Understanding the Electroporation of Cells and Artificial Bilayer Membranes

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A new physical mechanism is proposed to explain the electroporation of cells based on the electric-field-induced stretching of their curved membrane. The opening of pores simultaneously relaxes the surface tension leaving long-lived pores in the membrane. Semiquantitative results are proposed in good agreement with experimental observations on cells and artificial bilayer membranes. [S0031-9007(98)05862-1]

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Introducing exogenous macromolecules into a cell should not be an easy task since every cell is protected by a selective cytoplasmic membrane which prevents the unregulated crossing of essentially any large molecules. Still, this process is now performed on a daily basis in many cellular biology laboratories and biotechnology companies using usually much less sophisticated tactics than those developed by viruses or bacteria. The method employed is called *Electroporation* [1,2] and has become a widely used technique to incorporate various molecules (e.g., DNA and RNA fragments, proteins, antibodies, drugs, fluorescent probes, etc.) into many different types of cells (e.g., bacteria, yeasts, plant, and mammalian cells). It is a simple, flexible, and relatively nontoxic physical method which relies on the transient permeation of the cell membrane induced by the application of a strong electric pulse (typically $1-4$ kV/cm for 1 μs to a few ms). Large long-lived pores form [3], hence enabling large exogenous macromolecules, initially dispersed in the electroporation buffer, to enter into the perforated cells before the pores eventually reseal spontaneously after a few seconds to several minutes [1–3].

This Letter first reviews briefly some of the classical theories related to this now widespread technique. As most theoretical efforts have been focused on explaining the electroporation of flat membranes under constant mechanical tension [1,2], there appears to be little understanding about the formation of these large long-lived pores in actual cells, a qualitatively distinct problem. A simple physical mechanism is then proposed to account for this important biomedical technique. Although we are interested mainly in understanding the electroporation process of "real cells," part of our discussion also implies some novel results about the electroporation of a macroscopic (artificial) bilayer membrane.

The current theoretical understanding of the electroporation process relies on the well-known fact that the cell membrane behaves essentially as a local capacitor upon the application of an electric field across the dispersion of targeted cells. Hence, the electric potential drop over the cell size V occurs almost entirely across the cytoplasmic membrane of thickness d [4], leading to very large electric fields within the phospholipid bilayer $E_m \sim V/d$. Interestingly, most experimental studies agree that the electric potential threshold, above which electroporation of the cell membrane occurs, corresponds to a potential drop of about 1 V over the width of the bilayer.

Once the initial perforation of the membrane has occurred, the energy associated with the nucleation of a pore of radius r is classically written as [2]

$$E = 2\pi\gamma r - \Gamma\pi r^2, \tag{1}$$

where γ is the line tension at the pore rim and Γ is the surface tension in the membrane. At finite Γ, this predicts that the membrane is *unstable* against *unbounded* pore nucleation if the activation energy $\Delta E = \pi\gamma^2/\Gamma$ is provided. Although some electric-field-induced mechanisms have been suggested to stabilize the pore radius [5], this cannot explain, however, the stability of the perforated membrane on long time scales after the field has been turned off.

The onset of electroporation of *flat* artificial bilayers has been explained by the addition of an energetic contribution to Eq. (1) which destabilizes the subcritical micropores supposedly present in the membrane under constant mechanical tension (see, e.g., Chernomordik and Chizmadzehv in [2] and references therein). However, the relevance of this local energetic balance has been questioned [5] in light of the continuing dissipation under electric field in the region of the conducting pores.

Several authors have proposed an alternative description of the onset of electroporation based on an electromechanical instability crushing an elastic [6] or viscoelastic [7] homogeneous thin film modeling the membrane. This approach assumes that membrane rupture occurs as the compressive electrostatic stress $(\varepsilon_m E_m^2/2)$ grows larger than the *linear* (visco)elastic response of the thin film at strong compressional deformation. It is not clear, however, whether this *linear* and *continuous* model captures the actual physics of rupture of a real lipid bilayer.

More generally, it appears that these models of *flat* membrane under *constant mechanical tension* are not appropriate for describing the opening of pores in the *curved, closed* membrane of a cell. We will show in the remainder of this Letter that, because of its *curvature*, the cell membrane is in fact mechanically stretched to rupture under the

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application of a strong electric field. Since the cytoskeleton-generated membrane tension is normally some hundreds times smaller than the typical tension leading to membrane rupture [$\Gamma_{\text{rupt}} \simeq (1-5) \times 10^{-3}$ N/m], this process implies a strong transient electric-field-induced tension in the cell membrane. Such a tension arises because the membrane does not have enough time to exchange phospholipids with the natural reservoirs of the cell (at the very short time scales involved during electroporation) and therefore behaves essentially like an artificial vesicle with a *fixed number of phospholipids*. Once pores have been nucleated, their further opening after the end of the electric pulse is also expected to simultaneously relax the strong (electric-field-induced) membrane tension. This eventually leaves *long-lived* pores in the membrane, as we will discuss more quantitatively after first describing in some detail how the cytoplasmic membrane of an initially floppy cell becomes stretched and eventually ruptures under a short applied electric pulse.

We have to solve the general electrokinetic problem of a cell in an electroporation buffer submitted to an electric pulse. As shown below, the electric field generates noncanceling electric stresses at the curved membrane interfaces which tend to deform the shape of the cell. Since both the number of phospholipids in the membrane *and* the volume of the cell are essentially fixed at these very short time scales, the cell membrane stretches until some mechanical "equilibrium" is reached with the surface tension in the membrane balancing the sum of the normal (electric) stresses [8]. For simplicity we solve the electrokinetic problem for a spherical cell of external radius R and membrane thickness $d \ll R$, and we estimate the surface tension Γ at mechanical equilibrium using the Young-Laplace relation $\sigma_{rr} = 2\Gamma/R$. It is expected, however, that the results obtained with this simple spherical geometry capture the essential physics of the actual electrokinetic problem with a vesicle or a cell of spheroid shape. The cytosol, the cell membrane, and the electroporation buffer are described by uniform resistivities and permitivities: χ_i, χ_m, χ_e and ε_i, ε_m, and ε_e, respectively. For simplicity, we take $\varepsilon_e = \varepsilon_i = \varepsilon_{\text{water}} \simeq 10^{-9}$ F/m and we suppose that $\chi_m \gg \chi_i, \chi_e$, as expected for a poorly conductive membrane before electroporation. We further assume that the conductive phenomena dominate the dielectric ones in the cytosol and the electroporation buffer at the time scales involved for electroporation, i.e., $\chi_i^{-1} \gg \varepsilon_w \omega$ and $\chi_e^{-1} \gg \varepsilon_w \omega$. The electric potential obeys a Laplace equation in each medium (no charge density appears in a uniform medium) and hence takes the following classical form in spherical coordinates

$$\Phi_k(t, r, \theta, \phi) = \left(A_k(t) r + \frac{B_k(t)}{r^2}\right) \cos\theta \,, \qquad (2)$$

where $k = i, m, e$ represents internal, membrane, and external media, respectively. Assuming that a constant electric field $\vec{E}_0$ is applied along the $\theta = 0$ axis at $t = 0$, we find the time-dependent coefficients $A_k(t)$ and $B_k(t)$ by the Laplace transform technique and the use of the following appropriate boundary conditions: (i) finite potential at the origin and $\vec{E} \to \vec{E}_0$ at infinity; (ii) continuous potential at both membrane interfaces; (iii) variation in electric displacement equal to the surface charge density at each interface ($\varepsilon_2 E_{r2} - \varepsilon_1 E_{r1} = \Sigma_{12}$)); (iv) rate of increase of Σ_{12} equal to the net flow of charge at each interface ($\partial_t \Sigma_{12} = \chi_1^{-1} E_{r1} - \chi_2^{-1} E_{r2}$, neglecting the transport of charges along the interface [2]). Making use of the approximations, $\chi_m \gg \chi_i, \chi_e$ and $R \gg d$, we can find—after tedious but straightforward calculations—the radial and tangent components of the electric field at both membrane interfaces. They reach the following steady values after a short transient regime of time scale $\tau \sim \tau_m(2\chi_i + \chi_e)R/[(2\chi_i + \chi_e)R + 2\chi_m d]$, where $\tau_m = \varepsilon_m \chi_m$ (typically, $\tau \simeq 10^{-7}$ s for cells),

$$E_{ri}(R - d, \theta) \sim \frac{3E_0 \chi_i R \cos\theta}{(2\chi_i + \chi_e)R + 2\chi_m d} \,, \qquad (3)$$

$$E_{rm}(R - d, \theta) \sim \frac{3E_0 \chi_m R \cos\theta}{(2\chi_i + \chi_e)R + 2\chi_m d} \,, \qquad (4)$$

$$E_{\theta}(R - d, \theta) \sim -\frac{3E_0 \chi_i R \sin\theta}{(2\chi_i + \chi_e)R + 2\chi_m d} \,, \qquad (5)$$

and

$$E_{re}(R, \theta) \sim \frac{3E_0 \chi_e R \cos\theta (1 - 2d/R)}{(2\chi_i + \chi_e)R + 2\chi_m d} \,, \qquad (6)$$

$$E_{rm}(R, \theta) \sim \frac{3E_0 \chi_m R \cos\theta (1 - 2d/R)}{(2\chi_i + \chi_e)R + 2\chi_m d} \,, \qquad (7)$$

$$E_{\theta}(R, \theta) \sim -\frac{3E_0[\chi_i R + \chi_m d] \sin\theta}{(2\chi_i + \chi_e)R + 2\chi_m d} \,, \qquad (8)$$

where $\chi_i R$, $\chi_e R$, and $\chi_m d$ are the typical resistances (per unit surface at the scale of the cell) of the cytosol, the electroporation buffer, and the membrane, respectively.

The total electric stress σ^T applied to a membrane patch contained in a solid angle $d\theta \sin\theta d\phi$ is calculated from the Maxwell stress tensors at each interface, $\sigma_{kl} = \varepsilon(E_k E_l - E^2 \delta_{kl}/2)$. σ^T consists of one part σ^m proportional to ε_m coming from the (large) electric field in the membrane, and another part, σ^{ie}, proportional to ε coming from the electric field in the cytosol and the external medium. One finds, in particular, the following two expressions for the radial electric stress depending on the relative resistances of the membrane and the internal and external media:

(*i*) For $2\chi_m d \gg (2\chi_i + \chi_e)R$ and $d/R \ll \varepsilon_m/\varepsilon_w$, corresponding typically to the case of a cell *before* electroporation,

$$\sigma_{rr}^T \sim \sigma_{rr}^m \sim \frac{9}{4} \varepsilon_m E_0^2 \cos^2(\theta) \frac{R}{d} \,. \qquad (9)$$

(*ii*) For $\chi_m d \ll \chi_i R$ and $d/R \ll \varepsilon_w |\chi_e^2 - \chi_i^2| / \varepsilon_m \chi_m^2$, corresponding, for instance, to the case of giant artificial vesicles with very low internal ionic strengths

(see Kinosita *et al.* in [1]) or the situation of two immiscible fluids of different conductivities with no membrane separation [9],

$$\sigma_{rr}^{T} \sim \sigma_{rr}^{ie} \sim \frac{9}{2}\,\varepsilon_w E_0^2 \cos^2(\theta)\,\frac{\chi_e^2 - \chi_i^2}{(2\chi_i + \chi_e)^2}. \quad (10)$$

Hence, for the case of unperforated cells [i.e., Eq. (9)] we find that the radial stress is *proportional to the radius of curvature* of the membrane and *independent of the conductivities* of the cytosol or the electroporation buffer: It always tends to elongate the cell along the direction of the electric field (see, e.g., Schwan in [2]), whereas the deformation induced in the second case is prolate *or* oblate depending on the relative conductivities of the internal and external media [9]. At mechanical equilibrium under electric field, we therefore expect the following electric-field-induced surface tension in a cell membrane [the factor $\cos^2(\theta)$ has been omitted at this scaling level],

$$\Gamma \sim \sigma_{rr}^{T}\,\frac{R}{2} \sim \frac{9\varepsilon_m}{8d}\,(E_0 R)^2 \simeq 5 \times 10^{-3}(E_0 R)^2. \quad (11)$$

We want to emphasize that this electric-field-induced membrane tension is related to the closed, curved geometry of a cell. Indeed, for a flat membrane between identical electrolytes, there is an (accidental) cancellation of the total electric stress, and one can actually show [5] that the (in-plane) membrane tension *decreases* under electric field as expected for an incompressible membrane under normal (electrostatic) pressure. For $V = E_0 R \simeq 1$ V, which is usually regarded as the empirical estimate for the electroporation field, Eq. (12) gives $\Gamma \simeq 5 \times 10^{-3}$ N/m. This is indeed of the order of a surface tension likely to cause membrane rupture, $\Gamma_{\text{rupt}} \simeq (1\text{–}5) \times 10^{-3}$ N/m. More precisely, for a cell or vesicle under initial tension Γ_0 (induced, for instance, by osmotic stress or mechanically with a sucking micropipet [10]), we predict that the membrane ruptures for $\Gamma_0 \simeq \Gamma_{\text{rupt}} - 5 \times 10^{-3}(E_0 R)^2$, in good agreement with the experimental measurements of Needham *et al.* [10] on *spheroid* vesicles.

We now have to check that this mechanical equilibrium (i.e., $\sigma_{rr}^{T} \simeq 2\Gamma/R$) is indeed reached kinetically in these electroporation experiments. The time to deform a cell under the typical stress σ_{rr}^{T} [Eq. (9)] [11] can be estimated for a viscous fluid (i.e., the cytosol) as $\tau_{\text{eq}} \sim \eta_i/\sigma_{rr}^{T} \simeq 2 \times 10^{-6}$ s; as anticipated, it is short compared with the time needed to equilibrate with the phospholipid reservoirs of the cell, so that a large electric field (1–4 kV/cm) applied longer than a few microseconds should be enough to stretch and rupture the membrane of a cell, in good agreement with all experimental results [1,2].

We now come to the discussion of the (electric-field-induced) membrane tension relaxation by further opening of the nucleated pores once the electric field has been turned off. For clarity, we resolve the "instability" of the nucleated pores predicted from Eq. (1) with a simple heuristic approach assuming that the electric-field-induced pore nucleation has just occurred at the end of the electric pulse (i.e., no direct electric effect on pore growth). Let us then describe the opening of a pore of radius $r(t)$ and the simultaneous relaxation of the surface tension in a *finite* membrane under strong initial tension Γ_{ini}. At the linear order, the membrane tension is proportional to the relative increase of the membrane surface upon stretching. Hence, in a mean field approach, the surface tension in a perforated, finite membrane can be expressed as

$$\Gamma(r) = \Gamma_{\text{ini}}\,\frac{r_\infty^2 - r^2}{r_\infty^2}, \quad (12)$$

where r_∞ is the radius that the pores would "eventually" attain if their line tension was vanishingly small (in practice, however, the opening of large pores in cells is also likely to involve slower processes, as discussed below, once the initial pores have been stabilized by relaxation of the large electric-field-induced membrane tension). r_∞ depends on the initial tension Γ_{ini}, the membrane area S, and the number of (identical) pores n as $\Gamma_{\text{ini}} = K_s(n\pi r_\infty^2/S)$, where $n\pi r_\infty^2$ is the initial surface stretching and K_s is the elastic stretching modulus of the membrane ($K_s \simeq 0.2$ J/m^2 [13]).

Hence, the pore energy [Eq. (1)] becomes in a *finite* membrane under initial tension Γ_{ini},

$$\begin{aligned} E &= 2\pi\gamma r - \int_0^r \Gamma(r')2\pi r' dr' \\ &= 2\pi\gamma r - \Gamma_{\text{ini}}\pi\left(r^2 - \frac{r^4}{2r_\infty^2}\right), \end{aligned} \quad (13)$$

which now leads to an unstable local maximum at r_{uns} *and* an apparently stable pore radius, r_{eq}. Under strong initial tension and/or weak pore line tension (i.e., $r_\infty \gg \gamma/\Gamma_{\text{ini}}$), we find $r_{\text{uns}} \sim \gamma/\Gamma_{\text{ini}}$, as in the case with constant Γ_{ini}, and $r_{\text{eq}} \sim r_\infty[1 - \gamma/(2\Gamma_{\text{ini}} r_\infty)] \sim r_\infty$.

What are the time scales involved in the pore opening driven by the relaxing surface tension $\Gamma(r)$? As the membrane is significantly more viscous than the cytosol and the electroporation medium (i.e., $\eta_m > \eta_i, \eta_e$ see Ref. [14]), one finds that the work done by the tension $\Gamma(r)$ per unit time is initially dissipated in the radial plug-flow occurring inside the membrane around each pore of increasing radius $r(t)$. This problem has been studied recently by Debrégeas *et al.* [16] for the slow bursting of very viscous polymer films under constant surface tension (fixed by a peripheral reservoir of polymer chains). Their analysis can easily be extended to the case of a relaxing surface tension $\Gamma = \Gamma_{\text{ini}}(r_\infty^2 - r^2)/r_\infty^2$ and leads to the following energy dissipation balance between the viscous flow in the membrane at the scale of the pore radius r and the work performed per unit time by the surface tension:

$$\eta_m\left(\frac{\dot r}{r}\right)^2 r^2 d \sim \left(\Gamma_{\text{ini}}\,\frac{r_\infty^2 - r^2}{r_\infty^2}\right)r\dot r\,. \quad (14)$$

which eventually leads to

$$r(t) \sim \frac{r_\infty r_0}{\sqrt{r_0^2 + (r_\infty^2 - r_0^2)\exp(-2t/\tau_1)}}, \quad (15)$$

where $\tau_1 \sim d\eta_m/\Gamma_{\rm ini}$ and r_0 is the initial radius of the nucleated pores, $r_0 \geq \gamma/\Gamma_{\rm ini}$. We find that τ_1 is in the μs to tens of μs range for $\Gamma_{\rm ini} \simeq 10^{-4}$–$10^{-3}$ N/m near $\Gamma_{\rm rupt}$. If $\eta_i r_\infty > \eta_m d$ one finds that this initial dynamic regime crosses over for $r(t) > \eta_m d/\eta_i = r_c$ to a second one dominated by the dissipation in the surrounding fluid at the scale r. The time scale of the second dynamic regime is $\tau_2 \sim \eta_i r_\infty/\Gamma_{\rm ini}$, and the pore opening obeys

$$r(t') \sim r_\infty \frac{1 - a\exp(-2t'/\tau_2)}{1 + a\exp(-2t'/\tau_2)}, \qquad (16)$$

where $a = (r_\infty - r_c)/(r_\infty + r_c)$ and $t' = 0$ at the cross over between the two dynamic regimes.

Interestingly, for the case corresponding to a macroscopic finite bilayer initially stretched on a diaphragm [17] (e.g., $r_\infty = 10^{-5}$ m, $\eta_i = \eta_e = \eta_{\rm water} \simeq 10^{-3}$ Pa s, and $r_c \simeq 10^{-6}$ m), we predict that the (irreversible) electroporation process will start with a "very slow exponential" increase $r(t) \sim r_0 \exp(t/\tau_1)$ (with $\dot{r}|_{t=0} \sim r_0/\tau_1 \sim \Gamma_{\rm ini}/\eta_m \equiv 2 \times \Gamma_{\rm ini}$) in the first dynamic regime lasting for about $\tau_1 \ln(r_c/r_0) \simeq 2$–$5 \times \tau_1$ for $r_0 \simeq 10^{-8}$–10^{-7} m. Then, a catastrophic bursting of the film suddenly occurs at the cross over to the second dynamic regime (with $\dot{r}|_{t'=0} \sim r_\infty/\tau_2 \sim \Gamma_{\rm ini}/\eta_{\rm water} \equiv 10^3 \times \Gamma_{\rm ini}$). This abrupt transition happens possibly tens of microseconds *after* the end of the electric pulse and could easily be misinterpreted as the occurrence of pore nucleation after the end of the electric pulse. We note that such a sharp transitional feature has precisely been observed recently by Wilhelm *et al.* [17] while studying the macroscopic electroporation of artificial membranes (their data in Fig. 3 in Ref. [17] can be fitted with $\Gamma_{\rm ini} = 2.5 \times 10^{-4}$ N/m).

For the case of cells, we find $r_c \simeq 100$ nm similar to the mesh size of the underlying cytoskeleton network which suggests, together with the fact that the electric-field-induced membrane tension is also partially relaxed via reverse cell deformation (since $\tau_{\rm eq} \simeq \tau_1$), that the second dynamic regime is not generally reached, i.e., $r_\infty < r_c$. We note, however, that the "stable" pores we have found with this mean field heuristic model are actually expected to "coarsen" at longer time scales after this initial rapid relaxation of the electric-field-induced membrane tension. This is because the line tension of the small pores is not balanced by the average surface tension in the relaxed membrane ($\langle\Gamma\rangle \sim \langle\gamma/r\rangle$) which leads to their fast closure and to the simultaneous expansion of the larger pores. Finally, the slow pore closure is likely to be controlled by the cytoskeleton dynamics and possibly other (slow) biological regulation mechanisms.

In conclusion, a new mechanism is proposed to explain the electroporation of cells. It is based on an electric-field-induced stretching of the membrane and predicts that the electroporation of cells relies merely on the curvature and the high resistivity of their cytoplasmic membrane. In particular, the nucleation of pores is independent of the conductivity of the electroporation medium, which enables a very flexible use of this technique and might, in part, explain its great success with many types of cells [1,2]. In addition, we have also found that the elastic relaxation of the stretched membrane immediately after the pores nucleation may follow two qualitatively *and* quantitatively distinct dynamic regimes in good agreement with recent experiments on artificial bilayers [17].

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