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High electrical field effects on cell membranes

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Abstract

Electrical charging of lipid membranes causes electroporation with sharp membrane conductance increases. Several recent observations, especially at very high field strength, are not compatible with the simple electroporation picture. Here we present several relevant experiments on cell electrical responses to very high external voltages. We hypothesize that, not only are aqueous pores created within the lipid membranes, but that nanoscale membrane fragmentation occurs, possibly with micelle formation. This effect would produce conductivity increases beyond simple electroporation and display a relatively fast turn-off with external voltage. In addition, material loss can be expected at the anode side of cells, in agreement with published experimental reports at high fields. Our hypothesis is qualitatively supported by molecular dynamics simulations. Finally, such cellular responses might temporarily inactivate voltage-gated and ion-pump activity, while not necessarily causing cell death. This hypothesis also supports observations on electrofusion.

Keywords: Electroporation; High Field; Electrical Conductivity; Lipid Membrane; Molecular Dynamics

1. Introduction

Biological membranes are good electrical insulators and the main barrier for mass transport through cells. Their main function is to maintain ionic gradients and electrolyte homeostasis. Under physiological conditions, membranes resting potentials range from 30 mV in mammalian cells to −300 mV in some plant cells. If the transmembrane voltage reaches a critical level, the membrane becomes permeable to small ions like Cl^- or Na^+, independent of channel proteins. Lipid re-arrangement driven by the electric field and short-range dipole interactions, is thought to create conductive membrane pathways referred to as electroporation [1–3]. For example, Neumann et al. [3] had proposed a lipid head group permanent dipole reorientation mechanism, that would finally result in a so-called hydrophilic pore. Typically, a membrane voltage of about 1.0 V is necessary and nanosecond duration voltage pulses have been shown to yield pore radii on the order of 1 nm [4]. Pore formation is also relevant during cell fusion, drug release from liposomes, and passive transport of protons and hydrophilic compounds.

The notion central to the electroporation process is the gradual but well-organized, structural re-arrangement without any molecular detachment or break-up [2,5,6]. It has also been shown that the presence of localized membrane heterogeneities and defects (such as charged DPPS molecules) enhance the electroporation process [7]. Preferential electroporation at the anodic side can then result, and this has been demonstrated both experimentally [8,9] and through molecular dynamics (MD) simulations [7,10].

The traditional electroporation model seems to work well for relatively low-to-moderate electric fields (200 V/cm–5 kV/cm). However, it appears to be inadequate in explaining several of the observed phenomena at high electric field strengths. The various inadequacies with regards to experimental reports are listed below.

(i) The high-field experimental data indicates strong conductivity increases of the cell solution at the very beginning of a high-voltage pulse [11]. Our current experimental data sets (discussed in the next section) show conductance increases far exceeding values that

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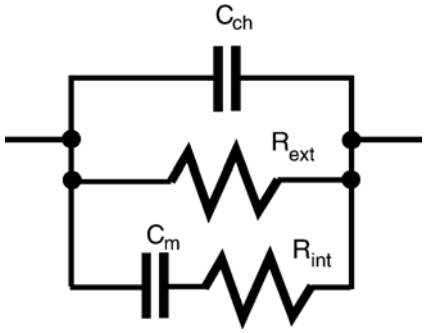

Fig. 1. Equivalent electrical circuit for the measurement chamber containing cell suspension.

might be predicted on the basis of simple membrane electroporation or dissolution. Also, such increases cannot be attributed to field-induced Wien effects.

(ii) The *fast decrease* in conductance [11], observed after the pulse cessation, is not compatible with pore shrinking or sealing [11]. The re-sealing process for electro-pores is known to be slow and can take up to several seconds to minutes [12]. An additional point of interest is the vesicle formation at the plasma membrane (i.e., blebbing) that has been observed [13]. This aspect is not addressed by the electroporation models.

(iii) Recent reports by Tekle et al. [14] show *loss of the phospholipid membrane* during high-voltage pulsing. This phenomenon was shown to occur in addition to a pore formation process. Their results demonstrated that up to 14 percent of the membrane surface could be lost upon electric field application.

(iv) Finally, it has been well documented that the application of electric pulses facilitates the penetration of large molecules through the membrane. This probably implies the creation of large pores. For example, in multi-lamellar systems such as the *stratum corneum*, contributions to structural changes arise from electro-thermally induced vesicle formation. However, electroporation theory, as applied to the *short-duration*, *high-intensity pulses*, predicts relatively small nano-pores [7,10]. Also, simple nano-pore formation alone cannot explain the transport of polyelectrolytes and DNA [15].

For quantitative studies of the macroscopic electric-field induced electroporative effects, one can measure the electrical conductance of a cell suspension under high-field conditions. Without electroporation or any other cellular effects, the overall conductance would remain unaffected. A 100% electroporation would refer to a complete replacement of the membrane by pores (or simply by the surrounding medium), thereby causing an appreciable change in measured conductance. It is expected that the measured conductance will lie somewhere between these two extremes.

In order to measure the impedance of cells, pulses of defined shape and amplitude can be applied to cell suspensions placed in a coaxial chamber. The reflected signal can then be recorded, and components of the electrical equivalent circuit as shown in Fig. 1, calculated from the Fourier-transform of the excitation and the reflected signal. In this equivalent circuit of Fig. 1, the cells are represented by a capacitor C_m for the cell membrane and a resistor R_{int} for the intracellular electrolytes including all the cell substructures. The extra-cellular electrolyte is modeled by a combination of a resistor (R_{ext}) and capacitor (C_{ch}). Recording the impedance data at frequencies fairly large compared to the characteristic β-dispersion frequency (inverse of the charging time constant for the cell membrane) ensures that the membranes are "electrically shorted". For mammalian cells the frequency range for this dispersion is in the range from 1 MHz to 10 MHz. The use of ultra-high frequencies would need to be avoided to ensure that the admittance was not dominated by capacitive contributions of the aqueous medium (C_{ch}). Since the capacitance of the electrolyte filled chamber in our experiments is approximately 48 pF, the typical 50 Ω resistance of our experimental chamber is reached at about 10 MHz. A good compromise for frequency dependent cell studies then, is the frequency range between 50 kHz and 20 MHz. One expects that measurements in this range will provide data on the upper limit for conductivity increases associated with electroporation.

2. Materials and methods

2.1. Cells

Jurkat cells were obtained from American Type Culture Collection (ATCC) and cultured in RPMI1640 medium, supplemented with 1% L-glutamine, 1% penicillin/streptomycin (all Mediatech Cellgrow, Herndon, VA), and 10% FBS (Atlanta Biologicals, Norcross, GA) at 37 °C with 5% CO_2. After harvesting in the log-phase, they were washed and re-suspended in osmotically balanced, buffered sucrose (7 mM PBS). For the experiments, different cell volume fractions, ranging from 1% to 15% were used.

2.2. Mice

Hairless mice (SKH-1) were purchased from Charles River Laboratories (Whilmington, MA) and anesthetized using 1.6% isoflurane. A skin fold of the mouse was placed between 5 mm diameter stainless steel plate electrode disks covered with a thin layer of 1% agar gel. To ensure a high conductivity, the gel was made of 150 mM PBS.

2.3. Impedance measurements

The impedance of the cell suspension was measured by means of time domain reflectometry (TDR). The cells were placed in a stainless steel coaxial chamber with a gap of 0.725 mm and a height of 5 mm. The inner diameter of the coaxial chamber was 5.97 mm while the outer diameter was 7.42 mm. A 25 ps rise-time, 60 mV square wave pulse was used for excitation. A digital oscilloscope (TDS7404, Tektronix, Beaverton, CA) monitored the sum of the incident and the reflected signal. The generator, the chamber, and the oscilloscope were matched to one another using a resistive network. From the recorded signals, the object

impedance in a frequency range between 16 kHz and 300 MHz was calculated. The maximum temporal resolution by using the memory segmentation of the oscilloscope was 500 μs.

2.4. High-voltage application

The pulse length of the Blumlein-configuration pulser was 100 ns and 300 ns, respectively, determined on the length of the cable that was used as pulse forming line [16]. The voltage and total current were monitored using an oscilloscope. A high-voltage switch, capable of switching within 10 ms from pulsing to impedance measurement, was used for disconnecting the chamber from the pulser immediately after the pulse was applied and connecting it to the impedance measurement system.

3. Experimental results

The measured conductance of the cell suspension, G, versus frequency is shown in Fig. 2. The conductance is constant up to frequencies of approximately 100 kHz, its value determined by the extra-cellular electrolyte. It increases with frequency beyond 0.2 MHz due to the β-dispersion and the dispersion arising from intracellular structures and stays constant in the range from 2 MHz to about 10 MHz. This range is characterized by the "electrical shorting" of the cell membrane. As previously mentioned, the highest conductance of a cell suspension is expected at frequencies for which the cell membranes are "electrically short-circuited". For this scenario, the measured net impedance is in effect determined by the parallel combination of resistors R_{int} and R_{ext} (Fig. 1). This frequency range is followed by a dramatic increase in conductance in the range past 30 MHz. This sharp rise in conductance is caused by the increasing influence of the overall capacitance of the chamber, which is roughly C_{ch}=48 pF in our set-up.

The conductance of the cell suspension during pulse application was measured by recording the current and voltage. For a 300 ns, 30 kV/cm high-voltage pulse; the current-to-voltage ratio (I/V) was obtained 200 ns after the onset of the pulse. This frequency independent value is shown as a dashed line in Fig. 2. The important point is that the conductance during the high-voltage pulse exceeds the value measured by impedance spectroscopy by at least 20% in a frequency range where the cell membrane is already "electrically shorted." Since electroporation permeabilizes cell membranes, the electrical conduction can only vary between that for "no electroporation," and that for an extreme scenario of complete membrane dissolution. Here, the measured conductivity under pulsed conditions is even larger than the high frequency "membrane dissolution" scenario. Also, the enhancement observed here cannot simply be accounted for by either membrane electroporation, the Wien effects [17], or field-induced water dissociation. This is because such effects can only contribute to a modest increase in conductance by about 3% [17].

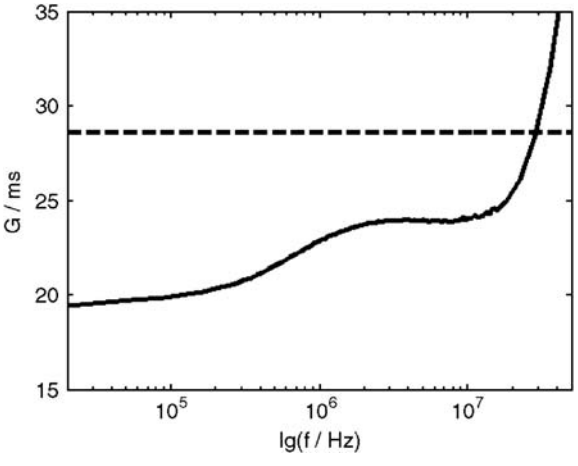

Fig. 2. Conductance of a Jurkat cell suspension (≈10^7 cells/mL) versus frequency. The dashed line indicates the measured conductance of the same sample 200 ns after the onset of a 300 ns, 30 kV/cm electrical pulse.

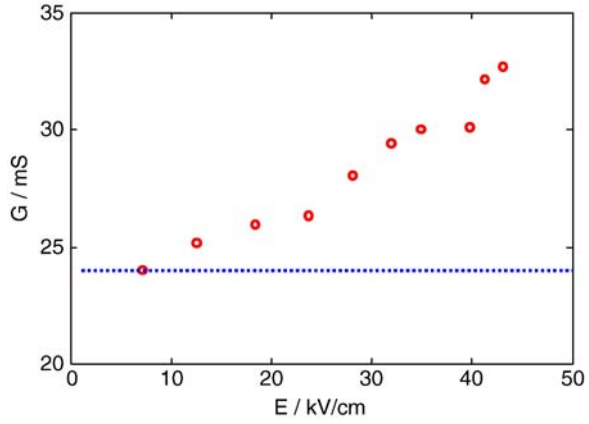

Fig. 3. Measured conductance versus the field strength for a Jurkat cell suspension (≈10^7 cells/mL). Only one pulse per experiment was applied and fresh suspension was always used.

The measured conductance can also not be attributed to any displacement current. The charging time constant for the water filled chamber is on the order of 1 ns. Since the measurements were taken at 200 ns into the 300 ns long pulse, displacement currents are negligible.

The conductance increase with field strength is shown in Fig. 3 for a Jurkat cell suspension. The measurements show an increase in conductance of up to 140% for the highest applied electric field strength of 45 kV/cm. For comparison, the conductance corresponding to the "shorted" regime obtained at zero dc bias from frequency domain measurements is also given. This large change with electric field cannot be accounted for by the Wien effects or by simple electroporation. Both the field distortion of counter ion clouds (i.e., the first Wien effect) and the field dissociation of weakly bound molecules and multipoles (i. e., the second Wien effect) only lead to very modest conductance increases. Electroporation alone would not increase the conductance beyond that expected for completely electroporated membranes. Hence, the measured conductivity increases suggest that either *new charge carriers are being created in a field-dependent manner, or existing immobile charges are being liberated by the applied voltage pulse*.

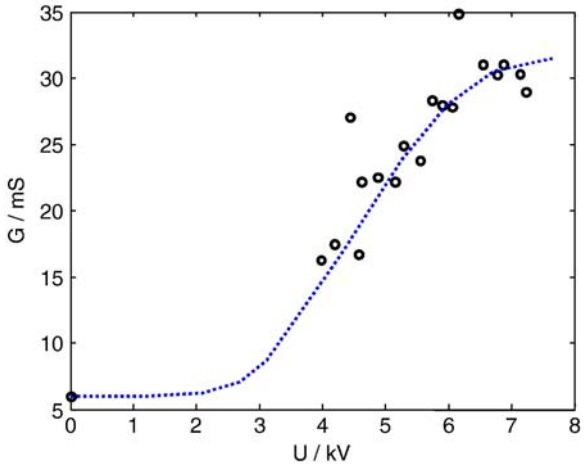

Fig. 4. The ratio I/V (here referred to as the conductance) during the pulse (at 200 ns) depending on the voltage applied to the skin fold of a mouse.

With in-vivo experiments on mice skin, the same general trend was found. Fig. 4 shows the results of current and voltage measurements at 200 ns (during the pulse) for a 300 ns pulse. Plotted is the current-to-voltage ratio versus electric field strength. The data show a conductance much higher than the high frequency impedance value. This latter is roughly 6.02± 2.11 mS and was obtained from impedance measurements at 10 MHz. It has been indicated at 0 V in Fig. 4, since the frequency measurements had no net "dc bias". The experiments were conducted by randomly changing the electrode positions at the mice, and measuring the current-to-voltage ratio, which is roughly the averaged impedance. Each dot in Fig. 4 denotes one single experiment. The dotted line has been inserted in Fig. 4 only serves as a guide indicating a probable conductance curve.

The ratio of conductance at the beginning (averaged from 1 ns to 10 ns) of a voltage pulse to its value at the end of the pulse (averaged from 80 ns to 90 ns) for mice skin is shown in Fig. 5. It is a plot as a function of applied field strength for a 100 ns pulse. The highest voltage applied was ~7 kV. Larger values were not used to avoid possible electric "flash-over" and arcing between the electrodes. The experimental results in Fig. 5 clearly show: (i) the application of an external voltage changes the system conductance. At lower fields (<5 kV/cm) the increase in conductivity is small (<5%), but it rises strongly for electric fields exceeding 5 kV/cm. (ii) The dramatic temporal changes occur over time scales that are on the order of 100 ns or less.

3.1. Model hypothesis for high-field membrane effects

Lipids at very low concentrations are stable as hydrated molecules in bulk water. With increasing concentration of lipids, they begin to form micelles. As the density is further increased, the micelles merge into double-layered membrane structures, and can even form vesicles spontaneously. Formation of a double layer at high lipid concentrations lowers the energy cost, which arises from repulsive forces between the hydro-carbon tails within a finite volume. Also, in a bilayer structure, the lipid molecules are able to randomly drift (the fluid mosaic model), favoring entropy increases.

Micelle structures at high lipid concentrations are only stable in an environment with low permittivity. Since water typically has a high permittivity, the micelles tend to merge into double-layered structures and vesicles in an aqueous medium. However, if the permittivity of water can be reduced, at least over localized regions that are in the immediate vicinity of micelles, then these entities would remain stable and exist in this form.

The creation of high electric fields is a process that can decrease the permittivity [18,19]. The relative permittivity (ε_r) is related to the mean dipole moment $<M>$ as [20]:

$$[(\varepsilon_r - 1)/3][(2\varepsilon_{RF} + 1)/(2\varepsilon_{RF} + \varepsilon_r)] = (4\pi/3)\left\{\left[<M^2> - <M>^2\right]/(3Vk_BT)\right\}, \quad (1)$$

where ε_{RF} is an effective reaction field permittivity, V the volume, k_B the Boltzmann constant, and T the temperature in Kelvin. The ε_{RF} term accounts for the finite-size of all simulations. Due to the finite simulation region, each dipole only interacts with entities that lie within a finite distance and contributions from far-lying regions are ignored. Such truncation can lead to physical errors that are corrected by invoking the effective reaction field permittivity ε_{RF}. Usually ε_{RF} can roughly be set to the permittivity ε_r under the continuum approximation. In any case, the individual dipoles tend to line-up when an external field is applied, thereby increasing the $<M>$ value. This naturally leads to a lowering of ε_r from Eq. (1) above. *Hence, the application of high external electric fields facilitates lowering of the permittivity, and so would favor membrane break-up into micelle structures*.

The following scenario can then be evoked. (i) The high externally applied electric fields causes electroporation and forces water inside the bilayer structure. (ii) Spatial confinement of water dipole molecules within the nano-pores effectively reduces the liquid permittivity. Such reductions in dielectric constant due to finite-size effects are well known [19,21], and arise from the inability of dipoles to effectively screen potential,

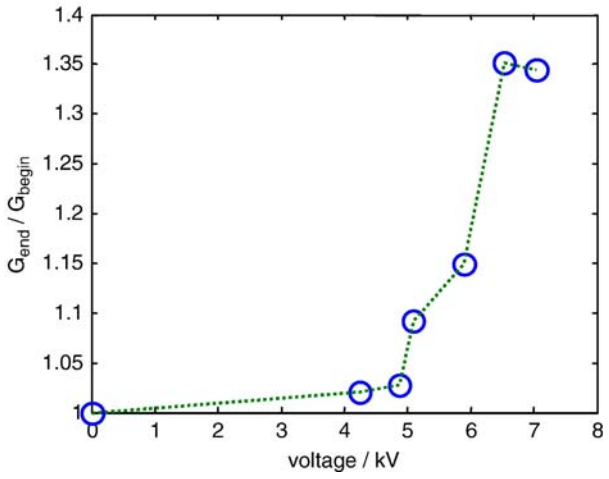

Fig. 5. Conductance ratio between ending (over 80–90 ns) and beginning (over 1 ns–10 ns) time for a 100 ns pulse.

as their free movement is curtailed. (iii) The high electric field near lipid membrane surfaces also facilitates the movement of charged molecules [e.g., phosphatidyl-serine (PS)] causing further structural re-arrangement. (iv) Since the permittivity (ε) decreases with electric field, a positive feedback mechanism would arise. Any field-induced reduction in permittivity would lead to further increases the local electric field since the displacement vector $\boldsymbol{D}=\varepsilon E$ is roughly constant. This local increase, in turn, would produce further reductions in permittivity. (v) The presence of a high electric field could also alter the electronic states and cause energy-level mixing through the Stark effect. It could become possible for the hybrid bonding states of one site to align with the anti-bonding energies at adjacent sites. Field-induced electronic transfer (e.g., hopping), leading to the weakening of molecular bonds could then follow as a naturally consequence. Hence, field-assisted fragmentation and micelle formation might be facilitated in the presence of high fields.

The recent application of ultrashort pulses (e.g., 10 ns duration voltage with a 1 ns rise and fall-times) to cells open up the possibility of membrane over-charging and the attainment of highly non-equilibrium conditions [22]. A transient "overshoot" in the transmembrane potential can result [4], leading to higher local electric fields than in conventional electroporation. Also, lower aqueous permittivities can be achieved [19,23]. Such overcharged porated membranes are very unstable, especially under highly non-equilibrium conditions. Hence, molecules in contact with the low-permittivity, aqueous medium might loosen and begin forming localized micelles. Micelles containing charged molecules (e.g., phosphatidyl-serine) derived from the membrane structure, would provide a net source of mobile charges within the aqueous medium. These micelles could contribute to the conductivity increases seen experimentally.

Since most cell membrane typically tend to be negatively charged, and consequently the micelles are likely to be negative entities, lipid molecules for micelle formation can be easier released at the anodic pole than at the cathodic pole of the cell. Experimental data by Tekle et al. [14] has indeed shown such a polarity effect with membrane loss preferentially at the anodic side. This has been confirmed by molecular dynamic simulations [7,10]. Furthermore, in this scenario, the conductivity enhancements would be expected to rapidly decrease following voltage turn-off. Such rapid conductivity decreases have been observed experimentally [11]. The conductivity slow-down would arise from scattering of charged micelles with the aqueous medium, and on-going self-organization into larger lipid structures.

For completeness, we would also like to point out that other possibilities for field-induced conductivity enhancements have been discussed in the literature. For example, the notion of protein release by electric fields of about 20 kV/cm, was first suggested several years ago by Neumann and Rosenheck [1]. This group probed the possibility of field-induced permeability changes, leading to the release of biogenic amines. This work was primarily experimental, and no specific process quantification or atomistic-level details were reported. Another possible source of conductivity increases in the aqueous media might arise from the efflux of ions (e.g., Potassium) from the cells upon electric-field induced poration. Such a possibility has also been discussed by several groups [24,25].

Here we take the view that the conductivity increases are due to membrane fragments that are essentially charged. The lowering of the local dielectric permittivity would favor micelle formation. This, however, is not necessary or critical, since the release charged fragments would contribute to conductivity modulation regardless of the shape, structure, or molecular organization. The earlier suggestion of charged proteins [1] is certainly a valid possibility. However, in the broadest sense, such protein detachment is naturally included within the present domain of charged molecular generation arising from membrane fragmentation. While we have not explicitly introduced proteins into our atomic level membrane simulations of this contribution, their detachment upon membrane fragmentation is implied and perhaps somewhat obvious. The second possibility of conductivity increase [24,25] due to the efflux of potassium ions from inside the cell after electroporation is not very significant in the present cases. Results from such a process would depend on the ionicity of the extra-cellular medium. At low ionic concentrations of the extra-cellular medium, one would expect the potassium efflux process to produce a stronger conductance enhancement. In our case, the experiments were carried out at several different background electrolyte conductivities and amphiphiles. The highest ionic extra-cellular content was considered. However, in our experiments, only amphiphiles showed a dramatic increase in conductivity.

3.2. Atomistic simulations

While the above hypothesis perhaps seems plausible, evaluations are necessary to provide simple "proof-of-concept" and to ascertain the overall likelihood of such events. Towards this end, MD simulations of cellular response to high electric fields were performed to probe the dynamical behavior. MD simulations rely on the application of classical Newtonian mechanics for the dynamical movement of ions and neutral atoms, taking account of the many-body interactions [26–28]. In our study, the GROMACS (Groningen Machine for Chemical Simulations) package in NpT ensemble [29] was used for the MD simulations of field-induced membrane effects. Details on our MD implementation for membranes in an aqueous medium, subject to external electric fields can be found elsewhere [7,10]. The dipalmitoyl-phosphatidyl-choline (DPPC) membrane was chosen with some embedded PS molecules, and the force fields for membrane molecular motion taken from the literature [27,28,30]. In all a total of 169,891 atoms were used comprising of 54,485 water molecules, 119 DPPC chains, 9 phosphatidyl-serine and 9 Na^+ ions for overall charge neutrality. The system was coupled using a semi-isotropic Berendsen pressure coupling of 1 atm with compressibility of 4×10^{-5} along the z direction, and zero along the x and y directions. A 323 K heat bath is chosen to retain the liquid phase of the membrane. The requisite time constants for pressure and temperature coupling were set to 1 ps and 0.1 ps, respectively.

Fig. 6 shows the initial configuration of a lipid bilayer structure at the start of MD simulations. The membrane was

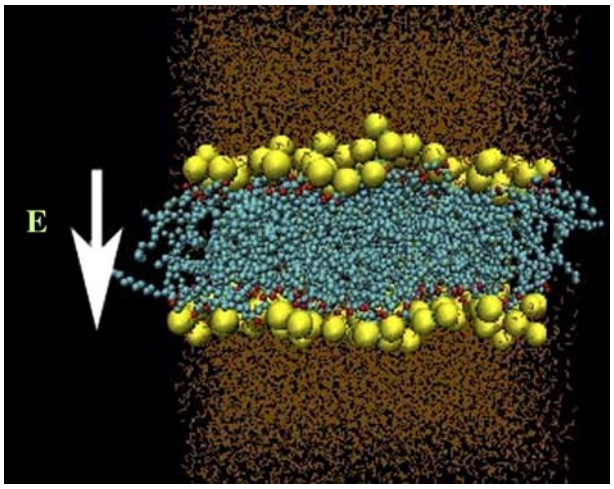

Fig. 6. The initial configuration of a DPPC lipid bilayer used for the MD simulations. Water is shown in brown, lipid headgroups in yellow and lipid chains in cyan.

comprised of a 8×8 square grid at the top and bottom surfaces. A constant, large electric field of 0.6 V/nm was imposed perpendicular to the membrane. The electric field value used here is large and this choice perhaps needs some explanation. The corresponding transmembrane voltage at this field is roughly 3 V. For traditional electroporation, the accepted potential values are on the order of 1 V [2]. However, for short-duration pulses highly non-equilibrium conditions exist, giving rise to transient overshoots in transmembrane voltage [31]. Hence, the use of membrane voltages larger than 1 V under transient conditions is not unphysical. Also, MD simulations are very computationally intensive and require small time steps in the femto-second range. Calculations of the dynamic response can only be carried out to tens of nano-seconds. In actual practice, membrane effects under a high external field could take as long as milliseconds to manifest. So here, a very high electric field was deliberately chosen to increase the driving force, and hence, produce a tangible outcome within tractable time scales. Since the underlying physics produced by the external field would be the same, the high value used here simply allows for the attainment of the final state at much shorter times — thus a "steered" result. Since the present focus is on the "proof-of-concept," and it is adequate to quantitatively demonstrate the final outcome via such "numerically accelerated testing."

A snapshot of the membrane patch shown in Fig. 7 at 1.85 ns reveals the membrane disruption and the onset of fragmentation. The headgroups are beginning to separate and move into the aqueous medium. Our results strongly suggest an intermediate state of a closed hydrophilic pore (HI), representing a non-conductive pre-pore on the pathway to a final HI pore. The fragmentation is more obvious in Fig. 8, which is a 2.05 ns snapshot from the MD simulations. The 0.6 V/nm field acting on a charged PS lipid molecule roughly corresponds to a 96 pN force that facilitates lipid extraction from the DPPC membrane. This force is slightly lower than previous estimates for bio-membrane rupture [32,33] based on pure mechanical stress. A slight lowering on the force requirement in this electric field context is perhaps to be expected based on the following qualitative aspects. Lipid bilayer membranes derive their resilience to structural dissolution, in part, by the hydrophobic interactions between lipid tails and the surrounding water. This interaction helps keep the lipid tails sequestered away from the water, maintains the structure and prevents membrane break-up. However, unlike pure mechanical stresses, the application of external electric fields creates nano-pores and lowers the local permittivity. Forced entry of water into the lipid bilayer system weakens the drive towards an organized structure. Collectively, electrostatic forces tugging on charged ions, the short-range dipole interactions, reductions in localized aqueous permittivity,

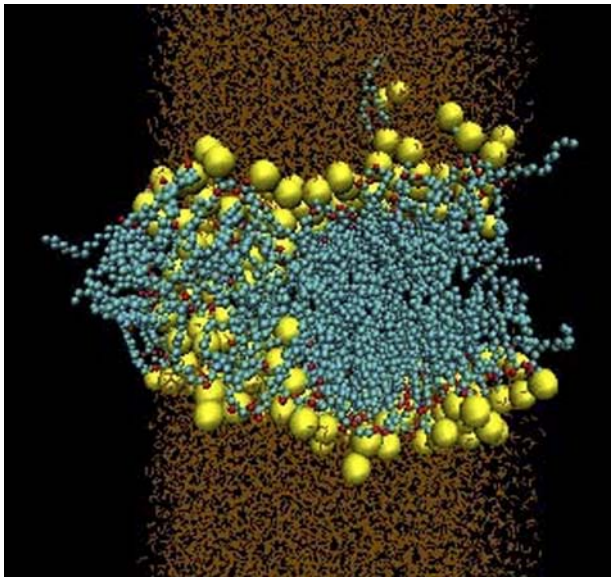

Fig. 7. Snapshot of the DPPC lipid bilayer patch at 1.85 ns. Slow membrane fragmentation is predicted during the electric pulse.

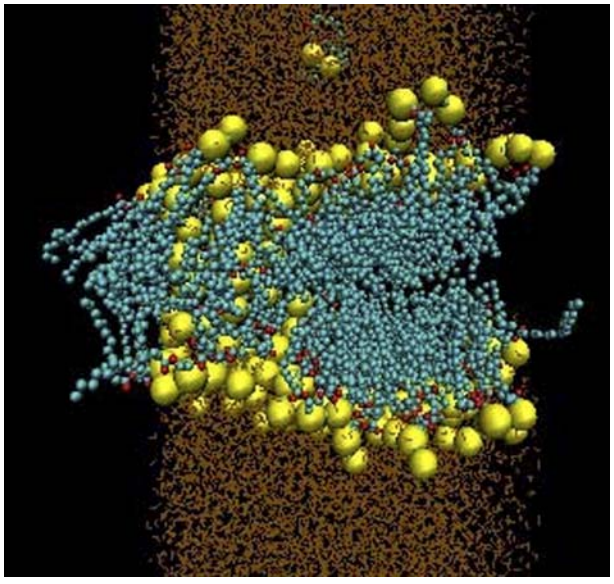

Fig. 8. A 2.05 ns simulation snapshot of the DPPC lipid bilayer patch with the voltage pulse applied. One lipid at the anode side (top) is seen to break loose.

and the possibility for increased entropy, all serve to facilitate lipid membrane fragmentation under strong external electric fields.

The above MD simulations clearly demonstrate the following aspects: (i) It is possible to get membrane rupture and fragmentation in response to a large externally applied electric field. In practice, such effects are expected to occur at the poles due to the highest electric fields in those regions. (ii) The effects are predicted to be stronger at the anodic side due to the existence of negatively charged molecular species within membranes. Such a clear polarity effects was previously shown by our group with MD simulations [7] and matched the experimental reports on PS externalization [8]. (iii) The ejection of lipid molecules from parts of the membrane has been shown to occur. Furthermore, such fragments are likely to cluster together and form micelles in the immediate vicinity of the parent membrane. (iv) Finally, though not shown here explicitly, it is conceivable that the uncharged micelles would tend to diffuse back and self-organize into the parent membrane. However, micelles containing charged entities would drift away due to the applied electric field, and also due to the mutual electrostatic repulsion between different micelles. Upon termination of the electric field, not all of such charged micelles would coalesce back due to the mutual electrostatic repulsion. Hence, a certain fraction of the membrane segment might be lost, in keeping with the reports of Tekle et al. [14].

4. Discussion

The molar conductivity of a suspension of micelles is higher than that of an aqueous medium alone. The collection of micelles formed due to the dis-integration of a charged membrane would itself be charged. A structural transition and membrane nanofragmentation in the presence of high electric fields would explain the unexpected high conductivities observed experimentally. The fast decrease in aqueous conductivity [11] would also be consistent with this picture, rather than the "pore-resealing" mechanism.

Under the present hypothesis, micelles could rearrange through self-reorganization after voltage pulse termination to re-constitute the membrane structure. Reorganization and pattern formations of membrane channels (or pumps) are ubiquitous in biological cells and tissues driven away from their equilibrium [34]. However, this does not necessarily imply that the entire membrane would fully recover. Instead, one might expect a number of small vesicles besides the recovered membrane, visible as "blebbing" at the membrane level. Also, this scenario does not necessarily imply "cell death" or irreversibility. If the area involved in micelle formation was small compared to the entire cell surface, it might be possible to "repair the membrane" through the action of transporter proteins functioning as flipases. Glycolipids, however, cannot flip back, since they need membrane recycling processes provided by the cell itself. Hence, it would appear logical that as long as the organnelle membranes were not destroyed by the voltage pulse, the plasma membrane could potentially be repaired within several minutes. Such cell-recovery following external voltage pulsing has been observed and is well documented. However, destruction of the nuclear membrane (or other inner membranes, e.g., the mitochondrial sites) would probably constitute severe biological disruption, leading to irreversibility and cell death.

In closing, we would like to point out that the present hypothesis also has relevance to the electrofusion phenomena. Electrofusion is believed to occur at the electroporated sites of adjacent cell membranes. However, several issues remain unexplained in this context. For example, the voltage needed across the two membranes for cell fusion is considerable higher than twice that required for electroporating a single membrane. Secondly, due to the existence of glycocalyx, it is very unlikely that the lipid structures of the fusing membranes actually touch each other. This makes lipid material exchange unlikely or difficult at best. Also, short-duration, higher voltage pulses are known to be more effective at cell fusion, rather than long-duration pulses.

All of the above could perhaps be understood in the context of the micellar formation hypothesis, by postulating that this process may be a key proponent of cell fusion. As discussed previously, the high fields might yield a diffuse distribution of lipid material, preferentially at the polar caps. This material can then redistribute back, after a short period of time (μs-range), into a single joint membrane. Since very high electric fields are necessary for micelle formation, the requirement of large external voltages would be logical for electrofusion based on the current hypothesis. The glycocalyx paradox would also be circumvented. The "ablation" of membrane materials from adjacent cell sites during the voltage pulsing, and their gradual inter-mingling during the recovery phase would produce field-assisted cell-fusion.

5. Summary

The physical phenomena of electrically stressing cells to high external voltages has been re-examined in the light of recent experimental data. It has been shown that under such conditions, significant increases in conductivity of the cellular medium can result, and that this conductivity rapidly decreases upon voltage termination. Such conductivity increases and the relatively fast conductivity turn-offs cannot be accounted for on the basis of simple electroporation alone.

A new hypothesis of localized membrane rupture and fragmentation on the "nanoscale" at high electric fields has been proposed. Its key feature is the transition from a double layer to membrane dis-organization, and partial fragmentation driven by a high external electric field. Such a response would lead to conductivity increases beyond the electroporative effects, and display a relatively fast turn-off with the external voltage. In addition, such fragmentation and material loss can be expected at the anode side of cells. This feature would be in keeping with the reports at high fields by the Tekle group [14]. Our hypothesis was qualitatively supported by MD simulations. Finally, such cellular responses might temporarily inactivate voltage-gated and ion-pump activity, though not necessarily leading to cell death. This hypothesis would also support several observations on electrofusion.

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