>
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<td>$10^7$</td>
</tr>
<tr>
<td>$V_{\text{m}}$ (V)</td>
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<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>$N$ (pores/mm²)</td>
<td>10</td>
<td>100</td>
<td>1000</td>
<td>10000</td>
<td>100000</td>
</tr>
</tbody>
</table>
### Figure 5-1: Spatial response: 100 ms, 0.01 $\text{MV/m}$ pulse. Electric potential, electric field magnitude, transmembrane potential, and pore density near the electrodes (a)–(d) during and (e)–(h) after a 100 ms, 0.01 $\text{MV/m}$ pulse with 10 µs rise- and fall-times. On each plot, 21 (for $\phi$) or 11 (for $E$, $V_m$, and $N_p$) contour lines are spaced evenly between the extreme values of the colorbar.
inner surfaces because the $E$ and $V_m$ are greater on the inner surfaces of the electrodes than on the outer surfaces.

$\phi$, $E$, and $V_m$ quickly decrease in the microseconds following the pulse (Fig. 5-2), while the slightly elevated $N$ at the electrodes persist for seconds (not shown; time scale too long).
5.1 Responses to Pulses of Varying Strength and Duration

Figure 5-3: Temporal response at points along centerline: 100 ms, 0.01 MV/m pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density at points along the centerline (y = 0 mm) in response to a 100 ms, 0.01 MV/m pulse with 10 µs rise- and fall-times. Positions, x, plotted (from blue to red) are −5 (anode surface), −3.38, −1.85, and 0 mm (midpoint between electrodes).

Temporal response at points along the electrode centerline

Figure 5-3 shows the response of tissue at four approximately linearly-spaced points between the anode and the tissue center. At all four locations, the φ and E reach their peak values at the end of the rise-time (10 µs) and then remain unchanged for the remainder of the pulse. The $V_m(t)$ profiles closely resemble the $\phi(t)$ profiles because the membrane charging time constant is significantly smaller than the pulse rise-time, and
$V_m(t)$ thus maintains quasi-steady-state during the pulse rise-time. $N$ slightly increases at the electrode surface in a linear fashion because the $N$ remains too low to significantly alter $V_m$. $N$ does not change at points farther from the electrode. $\phi$, $V_m$, and $E$ quickly decrease in the microseconds following the pulse, while the slightly elevated $N$ at the electrodes persist for seconds (not shown; time scale too long).

### 5.1.3 1 ms, 0.1 MV/m pulse

The response of tissue to the 0.1 MV/m, 1 ms pulse is more dynamic than the response to the 0.01 MV/m, 100 ms pulse because the electric field is sufficiently large to charge cell membranes throughout the region between the electrodes beyond the threshold for REB ($\sim 1$ V). For this pulse, the shifting voltage division that accompanies electroporation plays an important role in the overall tissue response.

**Spatial response**

Figure 5-4 shows the spatial tissue response to a 1 ms, 0.1 MV/m pulse with 1 $\mu$s rise- and fall-times. The pulse rise-time is 1 $\mu$s, and little electroporation occurs during this phase of the pulse except very near the electrodes (Fig. 5-4, 1 $\mu$s). Thus, $\phi$ and $E$ initially have the same appearance as in the 0.01 MV/m pulse (scaled by 10) (Fig. 5-1b). $V_m$ is elevated near the electrodes but remains low in the central tissue region.

By 5 $\mu$s, the region of electroporated tissue around the electrodes grows, and the sites of maximal $V_m$ move out from the electrodes (Fig. 5-4, 5 $\mu$s). On the time scale of this pulse, the conductive properties of the membrane primarily determine the membrane impedance. Consequently, as the region near the electrode electroporates, the impedance of the tissue decreases, which decreases $E$ near the electrodes and increases it in the central region of tissue, thereby increasing $V_m$ in the central tissue region.

As the pulse progresses, a wave of elevated $V_m$ moves outward from the electrodes into the
central region of tissue (Fig. 5-4b). A wave of electroporation accompanies the wave of elevated $V_m$ because $V_m$ exceeds the threshold for REB ($\sim 1$ V). $N$ remains significantly lower in the central region of tissue than near the electrodes because $V_m$ peaks at lower values in the central region of tissue (Fig. 5-4c, d). Thus, while the tissue impedance is decreased throughout the tissue between the electrodes, it is decreased most near the electrodes and least in the central region of tissue. Without electroporation (e.g. at a lower applied field strength), $E$ would be $\sim 10$ times larger at the electrode surface than in the center of the tissue. However, because of the gradient in tissue impedance, $E$ becomes very uniform between the electrodes by the end of the pulse (Fig. 5-4b, 999 µs).

Following the pulse, the potential difference between the electrodes is 0 V (Fig. 5-4e). However, the membranes of the tissue near the electrodes are still charged. The regions near the electrodes, which prior to the end of the pulse had had the highest $\phi$ (in magnitude), abruptly have the lowest $\phi$ (in magnitude). The relative maxima of $\phi$ (in magnitude) are then located in the tissue adjacent to the electrodes. Consequently, $\vec{E}$ changes direction and remains very large in magnitude near the electrodes, goes to zero at the points of maximal $\phi$ (in magnitude), and has relatively uniform, intermediate magnitude in the central region of tissue (Fig. 5-4f). Because $V_m$ is largely determined by $E$, the general appearance of $V_m$ after the pulse is similar to that of $E$. $\phi$, $V_m$, and $E$ quickly decay, particularly in the region between the electrodes where the tissue impedance is lowest (Fig. 5-4e, f, g). $N$ decays with a 1.5 s time constant (not shown; time scale too long).

Spatial response along electrode centerline

Figure 5-5 shows the tissue response to a 1 ms, 0.1 MV/m pulse along the electrode centerline ($y = 0$ mm). These results show the tissue response at the same time points as in the spatial results of Fig. 5-4.
During pulse

(a) Electric potential 
\[ \phi \ (V) \]

(b) Electric field magnitude 
\[ E \ (V/m) \]

(c) Transmembrane potential 
\[ V_m \ (V) \]

(d) Pore density 
\[ N \ (\text{pores}/\text{mm}^2) \]

(e) Transmembrane potential 
\[ V_m \ (V) \]

Time

- 1 µs
- 5 µs
- 31 µs
- 180 µs
- 999 µs
5.1 Responses to Pulses of Varying Strength and Duration

Figure 5-4: Spatial response: 1 ms, 0.1 MV/m pulse. Electric potential, electric field magnitude, transmembrane potential, and pore density near the electrodes (a)–(d) during and (e)–(h) after a 1 ms, 0.1 MV/m pulse with 1 µs rise- and fall-times. On each plot, 21 (for φ) or 11 (for E, V_m, and N_p) contour lines are spaced evenly between the extreme values of the colorbar.
Figure 5-5: Spatial response along centerline: 1 ms, 0.1 MV/m pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density along the centerline \((y = 0 \text{ mm})\) in response to a 1 ms, 0.1 MV/m pulse with 1 µs rise- and fall-times. Times shown (from blue to red) are 1, 5, 31, 180, and 999 µs during the pulse (solid lines) and 0, 0.6, and 6 µs after the pulse (dashed lines).

\(E\) and \(V_m\) quickly rise at the electrodes at the start of the pulse, and their profiles assume “scooped” appearances between the electrodes (Fig. 5-5a, b). As the threshold for REB is surpassed at the electrodes, \(N\) increases dramatically, decreasing the membrane impedance, thereby decreasing \(V_m\) (Fig. 5-5c, d). While the peak \(E\) remain at the electrodes, the peak \(V_m\) move outward from the electrodes, such that the \(V_m\) profile remains “scooped” at the tissue center and the distance between the \(V_m\) peaks decreases with time. A wave of pore creation accompanies the wave of elevated \(V_m\), but neither \(V_m\) nor
5.1 Responses to Pulses of Varying Strength and Duration

\( N \) reach as high of values as they did at the electrodes where \( E \) is largest. As the wave of electroporation moves toward the tissue center, the electroporated tissue in its wake has decreased impedance. On the time scale of electroporation for this pulse, the membrane impedance is dominated by its conductive properties. Thus, the voltage drop across the electroporated tissue nearer the electrodes is decreased and the voltage drop across the un-electroporated tissue center is increased, elevating \( E \) and \( V_m \) at the tissue center to values in excess of what they would reach without shifting voltage division. Note that without shifting voltage division, \( E \) and \( V_m \) at the tissue center (assuming no electroporation) would be 10 times their values in response to the \( 0.01 \, \text{MV}_m \) pulse, \( 51.2 \, \text{kV}_m \) and 0.555 V. Instead, \( E \) and \( V_m \) at the tissue center eventually peak at \( 95.4 \, \text{kV}_m \) and 1.03 V, nearly twice the magnitudes that a passive model would have predicted (Fig. 5-5b, c).

After the waves of electroporation from each electrode meet at the tissue center, \( E \) and \( V_m \) remain relatively constant spatially across the tissue center (Fig. 5-5b, c). \( V_m \) decreases slowly to \( 91.7 \, \text{kV}_m \) at the tissue center as pore creation continues throughout the pulse. At the end of the pulse, \( N \) is largest near the electrodes at \( 1.12 \times 10^{14} \, \text{pores/m}^2 \) and substantially smaller at the tissue center at \( 1.58 \times 10^{12} \, \text{pores/m}^2 \) (Fig. 5-5d).

After the pulse, \( \phi \) is forced to 0 V at the electrodes, dramatically changing the \( \phi(x) \) profile (Fig. 5-5a). While the peak \( \phi \) occur at the electrodes during the pulse, they occur at \( x = \pm 3.6 \) and \( \pm 6.6 \, \text{mm} \) after the pulse. Consequently, \( \vec{E} \) changes direction in the regions between the electrodes and peak \( \phi \), goes to zero at the peak \( \phi \), and is relatively constant across the tissue center (Fig. 5-5b). Because \( V_m \) is largely determined by \( E \), the \( V_m(x) \) profile follows the same general trends as \( E(x) \) (Fig. 5-5c). \( \phi \), \( V_m \), and \( E \) quickly decay, but \( N \) decays with a 1.5 s time constant (not shown; time scale too long).
Temporal response at points along the electrode centerline

Figure 5-6 shows the response of tissue at four approximately linearly-spaced points between the anode and the tissue center and nicely illustrates the shifting fields accompanying electroporation. The tissue at the electrode electroporates at the end of the 1 µs rise-time (Fig. 5-6d). $E$ at the electrode peaks at the end of the rise-time and then decreases as a greater proportion of the potential drops across the higher impedance, un-electroporated tissue nearer the tissue center. As $E$ drops at the electrode, it increases at the more central points (Fig. 5-6b). $V_m$ peaks and the onset of electroporation begins progressively later with distance from the electrode. Following electroporation, $V_m$ does not drop as precipitously near the electrode center as at the electrode, and pore creation continues at a slow rate at the points near the tissue center. $V_m$ drops to 0.86 V at the electrode because of the rise-time effect (Fig. 5-6c) (Sec. 4.3.6). After the tissue center begins to electroporate and decrease in impedance, some of the voltage shifts back toward the electrodes, and $E$ and $V_m$ slowly increase at the two points nearest the electrode. $\phi$, $V_m$, and $E$ quickly decay (Fig. 5-9a, b, c), but $N$ decays with a 1.5 s time constant (not shown; time scale too long).

The shifting of the voltage drop from the tissue near the electrodes to the tissue center dramatically affects the overall tissue response to the 0.1 MV/m pulse. A passive tissue model, in which the electric field magnitude does not change with time, would not predict significant electroporation at any of the three points closest to the tissue center because a passive tissue model would predict $E$ and, by extension, $V_m$ that are 10 times those in response to the 0.01 MV/m pulse (Fig. 5-3c), 0.943, 0.632, and 0.555 V. With an active model, it is clear that the $V_m$ actually peak at much larger values of 1.21, 1.10, and 1.03 V (Fig. 5-6c), thereby causing significant electroporation with $N$ reaching $5.22 \times 10^{12}$, $2.26 \times 10^{12}$, and $1.58 \times 10^{12}$ pores/m$^2$ (Fig. 5-3d). Note that $E \approx 10^5 \frac{V}{m}$ between the electrodes (Fig. 5-5b), precisely the magnitude for which voltage division was shown to be a major determinant of the spatial extent of electroporation (Sec. 5.1.1).
5.1 Responses to Pulses of Varying Strength and Duration

(a) Electric potential

(b) Electric field magnitude

(c) Transmembrane potential

(d) Pore density

Figure 5-6: Temporal response at points along centerline: 1 ms, 0.1 MV\textsubscript{m} pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density at points along the centerline (y = 0 mm) in response to a 1 ms, 0.1 MV\textsubscript{m} pulse with 1 \( \mu \)s rise- and fall-times. Positions, \( x \), plotted (from blue to red) are −5 (anode surface), −3.38, −1.85, and 0 mm (midpoint between electrodes).

5.1.4 10 \( \mu \)s, 1 MV\textsubscript{m} pulse

The response of tissue to the 1 MV\textsubscript{m}, 10 \( \mu \)s pulse is much more spatially extensive than the response to the 0.1 MV\textsubscript{m}, 1 ms pulse because the electric field is sufficiently large and the duration sufficiently long to charge membranes relatively far from the electrodes beyond the threshold for REB. Shifting voltage division plays a smaller part in the electroporation of the central tissue region because the electric field magnitude is larger.
and for the time scale on which the central tissue region electroporates, the dielectric properties of the membrane are significant and decrease the extent to which increased membrane conductance affects the total tissue impedance and voltage division. However, the response of more peripheral regions of tissue occurs over a longer time scale and at lower electric field magnitude, and shifting voltage division therefore significantly affects the overall spatial extent of electroporation in response to the $1 \text{ MV}_m$ pulse.

**Spatial response**

Figure 5-7 shows the spatial tissue response to a $10 \mu s$, $1 \text{ MV}_m$ pulse with $0.1 \mu s$ rise- and fall-times. The pulse rise-time is $0.1 \mu s$, and little electroporation occurs during this phase of the pulse except very near the electrodes. Thus, $\phi$ and $E$ initially have the same appearance as in the $0.01 \text{ MV}_m$ pulse (scaled by 100) (Fig. 5-1b). $V_m$ is elevated near the electrodes but remains low in the central tissue region.

The wave of elevated $V_m$ and pore creation quickly moves out from the electrodes, and by $1 \mu s$, the entire central tissue region is significantly electroporated (Fig. 5-7d). There is some shifting of voltage division from the electrode region to the central tissue region, but it has less of an impact than in the response to the $1 \text{ ms}, 0.1 \text{ MV}_m$ pulse because $E$ is sufficiently large to electroporate the central tissue with or without any added contribution from the decreased impedance of the tissue near the electrodes (Fig. 5-7b). However, the shifting voltage division does contribute to the electrical behavior and electroporation of the more peripheral regions of tissue.

By $1 \mu s$, all tissue near the electrodes is electroporated and the impedance of this region is decreased (Fig. 5-7d, $1 \mu s$). Thus, the proportion of the voltage drop across the tissue near the electrodes decreases and the proportion of the voltage drop across the more peripheral tissue must therefore increase. This effect is shown by the shift of the equi-electric field magnitude lines outward as the wave of electroporation moves outward.
This outward shift is primarily in the $y$-direction (Fig. 5-7b). In fact, the equi-electric field magnitude lines nearest the outsides of the electrodes actually shift in toward the electrodes toward the end of the pulse. The shift may be understood by considering the effect that electroporation has on the electric field. Initially, the electric field looks like that of a simple dipole; the field lines intersect the equipotential lines in Fig. 5-7a, 0.1 $\mu$s at right angles. As the tissue between the electrodes electroporates and the impedance of this region decreases relative to that of the more peripheral tissue, more electric field lines begin to squeeze into the low impedance region between the electrodes. In doing so, the lines become straighter and closer as they cross the line $x = 0$. The equipotential lines, which are normal to the electric field lines, must then rotate in toward $x = 0$, and indeed they do (Fig. 5-7a) as the pulse progresses. As the density of the electric field lines increases, $E$ also increases and causes electroporation. Near the outer edges of the electrodes, the field lines begin to preferentially turn toward the opposite electrode to remain in the lowest impedance tissue. Thus, the density of equipotential lines outside the electrodes decreases as these lines rotate toward $x = 0$, and $E$ decreases in these regions (Fig. 5-7a, b). The shifting electric field lines that accompany the tissue electroporation cause the electroporated region to become nearly circular and quite extensive by the end of the pulse (Fig. 5-7d). Based on the $V_m$ of the 0.01 $\text{MV}_m$ pulse, a passive tissue model would have predicted an electroporation footprint more like that at 1 $\mu$s (Fig. 5-7d, 1 $\mu$s). Another result of the shifting $E$ is the set of $V_m$ minima that develop outside the electrodes near $y = 0$ (Fig. 5-7c). These regions become highly electroporated early in the pulse when $E$ is large. If $E$ were held constant, $V_m$ would be expected to approach $\sim 1\text{ V}$ in these regions, but because $E$ decreases later in the pulse, the $V_m$ decrease to well below 1 $\text{ V}$ (Fig. 5-7b, c).

Following the pulse, the potential difference between the electrodes is 0 $\text{ V}$ (Fig. 5-7e). However, the membranes of the tissue near the electrodes are still charged. The regions near the electrodes, which prior to the end of the pulse had had the highest $\phi$
During pulse

<table>
<thead>
<tr>
<th>Time</th>
<th>0.1µs</th>
<th>0.315µs</th>
<th>1µs</th>
<th>3µs</th>
<th>9.9µs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric potential</td>
<td>φ (kV)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E (V/m)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>N (pores/µm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_m (V)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.1 Responses to Pulses of Varying Strength and Duration

After pulse

(e) Electric potential $\phi$ (V)

(f) Electric field magnitude $E_{\text{field}}$ (V/m)

(g) Transmembrane potential $V_{\text{m}}$ (V)

(h) Pore density $N$ (pores/m$^2$)

Figure 5.7: Spatial response: 10 $\mu$s, 1 MV/m pulse. Electric potential, electric field magnitude, transmembrane potential, and pore density near the electrodes (a)–(d) during and (e)–(h) after a 10 $\mu$s, 1 MV/m pulse with 0.1 $\mu$s rise- and fall-times. On each plot, 21 (for $\phi$) or 11 (for $E_{\text{field}}$, $V_{\text{m}}$, and $N$) contour lines are spaced evenly between the extreme values of the colorbar.
(in magnitude), abruptly have the lowest \( \phi \) (in magnitude). The relative maxima of \( \phi \) (in magnitude) are then located in the tissue adjacent to the electrodes. Consequently, \( \vec{E} \) changes direction and remains very large in magnitude near the electrodes, goes to zero at the points of maximal \( \phi \) (in magnitude), and has relatively uniform, intermediate magnitude in the central region of tissue (Fig. 5-7f). \( V_m \) decreases most quickly around and between the electrodes, where \( N \) is largest, and \( V_m \) decreases somewhat more slowly in the regions with elevated \( V_m \) that are still electroporating at the end of the pulse. \( \phi \), \( V_m \), and \( E \) quickly decay, particularly in the region between the electrodes where the tissue impedance is lowest (Fig. 5-7e, f, g). \( N \) decays with a 1.5 s time constant (not shown; time scale too long).

**Spatial response along electrode centerline**

Figure 5-8 shows the tissue response to a 10 \( \mu \)s, 1 MV/m pulse along the electrode centerline \((y = 0 \text{ mm})\). These results show the tissue response at the same time points as in the spatial results of Fig. 5-7.

\( E \) and \( V_m \) quickly rise at the electrodes at the start of the pulse and electroporate the tissue nearest the electrodes. A wave of elevated \( V_m \) moves out from the electrodes, leaving in its wake high \( N \) and \( V_m \) approaching \( \sim 1 \text{ V} \) (Fig. 5-8b, c, d). In contrast to the 0.1 MV/m pulse, \( E(x) \) changes little between the electrodes over the course of the pulse because the region becomes quite electroporated early in the pulse and the tissue impedance becomes a weak function of \( E \). Therefore, the impedance does not vary much differently (relatively) after electroporation than before it, leading to the unchanging \( E(x) \) between the electrodes with \( E \) at the electrodes \( \sim 10 \) times larger than \( E \) at the tissue center (Fig. 5-8b). Waves of elevated \( V_m \) and pore creation continue to move out from the outsides of electrodes for the duration of the pulse. The peak \( V_m \) and \( N \) decrease with distance from the electrodes because \( E \) decreases. For \( |x| > 10 \text{ mm} \), where \( E \approx 10^5 \frac{V}{m} \), shifting voltage division contributes to the large spatial extent of electroporation with \( E \) initially increas-
5.1 Responses to Pulses of Varying Strength and Duration

![Graphs of electric potential, electric field magnitude, transmembrane potential, and pore density along the centerline](image)

**Figure 5-8:** Spatial response along centerline: 10 µs, 1 MV/m pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density along the centerline (y = 0 mm) in response to a 10 µs, 1 MV/m pulse with 0.1 µs rise- and fall-times. Times shown (from blue to red) are 0.1, 0.315, 1, 3, and 9.9 µs during the pulse (solid lines) and 0, 0.3, and 3 µs after the pulse (dashed lines).

...ing and then decreasing following electroporation (Fig. 5-8b, d). This allows, over time, a sufficiently large $E$ to electroporate tissue to extend quite far beyond the electrodes. By the end of the pulse, $E(x)$ is quite uniform and $E \approx 10^5 \frac{V}{m}$ for $10 \text{mm} < |x| < 15 \text{mm}$, just as it was between the electrodes in response to the 0.1 MV/m pulse (Fig. 5-8b).

...pulse, $\phi$ is forced to 0 V at the electrodes, dramatically changing the $\phi(x)$ profile (Fig. 5-8a). While the peak $\phi$ occur at the electrodes during the pulse, they occur at
$x = \pm 3.6$ and $\pm 13.2$ mm after the pulse. The minima outside the electrodes occur much farther out than in response to the end of the $0.1 \text{ MV}_m$ pulse because of the greater extent of electroporation and decreased impedance in response to the $1 \text{ MV}_m$ pulse. As a result of the dramatic change in $\phi(x)$, $\vec{E}$ changes direction in the regions between the electrodes and peak $\phi$, goes to zero at the peak $\phi$, and is relatively constant across the tissue center (Fig. 5-8b). Because $V_m$ is largely determined by $E$, the $V_m(x)$ profile follows the same general trends as $E(x)$ (Fig. 5-8c). $\phi$, $V_m$, and $E$ quickly decay, but $N$ decays with a 1.5 s time constant (not shown; time scale too long).

**Temporal response at points along the electrode centerline**

Figure 5-9 shows the response of tissue at four approximately linearly-spaced points between the anode and tissue center. The tissue at the electrode electroporates at the end of the rise-time with $V_m$ peaking at 1.50 V at 77 ns and $N$ reaching $1.25 \times 10^{15}$ pores m$^2$ by the end of the pulse (Fig. 5-9c, d). The other points reach peak $V_m$ and electroporate in order of distance from the anode. At $x = -3.38$ mm, $V_m$ peaks to 1.42 V at 260 ns and $N$ reaches $2.45 \times 10^{14}$ pores m$^2$. At $x = -1.84$ mm, $V_m$ peaks to 1.40 V at 371 ns and $N$ reaches $1.65 \times 10^{14}$ pores m$^2$. At $x = 0$ mm, $V_m$ peaks to 1.40 V at 418 ns and $N$ reaches $1.47 \times 10^{14}$ pores m$^2$ (Fig. 5-9c, d). There is some shifting of voltage division between the electrodes, but it is much less significant than in response to the $0.1 \text{ MV}_m$ pulse. This is shown by slight peaks and subsequent decreases in $E(t)$ and associated changes in $\phi(t)$ (Fig. 5-9a, b). $\phi$, $V_m$, and $E$ decrease very quickly during the pulse fall-time in response to the negatively-oriented voltage division of the pulse end, and then they decrease somewhat more slowly as the membranes discharge (Fig. 5-9a, b, c). $N$ decays with a 1.5 s time constant (not shown; time scale too long).
5.1 Responses to Pulses of Varying Strength and Duration

(a) Electric potential

(b) Electric field magnitude

(c) Transmembrane potential

(d) Pore density

Figure 5-9: Temporal response at points along centerline: 10 µs, 1 MV/m pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density at points along the centerline (y = 0 mm) in response to a 10 µs, 1 MV/m pulse with 0.1 µs rise- and fall-times. Positions, x, plotted (from blue to red) are −5 (anode surface), −3.38, −1.85, and 0 mm (midpoint between electrodes).

5.1.5 100 ns, 10 MV/m pulse

The 100 ns, 10 MV/m pulse has simpler dynamics than the 0.1 and 1 MV/m pulses because the high frequency content of the pulse causes the tissue impedance to be determined largely by the dielectric properties of the membrane and the conductive properties of the aqueous electrolyte, and therefore shifting voltage division does not affect the spatial extent of electroporation. As such, high pore densities are generated in the tissue between and
around the electrodes, but peripheral tissue is unaltered.

Spatial response

Figure 5-10 shows the spatial tissue response to a 100 ns, 10 MV m pulse with 10 ns rise- and fall-times. By the end of the rise-time, a small region surrounding each electrode extensively electroporates and exhibits the rise-time effect (Sec. 4.3.6). During the rest of the pulse, this region maintains significantly higher $N$ and lower $V_m$ than the surrounding tissue not subject to the rise-time effect (Fig. 5-10c, d). $E$ remains large near the electrodes, though it does increase slightly in the central tissue region, because the dielectric properties of the membrane dominate its impedance. Therefore, the relative shift in the membrane impedance, and therefore the tissue impedance, is relatively minor on the time scale of the 100 ns pulse (Fig. 5-10c). As such, there is no dramatic shift of voltage division, as was seen in the responses to the 0.1 and 1 MV m pulses.

The large $E$ quickly charges the membranes around and between the electrodes, and waves of elevated $V_m$ and pore creation rapidly move out from the electrodes, leaving decreased $V_m$ and very large $N$ in their wakes (Fig. 5-10b, c, d). The spatial extent of electroporation is limited because of the short pulse duration. Longer 10 MV m pulses result in more circular electroporation footprints, like that in response to the 1 MV m pulse, that can be quite spatially extensive.

Following the pulse, the potential difference between the electrodes is 0 V (Fig. 5-10e). However, the membranes of the tissue near the electrodes are still charged. The regions near the electrodes, which prior to the end of the pulse had had the highest $\phi$ (in magnitude), abruptly have the lowest $\phi$ (in magnitude). The relative maxima of $\phi$ (in magnitude) are then located in the tissue adjacent to the electrodes. Consequently, $\vec{E}$ changes direction and remains very large in magnitude near the electrodes, goes to zero at the points of maximal $\phi$ (in magnitude), and has relatively uniform, intermediate
magnitude in the central region of tissue (Fig. 5-10f). \( V_m \) decreases most quickly around and between the electrodes, where \( N \) is largest, and \( V_m \) decreases somewhat more slowly in the regions with elevated \( V_m \) that are still electroporating at the end of the pulse. \( V_m \) becomes negative very briefly following the pulse in the regions very near the electrodes because of the negatively-oriented voltage division that accompanies the end of the pulse (Fig. 5-10g). \( \phi \), \( V_m \), and \( E \) quickly decay, particularly in the region between the electrodes where the tissue impedance is lowest (Fig. 5-10e, f, g). \( N \) decays with a 1.5 s time constant (not shown; time scale too long).

**Spatial response along electrode centerline**

\( E \) and \( V_m \) quickly rise at the electrodes at the start of the pulse and electroporate the tissue nearest the electrodes. A wave of elevated \( V_m \) moves out from the electrodes, leaving in its wake high \( N \) and \( V_m \) approaching \( \sim 1 \) V (Fig. 5-11b, c, d). \( E(x) \) changes little between the electrodes over the course of the pulse because the tissue impedance is primarily determined by dielectric properties of the membrane and the conductive properties of the electrolyte, both before and after electroporation, and \( E \) at the electrodes remains \( \sim 10 \) times larger than \( E \) at the tissue center (Fig. 5-11b). Waves of elevated \( V_m \) and pore creation continue outside the electrodes for the duration of the pulse. The peak \( V_m \) and \( N \) decrease with distance from the electrodes because \( E \) decreases, and the \( N \) drop off sharply for \( |x| > 10 \) mm (Fig. 5-11d).

After the pulse, \( \phi \) is forced to 0 V at the electrodes, dramatically changing the \( \phi(x) \) profile (Fig. 5-11a). While the peak \( \phi \) occur at the electrodes during the pulse, they occur at \( x = \pm 3.6 \) and \( \pm 9.1 \) mm after the pulse. As a result of the dramatic change in \( \phi(x) \), \( \vec{E} \) changes direction in the regions between the electrodes and peak \( \phi \), goes to zero at the peak \( \phi \), and is relatively constant across the tissue center (Fig. 5-11b). Because \( V_m \) is largely determined by \( E \), the \( V_m(x) \) profile follows the same general trends as \( E(x) \) (Fig. 5-11c). \( \phi \), \( V_m \), and \( E \) quickly decay, but \( N \) decays with a 1.5 s time constant (not
During pulse

(a) Electric potential
φ (kV)

(b) Electric field magnitude
$E (\text{V/m})$

(c) Transmembrane potential
$V_m (\text{V})$

(d) Pore density
$N (\text{pores/m}^2)$

(e) Tissue Electroporation

Time

- 5ns
- 10ns
- 21ns
- 43ns
- 90ns
### Figure 5-10: Spatial response: 100 ns, 10 MV/m pulse. Electric potential, electric field magnitude, transmembrane potential, and pore density near the electrodes (a)–(d) during and (e)–(h) after a 100 ns, 10 MV/m pulse with 10 ns rise- and fall-times. On each plot, 21 (for $\phi$) or 11 (for $E$, $V_m$, and $N_p$) contour lines are spaced evenly between the extreme values of the colorbar.
Figure 5-11: Spatial response along centerline: 100 ns, 10 MV m pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density along the centerline ($y = 0$ mm) in response to a 100 ns, 0.01 MV m pulse with 10 ns rise- and fall-times. Times shown (from blue to red) are 5, 10, 21, 43, and 90 ns during the pulse (solid lines) and 0, 0.3, and 3 µs after the pulse (dashed lines).

Temporal response at points along the electrode centerline

Figure 5-12 shows the response of tissue at four approximately linearly-spaced points between the anode and tissue center. The tissue at the electrode electroporates relatively early in the rise-time with $V_m$ peaking at 1.62 V at 3.4 ns and $N$ reaching $1.76 \times 10^{16}$ pores m$^{-3}$ by the end of the pulse (Fig. 5-12c, d). Because of the rise-time effect (Sec. 4.3.6), the $V_m$
5.1 Responses to Pulses of Varying Strength and Duration

(a) Electric potential

(b) Electric field magnitude

(c) Transmembrane potential

(d) Pore density

Figure 5-12: Temporal response at points along centerline: 100 ns, 10 MV/m pulse. (a) Electric potential, (b) electric field magnitude, (c) transmembrane potential, and (d) pore density at points along the centerline \( (y = 0 \text{ mm}) \) in response to a 100 ns, 10 MV/m pulse with 10 ns rise- and fall-times. Positions, \( x \), plotted (from blue to red) are \(-5 \) (anode surface), \(-3.38\), \(-1.85\), and \(0 \) mm (midpoint between electrodes).

of the tissue at the electrode drops to 1.15 V at 6.3 ns and then increases to 1.27 V by the end of the rise-time, after which it drops precipitously to 0.86 V. The other points reach peak \( V_m \) and electroporate in order of distance from the anode. At \( x = -3.38 \) mm, \( V_m \) peaks to 1.53 V at 17.2 ns and \( N \) reaches \( 2.43 \times 10^{15} \frac{\text{pores}}{\text{m}^2} \). At \( x = -1.84 \) mm, \( V_m \) peaks to 1.51 V at 27.8 ns and \( N \) reaches \( 1.66 \times 10^{15} \frac{\text{pores}}{\text{m}^2} \). At \( x = 0 \) mm, \( V_m \) peaks to 1.51 V at 32.2 ns and \( N \) reaches \( 1.47 \times 10^{15} \frac{\text{pores}}{\text{m}^2} \) (Fig. 5-12c, d). \( \phi \), \( V_m \), and \( E \) decrease very quickly during the pulse fall-time in response to the negatively-oriented voltage division of the
pulse end, and then they decrease somewhat more slowly as the membranes discharge (Fig. 5-12a, b, c). \( N \) decays with a 1.5 s time constant (not shown; time scale too long).

5.2 Total Tissue Current and Impedance

The total current that flows through tissue during an applied electric pulse is, in an absolute sense, largely a function of the electrical properties of the tissue and the geometric properties of the electrode system. Given a particular tissue system with particular transport properties and a particular electrode setup and applied electric pulse, the current that flows through the tissue during an applied electric pulse is a function of the frequency content of the pulse and the decrease in impedance that accompanies tissue electroporation.

In a clinical setting, the properties of the tissues will vary considerably among tissue types and locations within a particular tissue. Ideally, the pulse applied to a given tissue will be tailored to its electrical properties. One method of tailoring would be to use the electrodes to measure the electrical properties of the tissue.

Figure 5-13 shows the total current (per electrode length) that flows through the tissue (and electrodes) during pulses of four different strengths and durations. The simulations were performed on active (electroporating) tissue and passive (non-electroporating) tissue. In general, the current flowing in active and passive tissue is very similar on short time scales, in which the current is dominated by the dielectric properties of the tissue, and for all low magnitude pulses, in which electroporation is insufficient to appreciably change the tissue impedance.

The current drawn by the 100 ms, 0.01 \( \frac{MV}{m} \) pulse peaks at the end of the pulse rise-time because of the displacement current associated with the high frequency content (Fig. 5-
5.2 Total Tissue Current and Impedance

The total current (per electrode length) is shown as a function of time for four pulses for active (electroporating) (solid blue) and passive (dashed red) tissue models.

13a). The current then drops slightly to a steady value of $0.914 \frac{A}{m}$ for the rest of the pulse, during which the current is dominated by the conductive current. Because this pulse causes little electroporation, there is little change in current with time after the displacement currents contribution fades and there is very little difference between the current in the active and passive tissue simulations.

The current drawn by the 1 ms, $0.1 \frac{MV}{m}$ pulse peaks at the end of the pulse rise-time because of the displacement current associated with the high frequency content (Fig. 5-13b). The current then drops to a local minimum value of $14.8 \frac{A}{m}$ at 25 $\mu$s in the active
simulation while the current drops to a steady value of 9.14 $A_m$ in the passive simulation. While most of the tissue electroporation near the electrodes occurs early in the pulse (Fig. 5-4d), pore creation continues throughout the pulse, causing the current in the active simulation to increase slightly during the remainder of the pulse as the tissue electroporation further decreases the total tissue impedance. By the end of the pulse, the current in the active tissue simulations increases slightly to 15.4 $A_m$.

The current drawn by the 10 $\mu$s, 1 MV/m pulse peaks at the end of the pulse rise-time because of the displacement current associated with the high frequency content (Fig. 5-13c). The current then drops slightly in the active simulation, reaching 533 $A_m$ at the end of the pulse. The current in the passive simulation drops much further as the displacement current contribution decreases, reaching 91.7 $A_m$ at the end of the pulse.

The current drawn by the 100 ns, 10 MV/m pulse peaks at 12.5 kA/m at the end of the pulse rise-time because of the displacement current associated with the high frequency content (Fig. 5-13d). The contribution of displacement currents remains large throughout the pulse with the active simulation current dropping to 6.42 kA/m and the passive simulation current dropping to 6.10 kA/m by the end of the pulse.

Figure 5-14 is the counterpart to Fig. 5-13, showing the ratios of the currents in the active simulations, $I_t$, to those of the passive simulations, $I_{tp}$, or equivalently, the ratios of the impedances. Note that the blips at the ends of the rise- and fall-times are not significant. They result from the need to interpolate the time points of the passive simulations at the time points of the active simulations, and this can lead to significant errors at the pulse breakpoints. For all of the tissue responses, $\frac{I_t}{I_{tp}} \approx 1$ early in the pulse because the impedance is primarily determined by the dielectric membrane properties and/or because electroporation is minimal and not yet affecting the tissue impedance significantly. $\frac{I_t}{I_{tp}}$ remains $\sim 1$ throughout the 0.01 MV/m simulation because the electroporation in response
5.2 Total Tissue Current and Impedance

(a) 100 ms, 0.01 MV/m pulse

(b) 1 ms, 0.1 MV/m pulse

(c) 10 µs, 1 MV/m pulse

(d) 100 ns, 10 MV/m pulse

Figure 5-14: Tissue impedance ratio. The total current through passive tissue relative to the total current through active (electroporating) tissue as a function of time for four pulses. Note that the ratio of the passive tissue current to the active tissue current is equal to the ratio of the active tissue impedance to the passive tissue impedance.

to this pulse is minimal (Fig. 5-14a). $\frac{I_p}{I_{tp}}$ drops to 0.592 by the end of the 0.1 MV/m pulse after the high frequency components fade and the membrane impedance is primarily determined by its conductance, which increases due to electroporation (Fig. 5-14b). $\frac{I_p}{I_{tp}}$ drops furthest in response to the 1 MV/m pulse, reaching 0.172 by the end of the pulse, because of substantial electroporation and the decay of the high frequency components by the end of the pulse (Fig. 5-14c). $\frac{I_p}{I_{tp}}$ only drops to 0.950 by the end of the 10 MV/m pulse, despite extensive electroporation, because the membrane impedance is a minor
contributor to the total tissue impedance with the high frequency content of the 100 ns pulse (Fig. 5-14d).

5.3 Tissue Electroporation Regimes

The tissue responses have been presented for four pulses ranging widely in strength and duration. Figure 5-15 fills in the gaps by showing summary results over a wide range of pulse strengths and durations. The pulse magnitudes range from 0.01 to 10 MV m and have 100 ns duration and 10 ns rise-times. White log-spaced equi-energy lines ($E_{\text{app}}^2 t = \text{constant}$) indicate the wide range of tissue responses that can result from pulses of the same energy. The large magnitude, long duration combinations deliver tremendous energy and are unlikely to be compatible with tissue survival; they are included for completeness only.

Pulses with $E_{\text{app}} < \sim 10^5 \text{ V m}$ do not cause significant electroporation at the electrodes or any electroporation at the tissue center (Fig.5-15b) because $E$ is too small to charge the membranes past the $\sim 1$ V (Fig.5-15a, c). For these pulses, the current flows through the shunt pathways, except early in the pulse when the high frequency components of the pulse allow displacement currents to flow transcellularly (Fig.5-15d).

For pulses with $E_{\text{app}} \approx 10^5 \text{ V m}$, shifting voltage division becomes significant between the electrodes, and $E$ at the tissue center approaches $E_{\text{app}}$ after the tissue near the electrodes electroporates, shifting a greater fraction of the total voltage drop to the central tissue (Fig.5-15a, b). Pulses of this strength are just large enough to cause electroporation throughout the region between the electrodes because $E$ is large enough for $V_m$ to reach $\sim 1$ V (Fig.5-15a, c). Because of shifting voltage division, $V_m$ drops to $\sim 0.6$ V at the electrodes as the voltage falls primarily over the central tissue region (Fig.5-15c).
Pulses with $E_{\text{app}} \gg 10^5 \frac{V}{m}$ and sufficient duration electroporate the entire region between the electrodes (Fig. 5-15b). Following electroporation, $V_m$ drops to $\sim 1$ V at the tissue center. $V_m$ can drop to $< 1$ V at the electrodes for $E_{\text{app}} \sim 10^5 \frac{V}{m}$ because of shifting voltage division or for $E_{\text{app}} >\sim 3 \frac{MV}{m}$ because of the rise-time effect (Fig. 5-15c). There is also a somewhat abrupt increase in $N$ for $E_{\text{app}} >\sim 3 \frac{MV}{m}$ because of the rise-time effect. Early in the pulse, the membrane impedance is small because it is dominated by dielectric properties, and later in the pulse the membrane impedance is small because electroporation greatly increases the membrane conductance. Consequently, most current flows through, rather than around, the cells for $E_{\text{app}} >\sim 10^5 \frac{V}{m}$ (Fig. 5-15d). In general, very little changes after electroporation in response to pulses with $E_{\text{app}} >\sim 10^5 \frac{V}{m}$ because $V_m$ decreases to $\sim 1$ V, pore creation ends, and the system reaches a steady-state (Fig. 5-15). As such, there is little incentive to apply pulses that are much longer than the time needed to electroporate the central tissue region, at least for the purpose of creating pores. Longer pulses to may contribute to cell loading, or other secondary effects of interest.

### 5.4 Alternate Electrode Configurations

All of the results presented have varied the applied electric field duration, magnitude, and rise- and fall-times, while the electrode configuration has remained unchanged with electrode radii $r_e = 0.25$ mm and electrode spacing $L_e = 10$ mm. In this section, the four pulses of Sec. 5.1 are applied to tissue systems in which either the electrode radius or the electrode spacing is changed. Both significantly affect the spatial extent and uniformity of the tissue response.

Figures 5-16 and 5-17 show the tissue responses at the end of four pulses for three different electrode radii or electrode spacings. In Fig. 5-16, three electrode radii, $r_e$, are shown, 0.1, 0.5, and 2.5 mm, with the electrode spacing, $L_e$, held constant at 10 mm. In
(a) Electric field magnitude
\[ \frac{E_0}{E_{\text{app}}} \]

(b) Pore density
\[ N_0 \quad \text{(pores/m}^2) \]
Figure 5-15: Strength-duration summary results. (a) Electric field magnitude, (b) pore density, (c) transmembrane potential, and (d) transcellular current relative to total current (transcellular + shunt) for 100 ms pulses ranging 0.01–10 MV m with 10 ns rise-times. Subscript 0 denote quantities at the tissue center, and subscript e denote quantities at the electrode surface. On each plot, 11 contour lines are spaced evenly between the extreme values of the colorbar, and the white lines show log-spaced equi-energy lines ($E_{\text{app}}^2 t =$ constant).
### Tissue Electroporation

100 ms, 0.01 MV\_m pulse

<table>
<thead>
<tr>
<th>Electrode radius</th>
<th>Electric potential $\phi$ (V)</th>
<th>Electric field magnitude $E$ (V/m)</th>
<th>Transmem. potential $V_m$ (V)</th>
<th>Pore density $N$ (pores/m²)</th>
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<tr>
<td>0.1 mm</td>
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<td>2.5 mm</td>
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1 ms, 0.1 MV\_m pulse

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<thead>
<tr>
<th>Electrode radius</th>
<th>Electric potential $\phi$ (V)</th>
<th>Electric field magnitude $E$ (V/m)</th>
<th>Transmem. potential $V_m$ (V)</th>
<th>Pore density $N$ (pores/m²)</th>
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Figure 5-16: Spatial responses for three electrode radii. Electric potential, electric field magnitude, transmembrane potential, and pore density are shown for electrode radii of 0.1, 0.5, and 2.5 mm at the end of the pulse plateau for the following pulses: 100 ns, 0.01 MV/m; 1 ms, 0.1 MV/m; 10 μs, 1 MV/m; and 100 ns, 10 MV/m. The spacing between the electrodes (edge-to-edge) is 10 mm.
### 100 ms, 0.01 \( \text{MV}_\text{m} \) pulse

<table>
<thead>
<tr>
<th>Electrode spacing</th>
<th>Electric potential ( \phi ) (V)</th>
<th>Electric field magnitude ( E \left( \frac{\text{V}}{\text{m}} \right) )</th>
<th>Transmem. potential ( V_m ) (V)</th>
<th>Pore density ( N \left( \text{pores m}^{-2} \right) )</th>
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<td><img src="image3" alt="Transmem. potential" /></td>
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<td><img src="image10" alt="Electric field magnitude" /></td>
<td><img src="image11" alt="Transmem. potential" /></td>
<td><img src="image12" alt="Pore density" /></td>
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### 1 ms, 0.1 \( \text{MV}_\text{m} \) pulse

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<thead>
<tr>
<th>Electrode spacing</th>
<th>Electric potential ( \phi ) (kV)</th>
<th>Electric field magnitude ( E \left( \frac{\text{V}}{\text{m}} \right) )</th>
<th>Transmem. potential ( V_m ) (V)</th>
<th>Pore density ( N \left( \text{pores m}^{-2} \right) )</th>
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<td><img src="image14" alt="Electric field magnitude" /></td>
<td><img src="image15" alt="Transmem. potential" /></td>
<td><img src="image16" alt="Pore density" /></td>
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<tr>
<td>10 mm</td>
<td><img src="image17" alt="Electric potential" /></td>
<td><img src="image18" alt="Electric field magnitude" /></td>
<td><img src="image19" alt="Transmem. potential" /></td>
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<tr>
<td>40 mm</td>
<td><img src="image21" alt="Electric potential" /></td>
<td><img src="image22" alt="Electric field magnitude" /></td>
<td><img src="image23" alt="Transmem. potential" /></td>
<td><img src="image24" alt="Pore density" /></td>
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Figure 5-17: Spatial responses for three electrode spacings. Electric potential, electric field magnitude, transmembrane potential, and pore density are shown for electrode spacings of 2.5, 10, and 40 mm at the end of the pulse plateau for the following pulses: 100 ms, 0.01 MV/m; 1 ms, 0.1 MV/m; 10 µs, 1 MV/m; and 100 ns, 10 MV/m. The electrode radii are 0.25 mm. Note the size of the displayed region is scaled in proportion to the electrode spacing.
The electric field, in a relative sense, is determined by the ratio of the electrode radius to the electrode spacing. That is, if the radius, spacing, and applied field are all scaled equally, then the resulting spatial electric field magnitude will also scale by the same factor. Therefore, decreases in $L_e$ with $r_e$ held constant will produce the same qualitative changes as increases in $r_e$ with $L_e$ held constant. However, scaling will lead to absolute changes in the spatial extent of electroporation.

The changes in tissue response that result from changes in electrode geometry are best considered in light of how changing the electrode geometry changes the electric field magnitude. When $r_eL_e$ is small, the electric field lines are very dense at the electrodes, and $E$ is therefore very large at the electrodes and most of the potential drops across the tissue surrounding the electrodes. That is, there is significant spreading impedance associated with these electrode configurations. As $r_eL_e$ becomes larger, the electric field lines become less dense at the electrodes, and therefore $E$ becomes smaller at the electrodes and less of the potential drops across the tissue surrounding the electrodes. That is, the spreading impedance becomes less significant.

The changes in $E$ and $\phi$ that accompany changes in $r_eL_e$ are clearly illustrated by Figs. 5-16 and 5-17. As $\frac{r_e}{L_e}$ is increased, $E$ decreases at the electrodes and increases at the tissue center, and the equipotential lines spread out from the electrodes. The increased uniformity of $E$ between the electrodes results in more uniform $N$, and the increased distance from the electrodes at which $E$ is sufficiently large to cause electroporation also increases and results in more spatially extensive electroporation. Shifting voltage division still significantly affects the spatial extent of electroporation on long time scale in regions for which $E \approx 10^5 \frac{V}{m}$. In fact, with greater uniformity in $E$, regions with $E$ slightly too
small to cause significant electroporation require less of a voltage shift from neighboring tissue with $E$ just large enough to cause electroporation. Therefore, the spatial extent of electroporation can become quite large as a consequence of shifting voltage division, particularly as $\frac{r_e}{L_e}$ increases. This is especially true in response to the 1 MV m pulse because the regions at which $E \approx 10^5 \frac{V}{m}$ lie outside the electrodes and the pulse duration (10 µs) is long enough for shifting voltage division to occur.

Figures 5-18 and 5-19 examine the spatial uniformity of electroporation for $r_e$ ranging from 0.1 to 2.5 mm (Fig. 5-18) and $L_e$ ranging from 2.5 to 40 mm (Fig. 5-19). In each, subfigure (a) shows the pore density at the tissue center and at the electrode and (b) shows the ratio of these pore densities. Note that Figs. 5-18 and 5-19 look like reflections of each other over the regions of shared $\frac{r_e}{L_e}$. For all pulses and electrode configurations, $N$ at the electrode exceeds $N$ at the tissue center. However, as $\frac{r_e}{L_e}$ increases, they approach the same value. In the limit as $\frac{r_e}{L_e} \to \infty$ (i.e., as the electrodes become planar), $E_e \to E_{app}$ and $E_0 \to E_{app}$, and therefore $\frac{N_0}{N_e} \to 1$. In varying $r_e$ from 0.1 to 2.5 mm, $\frac{N_0}{N_e}$ shifts from $3.0 \times 10^{-4}$ to 0.98 for the 0.01 MV m pulse, $7.0 \times 10^{-4}$ to 0.584 for the 0.1 MV m pulse, 0.048 to 0.75 for the 1 MV m pulse, and 0.044 to 0.57 for the 10 MV m pulse. In varying $L_e$ from 2.5 to 40 mm, $\frac{N_0}{N_e}$ shifts from 0.93 to 7.2 $\times 10^{-5}$ for the 0.01 MV m pulse, 0.084 to 1.7 $\times 10^{-4}$ for the 0.1 MV m pulse, 0.38 to 0.0169 for the 1 MV m pulse, and 0.22 to 0.029 for the 10 MV m pulse.

For most clinical applications, uniformity of electroporation is desirable because it will result in uniformity of the biological response. Therefore, large $\frac{r_e}{L_e}$ are preferable. However, there are practical considerations that limit the size of this ratio. In electroporating a cancer nodule to facilitate uptake of an anti-cancer agent, for example, $L_e$ must be large enough to span the nodule. $r_e$ is limited by what patients will tolerate. Presumably, most would prefer smaller $r_e$. 
Figure 5-18: Pore density vs. electrode radius. (a) Pore density at the electrode (dashed lines) and at the tissue center (solid lines) and (b) pore density at the tissue center relative to pore density at the electrode at the end of the pulse plateau for the following pulses: 100 ms, 0.01 MV/m; 1 ms, 0.1 MV/m; 10 µs, 1 MV/m; and 100 ns, 10 MV/m. The electrode radii shown vary from 0.1 to 2.5 mm with the electrode spacing (edge-to-edge) fixed at 10 mm.

Figure 5-19: Pore density vs. electrode spacing. (a) Pore density at the electrode (dashed lines) and at the tissue center (solid lines) and (b) pore density at the tissue center relative to pore density at the electrode at the end of the pulse plateau for the following pulses: 100 ms, 0.01 MV/m; 1 ms, 0.1 MV/m; 10 µs, 1 MV/m; and 100 ns, 10 MV/m. The electrode spacings (edge-to-edge) shown vary from 2.5 to 40 mm with the electrode radii fixed at 0.25 mm.
Chapter 6

Discussion

The application of large, pulsed electric fields to biological systems is a unique method for altering their biochemistry, whether by facilitating transmembrane transport via pores or by other currently poorly understood mechanisms of coupling between large intracellular fields and biochemical events. Nonetheless, despite the widespread adoption of electroporation in research laboratories, it has not yet found a permanent home in clinical settings. This is due in part to the continued lack of knowledge about many aspects of electroporation and to the current lack of a compelling clinical application [45]. However, new, basic insights into the mechanisms of electroporation are emerging, largely as the result of molecular dynamics simulations, sophisticated spatially distributed electroporation models, and supra-electroporation experiments. As more is uncovered about the basic mechanisms of electroporation and other actions of large, pulsed electric fields, there is potential for the development of new, compelling therapies.

The results presented here provide comprehensive overviews of the electrical responses of cells and tissue to applied electric fields of widely varying strength and duration. The mechanistic, rather than empirical, basis of the models makes them robust and gives them additional predictive power. Additionally, because the models are based on the mesh transport network method, they can be easily extended in the future to account
Discussion

for pore expansion and molecular transport, which are essential for tight comparisons with experimentally measurable quantities and accurate prediction of important transport phenomena, from the uptake of drugs and genes to the loss of essential intracellular macromolecules.

This chapter begins with a brief discussion of the mesh transport network method (Sec. 6.1). The cell (Sec. 6.2) and tissue (Sec. 6.3) model methods and simulation results are then compared with published experimental and theoretical results, and the mechanisms of cell death in response to electroporation are discussed (Sec. 6.4). The chapter closes with a discussion of the model assumptions and future work (Sec. 6.5) and concluding remarks (Sec. 6.6).

6.1 Mesh Transport Network Method

The mesh transport network method (MTNM) presented here (Chap. 2) provides a robust framework for modeling complicated, spatially distributed, coupled transport phenomena. The method focuses on defining transport locally in terms of constitutive equations that can then be easily translated into equivalent circuits. The conservation principles imposed by Kirchhoff’s Current Law join the locally-specified constitutive equations into complete, spatially distributed models. Here the nonlinear equivalent circuits describing cell and tissue electroporation are simulated using Berkeley SPICE, though alternative simulation methods could also be used.

The fundamental approach of the MTNM was used previously by the transport lattice method (TLM) introduced by Gowrishankar and Weaver [34]. The MTNM is a generalization of the TLM to unstructured meshes. While the results of comparative simulations on passive [35] and active [36] systems have shown that the two methods produce similar results, the MTNM is more accurate and computationally efficient because it uses
unstructured meshes that respect the boundaries of all structures in the system and variably-sized elements that allow nodes to be optimally distributed throughout the system. For example, in the cell system mesh, the triangular elements resolve the 5 nm thickness of membranes but expand in size to have a triangle edge length of $\sim 5 \mu m$ at the system boundary, a difference of 3 orders of magnitude. The use of 5 nm rectangular elements in the TLM would require a prohibitively large number of elements.

The robustness of the MTNM will allow future models to incorporate added complexity, such as expanding pores and electrodiffusion, in a straightforward manner (see Sec. 6.5).

6.2 Cell Electroporation

Electroporation can be broadly categorized as conventional electroporation, in which pores form in the cell plasma membrane only, and supra-electroporation, in which pores form in membranes throughout the cell. The focus of the cell model simulation results presented here (Chp. 4) has been on supra-electroporation, about which less is known than conventional electroporation.

6.2.1 Conventional electroporation

Conventional electroporation, in which only the cell plasma membrane forms pores, became a widely used laboratory tool for loading cells with molecules, from small fluorescent dyes to large DNA plasmids, after initial demonstrations in lipid vesicles [49] and erythrocytes [2, 3] in the 1970s. The pore density distribution in pore radius space is expected to vary widely for pulses of varying strength and duration [41]. Short duration, large magnitude pulses are generally used for loading cells with small molecules, while long duration, small magnitude pulses are generally used for loading cells with large molecules, such as DNA.
Pore expansion is not included in the cell model presented here because the focus is on supra-electroporation, in which the pores do not expand significantly beyond the minimum pore radius (0.8 nm), and on the electrical response of the membrane, which is relatively insensitive to the pore population that forms because the transmembrane potentials across the membranes of actual cells are expected to approach $\sim 1$ V, whether by pore creation or pore expansion.

The conventional electroporation results are qualitatively comparable to those found by DeBruin and Krassowska [38, 47]. They developed the asymptotic model and used it to simulate the electrical response of a 50 $\mu$m radius spherical cell to smaller applied electric fields than those applied on the 10 $\mu$m radius cylindrical cell simulated here. For small magnitude pulses with long duration (substantially longer than the membrane charging time constant), the peak pore density and spatial extent of electroporation are primarily determined by the passive charging of the membrane and the peak transmembrane potential reached by each region of membrane before electroporating. For the 25 $kV/cm$ pulse simulated by DeBruin and Krassowska, the transmembrane potential of a passive 50 $\mu$m radius spherical membrane approaches $\frac{3}{2} E_{app} a \sin \Theta = 1.875 \sin \Theta$, while for the smallest pulse examined here, 100 $kV/cm$, the transmembrane potential of a passive 10 $\mu$m radius cylindrical membrane approaches $2 E_{app} a \sin \Theta = 2 \sin \Theta$. Thus, the responses of the cells to these pulses should be similar. In the DeBruin and Krassowska simulation, the polar pore density reaches $\sim 5 \times 10^{13}$ pores/m$^2$ and here the polar pore density reaches $\sim 2 \times 10^{14}$, which are consistent in light of the slight differences in the applied pulse magnitude, shape, and duration, and the differences in the electrical and geometric parameters. In both simulations, the hyperpolarized anodic cell pole electroporates before the depolarized cathodic cell pole, and the transmembrane potential approaches $\sim 1$ V after electroporation. The spatial extent of electroporation in response to the pulses is also similar.
The ultimate objective of a conventional electroporation model is the simulation of molecular transport within a spatially distributed cell system. Such a model will allow closer comparisons with experimental measurements and will have additional predictive power. Such a model will also require the simulation of pore expansion and solute transport by electrodiffusion. Both are straightforward extensions of the model presented here (see Sec. 6.5).

6.2.2 Supra-electroporation

In 2001, Schoenbach et al. first demonstrated that large magnitude, sub-microsecond pulses cause a cell response that is fundamentally different from the response to lower magnitude, longer duration pulses [13]. Since then, numerous papers have investigated the responses of cells to large magnitude, sub-microsecond pulses, and a variety of effects have been observed [13–24], such as caspase activation, cytochrome c localization, apoptosis induction, and phosphatidylserine translocation. Apoptosis induction has been seen in many of these experiments and is of particular interest because of its potential use in clinical applications.

Many experimental studies have hypothesized that ultrashort pulses perturb subcellular structures without perturbing the outer cell membrane because the measured intracellular fluorescence of propidium iodide (PI) and other fluorescent dyes is minimal following large magnitude, short duration pulses but not following conventional pulses [13, 15–17, 19–21, 23, 24]. This effect is often explained in terms of membrane charging time constants, which the authors claim are shorter for organelle membranes than the outer cell membrane because of their smaller sizes. However, because of the importance of dielectric membrane properties on short time scales, this assumption is incorrect. In fact, on short time scales the initial rate of membrane charging is independent of membrane radius [35, 50]. Moreover, aside from whatever parameter adjustments one may propose to make organelar membranes charge faster, the electrical properties
of the outer cell membrane are well established and theory and simulations show that transmembrane potentials greatly exceeding the threshold for reversible electrical breakdown (REB) (∼1 V) would be produced in the passive response to pulses applied in experiments [35,36,39,46,50], which are generally on the order of 5–15 MV/m. Furthermore, there is no mechanistic basis for why similar transmembrane potentials would have dramatically different effects on the cell membrane and the organelle membranes.

The alternative hypothesis supported by the simulations here and elsewhere [36,39,46] is that large magnitude, short duration pulses lead to supra-electroporation, in which minimum sized (0.8 nm) pores form in all membranes. A model of a cell membrane patch simulating the flow of individual ion species has shown that the small pores that form in response to large magnitude, short duration pulses are selective for small ions, whereas the larger pores that form in response to lower magnitude, longer duration pulses are less selective for small ions (unpublished result). This suggests that significantly less amount of fluorescent dye will be transported through the minimum size pores of supra-electroporation than through the larger pores of conventional electroporation, despite the greater fractional pore area associated with supra-electroporation. On the other hand, transport of calcium, which is significantly smaller than PI, should be less affected by pore size.

Experimentalists use different metrics for assessing the perturbations of the outer cell membrane and organelle membranes. The outer cell membrane integrity is assessed by the transport of PI or other fluorescent dyes, while the integrity of subcellular structures is assessed by calcium release and other events (e.g. caspase activation) that may not be directly related to the formation of pores in organelle membranes. While the increased conductance of membranes in response to transmembrane potentials exceeding 1 V is well established, there are likely other major biophysical mechanisms coupling large electric fields to biochemical changes within cells. PI is generally a good molecule for assessing
membrane integrity, but its transport through small pores is likely quite minimal, such that the signal-to-noise ratio is very low in the measurement of intracellular PI concentration. Calcium, on the contrary, would be assumed to face a smaller barrier to transport through pores in the endoplasmic reticulum.

Studies of phosphatidylserine (PS) externalization by Vernier et al. [22,51] are consistent with the supra-electroporation hypothesis. PS is a negatively-charged phospholipid normally located only on the intracellular side of the cell membrane. Vernier et al. applied 30 ns pulses of up to 3.5 MV/m magnitude to cells in suspension and observed asymmetric externalization of PS with significantly more PS externalization on the anodic side of the cell [51]. This is consistent with electrophoretic transport of negatively-charged PS through pores.

Recent molecular dynamics simulations further support the supra-electroporation hypothesis [52–55]. These models simulate the motions of individual membrane and electrolyte molecules in the presence of an externally applied electric field [52–54] or an imbalance of sodium ions [55]. These simulations generally apply or create relatively large transmembrane potentials of 2–3 V to increase the probability of pore formation on the order of nanoseconds because of the tremendous computational resources required for the simulations. In the small spatial regions simulated, the membranes form defects that become small pores within nanoseconds. The simulations have the potential to greatly enhance the as yet poorly understood dynamics of pore formation, and may provide better estimates of parameters used in continuum models, such as pore lifetime.

Supra-electroporation models can be categorized broadly as passive or active. Kotnik et al. created a passive model to thoroughly examine the transmembrane potentials of passive concentric spherical cell and organelle membranes in response to large magnitude, short duration trapezoidal pulses [50]. They derived analytical expressions for the
transmembrane potential as a function of frequency and position on the membranes. For cell (10 \( \mu \text{m} \) radius) and organelle membranes (3 \( \mu \text{m} \) radius) with the same electrical parameters, the organelle transmembrane potential is much less than the cell transmembrane potential for low frequencies (\(<\sim 0.1 \text{ MHz}\)) but approaches the cell transmembrane potential for higher frequencies (\(>\sim 1 \text{ MHz}\)). The transition occurs as the membrane impedance is increasingly determined by the dielectric membrane properties, which increases the intracellular electric field magnitude to almost as large as the extracellular field magnitude. Kotnik et al. explore the intracellular membrane electrical parameter space to demonstrate that for certain membrane parameters the organelle transmembrane potential can exceed the cell transmembrane potential by a factor of \( \sim 2 \) at particular frequencies [50]. They also examine the temporal cell and organelle transmembrane potentials in response to a trapezoidal 15 MV/m pulse with 1 ns rise- and fall-times and a 10 ns plateau, much like the supra-electroporation pulse examined here. Note that the applied electric field magnitude is not particularly important for passive models because the results scale linearly with the electric field magnitude. Because of the high frequency content of the short pulse, the transmembrane potentials of the cell and organelle are very similar for the duration of the pulse, reaching \( \sim 8 \text{ V} \) by the end of the pulse. Similarly, in the active model presented here the cell and organelle responses are very similar (Fig. 4-11), but because of electroporation the transmembrane potentials do not exceed \( \sim 1.7 \text{ V} \). Again adjusting the organelle parameters, Kotnik et al. are able to make the organelle transmembrane potential exceed the cell transmembrane potential such that the organelle transmembrane potential reaches \( \sim 26 \text{ V} \) by the end of the pulse while the cell transmembrane potential reaches \( \sim 8 \text{ V} \). This is offered as evidence that it may be possible to perturb the intracellular membranes without perturbing the cell membrane. Indeed, this may be true for very special pulses if the organelle membranes do in fact have electrical properties that allow them to charge faster than the cell membranes in response to pulses with high frequency content. However, there is no reason to think that this is a general, robust effect of large magnitude, short duration pulses. There is no
evidence that a cell membrane can withstand 8 V without perturbation. A more reasonable explanation for the apparent intracellular effects without measured changes in the cell membrane is that the experimental methods are indirect, limited by signal-to-noise ratio, and fundamentally different for the cell and organelle membranes.

A cylindrical cell model without organelles was used by Stewart et al. to validate and compare the transport lattice method (TLM) and mesh transport network method (MTNM) [35]. Analytical expressions were derived for the transmembrane potential as a function of frequency and position on the membrane and for the transmembrane potential in response to a step in the applied electric field. The TLM and MTNM were shown to both approximate the analytically derived transmembrane potential, but the MTNM was shown to be much more accurate and computationally efficient because of its use of variably-sized elements that align with structures in the domain [35].

Joshi et al. have created active cell electroporation models that use a Smoluchowski equation-based energy landscape method and concentric spherical membranes [56–63]. The simulations show that electroporation limits the transmembrane potential to $\sim 1.8$ V and that the pulse parameters determine the resulting pore density distribution in radius space. Further comparisons will not be made here because the methods and results of Joshi et al. are generally difficult to interpret and seem to exhibit dimensional errors (e.g. plots of $n(r, t)$ have dimensions of length$^{-2}$ rather than length$^{-3}$ in Refs. [57, 58, 61, 62] and the calculation of total pore area, $A_p$, appears dimensionally incorrect in Ref. [62] and more similar to the calculation of total pore circumference, $L_p$, by Neu and Krassowska [64]).

Stewart et al. [39], Gowrishankar et al. [46], and Smith et al. [36], have presented models of supra-electroporation based on the TLM [36, 39, 46] and the MTNM [36], and their simulations show supra-electroporation in response to large magnitude, short duration
pulses. The TLM model presented by Gowrishankar et al., which simulates the most biologically realistic system to date, has numerous organelles that are electroporated in response to large magnitude, short duration pulses. The TLM and MTNM models presented by Smith et al. use the same cell system as that used here but without resting potentials. As in the passive TLM and MTNM comparison, the MTNM produces more accurate results with much greater efficiency than the TLM, though the results of both models are quite similar generally, showing electroporation of the cell and organelle membranes [36].

6.3 Tissue Electroporation

Interest in tissue electroporation grew following the initial demonstration of electrochemotherapy by Okino et al. in 1987 [25]. Since then, Mir and colleagues have been the chief advocates of electrochemotherapy and other applications of tissue electroporation, such as DNA electrotransfer, publishing widely on these topics [7, 11, 26–31, 65–97]. The primarily experimental work by Mir et al. has been complemented by both the experimental and the theoretical work of Miklavcic and colleagues [70, 71, 75, 79, 86, 91, 97–117]. Numerous clinical trials of electrochemotherapy for the treatment of solid tumors have been conducted, most using intravenous or intratumoral injection of bleomycin followed by the application of electrical pulses [29]. Bleomycin, which does not easily pass through intact cell membranes, causes cell death by directly damaging DNA. Several thousand internalized bleomycin molecules are sufficient to cause slow cell death, and several million internalized molecules will cause quick cell death by apoptosis within minutes [29]. Bleomycin is attractive as an agent for electrochemotherapy because it does not freely pass through intact membranes but does pass though electroporated membranes. As such, low doses of bleomycin can be administered with reduced systemic side effects, but regions of tissue that are subsequently electroporated have a 300–700 fold increase in cytotoxicity [29]. Numerous clinical trials have shown electrochemotherapy to be an
6.3 Tissue Electroporation

effective palliative treatment for localized, non-metastatic tumors that requires only a single, outpatient procedure [29].

Tissue electroporation is also an effective non-viral means of introducing DNA into cells both in vitro and in vivo. Numerous applications have been suggested, including treatments for cancer, cardiovascular disease, monogenetic diseases, various tissue-specific diseases, and metabolic disorders, all of which have been examined in animal models [12,31].

Most clinical trials of electrochemotherapy have used planar electrodes and numerous pulses of \( \sim 120 \text{ kV/m} \) for \( \sim 100 \mu s \) [29] and most DNA electrotransfer experiments have used various electrode configurations and multiple pulses of \( \sim 20-100 \text{ kV/m} \) for \( \sim 5-100 \text{ ms} \) [10,30,31,78,81,84,91,118,119]. It is difficult to make detailed comparisons among the experiments because of the use of differing electrode configurations, tissues, and DNA plasmids. Nonetheless, in general, larger magnitude, shorter duration pulses have been used for drug delivery and lower magnitude, longer duration pulses have been used for gene delivery. This is consistent with the hypothesis that larger magnitude, shorter duration pulses create many relatively smaller pores with greater total area than lower magnitude, longer duration pulses, which are expected to be dominated by pore expansion [41]. Moreover, because DNA has a strong negative charge, its delivery benefits greatly from the continued electrophoretic force provided by long pulses [85,91]. It should be noted that there is evidence that DNA transport across the membrane may involve a multistep interaction with membrane that occurs on a time scale of minutes [9].

The tissue electroporation experiments and models produced by Miklavcic and colleagues [86,97,108,110,120] have been the most sophisticated to date and present data that are most easily compared with the tissue electroporation results presented here (Chp. 5). The early models used the finite element method (FEM) in a 3D passive model of tissue [86,108,110]. That is, given the conductivities throughout the system and
boundary conditions, the models solved the Laplace equation. In Refs. [108] and [110], complicated three dimensional systems comprising a number of different tissues, including those with anisotropic conductivities (i.e. tensor rather than scalar conductivities), were simulated and compared with magnetic resonance current density imaging results. The biological complexity represented by the models is impressive and the authors achieved their stated goal of examining the effect of changing the electrode orientation on the electric field. However, these models cannot illustrate the transient, dynamic effects of electroporation because they represent only the tissue conductivity (not permittivity), which was assumed not to change with time.

In Ref. [86], Miklavcic et al. presented another 3D FEM model of homogeneous passive tissue with needle electrodes. The electrodes are separated by 8 mm and electrode radii of 0.15, 0.35, and 0.55 mm are examined. The electric field magnitude contour plot in the plane perpendicular to the electrodes is qualitatively very similar to that predicted here in response to the 100 ms, 0.01 MV/m pulse (Sec. 5.1.2), which was essentially a passive simulation because electroporation was so minimal. Moreover, the Miklavcic et al. results, like those presented here, show that the electric field magnitude at the electrode surface decreases with increasing electrode radius and the magnitude at the tissue center increases with increasing electrode radius. Electrochemotherapy experiments with bleomycin were performed using an electrode configuration identical to that simulated. Eight 100 µs rectangular pulses separated by 1 s were applied for each electrode diameter with applied voltages of 860, 960, 1060, and 1360 V (i.e. applied field magnitudes of 107.5, 120, 132.5, and 170 kV/m). Drawings of the necrotic regions were shown for the 960 V pulse for all three electrode radii. The outlines of the necrotic regions roughly correspond to an electric field magnitude of 63.7 kV/m and transmembrane potential of 0.694 V. That transmembrane potentials significantly less than 1 V would lead to enough transmembrane bleomycin transport to induce apoptosis is somewhat surprising. However, smaller transmembrane potentials may contribute to transmembrane transport facilitating pore
expansion rather than pore creation. Additionally, if the pulses did cause shifting voltage division in the real tissue system, then the transmembrane potentials at which the membrane integrity was compromised may have been somewhat larger than the transmembrane potentials estimated based on the passive model.

The more recent tissue electroporation models by Miklavcic and colleagues [97, 120] use sequential FEM models to simulate the dynamic electrical responses of tissue to electroporation. The Sel et al. model [97] will be discussed here because it uses the same homogeneous tissue system and electrode configuration of Miklavcic et al. [86] described above. The model works by solving the Laplace equation in several steps. At each step the conductivity of the tissue is calculated as a function of the electric field magnitude of the previous step, and as the electric field magnitude increases, the conductivity increases. A sigmoidal curve for conductivity as a function of the electric field magnitude is fit using experimental data. This method of calculating the conductivity of the tissue as a function of the electric field is, in essence, the same as in the simple example in Sec. 5.1.1 used to explain shifting voltage division with the assumption of membrane impedance dominated by conductive membrane properties. In that simple example, the membrane was assumed to have a transmembrane potential of 1 V, independent of the electric field magnitude, and consequently, the tissue resistance in the limit of small electric field magnitude approached the resistance of the cell unit shunt and in the limit of large electric field magnitude approached the resistance of the cell unit without a membrane.

The electric field magnitude spatial plot presented by Sel et al. [97] for the response of tissue with 0.35 mm radius electrodes separated by 8 mm to an applied voltage of 520 V (65 kV m) is qualitatively similar to that in response to the 100 kV m pulse examined in Sec. 5.1.3. In both simulations, the electric field magnitude is initially much lower at the tissue center than at the electrodes, but as the tissue near the electrodes electroporates and decreases in impedance, the electric field magnitude increases at the tissue center
until it electroporates and shifts some of the elevated field back toward the electrodes. Sel et al. [97] performed tissue electroporation experiments for comparison with their simulations using the same methods used previously [86]. For each electrode radius, the current was measured in response to pulses of varying magnitude. In response to small pulses (e.g. $25 \, \text{kHz}$), the current peaks and then decreases and approaches a constant value. In response to larger pulses (e.g. $100 \, \text{kHz}$), the current peaks, decreases as the displacement currents give way to conductive currents, and then slowly increases as the tissue is electroporated. The current profiles are similar to that in response to the 1 ms, $100 \, \text{kHz}$ pulse shown in Fig. 5-13.

The sequential FEM method used by Sel et al. [97] has a number of shortcomings. First, the model has no intrinsic time dependence. Each FEM step leads to large swings in tissue conductance. The time to which each FEM step is purported to correspond is determined through fitting of empirical data. Second, on short time scales, electroporation is expected only to increase membrane conductance, but in the sequential FEM model the conductivity is calculated as a function of the electric field at each step. Therefore, when the voltage division shifts and the electric field magnitude decreases, so does the conductivity of the region. It would be more reasonable to calculate the conductivity as a function of the electric field magnitude and then use that value only if it is greater than the conductivity from the previous time step. Third, dielectric tissue properties are not considered. The dielectric properties of the membrane have been shown here to be significant in determining tissue impedance and voltage division when the pulse frequency content is high. Finally, it is not clear that the model has much predictive value because of its dependence on empirically determined relationships rather than fundamental mechanistic relationships. The time steps represented by the sequential FEM steps were determined \textit{a posteriori} by fitting the FEM data to experimental results. As such, it may not be possible to simulate other pulse strengths and durations without accompanying experimental data.
Miklavcic and colleagues have produced excellent models of electrical effects in tissue, in part because of their focus on creating models that are informed by or easily testable by experiments. In general, in modeling electric fields at the tissue scale, they have focused on macroscopic spatial complexity (i.e. 3D models with spatially varying electrical properties) and empirical relationships rather than microscopic complexity (i.e. macroscopic impedance determined by a microscopic model of membrane electroporation) and mechanistic relationships, while the model presented here focuses on the latter rather than the former.

Other models of tissue electroporation include those presented by Davalos et al. [95, 121], which use a sequential FEM-based method like that of Sel et al., and Ramos [122]. The Ramos model is somewhat similar to this model in that it is a multiscale Cartesian grid model that uses equivalent circuits as means of simulating nonlinear transport. The electroporation model is based on an empirical electroporation conductance expression. Currently Ramos has only published one very short paper on the model and its simulation results [122], which is insufficient for making comparisons with the tissue results presented here.

6.4 Cell Death

Cell death is a key variable in assessing the outcomes of electroporation. While the very goal of some electroporation-mediated therapies is in fact cell death (e.g. electrochemotherapy), the desired mode of cell death is apoptosis, not necrosis. The fundamental mechanisms contributing to cell death are not well understood and therefore cell viability cannot be directly modeled. However, some of the causes of death are understood in a general sense, and, to that end, cell and tissue models can provide general insights about the likelihood of cell survival following electroporation.
6.4.1 Conventional electroporation

Unintended necrotic cell death is a potential negative outcome of electroporation. For the purpose of loading cells, the uptake efficiency of surviving cells must be considered in light of the fractional cell survival. An optimal electroporation protocol will result in high uptake and relatively little cell death. Unfortunately, high uptake and cell survival are generally at odds with one another [87]. While the exact mechanisms of cell death are not well understood, unintentional necrosis (i.e. not secondary to the purposeful uptake of toxic molecules like bleomycin) probably stems from three sources: loss of intracellular contents, thermal insults, and toxic electrochemical byproducts [123].

It stands to reason that any pulse that creates a pore population that allows significant transfer of molecules from the extracellular to the intracellular space will also allow the reciprocal transfer of molecules from the intracellular to the extracellular space. This is especially true for conventional electroporation, in which pores are expected to expand to sizes that allow transmembrane transport that is relatively indiscriminate based on molecular size. While some limited studies have shown relationships between either delivered charge or energy and uptake and cell viability, a more comprehensive examination by Canatella et al. did not find simple relationships among these quantities [124]. This is not surprising given the very different pore populations and associated molecular selectivity expected in response to pulses that vary significantly in strength and duration [41]. Cells in tissue may have some level of protection against death by loss of intracellular contents because the limited volume of the extracellular space surrounding cells may lead to chemical equilibrium across the membrane without much total loss from the cell interior [125]. For loading cells with small molecules, simulations may help select pulses that will create pores just large enough to facilitate the transfer of the small molecule without allowing reciprocal transport of larger macromolecules out of the cell. Unfortunately, similar optimization based on pore selectivity may not be possible for DNA electrotransfer given the large pore sizes presumably required for DNA transport.
Thermal damage is known to be a cause of cell death by necrosis, though its role in determining cell viability following electroporation is not entirely clear. The magnitude and duration of the temperature rise vary strongly with the pulse strength and duration and the experimental setup. Most studies cite the temperature rise after electroporation as that of the electrolyte. However, the power dissipation at the micro-scale is largely determined by the pulse frequency content and the electrical properties of the membrane and electrolyte [87]. Therefore, while the spatially-averaged temperature increase may be relatively small, there may be very large, transient temperature increases at the scale of cells that lead to significant damage. Thermal damage is also of particular concern in tissue systems because electric field magnitude tends to be particularly large in the tissue surrounding the electrodes [121].

A final potential cause of cell death following electroporation is the release of toxic byproducts from the electrodes [123], which is often neglected by experimenters [126]. Tomov et al. showed that ferrous ions may be released from stainless steel electrodes and preliminary experiments by Krassowska et al. demonstrated a statistically significant increase in cell survival in response to pulses applied by gold, rather than stainless steel, electrodes [123].

Cell viability, while a critical metric in cell and tissue electroporation, cannot be directly assessed within electroporation models because too little is known about fundamental mechanisms of necrotic cell death in response to electroporation. However, models with pore expansion may begin to assist in pulse optimization for loading of particular molecules while minimizing the loss of intracellular macromolecules that cause result in cell death. The transport lattice method was used to examine thermal heating on the cell level by Gowrishankar et al. [34], and the same could be done to examine thermal effects in the cell and tissue models presented here. Simulation results could then predict
whether heating should be a concern for a particular pulse.

### 6.4.2 Supra-electroporation

Supra-electroporation is fundamentally different from conventional electroporation in its creation of many small pores in both cell and organelle membranes, rather than fewer larger pores in the cell membrane only. Therefore, while supra-electroporation may lead to cell death via thermal effects or toxic electrochemical by-products just like conventional electroporation, the contribution of the loss of intracellular contents to cell death may be quite different. Additionally, supra-electroporation may lead to cell death, whether necrotic or apoptotic, through perturbations of intracellular structures by large electric fields.

Gowrishankar et al. have noted that while the total pore area that results from supra-electroporation is 2–3 orders of magnitude larger than that of conventional electroporation, the pores are all \( \sim 0.8 \text{ nm} \), and therefore only ions and small molecules are transported through the pores [46]. Thus, the many proteins and macromolecules inside of cells are expected to be retained, reducing the likelihood of necrosis.

However, cell death may come by other means. Numerous studies have shown that cells may undergo apoptosis in response to supra-electroporation [13–19,22], though the mechanisms are still poorly understood. In contrast to conventional electroporation, supra-electroporation results in intracellular electric field magnitudes that are approximately the same as the applied electric field magnitude, potentially leading to new mechanisms for coupling between the large fields and the biochemistry of the cell and organelle interiors. Candidate mechanisms suggested by Gowrishankar et al. include phospholipid translocation, direct DNA damage, and calcium release from the endoplasmic reticulum through pores or voltage gated channels [46].
Supra-electroporation-mediated apoptotic cell death may prove clinically useful, depending in part on the biological variability of this outcome. For example, some tumors may lack the functioning tumor suppressor genes that initiate apoptosis in response to particular cellular insults. However, it is also possible that supra-electroporation may couple to later-stage pathways of apoptosis that are more robust. In any case, much remains to be learned about the mechanisms of apoptosis, both generally and in response to large magnitude, short duration pulsed electric fields, but the potential for agentless apoptosis induction is intriguing.

6.5 Assumptions and Future Work

Despite the assumptions made by the cell and tissue models, they are quite robust in characterizing the electrical responses of cells and tissue to large pulsed electric fields. While the nature of the pore populations that develop in membranes in response to large applied electric fields may vary, the tendency of the membrane, whether by pore creation or expansion, is to quickly reduce the transmembrane potential to $\sim 1$ V. As such, the electrical predictions made are somewhat independent of the details of the pore populations that form. Nonetheless, electroporation-based clinical applications will require understanding of more than just the electrical responses of cells and tissue. Specifically, biological effects and medical interventions are likely to depend on the pore size distribution, molecular transport, and mechanisms by which large fields can alter cell biochemistry. Therefore, future models will address fundamental questions involving the molecular nature of pores, biological realism, and coupled molecular transport.

6.5.1 Fundamental characterization of electroporation

The electroporation models and parameters were originally developed to explain conventional electroporation, in which pore densities are quite low relative to those of supra-
electroporation. Thus, there is an emerging question as to the limits of current electroporation theory and, indeed, the fundamental molecular structure of a pore. Given a large enough pulse, for example, the asymptotic model can predict total pore areas that exceed the membrane area, which clearly is not possible. There are, perhaps, simple ways to extend the current electroporation theory, such as modifying the differential equation for pore creation (Eq. 3.3) such that pore creation strongly decreases at high pore densities. Any such changes must, however, be based on new insights from experimental results and fundamental theory and simulation, such as molecular dynamics, not on simple “fudge factors”.

The highest pore densities resulting from the detailed cell simulations were \( \sim 5 \times 10^{16} \) at the cell membrane poles in response to the 10 ns, 10 MV/m pulse (Fig. 4-11b). In the summary results showing the polar \( N \) vs. \( E_{\text{app}} \) at the end of 10 ns pulses (Fig. 4-12b), \( N \) reaches \( \sim 10^{17} \text{ pores/m}^2 \) in response to 30 MV/m applied pulses. These pore densities correspond to fractional pore areas of 0.1 and 0.2. Furthermore, given the assumed toroidal conformation of hydrophilic pores, essentially the entire membrane areas in the regions of such high pore density are significantly structurally perturbed in response to these pulses. As such, the results should be viewed with the qualification that in the limit of high pore densities, the physical behavior of a membrane may vary significantly from that predicted, and, indeed, the very nature of what a pore is on such short time scales and at high pore densities may be very different from the nice toroidal pores typically conceptualized. It may also be that the molecular structure of pores does not change on shorter time scales or at higher pore densities, but rather there are self-limiting features of electroporation that are not captured by current models.

One molecular feature of pores that is not accounted for in the models presented here is the change in membrane permittivity that accompanies large pore densities. The conductivities of lipid and electrolyte differ by many orders of magnitude, which is why the
membrane conductance drastically increases when it electroporates. In contrast to the conductances, the lipid and electrolyte permittivities differ by only an order of magnitude. Nonetheless, the increased aqueous area that accompanies supra-electroporation presumably alters the membrane permittivity and impedance at high frequencies. For example, Fig. 4-5 shows that at very high pore density ($\sim 10^{17}$ pores/m$^2$), in contrast to at lower pore density, the voltage drop across the membrane is lower at low frequency than at high frequency. In fact, if the membrane permittivity is altered to account for the increased membrane permittivity that accompanies electroporation, the membrane impedance is always lower at high frequency than at low frequency, regardless of the pore density.

As the understanding of what pores are on a fundamental molecular level changes, so will the future generations of the models presented here. Fortunately, the MTNM, on which the models are based, is quite robust and will easily accommodate new electroporation models.

6.5.2 Modeling pore expansion and molecular transport

The transmembrane transport of large molecules is sensitive to pore size. One would expect, for example, that more large molecules would pass through a few large pores than through many smaller pores of the same total area because of the decreased interaction between the interior of the large pores and the molecules. The original asymptotic model, in which pores are assumed to be created at and maintained at the minimum pore radius of 0.8 nm, is robust in modeling electrical phenomena because pores reduce the transmembrane potential to $\sim 1$ V, whether by pore creation or expansion. However, in coupling an electrodiffusion model to the electrical model, the pore size distribution becomes important in predicting the electrodiffusive transport across cell membranes.

A model incorporating expanding pores has previously been coupled to the asymptotic
model and used to predict the uptake of DNA [41]. The expansion of pores was calculated based on the electrical force expanding pores [127]. A similar approach could be coupled to the current model. Alternatively, the Smoluchowski equation (Eq. 3.2) can be used with the source term replaced by that of the asymptotic model (Eq. 3.3), and pore expansion modeled on a small subcircuit associated with each membrane node pair. This implementation is straightforward because the Smoluchowski equation has the same form as the electrodiffusion equation (Eq. 2.62). In 1D electrodiffusion, the node voltages in the equivalent circuit correspond to concentrations at associated positions in physical space. In modeling the Smoluchowski equation using an equivalent circuit, each node voltage corresponds to a pore density at a particular pore size. Thus, the evolution of a pore population can be considered as electrodiffusion in pore radius space.

Given an electroporation model with expanding pores, the transport of solute may be modeled using an equivalent circuit for electrodiffusion, as described in Chp. 2. A separate circuit from the primary electrical circuit is used to simulate the movement of solute in response to the gradients in concentration and electric potential. At the membrane, the model must be modified to account for the dependence of transmembrane transport on the pore population.

Models with expanding pores and electrodiffusion will be powerful tools in understanding published experimental results and in prediction of optimal pulses for maximizing the uptake of specific molecules both in vitro and in vivo.

6.5.3 Biologically realistic models

The cell model used here has many of the features of a cell, including organelles and resting potentials, but to the eye of a biologist, it is still a very simplistic collection of circles. Cells are very structurally complex, and a model could never hope to resolve all of the structural complexity. However, cells with considerable structural complexity includ-
ing many organelles (golgi apparatus, endoplasmic reticulum, mitochondria, etc.) may be modeled with the current methods. Circular membranes were used here to facilitate data presentation and comparisons with analytically-derived expressions for membrane charging. However, more complicated structures without symmetry can easily be meshed and simulated. Currently, the meshing algorithm requires the same fixed node spacing on all membranes, and this will be modified to allow more efficient node allocation in cell systems with very small organelles that require very small membrane node spacings. Biologically-realistic cell models will help electroporation research establish a stronger footing with those in the biological research community. Additionally, the responses of organelles to applied electric fields will suggest new candidate biophysical mechanisms for electro-manipulation of cells.

The tissue model used here has impedance that is quite similar to the impedances of actual tissues (Fig. 3-10). However, the tissue is homogeneous. Altering the central region of tissue to have properties similar to those of a tumor would provide a compelling model for electroporation and drug delivery to a tumor. The transport properties of tumors have been extensively studied by Jain and colleagues [128–136], and their papers would serve as an excellent starting point for developing a tumor tissue model.

### 6.5.4 Alternative pulses and electrode configurations

The cell and tissue responses to single, trapezoidal pulses have been explored here. For the purpose of understanding the fundamental features of electroporation, experiments and simulations should focus on using simple, single-pulse protocols because this reduces the number of variables contributing to observed outcomes. Nonetheless, for the purpose of developing new biotechnologies and medical therapies, other pulsing protocols should be explored because they may be better for a given application [5,48,87,90,92,105,137–141]. For example, Sukharev et al. showed that a two-pulse protocol, in which a large magnitude, short duration ($\sim 10 \mu s$) pulse is used to electroporate cells and a smaller magnitude,
longer duration (∼100 ms) pulse is used to facilitate transmembrane transport of DNA, is advantageous in transfecting cells because it limits the applied energy while still creating pores and contributing significantly to electrodiffusion of DNA. This experimental result was later examined and replicated in silico by Smith et al. [41].

Multiple-pulse protocols and more complicated electrode arrangements have often been used in tissue systems. For example, hexagonal electrode arrays, which can apply multiple pulses in various directions, have been shown to be effective in drug delivery and gene delivery [137, 138]. Such pulse protocols and electrode arrangements can be studied with the current tissue model by making relatively few modifications. They were not examined here because the focus of this thesis is on explaining fundamentals, such as shifting voltage division in tissue systems, the details of which may be more difficult to analyze in more complicated systems.

6.5.5 3D models

The models presented here are 2D. The MTNM extends to 3D, and therefore the models could be recast in 3D, but because of the limited added benefit and increased complexity of 3D models, the extension to 3D is, in most cases, a lower priority than making the other improvements described here. One exception is the examination of out-of-plane effects in tissue electroporation. The 2D model assumes that the depth of the tissue system is greater than the electrode separation such that out-of-plane effects are negligible. This may not be reasonable in some clinical scenarios. Moreover, as the 10 µs, 1 MV m pulse demonstrates, shifting voltage division can lead to perturbed tissue quite far from the electrodes. This may be a concern clinically if the tissue deep to the target tissue is sensitive to electroporation.
The models and simulations presented here offer new insights into the electrical responses of cells and tissue to large, pulsed electric fields. The simulation results are consistent with experimental results, and the mechanistic bases of the models make them robust and predictive. The models will be powerful tools in evaluating potential clinical applications, and will become more so as they become increasingly sophisticated, simulating pore expansion and molecular transport.

The cell model simulations demonstrate the fundamentally disparate responses of cell and organelle membranes to low magnitude, long duration pulses and large magnitude, short duration pulses. The former involves electroporation of the outer cell membrane only and provides a means of transporting molecules into cells, while the latter involves electroporation of nearly all cell and organelle membrane regions and resulting selective transmembrane transport of small ions and leading to biochemical events that can result in apoptosis. Both may be utilized in future biotechnologies and therapies based on drug and gene delivery or direct apoptosis induction secondary to supra-electroporation.

The tissue model simulations examine the responses of homogeneous tissue to conventional pulses used for drug delivery as well as large magnitude, short duration pulses that have not previously been used clinically or been simulated. The mechanistic basis of the tissue model allows the shifting voltage division that accompanies tissue electroporation to be studied in detail for the first time. Moreover, because the model uses a multiscale approach and explicit membrane electroporation model, future models can be extended to account for molecular transport of drugs and genes, which is out of the reach of passive and empirically-based tissue models. As the mechanisms of electroporation are studied at the cell level and new applications are suggested, the tissue model will provide a powerful framework for understanding how these applications can be transferred from the
test tube to the body.

The cell and tissue models presented here will be extended in future models to account for pore expansion and molecular transport. The flexibility of the mesh transport network method makes these and other extensions straightforward and will allow increasingly sophisticated and biologically realistic models of cells and tissue. These models will enhance our knowledge of the fundamental responses of cells and tissue to large, pulsed electric fields and open the door to new electrically-based therapies.
References


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