Effects of solution conductivity on macropore size dynamics in electroporated lipid vesicle membranes

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A B S T R A C T

Using fast imaging microscopy, we investigate in detail the expansion of micron-sized pores occurring in individual electroporated giant unilamellar vesicles composed of the phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). To infer pore dynamics on the electrodeformed and electroporated vesicles, we develop a computational approach and provide for the first time a direct evidence of quantitative agreement between experimental data and the well-established theoretical prediction of Smith, Neu and Krassowska (SNK). The analysis we describe also provides an extension to the current theoretical literature on how the conductivity ratio of the internal and the external vesicle solution plays a determinant role in the definition of the electrical force driving pore expansion kinetics.

1. Introduction and motivation

Cell electropermeabilization (EP) sets the basis of a number of novel therapeutic strategies, including electrochemotherapy and tumor ablation, drug and gene delivery and cell transfection, to mention a few. These applications motivate the current approach in understanding the EP mechanisms of biological cells and lipid vesicles [1–23]. In these settings, there has been a longstanding debate over the mechanism of pore opening and expansion across cell membranes and/or lipid bilayer. Strong electric fields can trigger phase transitions in phospholipid bilayers and lead to the formation of hydrophilic aqueous pores. However, despite the large amount of work dedicated to set solid theoretical groundings to vesicle and lipid bilayers [24–27] the precise physical mechanisms underlying the generation and expansion of these local defects remain an open question. This challenge is further compounded by the current lack of knowledge of the role of the membrane solution environment which eventually impacts significantly pore nucleation and expansion processes in vesicle and cell membranes [13–14]. In particular, the role of conductivity conditions across the membrane has not been resolved even though conductivity asymmetry is inherent to plasma membranes. In general terms, vesicle membranes can be treated as incompressible fluid interfaces and have significant promise as an arena for investigating multiscale and multiphysics cell membrane EP models. Additionally, there does seem to be (at least) a consensus that the pore size and transmembrane potential, $V_m$, are two critical variables in determining EP dynamics, but little is known about the contribution from each cue individually, see e.g. Refs. [13–14]. Current strategies for visualizing the time-dependent behavior of individual electropores in vesicles rely on detecting the dynamics of solute flow between the internal and external volumes and imaging the membrane itself [17–23,28–33], or using optical single-channel recording and fluorescence imaging of bilayers [11].

In light of the above, this work considers a tractable geometric configuration of lipid vesicles for which the electrodeformation (ED) and electroporation phenomena can be simultaneously studied. For this purpose, our analysis is based on the computation of the Maxwell stress tensor (MST) to evaluate the electromechanical forces that induce both membrane deformation [34] and expansion of embedded pores [15]. On the experimental side, the electropore expansion velocity is characterized by studying the dynamics of solute flow between the internal and external volumes of giant unilamellar vesicles composed of POPC. We find that the conductivity ratio of the inside and the outside vesicle solution impacts significantly the expansion velocity of a macropore occurring in electrodeformed and electroporated vesicles. We provide both numerical and analytical support to these findings which are in quantitative agreement with our experimental results. The electrical force needed to expand a transient macropore is computed as a function of the local transmembrane potential and is compared with the experimental data of pore expansion velocities when the conductivity ratio of the internal and the external vesicle solutions is varied over two
orders of magnitude. We find that the quadratic dependence of the electric force expanding the pore on the transmembrane potential, $V_m$ is conserved as in the Smith, Neu and Krassowska (SNK) model [15]. On the numerical side, we use finite element simulations in order to quantify the electromechanical behavior of electropermeabilized lipid vesicle membranes [34–36].

2. Background

First theories of lipid membrane electrical breakdown have described the EP phenomena as a stochastic process during which structural defects, presented as aqueous pores [26], form in the membrane when subjected to a sufficiently high transmembrane potential [24–26,37]. Under such steady transmembrane potential, pore nucleation and expansion dynamics were suggested to follow a two-stage process: first nucleating as small cylindrical non-conducting pores that expand in the membrane, then becoming conductive pores as they exceed a threshold size (roughly 0.5 nm) as a result of their expansion. This expansion process would eventually result in membrane rupture, characterized by an increase in membrane conductivity over several orders of magnitude [24–26,37]. In these settings, a theoretical description of the EP phenomena was proposed in [26] based on thermodynamics and in which the overall pore population obeys the Smoluchowski equation.

$$\frac{dh(r,t)}{dt} = D \frac{\partial}{\partial r} \left( h(r,t) \frac{\partial}{\partial r} U(r,V_m) \right)$$

Here $h(r,t)$ denotes the pore density distribution function, where the number of pores per unit area with a size comprised between $r$ and $r+dr$ is $h(r,t)dr$ [26]. $D$ is the diffusion coefficient of pore radius in the space [26], $k$ is the Boltzmann constant, $T$ is the absolute temperature, $t$ represents time, and $U(r,V_m)$ represents the shift in membrane free energy due to the presence of a pore in the membrane, defined as.

$$U(r,V_m) = -2 \pi \gamma + (V_m^2 + 2 \pi \sigma) r$$

where $\gamma$, $C$ and $\sigma$ respectively account for pore edge tension, membrane capacitance per unit area and lipid-water interfacial surface tension [26]. It is also important to notice that Eq. (1) has no analytical solution, and therefore the density distribution of pores in the membrane must be solved numerically [32,37,38]. In addition, the large computational time required for solving Eq. (1) on tractable space and time scales constitute serious drawbacks [38]. To tackle these issues, Neu and Krassowska conducted a thorough analysis in [38] and proposed an asymptotic model to the one presented above, which simplifies the partial differential equation (PDE) of Eq. (1) in two independent ordinary differential equations to describe the time evolution of pore density and pore radii.

$$\frac{dN}{dt} = a \exp\left(\frac{V_m^2}{V_m^2}\right) \left(1 - \frac{N}{N_0 \exp\left(qV_m^2/V_m^2\right)}\right)$$

and

$$\frac{dV}{dt} = D \frac{\partial}{\partial r} U(r,V_m).$$

Here $N$ is the pore density, $a$ and $q$ are pore creation rate constants, $V_m$ is the electroporation threshold transmembrane potential and $N_0$ is the initial number of defects [38]. The expression $U(r,V_m)$ for conducting pores ($r > 0.5$ nm) has been extensively debated in the literature, with particular emphasis on the definition of the energy term related to the induced transmembrane potential $V_m$ [15,25–27,37].

From the perspective of today’s understanding, the approach of SNK [9], which consisted in defining the $V_m$ related energy term from the integration of the electric stresses (MST) over the internal boundaries of a toroidal pore [15], has been by far the most reemployed numerical model for cell membrane EP simulation over the past two decades [4–5,10,38–49]. An important remark here is that imbalances in the conductivities of media surrounding the membrane were neglected, i.e., only the case $\Lambda = 1$ was investigated in the SNK model [9], while the vast majority of membrane EP simulation studies have focused their efforts on modeling the response of systems showing conductivity imbalances of one order of magnitude between the media surrounding the membrane [4–5,10,38–49]. In the SNK model, $U(r,V_m)$ is defined as.

$$U(r,V_m) = \sum_{i=1}^{4} U_i = V_m^2 f_{SNK}(r) + \frac{2 \pi}{r} \left(\frac{r^2}{2 \pi \sigma} + 2 \pi \sigma f_{SNK}(r) - 2 \pi \gamma \right)$$

Here $f_{SNK}(r)$ represents a force per unit square voltage [15], $\beta$ denotes the steric repulsion energy, $r^*$ is the minimum pore radius, and finally, $\sigma_{eff} = 2 \pi \sigma f_{SNK}(r)$ is the effective tension of the porated membrane felt by the pore of radius $r$ [50], where $\Lambda_p$ and $A$ correspond to the area occupied by the pores and the total membrane area, with $\sigma$ and $\sigma_0$ defined in Table 1.

The first term of Eq. (2) accounts for the electric force induced by the local value of $V_m$; the second represents the steric repulsion of lipid heads, the third, for the edge tension opposing the expansion of the circumference of the pore, and the fourth introduces the surface tension of the membrane [15,38]. Here, we reproduce the numerical method of SNK and extend their model to compute the $V_m$ dependent energy term of Eq. (5) as a function of $\Lambda$. In the SNK model, the first term of Eq. (5) takes the form [15].

$$f_{SNK}(r) = f_{\Lambda}(r) = \frac{1}{r + r_1} \left(\frac{r + r_3}{r + r_2} - \frac{r}{r + r_2}\right)$$

This expression was obtained by SNK as a phenomenological approximation of their numerical computations where $f_{\Lambda}(r)$ is the maximal radial force per unit squared Volt, and $r_1$ and $r_2$ are two constants [15]. Here, we derive a $\Lambda$ dependent extension of their model, also based on a similar approximation of our numerical results which has a different analytical expression, namely.

$$f_{\Lambda}(r) = f_{\Lambda}(r) = \frac{1}{r + r_1} \left(\frac{r + r_3}{r + r_2} - \frac{r}{r + r_2}\right)$$

where $f_{\Lambda}(r)$, $r_1$, $r_2$, $r_3$ and $\chi$ are constants which depend only on $\Lambda$ (discussed below and summarized in Table 2).

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Numerical value</th>
<th>Fig.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane total thickness</td>
<td>$d_m$</td>
<td>$2.7 \times 10^{-3}$</td>
<td>6</td>
<td>[17]</td>
</tr>
<tr>
<td>Membrane dielectric thickness</td>
<td>$d_{m0}$</td>
<td>$1.7 \times 10^{-10}$</td>
<td>1</td>
<td>[15]</td>
</tr>
<tr>
<td>Internal medium permittivity</td>
<td>$\varepsilon_{int}$</td>
<td>$4.4 \times 10^{-4}$</td>
<td>1</td>
<td>this work</td>
</tr>
<tr>
<td>Internal medium conductivity</td>
<td>$\lambda_m$</td>
<td>$1.7 \times 10^{-11}$</td>
<td>1</td>
<td>[15]</td>
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<tr>
<td>Membrane permittivity</td>
<td>$\varepsilon_m$</td>
<td>$1.4 \times 10^{-12}$</td>
<td>1</td>
<td>[15]</td>
</tr>
<tr>
<td>Membrane conductivity</td>
<td>$\lambda_m$</td>
<td>$1.4 \times 10^{-12}$</td>
<td>1</td>
<td>[15]</td>
</tr>
<tr>
<td>External medium permittivity</td>
<td>$\varepsilon_{ext}$</td>
<td>$1.4 \times 10^{-12}$</td>
<td>1</td>
<td>[15]</td>
</tr>
<tr>
<td>External medium conductivity</td>
<td>$\lambda_{ext}$</td>
<td>$1.4 \times 10^{-12}$</td>
<td>1</td>
<td>[15]</td>
</tr>
<tr>
<td>Temperature</td>
<td>$T$</td>
<td>$300$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>Diffusion coefficient for pore</td>
<td>$D$</td>
<td>$5 \times 10^{-14}$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>radius</td>
<td>$m$</td>
<td>$1.4 \times 10^{-12}$</td>
<td>1</td>
<td>[15]</td>
</tr>
<tr>
<td>Initial surface tension</td>
<td>$\sigma_0$</td>
<td>$10^{-6}$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>Steric repulsion energy</td>
<td>$\beta$</td>
<td>$1.4 \times 10^{-12}$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>Energy of hydrocarbon-water</td>
<td>$\sigma'$</td>
<td>$2 \times 10^{-12}$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>interface per unit area</td>
<td>$\sigma'$</td>
<td>$2 \times 10^{-12}$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>Minimum size of hydrophilic</td>
<td>$r^*$</td>
<td>$0.5 \times 10^{-8}$</td>
<td>5</td>
<td>[51]</td>
</tr>
<tr>
<td>pore at $V_m = 0$ V</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
the electropermeabilization of giant unilamellar vesicles. Then, we describe the computational procedure for assessing the macropore size dynamics in electropermeabilized vesicles.

3.1. Vesicle preparation, pulse application and resolving the pore dynamics with video microscopy

Giant unilamellar vesicles (GUVs) of palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC) (Avanti Polar Lipids, Alabaster, AL) were prepared by using the electroformation method [52–53], for which 10 μL of a 4 mM of lipid solution prepared in chloroform is spread on the surfaces of a pair of indium tin oxide-coated, conductive glasses (Delta technologies Lt., Germany). Then, the glasses were kept in desiccator for 2 h to remove all traces of the organic solvent. The two glasses were then placed with their conductive sides facing each other and separated by a 2 mm-thick Teflon frame to form a chamber. The chamber was filled with a 200 mM sucrose (Merck, Germany) containing NaCl at concentrations up to 1 mM (Merck, Germany) depending on the desired internal vesicle conductivity. The chamber was then connected to a function generator (Agilent, Germany) and an alternating current (AC) of 1 V with a 10 Hz frequency was applied for 1 h. After harvesting the vesicle solution from the electroswelling chamber, it was 10-fold diluted in 200 mM of glucose solution (Merck, Germany). The external solution also contained between 0 and 1 mM NaCl to adjust the desired external conductivity. We note that the sugar asymmetry between the interior and the exterior of the vesicle enhances the optical contrast of phase contrast images due to refractive index difference of glucose and sucrose solutions and stabilizes the vesicle position onto the bottom of the electroporation chamber due to the higher density of the sucrose solution in the GUV interior. The osmolarities of sucrose and glucose solutions were measured with a freezing point osmometer Osmatop 3000 (Gonotec GmbH, Germany) and matched to avoid osmotic pressure effects. The conductivities of the sucrose and glucose buffers were measured with SevenEasy Conductivity Device (Schwerzenbach, Switzerland). The results of conductivities are summarized in Table 3.

The electroporation chamber, purchased from Eppendorf (Eppendorf electrofusion chamber, Hamburg, Germany), consisted of a Teflon frame confined above and below by two glass plates through which observation is possible. A pair of parallel electrode wires (92 μm in radius) is located at the lower glass at a distance of 475 ± 5 μm. The spacing between the electrodes is important for defining the field strength at the location of a selected vesicle above the floor of the chamber. Assuming that the electrodes are perfect cylinders, the distance between the electrodes right at the bottom glass is 674 μm. Because the exact location of the vesicle center of mass above the glass cannot be precisely defined, a nominal gap distance of 500 μm between the electrodes is used, which may induce an error of ~10 % for the electric field strength. The chamber is attached to a jitech pulse generator GHT_Bi500 (jitech, l’Union, France), which generates square-wave direct current (DC) pulses. The pulse strength and duration ranges from 8 to 26 V (0.16 ±

Table 2
Fit parameters used for the electric force contribution in \( U(r, V_m) \), i.e. \( F_p = \frac{V_m}{r} \Lambda \left( \frac{1}{r + \beta} \right) \), as a function of the conductivity ratio \( \Lambda \). Here, the notation (1;1/1) means that \( f_s = f_{1/1} \).

<table>
<thead>
<tr>
<th>( \Lambda )</th>
<th>( F_{max} ) (mN/V²)</th>
<th>( r_1 ) (nm)</th>
<th>( r_2 ) (nm)</th>
<th>( r_3 ) (nm)</th>
<th>( \chi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (SNK)</td>
<td>0.69</td>
<td>0.23</td>
<td>1.18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(2;1/2)</td>
<td>1.008</td>
<td>0.242</td>
<td>1.221</td>
<td>1.828</td>
<td>0.399</td>
</tr>
<tr>
<td>(3;1/3)</td>
<td>1.599</td>
<td>0.207</td>
<td>1.179</td>
<td>1.544</td>
<td>0.680</td>
</tr>
<tr>
<td>(4;1/4)</td>
<td>1.806</td>
<td>0.179</td>
<td>1.079</td>
<td>1.405</td>
<td>0.758</td>
</tr>
<tr>
<td>(5;1/5)</td>
<td>1.930</td>
<td>0.157</td>
<td>0.994</td>
<td>1.291</td>
<td>0.803</td>
</tr>
<tr>
<td>(6;1/6)</td>
<td>2.186</td>
<td>0.142</td>
<td>0.934</td>
<td>1.183</td>
<td>0.846</td>
</tr>
<tr>
<td>(7;1/7)</td>
<td>2.387</td>
<td>0.130</td>
<td>0.880</td>
<td>1.097</td>
<td>0.873</td>
</tr>
<tr>
<td>(8;1/8)</td>
<td>2.412</td>
<td>0.121</td>
<td>0.829</td>
<td>1.032</td>
<td>0.887</td>
</tr>
<tr>
<td>(9;1/9)</td>
<td>2.356</td>
<td>0.113</td>
<td>0.781</td>
<td>0.980</td>
<td>0.893</td>
</tr>
<tr>
<td>(10;1/10)</td>
<td>2.373</td>
<td>0.107</td>
<td>0.744</td>
<td>0.931</td>
<td>0.902</td>
</tr>
</tbody>
</table>

Fig. 1. The predicted change in the four contributions of the force acting on the pore \( U(r, V_m = 1V) \) as a function of pore radius for different values of the electrical conductivity ratio \( \Lambda \) of the inside and the outside vesicle solution. \( F_p \) denotes the electromechanical force, i.e. the \( V_m \) dependent contribution to the \( U(r, V_m = 1V) \) energy term. Note that for the electric force (1;1/1) means that \( f_s \equiv f_{1/1} \). The parameterized analytic function \( f_s(r) \) (solid lines) are shown to match well with the numerical solution (solid symbols). For this calculation we assume \( e_{all} = 10^{-4} \text{N/m} \) and \( \beta = 24 \text{pN} \). The vertical red dotted line represents the limit of our experimental resolution (values of \( r \) below this limit are not detected experimentally).

Eq. (5) which are plotted as a function of pore radius, where simulation data points are compared with Eq. (7).

The curve \( \Lambda = 1 \) (SNK) shows the match between our results and the ones from the SNK model. It is also discernible that the \( V_m \) dependent component dominates over the other contributions in the entire range of \( r \) investigated, where a variation of one order of magnitude of the value of \( \Lambda \) impacts the magnitude of the \( V_m \) dependent component of \( U(r, V_m) \) up to a factor 3. Consequently, the combination of Eqs. (4) and (7) suggest a dependence of pore expansion dynamics on conductivity imbalances between membrane surrounding media. Table 2 lists the material properties for cells deduced from consolidated literature data to obtain Fig. 1, and includes details of the numerical method used to compute \( f_s \), which are completed by Fig. 3.

3. Methods

Before proceeding with our method for inferring the electrical force on a macropore, we pause to discuss how the experimental results of this study should be interpreted in the light of previous work dealing with the electropermeabilization of giant unilamellar vesicles. Then, we describe the computational procedure for assessing the macropore size dynamics in electropermeabilized vesicles.

Table 3
Composition and conductivity values of the vesicle internal and external solutions for the experimental conditions of Figs. 3-5.

<table>
<thead>
<tr>
<th>Conductivity ratio, ( \Lambda )</th>
<th>Concentration and conductivity of vesicle interior solution</th>
<th>Concentration and conductivity of vesicle exterior solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>200 mM sucrose, 4.35 ± 0.65 μS/cm</td>
<td>200 mM glucose and 1 mM NaCl, 56.68 ± 0.63 μS/cm</td>
</tr>
<tr>
<td>0.14</td>
<td>200 mM sucrose, 4.35 ± 0.65 μS/cm</td>
<td>200 mM glucose and 0.5 mM NaCl, 32.10 ± 0.08 μS/cm</td>
</tr>
<tr>
<td>1</td>
<td>200 mM sucrose, 4.35 ± 0.65 μS/cm</td>
<td>200 mM glucose, 4.31 ± 0.35 μS/cm</td>
</tr>
<tr>
<td>4.6</td>
<td>200 mM sucrose and 0.5 mM NaCl, 33.69 ± 0.04 μS/cm</td>
<td>200 mM glucose, 4.31 ± 0.35 μS/cm</td>
</tr>
<tr>
<td>6.6</td>
<td>200 mM sucrose and 1 mM NaCl, 62.46 ± 2.46 μS/cm</td>
<td>200 mM glucose, 4.31 ± 0.35 μS/cm</td>
</tr>
</tbody>
</table>
0.016 to 0.52 ± 0.052 kV/cm), and 10 to 50 ms, respectively.

An inverted microscope Zeiss Axiovert 200 (Jena, Germany) equipped with a Ph2 20x/0.4 objective is used to monitor GUVs in phase contrast mode. An ultra-high-speed digital camera v2512 (Phantom, Vision Research, New Jersey, USA) is mounted on the microscope and connected to a computer. Image sequences are acquired at 20,000 fps which corresponds to a sampling rate of 50 µs, with resolution of 1.42 pixels/µm. The sample illumination is achieved with a mercury lamp. The onset of pulse application is defined as one frame (50 µs) before visible vesicle deformation occurs. In order to compute the transmembrane potential of the vesicles in their deformed state, the image sequences of a total of 41 vesicles containing a single expanding pore are processed via a contour recognition numerical method [54] which allows us to extract the values of the long and short semi-axis of the vesicles. Pore radii are computed using a lab developed pore edge detection software, PoET based on a numerical method for image processing described in Ref. [28] with a precision of the order of 0.5 µm.

The data are obtained with electropermeabilized giant unilamellar vesicles composed of POPC of radii ranging from 14 to 54 µm filled with 0.2 M sucrose solution and immersed in a 0.2 M glucose solution to stabilize the vesicles osmotically and facilitate pore imaging under phase contrast microscopy. Above a critical \( V_m \) close to 1 V for tensionless vesicle membranes [17], the formation of macropores (diameter in the range 0.7–15 µm) with lifetimes of up to a few hundreds of milliseconds is detected. The evolution of the size of a macropore can be individually observed under phase contrast microscopy by tracking the interrupted vesicle contour visualized from difference in refractive indexes between the inner sucrose and external glucose solutions, and detecting its radius using an automated method developed in [28]. As all observed pores are located at the vesicle pole (\( \theta = 0 \)) we finally assume that \( V_m = V_m(\theta = 0, t_p) \), takes a constant value in the time interval over which \( \theta \) is measured, here \( t_p \) represents the time at which nucleation starts.

### 3.2. Computational procedure for assessing macropore size dynamics in electropermeabilised vesicles

Our method follows the same numerical procedure as used by SNK in [15], and therefore, we take an electrostatic approach in computing the MST. We also assume that the membrane can be regarded as an electrically linear, homogeneous isotropic and continuous medium. Fig. 2 shows the 2D axisymmetric configuration comprising four domains: internal fluid, membrane, external fluid and pore, where the size of the system can be scaled in terms of the pore radius \( r \). Dirichlet boundary conditions on top and bottom horizontal boundaries are applied, such as \( V(z = 0) = 0 \) V and \( V(z = 40r) = 1 \) V, to analyze the impact of \( V_m \).

Within each domain, the following set of equations is solved.

\[
\nabla J = Q \tag{8}
\]
\[
J = \lambda E \tag{9}
\]
\[
E = -\nabla V \tag{10}
\]
\[
T = \varepsilon \varepsilon_0 (E \times E - 1/2 E^2) \tag{11}
\]

where \( \lambda, \varepsilon, V \) and \( Q \) account respectively for the electrical conductivity, permittivity, electric potential and current density, whereas \( J, \ T, \ and \ E \) refer respectively to the surface current density, Maxwell stress tensor, and electric field. Additionally, electric insulation boundary conditions, i.e. \( n \cdot J = 0 \), are applied on the vertical faces of the system while current conservation boundary conditions are applied to all other interfaces. The conductivity of the solution filling the pore is defined as a function of the conductivities of the internal and external solutions, and the vertical coordinate \( z \).
\[ \lambda_p = \lambda_{\text{ext}} - \left( \lambda_{\text{ext}} - \lambda_{\text{in}} \right) \left( \frac{z + 20r}{h} - \frac{1}{2} \right) \]  

(12)

where \( \lambda_{\text{ext}} \) and \( \lambda_{\text{in}} \) are respectively defined as

\[ \lambda_{\text{ext}} = \frac{\lambda_{\text{ac}} \lambda_{\text{a}}}{2(1 + 2d_a/(\pi r))} \]  

(13)

\[ \lambda_{\text{in}} = \lambda_{\text{a}} + \frac{\lambda_{\text{ac}} - \lambda_{\text{a}}}{2(1 + 2d_a/(\pi r))} \]  

(14)

A technical point to be made here is that Eq. (11) allows to compute the MST and the electric force \( F_p \) acting on the pore in the membrane plane which is defined as the integral of the normal component of Maxwell stresses over the surface \( \partial \phi \) of the pore (with reference to Fig. 2) as:

\[ F_p = \int_{\partial \phi} n \cdot (T_{(1)} - T_{(0)}) dS \]  

(15)

As a side remark, we note that the resting potential (\( dv/dt = 0 \)) is most polarized cells and vesicles is set to \(-70 \text{ mV} \).

From a computational perspective, the numerical analysis was performed based on finite element method computations via a cluster computer (262 GB RAM, Intel® Xeon® 2.2 GHz (48 CPUs) processor) using the COMSOL simulation package [55]. The mesh is constructed using COMSOL built-in extremely fine element size parameter setting for all domains. Eqs. (SI-S4) are solved using quadratic shape functions within triangular finite elements. Since the size of the system scales with the pore radius \( r \) ranging from 0.51 nm to 100 nm, the number of elements discretizing the pore domain is respectively of 577 up to 652, from 5541 up to 1967 elements for the membrane domain, from 94 up to 42 nodes for pore surface, and from 18,506 to 35,924 for the entire system leading to the resolution of respectively 37,315 up to 72,205 degrees of freedom which represents to computational times of respectively 21 s up to 36 s.

3.3. Matching the framework of the experimental and the numerical data

In order to shed light on the experimental validity of the foregoing results, we set a few analytical simplifications of the general theory presented in section II and restrain the theoretical frame of our analysis for micron-sized pores, which correspond to the limit of camera resolution. From a computational perspective, the numerical analysis was performed based on finite element method computations via a cluster computer (262 GB RAM, Intel® Xeon® 2.2 GHz (48 CPUs) processor) using the COMSOL simulation package [55], the mesh is constructed using COMSOL built-in extremely fine element size parameter setting for all domains. Eqs. (SI-S4) are solved using quadratic shape functions within triangular finite elements. Since the size of the system scales with the pore radius \( r \) ranging from 0.51 nm to 100 nm, the number of elements discretizing the pore domain is respectively of 577 up to 652, from 5541 up to 1967 elements for the membrane domain, from 94 up to 42 nodes for pore surface, and from 18,506 to 35,924 for the entire system leading to the resolution of respectively 37,315 up to 72,205 degrees of freedom which represents to computational times of respectively 21 s up to 36 s.

Due to the large experimental variability of the pore edge tension found for POPC (and \( \Lambda = 1 \)) in the literature, our numerical results are obtained by making use of an average value of \( r = 24 \text{ pN} \) [17-23,56].

Ultimately, our goal is to see how well SNK’s model fits with the observed data. While there has been significant theoretical treatment of planar membranes (e.g. SNK [15]) there has been little study combining theoretical and experimental approaches of cell and vesicle membranes with other common shapes (spheroids, ellipsoids) during pore expansion [10,39]. Moreover, the collective nature of SNK’s model, i.e. coupling of individual pores through the membrane lipid-water interfacial tension [50], is still very much unknown, and a wide variety of direct and indirect detection experiments are actively searching for evidence of post-pulse collective membrane resealing kinetics [11-12,29,31].

Fast digital imaging [17-23] offered insight into the deformation and permeabilization of giant unilamellar vesicles subjected to electric pulses of varying strength/duration. The aspect ratio (defined as the ratio of semi-major axis \( b \) to semi-minor-axis \( a \) of the ellipsoid) of the ellipsoidal deformation for a vesicle (initially spherical) represents a reliable metric for the underlying morphology of the vesicle subjected to electric pulses as it allows to define the transmembrane potential from analytical considerations. Recently [57], there has been an increased emphasis on the role anisotropy may play in the broad set of phenomena described above. When \( b/a \) is large at elevated \( V_m \), EP dominates, and maximum membrane deformation coincides with maximum pore aperture. The transmembrane potential for an ellipsoidal membrane can be evaluated [57] as:

\[ V_m(\theta, t) = \frac{b^2 - a^2}{\sqrt{a^2 \cos^2(\theta) + b^2 \sin^2(\theta)}} \left( \frac{\Lambda \cos(\theta)}{1 - \exp(-((t - \tau)/\tau))} \right) \]  

(17)

where \( E_0, \tau_0, \tau \) and \( \theta \) respectively represent the electric field intensity, time, the time of electric pulse onset, the membrane charging time and the angle between membrane surface normal vector and the direction of the field. Using Eq. (17) for the transmembrane potential, and by defining the value of \( \tau \) to be the first instant at which a pore is detectable, we can now proceed with comparing theoretically predicted pore expansion rates with experimentally obtained data.

As an illustrative example, Fig. 3 shows the aspect ratio of an electropropelled vesicle with a nominal applied field strength of 0.24 kV/cm, and \( \Lambda = 6.6 \). The initial diameter of the vesicle was 23.2 \text{ nm} and after 50 ms, it was deformed into a prolate spheroid with \( b/a = 1.8 \). The maximum shape deformation is attained close to the pulse end.

4. Results and discussion

In the upper top panel of Fig. 4 we show the ED and EP of a POPC vesicle characterized by phase contrast microscopy images (the data correspond to the same vesicle shown in Fig. 3). The membrane charging stage starts with the application of the electric pulse to the initially spherical vesicle followed by several electrodeformed states of the ellipsoidal membrane along the direction of the applied field. The macropores are also visualized in the snapshots of the lower top panel using our detection software [28]. The bottom panel of Fig. 4 shows the time evolution of the pore radius following our measurement protocol [28].

The guiding principle of the analysis we propose is this: in order to unravel the impact of conductivity conditions on the rate of expansion of the macropores considered in this study, we focus on the early stages of pore growth where the assumption of a quasi-constant value of \( V_m \) is valid, and defined from Eq. (17). We generated a set of data comprising the pore expansion slopes of all porated GUvs and selected only those for which a single macropore is nucleated in the membrane (Fig. 4). Fig. 5 shows the rate of pore expansion as a function of \( V_m^2 \). The dashed lines in Fig. 5 represent the prediction of the extended SNK model for \( \frac{b}{a} \) at a given value of \( \Lambda \). On the experimental side, the linear dependence between the rate of pore expansion with \( V_m^2 \) can be clearly seen from this graph, and more importantly these trends coincide with the theoretical predictions of Eq. (7). For all data, the error bars in Fig. 5 represent experimental error associated with estimation of the slope \( \frac{b}{a} \) and \( V_m^2 \) intercept related to the 50 \text{ ms} time scale of camera sampling. These observations lead to two important conclusions. First, the \( V_m \) dependent energy term of the SNK model (\( \Lambda = 1 \) in Fig. 5) appears to fall quantitatively in the range of the experimental data presented in this study. Second, the agreement between data and experiments in Fig. 5 for the case \( \Lambda \neq 1 \) clearly establishes the importance of \( \Lambda \) in the process of pore expansion, it also suggests the existence of a symmetry in the impact of solution conductivity on pore expansion, i.e. \( f_\Lambda = f_1/\Lambda \), which suggests
that pore expansion does not discriminate between conditions of inverted inside vs outside vesicle media conductivity.

The observed broad range of \( V_m \) values shown in Fig. 5 can be related to the initial mechanical tension of the membrane, which is expected to lower the EP threshold of the membrane [17-23,58]. This can also be confirmed by analyzing the linear dependence (Fig. 6) of the relative increase in membrane surface area induced by the application of an electric field, \( \alpha = \frac{A_{el}}{A_i} \), on the electrical tension (which is a function of \( V_m^2 \)) induced in the membranes, where \( A \) and \( A_i \) respectively correspond to the surface area of the deformed vesicle and the area of a sphere of equal volume.

Based on the original studies by Helfrich [59-62], the mechanical deformation and apparent area increase of a vesicle deformed by an electric field can be presented as:

\[
\alpha_{el} = \frac{8\pi\epsilon_0}{kT} \ln \left( \frac{\sigma_0 + \sigma_{el}}{\sigma_0} \right) + \frac{\sigma_{el}}{K}
\]

(18)

where \( \sigma_0 \) is the initial tension of the vesicle, \( \sigma_{el} \) is the electrical tension induced in the membrane when the vesicle is exposed to the electric field [37,58], \( k \) is the membrane bending stiffness (\( k \) is on the order of 30 \( kT \) for POPC [63-64]), and \( K \) is the bulk elastic modulus of the membrane (here \( K \) is expected to be close to 0.2 N/m [64-65]). It has also been argued by Needham and Hochmuth [58] that if the electrical tension is much larger that the initial tension and if membrane incompressibility is assumed, then the total tension is:

\[
\sigma = \sigma_0 + \sigma_{el} = (\sigma_0 + \sigma_{el})K
\]

(19)

where

\[
\sigma = \epsilon_0 \epsilon \left( \frac{h}{2h} \right) V_m^2
\]

(20)

is the electromechanical tension, \( \epsilon \) is the permittivity of the membrane, \( \epsilon \) the vacuum permittivity, \( h \) is the total bilayer thickness, \( h \) is the dielectric thickness, \( h_i \) 2.7 nm [58]. By contrast with Ref. [58], where the initial tension \( \sigma_0 \) is induced via a micropipette aspiration pressure which has for effect to increase the area of an initially unconstrained vesicle by \( \sigma_0 \), our vesicle preparation protocol does not permit a full control over the initial area-to-volume ratio of individual vesicles, thus resulting in vesicles with a broad range of initial tensions \( \sigma_0 \) in our experiments. Consequently, a decrease in the initial area-to-volume ratio increases the tension in the membrane, similarly as an isotropic swelling of the vesicle, which can be described by an initial isotropic area increase of \( \sigma_0 \). More significantly, the relative increase in area \( \alpha_{el} \) induced by the electric field relies on \( A_i \) which is a function of the encapsulated volume, and thus of \( \sigma_0 \). For a given value of the electrical tension \( \sigma_{el} \), we argue below that the relative increase in membrane area induced by the electric field can be expressed as.
where \( a_d(\sigma_0 \approx 0) \) accounts for \( a_d \) in the case an initially tensionless vesicle, i.e. a vesicle that encapsulates a sufficiently small volume of water so that thermal fluctuations would induce a visible flickering of its membrane surface. For that purpose, we consider the dependence of the relative area increase induced by the application of the field.

\[
a_d = \frac{A - A_i}{A_i^0} \tag{21}
\]

on the initial isotropic area increase of \( a_0 \). We define \( a_0 \) as

\[
a_0 = \frac{A_i - A_0}{A_0} \tag{22}
\]

where \( A_i \) and \( A_0 \) respectively represent the initial apparent area of the vesicle and the initial apparent area of the same vesicle in the case of \((\sigma_0 = 0)\). Based on this definition \( a_d \) can be written as

\[
a_d = \frac{A - A_i}{A_i^0} = \frac{A - \sigma_0 A_0}{A_0 (\alpha_0 + 1)} - 1 \tag{23}
\]

In the case of a negligible initial tension, i.e. \((\sigma_0 = 0)\), we have \( a_0 = 0 \) where,

\[
a_d(\sigma_0 = 0) = \frac{A}{A^0} - 1 \tag{24}
\]

Using this relation we get,

\[
a_d = \sigma_0(\sigma_0 = 0) - a_0 \approx a_d(\sigma_0 = 0) - a_0 \tag{25}
\]

as \( a_0 \ll 1 \) in our experiments.

In this context, Fig. 6 was plotted by selecting a vesicle exhibiting a flickering membrane as a reference (blue data) to compute \( a_d(\sigma_0 \approx 0) \) as a function of \( \sigma_0 \). The electrical tension was gradually increased at values above 1 mN/m in order to set our analysis within the boundaries of membrane stretching regime \([63-65]\) (i.e. corresponding to the case \( \sigma_0 \ll a_d \)), and every data point abscissa was translated by \( a_0 \). Then, the data were fitted using the relation \( a_d = a_d(\sigma_0 \approx 0)K \) to compute the stretching modulus \( K \), yielding a value of \( 0.35 \pm 0.07 \) N/m which is consistent with typical values reported in the literature \([63-66]\). The values of the initial tensions range from 0.03 mN/m to 4.8 mN/m.

5. Concluding remarks and perspectives

To summarize, the comparison between the experimental observations of the dynamic behavior of vesicle pores under the application of an electric field with an extended version of SNK’s model, showed that the conductivity ratio of the inside and the outside vesicle solution has significant and distinctive influence on the rate of change of macroscopic expansion in electropermeabilized lipid vesicles. We also highlighted the symmetry of these effects with respect to the conductivity ratio, where our results suggest that EP efficiency should be maximized as the conductivity ratio approaches the value \( \Lambda = 1 \). Another novelty of our study is given by a precise and simple analytical adaptation of the SNK model, allowing to reproduce accurately the predictions of our conductivity dependent EP model.

Given these observations, it is useful to indicate the aspects in which the analysis derived in this study can capture the essence of electro-deformed and electropermeabilized cells, notably the pore density dynamics in the membrane. Do the electromechanical properties of a cell membrane look like the properties of a stretched lipid bilayer? We first note that intact cell membranes contain many features not found in artificial lipid bilayers, e.g. an actin network in the cytoskeleton, whose presence was reported to prevent the formation of macropores in lipid vesicle membranes and inhibit large deformations \([29]\). Another feature that differs from artificial lipid bilayers is the presence of proteins in the membrane which are likely to alter the mechanical properties of the membrane \([71]\). Secondly, phospholipid vesicles are often used as model systems to study the electromechanical properties of living cells \([39]\), and from the perspective of our theoretical predictions, the trends observed for large pores should be valid also for nanometric pores, which would constitute an interesting basis for the interpretation of cell EP efficiency variations induced by changes in extracellular conductivity reported in the literature \([13-14]\). For comparison with biological cells, \( \Lambda \) varies over a range of values 0.1–1 \([14,39,56-57,67]\) and we believe that a combination of the present theory with measurements of electroporation efficiency could constitute a novel method for inferring cytoplasm conductivity. We expect our analysis will be useful to characterize the electromechanical properties of living cells, and will allow improving the current disagreement between the current state of the art numerical models and the observed impacts of \( \Lambda \) on electroporation efficiency \([13]\). A number of points still remain to be investigated, regarding the fundamental issue of the large number of small pores with a random distribution which is more relevant for realistic defective conditions in an electropermeabilized cell \([10-11,39]\). One important question in this regard is how a SNK generalization can be implemented to deal with the intracellular mechanical changes. This connection needs to be understood in (at least) two different ways. First, there is the problem of understanding strain and stress at interfaces in-between the different phases of the cell \([68-73]\). And second, there is the solid phase of the cytoplasm (cytoskeleton network and macromolecular crowders) in eukaryotic cells which is known to provide structural support and mechanical stability, and play a fundamental role in controlling the rate at which the cell can be deformed \([68-73]\). The importance of strain engineering might prove crucial for the design of technologies like vesicle-based biosensors and artificial cells acting as drug delivery carriers. That is a formidable project, extending well beyond what is conventionally considered multiphysics \([39]\).

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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