1. Introduction

Electroporation subjects cells to strong (~kV/cm), short (~μs-ms) pulsed electric fields that are of sufficient strength to disrupt the plasma membrane. This disruption, which can be either transient or permanent, renders the membrane permeable to exogenous materials, and for this reason electroporation is a widely-used technique to insert molecules into the cytoplasm of cells [2–7]. The technique finds application in such diverse areas as cell transfection, drug delivery, gene and cancer therapy, synthetic biology, stem-cell research, and many others [8].

Over the past three decades, significant theoretical progress has been made that has increased the mechanistic understanding of electroporation. Most of these modeling efforts have been devoted to mammalian cells [9]. By contrast, the development of analytical and numerical models of electroporation in bacteria has been relatively scarce. Nevertheless, electroporation is performed on bacteria for many important practical applications such as food sterilization [10], engineering organisms to serve as catalysts for microbial fuel cells [11], the use of bacteria as model organisms to study diseases [12,13], providing a higher-efficiency alternative to heat shock transformation [14], and many more [8]. Improving the fundamental understanding of the effects of bacterial physiology on electroporation will broaden the applicability of this technique and could enable genetic engineering of intractable or difficult-to-transform bacteria.

Genetic engineering and synthetic biology show tremendous promise to benefit humanity in various ways, including providing alternative fuels [15], production of new materials [16], bioremediation of mercury pollution or oil spills [17,18], treating diseases such as Crohn’s disease [19] and cancer [20], and more [21,22]. Gene electrotransfer is the most effective methodology for genetically engineering gram-positive and gram-negative bacteria [8]. The transformation efficiency decreases with increasing cell wall thickness and typically results in $10^8$–$10^{10}$ CFU/μgDNA (colony-forming units per microgram of DNA) in gram-negative bacteria and $10^5$–$10^7$ CFU/μgDNA in gram-positive bacteria with plasmid DNA [8,23]. One important consideration in genetic transformation is the molecular form of the nucleic acid used, which could affect the fitness of the microbe. The optimal electric field strengths vary with bacterial species and even strains, but generally range between 5 and 20 kV/cm with pulse durations of milliseconds [8,23]. Protocols to perform electroporation-mediated gene transformation on bacteria are currently developed primarily by trial and error, a process that is time-consuming and entirely empirical. Thus, there is a need for greater exploration of the physics underlying the contribution of the cell envelope on bacterial electroporation.

Past theoretical efforts, validated by experiments, have provided key insights about the electroporation process. It has been experimentally...
observed that the membrane only becomes permeable when the trans-membrane voltage \( (V_{\text{m}}) \) reaches a certain threshold, typically between 200 mV and 1 V [24,25]. At this condition, it is widely accepted that hydrophilic pores form on the membrane that fill with aqueous media and allow translocation of extracellular or intracellular materials into or out of the cell [26–31]. Although the dynamics of the pore formation process are well described by the Smoluchowski equation [32–34], many modern numerical models of the process are based on the asymptotic model of electroporation derived by Krassowska, Neu and co-workers [35,36]. This model was applied in 1999, by DeBruin & Krassowska, to study the electroporation of a single spherical cell and identify the effects of cell rest potential, electric field strength, and ion concentrations [37,38]. In 2004, Smith et al. presented a simple model for the evolution of pore radii in a planar bilayer membrane undergoing electroporation and identified three criteria that pores must satisfy to be eligible to accept foreign DNA [39]. Building on this and previous work, Krassowska & Filev [1] provided a full model of electroporation in a single 50-μm-diameter cell, in which the spatial and temporal dependence of the electroporation statistics (pore radius, pore number, membrane conductance, etc.) were given equal consideration.

More recently, Li & Lin provided a numerical model of molecular uptake via electroporation which simulated the transport of calcium ions into a Chinese hamster ovary (CHO) cell using the Nersten-Planck system of equations [7]. A first step was taken in modeling the effects of bacterial physiology on electroporation by Piggot et al., who conducted molecular dynamics (MD) simulations of both a gram-positive and a gram-negative cell undergoing electroporation [40]. Due to the extremely fine spatial and temporal resolution and computational cost inherent to MD, their simulations were limited to a small patch of membrane and an extremely short duration (<1 μs) of the simulated pulse. There is thus a need for computational models that specifically take aspects of bacterial physiology into account on the whole-cell level, in order to enable more-informed preparation of electroporation protocols for bacteria.

In contrast to rigid colloidal particles, the interface between a bacterial surface and the outer electrolytic domain is not a rigid boundary but a diffuse, ion-permeable charged layer commonly known as the “soft layer” [41]. For instance, *Escherichia coli*, which is gram-negative, has a negatively charged thin lipopolysaccharide (LPS) layer of 1–3 nm thickness outside its rigid plasma membrane. *Staphylococcus aureus*, which is gram-positive, has a negatively charged peripheral peptidoglycan layer of thickness 20–100 nm. Other examples of surface appendages, which may be classified as a part of the soft layer include flagella, capsules and pili [42]. *E. coli* uses flagella to swim toward regions of greater nutrient concentration, e.g. via chemotaxis [43]. Pili provide microbes with sensory and tactile information, which allows them to adhere to animal cells and enables DNA exchange with other bacteria during conjugation.

In describing the soft layer, the word “soft” refers not to mechanical softness but rather to the fact that the layers are ion-permeable and to some extent hydrodynamically permeable. Consequently, the ion transport in and around the soft layer is usually described by a Poisson-Nernst-Planck-based electrokinetic formalism commonly referred to as soft-layer electrokinetics. Based on the pioneering work done by Ohshima and others [44,44,45], soft-layer electrokinetics has been widely used to understand the surface interfacial properties of bacteria through standard colloid science-based measurements. Sonahara et al. [46] applied Ohshima’s soft layer electrokinetic theory to obtain the soft layer fixed charge density \( \rho_{\text{soft}} \) and the soft layer hydrodynamic penetration length \( 1/N_{\text{app}}^{\text{fl}} \) for *E. coli* and *S. aureus*. They measured the electrophoretic mobilities of these organisms and concluded that the cell wall of the gram-positive *S. aureus* is more rigid and less permeable compared to that of the gram-negative *E. coli*. In their review, Duval and Gaboriaud provided a comparison of \( 1/N_{\text{app}}^{\text{fl}} \) for various gram-positive and gram-negative bacterial strains using electrokinetic measurements [47].

In the absence of an external electric field, the concentration of positive and negative ions within and outside the soft layer is described by Donnan equilibrium. For a negatively charged soft layer, electroneutrality requires that positive ions be in excess in the soft layer compared to the bulk electrolyte, and the opposite is true for negative ions. This mismatch in the ion concentrations within and outside the soft layer establishes an electrical potential profile, which changes from a negative value inside the soft layer to zero in the bulk electro-neutral electrolyte [41]. The region of potential decay, characterized by a net charge density, is known as the electric double layer (EDL) or Debye layer in the electrokinetics literature [48]. The characteristic thickness of the EDL, known as the Debye length \( (\lambda_{D}) \), is related to the nominal electrolyte concentration \( C_0 \) by the expression.

\[
\lambda_D = \sqrt{\frac{e^2 e_0 k_B T}{2 \pi e_0^2 e^2 C_0}}
\]

where \( e_0 \) is the permittivity of free space, \( e_i \) is the relative permittivity of water, \( k_B \) is Boltzmann’s constant, \( T \) is the absolute temperature, \( z \) is the valence for a symmetric \( xz \) electrolyte and \( e \) is the elemental charge.

The Debye length for a 1 M monovalent electrolyte is roughly 0.3 nm, which is much smaller than a soft layer of thickness of tens of nanometers as is the case for gram-positive bacteria. Therefore, in the absence of an electric field, the bulk of the soft layer is electroneutral and is characterized by a uniform potential known as the Donnan potential \( (\phi_D) \) [41],

\[
\phi_D = \sinh^{-1}\left(\frac{\rho_{\text{app}}}{2\lambda_D^2}\right)
\]

where \( F \) is Faraday’s constant. Eq. (2) assumes complete dissociation of ionogenic groups within the soft layer. For incomplete dissociation \( \rho_{\text{app}} \) can be a function of buffer pH and ionic strength [49,50].

Under the influence of an external electric field, the electric double layer within and outside the soft layer polarizes. This polarization is a result of the balance between tangential diffusive and electromigrative ionic fluxes (neglecting advection) within and outside the soft layer. When the electric field is weak, this double layer polarization does not result in significant gradients in the bulk electrolyte concentration and the length scale of these concentration gradients is limited to a distance of the order of the Debye length \( \lambda_D \) from the particle surface. The effect of double layer polarization on soft particle electrophoresis has been studied in the weak-field limit [50–53]. Dingari and Buie [42] demonstrated that double-layer polarization results in significant differences in polarizability (quantified by the induced dipole moment coefficient) for two strains of *S. salivarius*: fibrillated and unfractillated. When the electric field is strong, i.e., \( E_{\text{app}} \gg k_B T/ea \), here \( E_{\text{app}} \) is the applied electric field and \( a \) is the particle radius, the double layer polarization is strong enough to create gradients in bulk electrolyte concentration in order to preserve electro-neutrality in the bulk. These concentration gradients generate a diffusio-osmotic slip around the particle, which opposes the electro-osmotic flow within the electric double layer. This retardation in particle electrophoresis due to concentration polarization has received significant attention in the context of ideally polarizable particle electrophoresis [54–56]. Similar conclusions were drawn recently by Bhattacharya and De [57] who numerically modeled strong-field soft particle electrokinetics with an additional effect of a weakly polarizable core. Since electroporation requires electric fields well above the strong-field threshold, it is natural to expect that double layer polarization effects in the soft layer can significantly affect the outcome of electroporation.

To create pores via electroporation, the electric field must be strong enough to impose a voltage difference across the plasma-membrane of roughly 1 V. Assuming \( a = 1 \mu m \) for bacteria, \( E \sim 10 \text{ kV/cm} \), which is close to the value suggested by electroporation protocols for bacteria. This can be validated by a more rigorous analysis leading to the Schwan equation.
where \( V_m \) is the voltage difference across the plasma membrane, commonly referred to as the transmembrane voltage, \( \tau = \alpha C_m \left( \frac{1}{\tau_i} + \frac{1}{\tau_r} \right) \) is the characteristic RC charging time scale for the plasma membrane, \( C_m \) is the membrane capacitance, \( \alpha_i \) is the intra-cellular conductivity and \( \alpha_r \) is the extra-cellular conductivity and \( V_{rest} \) is the rest-potential for the cell. The Schwan equation calculates \( V_{m} \) assuming no pore formation and assumes constant intracellular and extracellular conductivities. However, for bacteria with soft layers exposed to such high electric fields, there is a significant amount of double layer polarization. Thus, the voltage across the membrane that is established in response to an electroporation pulse will depend on the soft layer properties (soft layer charge density) and the buffer conditions. The former property is related to bacterial physiology while the latter is an experimental parameter, which can be chosen wisely with the help of computational analysis.

In this work, we perform finite-element simulations of a gram-positive bacterium undergoing electroporation in a uniform electric field (Fig. 1). We account for the presence of the soft layer and consider several different thicknesses. We numerically estimate the size and number density of the pores formed on the membrane for each different condition. This theoretical study is intended to inform the design of favorable electroporation protocols for bacterial strains and enable the prediction of experimental conditions for bacteria previously considered intractable or difficult-to-transform with electroporation.

2. Theory

We consider a solitary, gram-positive bacterium that is subjected to a 100-μs square-wave electrical pulse. The computational domain is a two-dimensional slice of the three-dimensional axisymmetric problem and consists of two subdomains: the intracellular domain (cytoplasm) and the extracellular domain containing both the soft layer (which includes the appendages expressed on the outer cell surface such as those mentioned above) and the bulk solution (Table 1). Using a Poisson-Nernst-Planck-based formalism, we describe the influence of ion transport in the polyelectrolyte (soft) layer around the bacterial cell envelope on the electroporation characteristics, specifically the transmembrane voltage and pore statistics.

In this work, we treat the density of bound charge in the soft layer as spatially uniform, which effectively neglects the presence of the periplasmic space between the plasma membrane and the peptidoglycan layer. It is generally accepted that the periplasmic space is much smaller in gram-positive bacteria (considered in the present work) than in gram-negative bacteria; recent cryo-electron microscopy studies have directly visualized the cell wall structure of several gram-positive bacteria, supporting this notion [60,61]. A valuable future study could include the periplasmic space and outer membrane [61] as additional electrolyte layers (qualitatively similar to the cytoplasm), quantifying the effect of more complex envelopes on electroporation and the extent of cell wall disruption [62]. The intracellular electric potential \( \phi_i \) is governed by the Laplace equation, given as

\[
\nabla \cdot (s_{cyto} \nabla \phi_i) = 0
\]

(4)

where \( s_{cyto} \) is the cytoplasm electrical conductivity.

The extracellular potential \( \phi_e \) and the concentrations of positive (\( c_+ \)) and negative (\( c_- \)) ions in the electrolyte are given by the Poisson-Nernst-Planck equations as

\[
-\varepsilon_0 \nabla^2 \phi_e = \frac{ze}{\kappa_b} \left( c_+ - c_- + \rho_{soft} f(r) \right)
\]

(5)

\[
\nabla \cdot \left( D_e \nabla c_\pm + D_{fl} \frac{ze}{\kappa_b} c_\mp \nabla \phi_e + c_\pm \mathbf{u} \right) = \frac{\partial c_\pm}{\partial t}
\]

(6)

Here \( \varepsilon_0 \) is the extracellular dielectric permittivity (assumed to be spatially invariant and equal to that of water), \( e \) is the fundamental charge, \( D_e \) are the diffusivities of positive and negative ions, \( z \) is the valence of ions assuming a symmetric electrolyte, \( \kappa_b \) is Boltzmann’s constant, \( T \) is the absolute temperature, \( \mathbf{u} \) is the flow field that can be described by the Stokes equation, \( \rho_{soft} \) is the fixed charge density of ionogenic groups within the polyelectrolyte (soft) layer around the bacterium and \( f(r) \) denotes the spatial variation of the polyelectrolyte groups concentration, with \( r \) indicating the perpendicular distance from the plasma membrane to an arbitrary point within the soft layer. Here \( f(r) = 1 \) within the soft layer and \( f(r) = 0 \) outside the soft layer. The three terms on the left-hand side of Eq. (6) denote respectively the diffusion, electromigration, and advection-driven fluxes of ions in the Nernst-Planck equation.

### Table 1

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Description</th>
<th>Value [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_{app} )</td>
<td>Applied electric field</td>
<td>12.5 kV/cm [63]</td>
</tr>
<tr>
<td>( \kappa_{cyto} )</td>
<td>Cytoplasm conductivity</td>
<td>0.1 S/m [68]</td>
</tr>
<tr>
<td>( \kappa_{cyto} )</td>
<td>Cytoplasm dielectric constant</td>
<td>61 [68]</td>
</tr>
<tr>
<td>( V_{rest} )</td>
<td>Rest potential</td>
<td>0 V</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>Pore creation rate coefficient</td>
<td>( 10^6 ) m$^{-2} \cdot$ s$^{-1}$ [37]</td>
</tr>
<tr>
<td>( \rho_{soft} )</td>
<td>Equilibrium pore density</td>
<td>( 1.5 \times 10^9 ) m$^{-2}$ [37]</td>
</tr>
<tr>
<td>( V_{mp} )</td>
<td>Characteristic transmembrane voltage for electroporation</td>
<td>0.258 V [37]</td>
</tr>
<tr>
<td>( \kappa_m )</td>
<td>Membrane dielectric constant</td>
<td>10.8 [68]</td>
</tr>
<tr>
<td>( h_{m,0} )</td>
<td>Membrane thickness (without pores)</td>
<td>( 5 \times 10^{-10} ) m</td>
</tr>
<tr>
<td>( h_r )</td>
<td>Membrane thickness</td>
<td>2.5 nm [37]</td>
</tr>
<tr>
<td>( r_i )</td>
<td>Initial pore radius</td>
<td>0.8 nm [37]</td>
</tr>
<tr>
<td>( r_p )</td>
<td>Minimum hydrophilic pore radius</td>
<td>0.51 nm [37]</td>
</tr>
<tr>
<td>( r_{p,0} )</td>
<td>Constant for pore radius evolution</td>
<td>0.97 nm [1]</td>
</tr>
<tr>
<td>( r_r )</td>
<td>Constant for pore radius evolution</td>
<td>0.31 nm [1]</td>
</tr>
<tr>
<td>( \sigma_0 )</td>
<td>Tension of lipid bilayer without pores</td>
<td>( 10^{-16} ) N/m [1]</td>
</tr>
<tr>
<td>( \sigma_r )</td>
<td>Tension of lipid bilayer without pores</td>
<td>( 10^{-16} ) N/m [1]</td>
</tr>
<tr>
<td>( \beta )</td>
<td>Lipid bilayer steric repulsion constant</td>
<td>( 1.4 \times 10^{-19} ) [1]</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>Lipid bilayer line energy constant</td>
<td>( 1.8 \times 10^{-11} ) [1]</td>
</tr>
<tr>
<td>( \beta_{max} )</td>
<td>Lipid bilayer electrical force constant</td>
<td>( 0.7 \times 10^{-9} ) N/V$^{-2}$ [1]</td>
</tr>
<tr>
<td>( \rho_{soft} )</td>
<td>Concentration of fixed charge groups within the soft layer</td>
<td>1 nM [47]</td>
</tr>
<tr>
<td>( \rho_{peptidoglycan} )</td>
<td>Peptidoglycan elastic modulus</td>
<td>10 MPa [69]</td>
</tr>
<tr>
<td>( \rho_{peptidoglycan} )</td>
<td>Peptidoglycan shear modulus</td>
<td>( E_{peptidoglycan} )</td>
</tr>
<tr>
<td>( T_0 )</td>
<td>Ambient temperature</td>
<td>298.15 K</td>
</tr>
<tr>
<td>( D_{ion} )</td>
<td>Ionic diffusivity</td>
<td>( 1.6 \times 10^{-9} ) m$^2$/s</td>
</tr>
<tr>
<td>( z )</td>
<td>Ionic valence</td>
<td>( \pm 1 )</td>
</tr>
</tbody>
</table>
At the interface of the lipid bilayer and soft layer, we assume the ionic flux boundary conditions given by Li and Lin [7]. These ionic fluxes account for the diffusive and electromigrative fluxes through the nanopores as functions of intracellular and extracellular ionic conductivities. Specifically, we enforce

\[ \hat{h} \cdot \mathbf{N}_i = F_z \]  

(7)

at the interface of the lipid bilayer and soft layer, where \( \mathbf{N}_i = -D_i \nabla c_i \) is the sum of diffusive and electromigrative ionic flux components. Here

\[ F_z = \rho_p \frac{D_z \left( P_e z \ln(\gamma) (\gamma - 1) (C_{s,soft} - C_{s,1}) \exp(P_e z) \right)}{\ln(\gamma) (\gamma - \exp(P_e z))} \]  

(7a)

Here \( P_e z = -w_{z,1} \frac{F z V_{app}}{D_z} \), where \( w_{z,1} \) is the ionic mobility obtained from the Stokes-Einstein relation as \( w_{z,1} = D_z / RT \). \( \gamma = s_{s,soft}(r, t)/s_i(r, t) \) is the ratio of ionic conductivities on either side of the lipid bilayer, \( s_{s,soft}(r, t) \) is the ionic conductivity on the soft layer side and \( s_i(r, t) \) is the ionic conductivity on the intracellular side, both of which are functions of position and time, and \( \rho_p \) is the local pore area fraction, defined as

\[ \rho_p(r, t) = \frac{\sum_{\phi} \left( \pi r^2 \right)}{dA(r)} \]  

(7b)

The quantity \( \rho_p \) is the ratio of total pore area summed across \( K(r, t) \) pores in a differential membrane patch to the area of the differential membrane patch \( dA(r) \).

The external electric field is included through boundary conditions on the top and bottom boundaries of the simulation domain:

\[ \phi_{top} = \frac{V_{app}}{2}, \phi_{bottom} = -\frac{V_{app}}{2}, \]  

(8)

where \( V_{app} \) is the applied potential difference. In this work, the applied voltage and boundary spacing are chosen such that the model bacterium is exposed to a spatially uniform electric field of magnitude...
12.5 kV/cm, which is a generally-accepted value for electroporation-induced genetic transformation of many bacteria, including both gram-positive and gram-negative strains such as E. coli, Pseudomonas syringae, Corynebacterium glutamicum, and others [63].

The controlling parameter for electroporation is the trans-membrane voltage ($V_m$), which is defined as the voltage across the plasma membrane,

$$V_m = (\phi_i - \phi_e)_{\text{membrane}}$$  \hspace{1cm} (9)

We assume that the (unporated) plasma membrane is a dielectric between intracellular and soft layer domains of high permittivity compared to that of plasma membrane, and thus

$$\phi_i \approx \phi_e$$

Eq. (10) effectively assumes that the charge per unit area on either side of the plasma membrane is equal in magnitude ($C_m V_m$) and opposite in sign (as in a capacitor). Here $C_m$ is the plasma membrane specific capacitance.

The trans-membrane current density $J$ from the intracellular domain is given as

$$J(t) = -n \cdot (s_{cytoplasm} \nabla \phi_{cytoplasm}) = \frac{V_m s_{membrane}}{h} + I_{pore} + C_m \frac{dV_m}{dt}$$  \hspace{1cm} (11)

Here $h = 5$ nm is the thickness of the plasma membrane, $s_{membrane}$ is the plasma membrane conductivity in the absence of pores and the pore current $I_{pore}$ is given as

$$I_{pore} = \frac{2\pi \gamma s_{soft} V_m}{n r_p + 2h}$$  \hspace{1cm} (12)

Here $s_{soft}(r_i)$ is the approximate conductivity of the aqueous solution filling the pores, where $s_{soft}$ is the soft layer conductivity at the soft layer-lipid bilayer interface (which generally varies with position $r_i$ on the surface) and $r_p$ is the instantaneous pore radius. Eq. (12) is obtained by substituting the extracellular conductivity with the soft layer conductivity in the analogous expression derived for mammalian cell electroporation by Li and Lin [64]. The fundamental assumption in their work is that pore current is predominantly Ohmic owing to large membrane thickness compared to the pore radius. Here $s_{soft}(r_i)$ is defined as

$$s_{soft}(r_i) = 2z^2 \Lambda_s C_s (r_i) + z^2 \Lambda_c C_c (r_i)$$

where $z$ is the valence of the positive/negative ions (again assuming a symmetric electrolyte and $\Lambda_s$ is the molar conductivity of positive/negative ions.

The value of $V_m$ governs the rate of pore creation and destruction, which is assumed to obey the ordinary differential equation derived previously by Krassowska, Neu and coworkers [35,65],

$$\frac{dN}{dt} = \alpha \exp(V_m/V_{cp}) \left[ 1 - \frac{N}{N_{eq}(V_m)} \right]$$  \hspace{1cm} (14)

Here $\alpha$ is a constant related to the average pore creation rate, $V_{cp}$ is a characteristic voltage for electroporation (related to the empirically-determined critical voltage for electroporation $V_{cp} \approx 1$ V using $V_{cp} = V_{crit}/4$) [37], and $N_{eq}$ is the equilibrium pore density. This equation is solved at each point on the plasma membrane, and at any given time the total number of pores on the cell membrane is given as the surface integral of the pore density, rounded to the nearest integer.

$$N_{pores}^{membrane} = \int \int N \ dA$$  \hspace{1cm} (15)

The size of the pores is expected to be a key parameter in determining the efficacy of a given electroporation experiment [39]. Thus, we also track the time evolution of the radii of the pores by solving the following equation for each individual pore created on the cell membrane:

$$\frac{dr_p}{dt} = \frac{D}{r_p^2} \left\{ \frac{V_m^2 F_{\text{max}}}{1 + r_p/(r_p + r_i)} + 4\beta \frac{r_p}{r_i} - 2ny + 2n\sigma_{eff} r_p + F_{\text{elastic}} \right\}$$  \hspace{1cm} (16)

In Eq. (16), $D$ is an effective diffusivity for the pore radius and is set to $5 \times 10^{-14}$ m$^2$ s$^{-1}$ [1,34], $F_{\text{max}}$ is the maximum electric force for $V_m = 1$ V [1], $r_i$ and $r_p$ are constants [70], $\beta$ is the steric repulsion energy [1], $r_i$ is the minimum radius of hydrophilic pores [1], and $\gamma$ is the energy per unit length of the pore’s edge [1]. Each term on the right hand side of Eq. (16) represents a different physical mechanism that can expand or

![Fig. 3. Dimensionless positive ion concentration, non-dimensionalized with the bulk electrolyte concentration $C_{00}$ around a rod-shaped gram-positive bacterium with a 50-nm-thick soft layer at $t = 100 \mu$s after the application of a 12.5 kV/cm electric field pulse at (a) 0.1 mM, (b) 1 mM, (c) 10 mM and (d) 100 mM buffer concentrations. The figure demonstrates that concentration polarization around the cell envelope is significantly higher at 0.1 mM buffer concentration compared to other buffer concentrations. We computed the log$_{10}$ of the scaled positive ion concentration in order to show the effect of scaled concentration polarization and pore current across four orders of magnitude (0.1 mM–100 mM).](Image 312x110 to 536x275)
shrink pores. The first term represents electrical stress on the membrane; the second, steric repulsion among lipid heads lining the pores; the third, the line tension along the pore perimeter that tends to shrink the pores; the fourth, the effective interfacial tension between the membrane and solution; and the fifth, the elastic force exerted by the peptidoglycan layer, which tends to oppose pore expansion. The term

$$
\sigma_{eff} = 2\sigma' - \frac{2\sigma' - \sigma_0}{1 - \rho_p} \rho_p/C_1/C_2 \left( \frac{1}{r_0} \right)
$$

represents the effective interfacial tension between the membrane and the soft layer, which is assumed to be partially impregnated with fluid. Here $\sigma'$ is the tension of the hydrocarbon-water interface, and $\sigma_0$ the tension of the lipid bilayer without pores. In this study, we assume that the bacterium is rigid enough that changes in surface area due, e.g., to electrically-induced cell deformation can be neglected. Here $F_{\text{elastic}} = \sigma_{pore}(2\pi r)$ is the elastic force exerted by the peptidoglycan layer on the expanding pore in the lipid bilayer. The quantity $\sigma_{pore}$ is defined by the expression.

$$
\sigma_{pore} = \sigma_{\text{peptidoglycan}} = \left( -\frac{1}{2} + \frac{1}{2} \frac{r_p}{r_0} \right) - \log \left( \frac{r_p}{r_0} \right).
$$

Which describes the radial force per unit area required to expand a cylindrical pore against elastic forces in a domain of shear modulus.
The expression given by Eq. (18) is derived from rubber elasticity considerations [66] by assuming that the peptidoglycan layer exhibits elasticity with large strains as reported in the literature [67] (see Appendix A for details).

The simulations are performed in COMSOL Multiphysics 5.1 (Burlington, MA). A finite-element mesh consisting of 30,000–50,000 elements is used. The pore radii on each membrane patch are tracked using a MATLAB script, and the two programs are connected via COMSOL LiveLink™ for MATLAB. The total number of elements depends on the thickness of the modeled soft layer, since the mesh is extremely fine in that region to accurately resolve the conductivity and electric field gradients. Throughout the Results section, all electroporation statistics are shown 100 μs after the pulse is applied, unless otherwise noted. The magnitude of the electric field is constant during the pulse and is equal to 12.5 kV/cm (i.e., the pulse is a square wave). In all the simulations reported here, the bacterium is assumed to be aligned parallel to the direction of the electric field [71]. In practice, bacteria can have an arbitrary orientation prior to application of the electric field, but it is well-documented that prolate cells align parallel to an applied electric field [72–74] on a timescale of order 1 ms [75]. It was experimentally observed that bacteria re-orient to be parallel to the field lines on a time scale shorter than 1 ms in response to a 7-ms-long 2 kV/cm pulse [75]. Thus, for simplicity we assume that the bacterium is parallel to the field for the entire duration of the pulse. Finally, in this study we considered an individual cell suspended in an infinite electrolyte medium. Therefore, the effect of neighboring cells was not included here but should be considered when modeling electroporation of bacteria in biofilms or cells in tissues. This would be similar to the work of Pucihar et al. [76] in Chinese hamster ovary cells in which the electrical conductivity is non-homogeneous.

3. Results

In this work, we perform a parametric numerical study on the effects of the soft layer’s presence on electroporation of a generic gram-positive bacterium. We consider a rod-shaped bacterium with a soft layer thickness of tsoft in a buffer with bulk electrolyte concentration C0. We expect these two parameters (tsoft and C0) to most directly affect the pore statistics relevant to intracellular delivery of exogenous materials into bacteria. Tables 1 and 2 show the values of the constants and (variable) parameters considered in this work, respectively. We have assumed that the rest potential Vrest is zero to highlight the asymmetry in the transmembrane voltage distribution between the north and south poles (Fig. 1) arising only from concentration polarization. Our goals are to determine the conditions that are most conducive for successful delivery of foreign materials (e.g. plasmid DNA) into a bacterium during the electroporation pulse, and to assess the effect of the bacterium’s soft layer on those conditions.

Fig. 2 shows the positive ion concentration in the 50-nm soft layer and fluid surrounding a bacterium at bulk electrolyte concentrations of 0.1 mM (Fig. 2a–c), 1.0 mM (Fig. 2d–f), 10 mM (Fig. 2g–i) and 100 mM (Fig. 2j–l) at the end of the 100-μs pulse. The electric field drives surface conduction of positive ions parallel to the membrane, such that cations are depleted from the pole of the cell facing the positive electrode (top of all panels in Fig. 2), henceforth referred to as the “north pole.” As a result of surface conduction, cations accumulate at the pole facing the negative electrode (“south pole,” at the bottom of each panel in Fig. 2). This tangential ion flux in the soft layer results in concentration polarization in the bulk electrolyte, in order to preserve electroneutrality outside the soft layer. The amount of concentration polarization depends on the relative soft layer conductivity compared to the bulk. Thus concentration polarization increases at lower bulk electrolyte concentrations as seen in Fig. 2a–f compared to Fig. 2g–l.

These figures also depict the effect of pore current during the electroporation process. In Fig. 2a–c and d–f there is a net positive ion flux from the cytoplasm to the soft layer, whereas in Fig. 2g–i and j–l there is a net positive ion flux from the soft layer to the cytoplasm. This can be seen from a slight local cation concentration enhancement at the poles (soft layer–cytoplasm interface) of the cell in Fig. 2a–c and d–f and slight local cation concentration depletion near the poles in Fig. 2g–i and j–l.

Fig. 3 shows the dimensionless positive ion concentration at the end of electroporation pulse i.e., t = 100 μs, non-dimensionalized by the bulk electrolyte concentration C0. The figure demonstrates that the concentration polarization around the cell envelope is significantly higher at the lowest buffer concentration of 0.1 mM (Fig. 3a) compared to other buffer concentrations (Fig. 3b–c). We present the scaled results in log10 scale in order to illustrate results across four orders of magnitude.

![Fig. 7. Pore density (1/μm²) vs. position (μm) along the membrane of a rod-shaped bacterium for (a) 0 nm (b) 50 nm and (c) 100 nm soft layer thicknesses at various buffer concentrations. The abscissa (arc length) is the distance along the cell envelope as shown in Fig. 1. This figure shows the influence of concentration polarization on the number of pores formed during the electroporation of this bacterium.](Image)
of variation in buffer concentration. The maximum positive ion concentration polarization exhibits an approximate 19-fold enhancement at the 0.1 mM electrolyte concentration. A mild enhancement is also detected at the 1.0 mM with an approximate 3.25-fold increase in positive ion concentration in comparison with the background electrolyte concentration. All four electrolyte concentrations evaluated exhibit a minimum cation concentration depletion ranging between $0.2 \times C_0$ and $0.4 \times C_0$.

The tangential ionic flux within and outside the soft layer alters the distribution of electric field intensity near the cell membrane, and thus affects the transmembrane voltage ($V_m$). Fig. 4 demonstrates the temporal evolution of $V_m$ as a function of position along the membrane for the case of a 50 nm soft layer thickness bacterium. The variable “arc length” refers to the distance along the surface of the membrane, with 0 $\mu$m corresponding to the north pole and 2.28 $\mu$m to the south pole. The transmembrane voltage plot is qualitatively different before (capacitive charging) and after pore formation. During the capacitive charging phase ($t = 1 \mu$s), the transmembrane voltage plot reaches a maximum (minimum) value at south (north) pole. After pore formation, there is a decrease in the transmembrane voltage at the poles due to pore current. The transmembrane voltage profile is qualitatively similar for other soft layer thicknesses and bulk electrolyte concentrations. Along the cylindrical portion of the cell (away from the poles), $V_m$ varies linearly, reflecting the constant applied electric field, and thus linear potential variation, outside the cell.

Fig. 5 shows the influence of bulk electrolyte concentration on transmembrane voltage profile along the cell envelope. In order to show a stark contrast, we have chosen bulk electrolyte concentrations of 0.1 mM (Fig. 5a and c) and 100 mM (Fig. 5b and d). At 0.1 mM bulk electrolyte concentration, the concentration polarization around the bacterial cell envelope is significantly higher than at 100 mM bulk electrolyte concentration because of higher concentration of soft layer charge groups relative to the background electrolyte. Therefore, one can see that the transmembrane voltage plots are similar for 50 nm and 100 nm soft layer thicknesses at 100 mM bulk electrolyte concentration (Fig. 5b and d). However, they are qualitatively very different at 0.1 mM bulk electrolyte concentration (Fig. 5a and c). In Fig. 5c (100 nm soft layer thickness, 0.1 mM bulk electrolyte concentration), the concentration polarization around the cell envelope reduces the transmembrane voltage magnitude to such an extent that there is no pore formation. In this case the transmembrane voltage is reminiscent of capacitive charging of the membrane, described by the Schwan equation. These results suggest that it is harder to successfully electroporate cells at lower buffer concentrations.

Fig. 6 shows the influence of soft layer thickness on transmembrane voltage profile along the cell envelope (at $t = 100 \mu$s) for soft layer thicknesses of 0 nm, 50 nm and 100 nm. Note that, we have assumed the same mechanical properties for these three soft layer thicknesses. Therefore the 0 nm soft layer case corresponds to a soft layer with no charge but the same mechanical properties as that of 50 nm and 100 nm soft layers. For the case of 0 nm soft layer thickness, the transmembrane voltage distribution is symmetric about $V_m = 0$, i.e., the transmembrane voltages at north and south pole are equal in magnitude and opposite in sign. However, this symmetry breaks down for 50 nm and 100 nm soft layer thicknesses, due to soft layer electrokinetics and different pore currents at both the poles. Soft layer

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Number of pores at the northern half and south half of the bacterial cell envelope.</th>
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<tbody>
<tr>
<td></td>
<td>0 nm</td>
</tr>
<tr>
<td></td>
<td>North</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>10</td>
</tr>
<tr>
<td>1 mM</td>
<td>22</td>
</tr>
<tr>
<td>10 mM</td>
<td>26</td>
</tr>
<tr>
<td>100 mM</td>
<td>6</td>
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Fig. 9. Average pore radius, averaged over multiple pores in a membrane patch of area 0.0091 $\mu$m$^2$, at the north (solid line) and south (dashed line) poles as a function of time for 0 nm soft layer thickness at various buffer concentrations as shown in each figure. In the legend of each graph, the number in parentheses denotes the number of pores in the membrane patch, each having an area 0.0091 $\mu$m$^2$. The pore radius grows with time because of the applied electric field (to dissipate the imposed Maxwell stress on the membrane). This pore radius growth rate is opposed by the peptidoglycan layer elastic force, which tends to hold the membrane together.

![Fig. 9](image-url)
electrokinetics drives the positive ions (which are in excess inside the soft layer) toward the south pole, thereby decreasing the conductivity near the north pole. This reduced conductivity within and outside the soft layer at the north pole reduces the magnitude of the transmembrane voltage at the north pole. Therefore one can see that the transmembrane voltage is smaller in magnitude at the north pole (arc length = 0) compared to the south pole (arc length = 2.28 μm) in Figs. 4, 5 and 6. However, an exception to this occurs for the case of 50 nm soft layer thickness and 0.1 mM buffer concentration (Figs. 5a and 6b) due to pore current. Here the pore current at the south pole (from soft layer to cytoplasm due to enhanced local conductivity) reduces the local transmembrane voltage magnitude and pore current at the north pole (from cytoplasm to soft layer due to depleted local conductivity) increases the local transmembrane voltage magnitude. Therefore, the transmembrane voltage at the north pole is higher in magnitude compared to the south pole in this case.

The transmembrane voltage $V_m$ is the driving parameter for pore nucleation and growth. Physically, pores nucleate in response to the (Maxwell) stress exerted on the membrane by the electric field, which drives re-organization of the lipid heads to form stable, conducting, hydrophilic pores [35,77–79] whose sizes vary to minimize the overall free energy of the membrane. Mathematically, this process is modeled by Eq. (14), according to which the rate of pore creation depends exponentially on the square of $V_m$. Fig. 7 shows the pore density as a function of position along the bacterial membrane at the end of a 100-μs pulse. As expected, pores nucleate primarily on the poles of the cell, where the transmembrane voltage reaches the generally accepted threshold value of 1 V. In Fig. 7a, in the absence of soft layer electrokinetics, the
pore density distribution is symmetric from north pole to south pole. Concentration polarization breaks the symmetry between north and south poles due to the change in local conductivities near the poles.

In the cases considered in this study, an increase in bulk conductivity leads to the formation of more pores. This trend is consistent with that found by Ivorra et al. [80], who showed numerically that a less conductive medium requires a greater electric field in order to achieve the same level of permeabilization. It has also been observed experimentally that the electric field magnitude required to achieve a specific electroporation effect decreases with an increase in extracellular conductivity [80], e.g., permeabilization of green algae cells [81], 50% fibroblast survival after a train of eight 100-μs pulses [82], and gene expression in mesenchymal cells after a train of eight 100-μs pulses [83]. The reduction in pore density due to concentration polarization is maximum for the 100 nm soft layer thickness at the lowest buffer concentration as seen from Fig. 7c.

Fig. 8 shows the total number of pores created on the cell membrane for the case of no soft layer ("0 nm") and two finite soft layer thicknesses (50 nm and 100 nm), at four different background electrolyte concentrations. At low buffer concentration (0.1 mM), the tangential ionic flux within the soft layer reduces the transmembrane voltage and inhibits pore formation for the 100 nm soft layer. This is because at low buffer concentration, the electrical double layer is relatively thick and thus the effects of surface conduction are significant. However, at high bulk electrolyte concentrations (>10 mM) the pore current increases due to the increase in pore conductivity (which is the logarithmic mean of bulk electrolyte conductivity and intracellular conductivity) reduces the transmembrane voltage and lowers the number of pores. Table 3 shows the number of pores formed at the northern and southern halves of the cell. (It should be noted that the values provided in this table are meant to be taken as order-of-magnitude estimates, and the error in these values could be non-negligible.) For the case of 0 nm soft layer, in the absence of concentration polarization, the number of pores is the same at both the hemispheres, as expected. For 50 nm and 100 nm cases, concentration polarization causes an asymmetry in the pore number at the northern and southern hemispheres. At low bulk electrolyte concentrations (0.1 mM and 1 mM), the local conductivity enhancement due to concentration polarization at the south pole leads to an increase in the pore number. However, at high bulk electrolyte concentrations (10 mM and 100 mM) an increase in pore current dominates this effect and locally opposes pore formation. Therefore, an opposite trend is observed for 10 mM and 100 mM cases. Thus, there is an optimal ion concentration that maximizes the number of pores formed. It is likely that this optimal concentration varies for bacteria with different shapes, soft layer thicknesses, and soft layer conductivities [84]. Also, at high electrolyte concentrations (100 mM), the electrical double layer is negligibly thin and thus the soft layer electrophoretic rate is negligible effect on pore formation. As a result, the total number of pores is unaffected by the presence of the soft layer. Thus it is possible that this model could be used to design protocols for new bacteria, such that the number of pores created is maximized, increasing the likelihood of successful insertion of exogenous material.

Figs. 9, 10 and 11 shows pore radius evolution for three soft layer thicknesses 0 nm (Fig. 9), 50 nm (Fig. 10), and 100 nm (Fig. 11) at various bulk electrolyte concentrations. For each test case, Figs. 9–11 show the pore radius evolutions of the average pore radius, averaged over multiple pores in a membrane patch of area 0.0091 μm² at the north and south poles, respectively. The number of pores in this membrane patch is shown in parentheses in the legend for each graph. For each soft layer thickness, in general, increasing the bulk electrolyte concentration increases the rate of pore radius evolution. This is consistent with the fact that the electrical driving force for pore radius growth is proportional to the square of the transmembrane voltage (Eq. (16)), which increases with increasing bulk electrolyte concentration. For the case of zero soft layer thickness, pores at north pole and south pole follow the same pore radius evolution trend because of the symmetry between the pore formations at north and south poles. (This is also true for pores at north-east and south-east locations.) However, this is not true for 50 nm and 100 nm soft layers, where in general (except in Fig. 10a, discussed later) pores at the north pole have slightly lower radius compared to pores at the south pole. This is because concentration polarization lowers the local conductivity at the north pole, which lowers the electrical driving force for pore radius evolution. In Figs. 9b, 10b and 11a, which correspond to the 1 mM buffer concentration, the pores almost reach an equilibrium value at the end of 100 μs, which is due to the balance between electrical force on the pore and the elastic retarding force due to the peptidoglycan layer. At the lowest buffer concentration of 0.1 mM for the 50 nm soft layer thickness (Fig. 10a), the pores generally form at very different time instants due to significant concentration polarization.

Therefore, they follow very different pore radius evolutions. In general, at higher concentrations the pores tend to nucleate at roughly the same time. We note that the radii shown on each pole actually represent the average of multiple individual pores on the discrete membrane patch, which all have nearly exactly the same radius. From Figs. 9–11 we can conclude that higher bulk electrolyte conductivity in general results in larger pore growth rate. However, at the highest buffer concentration of 100 mM, significant pore current results in a lower number of pores compared to 1 mM and 10 mM buffer concentrations. So optimal choice of bulk electrolyte concentration for effective transformation based on our simulations would be in the range 1 mM–10 mM.

It is well-known in the electroporation modeling community that quantitative experimental validation of model predictions is essentially impossible (for the time being). The pores that are posited to nucleate in response to an electroporation pulse are too small and too short-lived to be observed with state-of-the-art optical or electron microscopy. Furthermore, molecular dynamics studies of electroporation are limited to sub-microsecond durations and patches of membrane on the order of nanometers in size. To make theoretical progress toward increasing the physical understanding of electroporation, approximations are necessary.

We expect the main value of this study to lie in the interpretation of the predicted trends in pore size and number as a function of soft layer thickness and buffer concentration. Several of the predictions made in this work, such as the existence of an optimal concentration for pore formation, should be experimentally observable. For example, it is relatively straightforward to perform electroporation experiments at different buffer concentrations and to observe the effect of concentration on uptake of dye molecules, proteins, or DNA. Similarly, bacteria with different soft layer properties can be subjected to the same electroporation conditions [84] and the difference in molecular uptake should be measurable.

This work represents a first step in a new direction, of modeling electroporation in bacteria on the whole-cell level, to increase the community’s understanding of the factors that govern the success of bacterial electroporation experiments.

4. Conclusions

This work demonstrates that in gram-positive bacteria the soft layer alters the spatial distribution of electric field intensity near the cell membrane such that the subsequent pore dynamics are affected. As a result, the transmembrane voltage distribution is altered, and thus the number and size of the pores created are different compared to their values for cells without a soft layer. The implication of this is that for bacteria with thick and/or conductive soft layers, conditions favorable for inserting DNA by electroporation may be more difficult to ascertain. In some cases, this may contribute to the difficulty in successfully performing electroporation on certain species of bacteria. Thus, for
difficult-to-transform or intractable organisms, one should consider bulk electrolyte and soft layer properties when exploring conditions that lead to successful electroporation.

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Appendix A

References


Fig. 12. A schematic of lipid bilayer and peptidoglycan (top) without and (bottom) with the formation of a pore in the lipid bilayer. The formation of pore stretches the peptidoglycan chains, which in turn exerts a force on the pore resisting its expansion.

Fig. 12 shows the lipid bilayer and peptidoglycan layer combination in the absence and presence of a hydrophilic pore in the lipid bilayer. Due the pore expansion, the peptidoglycan layer chains get stretched. The radial elastic force on the peptidoglycan layer due to a pore in the lipid bilayer of size \( r_p \) is approximated by the radial force required to expand a pore in an elastomer from an initial radius \( r_0 \) to final radius \( r_f \). The initial void size in the peptidoglycan layer which is \( \sim 2 \) nm. The derivation is given in the appendix of the paper by Cai et al. [66], which deals with the osmotic collapse of a void in an elastomer. The radial stress on the peptidoglycan chains close to the lipid bilayer pore are given as

\[
\sigma_{\text{peptidoglycan}} = \left( \frac{1}{2} + \frac{1}{2} \frac{r_f^2}{r_0^2} \right) - \log \left( \frac{r_f}{r_0} \right)
\]

Consequently, the radial force exerted by the peptidoglycan chains on the lipid bilayer pore is given as

\[
F_{\text{elastic}} = \sigma_{\text{peptidoglycan}} (2\pi r_p h)
\]