Numerical analyses of electroporation-mediated doxorubicin uptake in eukaryotic cells: role of membrane cholesterol content

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Electroporation or electropermeabilization is a biophysical process involving enhanced permeability of biological cell membrane due to the application of an electric field of very short duration. Since its inception in the early 1970’s, the technique has been utilized widely in biomedical research and applications including gene transfection and electrochemotherapy of cancer. Past theoretical models of cell electroporation considered approximations which made the predicted results very different from the experimental descriptions of poration, especially for electrochemotherapy applications. Present work is a theoretical formulation and numerical implementation of small molecule (Doxorubicin) uptake during electroporation of a mammalian cell with cholesterol-containing membrane. Here, we explore the effects of changes in membrane cholesterol content on electroporation pore dynamics and uptake of small molecules.

Keywords: Cholesterol content, Doxorubicin, Electroporation, Pore dynamics

Electropermeabilization or electroporation is a physical process involving enhanced permeability of biological cell membrane due to the application of high voltage electric pulses of very short duration\(^1\)^.\(^5\) The external applied electric field causes structural changes in the cell membrane leading to the formation of aqueous pathways or micro-pores in the lipid bilayer. The opening of such channels/pores enables exogenous ions and molecules like drugs and DNA to move into the cell. While the application of short duration, low amplitude electric pulses creates reversible electroporation as pores close within milliseconds, high voltage and long duration pulses result in irreversible portion and subsequent membrane breakdown. Research reveals that application of ultra-short high voltage pulses can also cause irreversible damage. Since the 1970s, the technique has found a wide range of applications in biology and medicine including gene transfection, gene therapy, vaccine delivery against viruses like Influenza and Hepatitis C in animals have generated 3-5 fold greater immune response compared to normal intravenous injections\(^23\)-\(^25\).

Electroporation phenomenon has been studied at various levels from planar lipid bilayers, single cells, cells in suspensions, to tissues in animals (in vivo experimentations). Though a wide range of experimental studies on the mechanism of electroporation exists\(^26\)-\(^32\), theoretical studies on the biophysical basis of electroporation phenomenon are sparse. Theories of electroporation provided by Neu et al.\(^31\) and Smith et al.\(^27\) throw considerable insight into the physical forces involved in the process. However, these theories are not free from limitations. As these are based on assumptions and approximations, predictions sometimes yield more dramatic results than observed in experiments, especially in case of mammalian cells and at higher applied electric fields \(\geq1\text{kV/cm}\)^.\(^27\). Unlike earlier studies, Shil et al.\(^5\) have attempted a more realistic theoretical explanation by assuming a cholesterol-containing membrane along with DPPC.

To the best of our knowledge, theoretical studies exploring the effects of variation in membrane

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Abbreviations: DOX; Doxorubicin
cholersterol-content on mammalian cell electroporation and electroporation mediated cellular drug uptake does not exist, when studied with parameters in the electro-chemotherapy applications range. The present study is an attempt to understand theoretically (and based on simulations) the biophysical basis of cell electroporation incorporating effects of variation in membrane cholesterol-content i.e. to understand how the changes in membrane composition (in terms of cholesterol-content) alter pore formation and affect the uptake of a small molecule.

**Materials and Methods**

**Theoretical formulation**

**Pore dynamics**

Reversible electroporation process in a cell is a two-stage process. The first stage involves the formation of transient aqueous pores due to the application of electric field leading to enhanced permeabilization of the cell membrane. The second stage is the process of resealing of cell membrane at the end of the electric pulse application. In general, the transmembrane potential generated by the applied electric field in a biological cell is a key parameter for effective electroporation. When a biological cell of radius, \( R_{cell} \) is suspended in solution and exposed to an electric field \( E_c \), then the induced transmembrane potential of the cell membrane is described by the Schwan equation:

\[
\phi_m = 1.5 R_{cell} E_c \cos \theta \quad \text{Eqn 1.1}
\]

Here, \( \theta \) is the angle, indicated by the Figure 1A.

This is obtained by solving Laplace’s equation (with physiological values of parameters) with the assumption that the biological cell is a non-conducting sphere with equipotential inner side and the cell membrane being an infinitesimally thin insulator. It is derived from this equation that at \( \theta = 0 \) and \( \theta = \pi \) (known as ‘poles’), the value of \( \phi_m \) becomes extremum and is dependent solely on cell size \( (R_{cell}) \) and electric field \( E_c \). The value of \( \phi_m \) changes with \( \theta \) implying that at any given point in time during the application of the electric field different regions of the cell surface will experience different values of \( \phi_m \).

Though the theoretical aspect of electroporation phenomenon in a spherical cell with cholesterol-containing membrane has been established, a brief description of the mechanism of the portion is given below.

Pores created with radius \( \geq 0.8 \) nm (critical radius) can expand under the influence of the applied electric field. It is assumed that the pores are created with a rate

\[
\frac{dN}{dt} = \alpha e^{(\phi_m/\phi_p)^2} \left( 1 - \frac{N}{N_{eq}(\phi_m)} \right)
\]

**Eqn 1.2**

where \( \phi_p \) is the voltage of electroporation, \( \phi_m \) is the transmembrane potential, \( N \) is the pore density,

\( \alpha = 1 \times 10^9 \text{ m}^{-2} \text{s}^{-1} \) is the creation rate coefficient. \( N_{eq} \) is the equilibrium pore density defined as

\[
N_{eq}(\phi_m) = N_0 e^{q(\phi_m/\psi_p)^2}
\]

With \( N_0 \) is the equilibrium pore density at \( \phi_m = 0 \), \( r_m = 0.8 \text{ nm}, r^* = 0.51 \text{ nm} \) (minimum possible radius of a hydrophilic pore) and \( q = (r_m/r^*)^2 \) as described by Shil et al. and Smith et al.

Once formed, the pores evolve under the influence of the applied electric field with the radius \( r_j \) \( (j = 1, 2, 3 \ldots n, \text{ accounting for } n \text{ pores}) \) changing at a rate

\[
\frac{dr_j}{dt} = -\frac{D_p}{k_B T} \frac{\partial W}{\partial r_j}
\]

**Eqn 1.3**

where \( k_B \) is the Boltzmann constant, \( W \) the bilayer energy, \( D_p = 5 \times 10^{-14} \text{ m}^2 \text{s}^{-1} \) is the diffusion coefficient for the pore radius and \( T \) the absolute temperature.

Cell electroporation was simulated based on the theory of electroporation published elsewhere with suitable modifications as to account for the variation in membrane cholesterol content. Briefly, the equation describing the bilayer energy was modified as

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**Fig. 1—Spherical cell divided into segments as: \( \theta = 0 \) to \( \theta = \pi/8 \) (segment S1), \( \theta = \pi/8 \) to \( \theta = \pi/4 \) (segment S2), \( \theta = \pi/4 \) to \( \theta = 3\pi/8 \) (segment S3) and \( \theta = 3\pi/8 \) to \( \theta = \pi/2 \) (segment S4)(A) and; artistic impression of the segments (B).**
\[ W = \sum_{j=1}^{n} \left( \beta \left( \frac{r_j}{r_j^0} \right)^4 - 2 \pi r_j \zeta + \int_0^{r_j} F(r_j, \phi_m)dr - \pi \sigma_{eff} (A_j) r_j^2 \right) \]

Eqn. 1.4

Where \( r_j \) is the radius of the \( j^{th} \) pore formed due to the application of electric field.

In the equation 1.4, the first term represents the static repulsion of the lipid heads (\( \beta = 1.4 \times 10^{-19} \) J). The second term of Eqn (1.4) represents the edge energy of the pore perimeter with the line tension \( \zeta \), defined as:

\[ \zeta = \pi \kappa \left( \frac{1}{h} - \psi c_0 \right) \]

Eqn 1.5

where \( \kappa = 2.7 \times 10^{20} \) is the bending modulus of the membrane, \( h = 5 \) nm is the membrane thickness and \( \psi \) is the mole fraction of cholesterol in the membrane (also termed as membrane cholesterol content) and \( c_0 \) being the curvature for cholesterol molecule (\( c_0 = -0.9 \) nm\(^{-1}\)). It should be noted that we assume the cell membrane to be composed of Dipalmitoyl-sn-glyco-3-phosphocholine (DPPC) and cholesterol.

The third term of Eqn 1.4 represents the energy contribution due to transmembrane potential and the last term accounts for the effect of pores in the membrane.

Once the pores are formed and expand, their presence affects the transmembrane potential which is calculated by:

\[ C_m \frac{d\phi_m}{dt} + \left( \frac{1}{R_S} + \frac{1}{R} \right) \phi_m + I_p = \frac{\phi_m}{R_S} \]

Eqn 2

where \( C_m \) is the membrane capacitance, \( I_p \) is the current through the pores. As described elsewhere\(^{27} \), \( R = R_m/A \) is the membrane resistance with \( R_m = 0.523 \) \( \Omega \)m\(^2\) and \( R_S \) is 100 \( \Omega \) in series resistance of the experimental setup (electroporated devices, buffer etc.). These constants are assumed to be same for all segments (S1, S2, S3, and S4) (Fig. 1) so as to maintain the physical integrity of the cell.

When the electric field is turned off, the \( \phi_m \) becomes 0 and the pores start shrinking. Subsequently, rescaling of the membrane is completed. Eukaryotic cells electroporated in the range of applied electric field \( E = 0.8 \) kV/cm to \( E = 1.8 \) kV/cm (electrochemotherapy applications range) undergo complete membrane rescaling and restoration of original transmembrane potential between 5-15 min (average \( \sim \)10 min) from the end of pulsation at \( \sim \)25°C. However, the efficacy of rescaling depends on temperature, the amplitude of applied electric field and pulse duration\(^{15} \). Neu et al.\(^{31} \) provided a theoretical explanation of cell rescaling process in great details. It is understood that rescaling is a two-step process: Firstly, upon withdrawal of the electric field, the pores rapidly shrink to the minimum energy radius \( r_m \approx 0.8 \) nm. Secondly, the pores that shrank to \( r_m \) disappear due to lipid fluctuations and rearrangements and the original bilayer configuration is restored. It has been established that the mean rescaling time constant for pores is \( \sim 3 \) sec\(^{34} \) based on parameters used in the study. Complete restoration of the total membrane (to original transmembrane potential) may take few minutes\(^{35,36} \).

**Molecular uptake during electroporation: (Diffusion of DOX)**

To study molecular uptake during electroporation, the widely used anticancer drug Doxorubicin\(^{10} \) (DOX) was chosen as a suitable candidate. To model the cellular uptake of DOX through the pores in the polar regions, we assumed: (i) the aqueous pores (toroidal in shape) were uniform in size for each segment of the cell; (ii) diffusion is the dominant mode of molecular transport both across the membrane and within the cell and (iii) diffusion occurs in the direction perpendicular to the plane of the membrane (1-dimensional movement of molecule through the pore)\(^{18} \) as shown in Figure 2.

If \( D \) be the diffusion coefficient of the drug in dilute solution, then the distance traveled by the molecule during time \( T \) is given by:

\[ x = 2 \sqrt{DT} \]

Fig. 2 — Diffusion of molecules through pores during electroporation
The equation governing intracellular diffusion in a permeabilized membrane (dilute solutions) is given by:

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \text{for} \quad 0 \leq t \leq T \quad \text{(Eqn.3.1)}
\]

where \( t \) is time, \( D \) is the diffusion coefficient, \( C \) is an intracellular concentration of small molecule and \( x \) is the distance travelled by the molecule as defined by Fig. 2.

The initial and boundary conditions describing the process are

\[
-\frac{\partial C}{\partial x} = \frac{P}{D}(C_0 - C) = b(C_0 - C) \quad \text{(Eqn. 3.2)}
\]

at \( x \to \infty \) we must have \( C=0 \) \quad \text{(Eqn. 3.3)}

and, at \( t=0, C=0 \) \quad \text{(Eqn 3.4)}

Where \( C_0 \) is the concentration of drug molecule in an extracellular medium, and

\[
b = \frac{P}{D} = \frac{\alpha \varphi}{\delta}
\]

is the ratio of permeability coefficient \( (P) \) and diffusion coefficient \( (D) \) with \( \alpha \) as the total area available for diffusion, \( \varphi \) is partition coefficient for the drug molecule and \( \delta \) is membrane thickness.

Analytical solution of equation 2.1-2.2 gives the intracellular concentration of drug as,

\[
C = C_0 \left[ \text{erfc} \left( \frac{x}{2 \sqrt{Dt}} \right) - \exp \left( bx + b^2 Dt \right) \text{erfc} \left( \frac{x}{2 \sqrt{Dt}} + b \sqrt{Dt} \right) \right] \quad \text{(Eqn 3.5)}
\]

where \( \text{erfc} \) is the complementary error function\(^{18,37} \).

To obtain numerical solutions to these equations, parameters like partition function \( (\varphi) \), the total area of diffusion and \( b \) were determined as follows:

**Area available for diffusion**

This is the total area in the permeabilized membrane through which diffusion of drug molecules occurs. As it became clear from the first part of the study (cell electroporation simulations) that pore formation occurs in the S1 and S2 segments of the cell, we estimated the area for diffusion as follows.

\[
\alpha = (\text{Average Area of a pore in S1} \times \text{number of pores in S1}) + (\text{Average Area of a pore in S2} \times \text{number of pores in S2})
\]

Total area for diffusion in the whole cell=2 \( \alpha \), accounting for both halves of the cell.

**Partition coefficient \( \varphi \)**

For the drug molecule entering the cell through open pores by diffusion, the partition coefficient\(^{18} \) is defined as

\[
\varphi = (1 - \lambda)^2 \quad \text{(Eqn 4.1)}
\]

with

\[
\lambda = \frac{\text{Diameter of drug molecule}}{\text{Diameter of pore}}
\]

Since it became clear from the first part of the study that S1 and S2 segments of the cell have pores of different dimensions, we calculate the values of \( \lambda \) separately.

\[
\hat{\lambda}_1 = \frac{\text{Diameter of drug molecule}}{\text{Average pore diameter in S1}}
\]

\[
\hat{\lambda}_2 = \frac{\text{Diameter of drug molecule}}{\text{Average pore diameter in S2}}
\]

Hence, \( \varphi_1 = (1 - \hat{\lambda}_1)^2 \) and \( \varphi_2 = (1 - \hat{\lambda}_2)^2 \).

To avoid complication in computing while generating numerical solutions for equation 3.5, we have designed an effective partition coefficient \( \varphi \) for the drug, applicable to all pores covering S1 and S2 segments. If \( n_1 \) be the number of pores in S1 segment and \( n_2 \) in the S2 segment, then the effective partition coefficient for the drug (for all pores in S1 and S2 segments) is given by

\[
\varphi = (n_1 \varphi_1 + n_2 \varphi_2) / (n_1 + n_2) \quad \text{Eqn 4.2}
\]

This is a weighted average of the partition coefficients from S1 and S2. This value of \( \varphi \) was considered for the simulation of drug diffusion in both the segments S1 and S2 of the cell.

**Computational method**

Computer programs are developed in MATLAB for simulations of cell electroporation dynamics as well as for to study the dynamics of diffusion of doxorubicin.

In the first phase, the numerical solutions were obtained for the pore dynamics of electroporation.

**Cell electroporation-pore dynamics:**

For each case, simulations were carried out for a time duration of 40 \( \mu \text{s} \) divided into (i) the time for...
application of an electric pulse of 20 µs and (ii) post-pulse duration of 20 µs. Also, it is assumed that at
\( t=0 \), transmembrane potential \( \phi_m=0 \), initial pore density \( N=N_0=1.5 \times 10^9 \) m\(^{-2} \) and number of pores \( n=0 \).

For PS cell (with experimentally determined cell radius ~9 µm), simulations were performed with fixed
applied electric field of \( E_e=1.0 \) kV cm\(^{-1} \) and varied membrane cholesterol content (range 15% to 29%
mole fraction). The total time for simulation is divided into small time steps, each of duration
0.2 ns = 2 \times 10^{-4} \) µs.

**Dynamics of drug uptake:**

To study molecular uptake during electroporation, the widely used anticancer drug Doxorubicin (DOX) was
chosen as a suitable candidate. The molecular dimension of DOX being smaller than that of pores, molecular
transport was considered as diffusion in dilute solutions. Hence, the diffusion coefficient of DOX in dilute
solutions \( D=1.57 \times 10^{-10} \) m\(^2\)/s is used.

A computer program was developed in MATLAB
and numerical solutions to equations 3.1-3.5
(incorporating equations in 4.1-4.2) were obtained.
The concentration of DOX in the extracellular matrix
\( (C_0=6.3 \times 10^4 \) kg/L) is derived from experimental
doses of the drug from \textit{in vivo} experiments reported earlier.

**Estimating molecular dimensions of Doxorubicin:**

The known 3D structure information of DOX was
obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/, CID: 31703). Visualization of the structure and estimation of the molecular parameters were carried out using CHIMERA 1.8.1. 3D structure display and rendering of images was carried out using Discovery Studio Viewer
v.3.5 available at the authors’ laboratory.

**Study design**

Formulation of the mathematical model is based on
earlier work of Shil \textit{et al.} \(^5\), with suitable modifications
to accommodate variation in membrane cholesterol
content. Numerical solutions of cell electroporation
were obtained using the cell geometry parameters as
inputs in computer simulations of the model developed.
For each cell, simulations were carried out for three
different values of membrane cholesterol content.

Real cell geometry was determined experimentally
by studying THP1 and PS cells (grown by cell-culture
techniques) using microscopy methods and parameter
values used as inputs for computer simulations. For
the ease of computation and to account for the surface
distribution of pores, the cell has been divided into
segments S1, S2, S3 and S4 (Fig. 1).

In each case, outputs generated included a number of pores formed, pore dimensions and distribution of pores on the cell surface (segment-wise distribution).
From this data total permeabilized area (through
which diffusion of the drug was possible) was calculated.

Utilizing data generated above as input parameters,
diffusion of Doxorubicin into the electroporated cell
was then simulated using another formulation
(described below, Section 2.3.2). All simulations were
performed by developing programs in MATLAB.

**Results**

In the present study, the effects of changes in
membrane cholesterol content on electroporation pore
dynamics as well as uptake of doxorubicin in
mammalian cells have been studied. In the first phase,
a mathematical formulation was developed to
describe the dynamics of poration and pore
distribution on the cell for a membrane containing
cholesterol. In the second phase, a mathematical
model was developed to describe the drug uptake
during electroporation. Information of pore
dynamics \textit{viz.} pore radius, number of pores, etc. obtained as output from the first phase of the study
were used to as inputs for simulations for drug
uptake. Parameters from two different cell lines
(namely PS cells and THP1 cells) with distinct
geometries have been used for the numerical
implementation (simulations). The results are
discussed below.

**Cell with radius 9 µm (PS cells)**

Simulations of electroporation in PS cells
(radius ~9 µm) were carried out for the electric field
of 1 kV cm\(^{-1} \) considering three different cases of
membrane composition with cholesterol content as
15%, 20% and 29% mole-fraction (assumed values).
The total time interval is divided into two parts: a)
pulse application for 20 µs and b) post-pulse
duration of 20 µs. In each case, electroporation was
evaluated in terms of transmembrane potential
\( \phi_m \), average pore radius and pore count \textit{(vs. time)}
for four segments of the cell.

Results for these parameters are summarized in
Fig. 3. In each case, pore formation (poration)
ocurred in the segments S1 and S2 both of which
developed higher \( \phi_m \). However, no poration was

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\( N = N_0 = 1.5 \times 10^9 \) m\(^{-2} \) and number of pores \( n = 0 \).
observed in the S3 and S4 segments which developed lower values of $\phi_m (\leq 0.8 \text{ V})$. Large numbers of large pores are formed in the S1 region and a small number of smaller pores are formed in the S2 region for all values of cholesterol content.

Comparison of data from all the three cases (different membrane cholesterol content) revealed that the number of pores in both S1 and S2 segments rise significantly with the increase in cholesterol content from 15% to 29% mole-fraction (Fig. 3A & B). While the pore count increases from 14650 to 15478 for the S1 segment, for segment S2 it increases from 431 to 460. However, the average pore radii decreased from 33.2 to 31.7 nm for S1 region and from 26.1 to 22.6 nm for the S2 region with increasing cholesterol content (Fig. 3C).

Overall, for fixed cell geometry (radius of 9 µm), the increase of membrane cholesterol content from 15% to 29% mole-fraction resulted in an increase in pore count by 6% and a decrease of average pore radii by ≥5%.

**Cell with radius 6 µm (THP 1 cells)**

Simulations of electroporation in THP 1 cells (radius ~6 µm) were carried out for the electric field of 1.5 kV/cm considering three different cases of membrane composition with cholesterol content as 15%, 20% and 29% mole-fraction in a fashion similar to PS cells Fig.4.

In each case, cell electroporation was evaluated in terms of transmembrane potential $\phi_m$, average pore radius and pore count vs time for four segments of the cell. Results for these parameters are summarized in Fig. 5. Poration was observed in the segments S1 and S2 both of which developed higher $\phi_m$, but no poration was observed in the segments S3 and S4 which developed lower values of $\phi_m (\leq 0.8 \text{ V})$.

For each case of cholesterol content, simulation for the THP 1 cells (radius 6 µm) revealed the formation of larger pores in the S1 region compared to pores of smaller dimension in the S2 region. It was observed that the number of pores in both S1 and S2 segments rises significantly with the increase in cholesterol content from 15% to 29% mole-fraction (Fig. 6 A & B). While the pore count increases from 7812 to 8228 for the S1 segment, for segment S2 pore count increases from 153 to 157. However, the average pore radii decreased from 32.6 nm to 31.2 nm for S1 region and from 26.7 nm to 25 nm for the S2 region (Fig. 6 C & D).

It should be noted that simulations for THP1 cells with the applied electric field of 1-1.4 kV/cm did not show the formation of pores. Effective electroporation (pore formation) was obtained with $E_e \geq 1.45$ kV/cm. Hence, for simulations with THP1 cells, $E_e=1.5$ kV/cm is used. This is consistent with well-established fact that cells with smaller radii need higher values of an applied electric field for electroporation 

**Molecular diffusion during electroporation**

In the next phase, dynamics of drug uptake during electroporation has been studied using parameters of PS cells Fig.4 (radius ~9 µm) and THP1 cells (radius ~9 µm). Three different cases
were considered depending on the membrane cholesterol content as 15%, 20% and 29% mole-fraction.

Molecular structure information was downloaded from the PubChem database and visualized in Discovery Studio viewer v3.5, an advanced Bioinformatics software tool for visualization of molecular structures. Molecular dimensions were determined by using CHIMERA1.8.1. The molecule is considered cylindrical with a mean diameter of 10 Å and length of 14.7 Å (Fig. 7). For numerical solutions of Eqn 2.1-2.5, the extracellular concentration of DOX was considered as $C_0 = 6.3 \times 10^{-4}$ kg/L, as derived from drug doses used in in vivo electrochemotherapy studies\textsuperscript{10}. Considering the molecular dimensions of DOX (14.7 Å) very much smaller than the dimension of the pores ($\geq 25$ nm), it was assumed that the transport of DOX through pores was diffusion in dilute solutions. Hence, the diffusion coefficient, $D = 1.57 \times 10^{-10}$ m$^2$/s was used\textsuperscript{38}.

The results are summarized in Table 1. Briefly, for cells with $R_{\text{cell}} = 9$ µm (PS cells), with the increase in membrane cholesterol content from 15% to 29% the total area available for diffusion decreases from $1.03 \times 10^{-10}$ m$^2$ to $0.985 \times 10^{-10}$ m$^2$ resulting in a decrease in intracellular DOX concentration from $7.07 \times 10^{-14}$ kg/L to $6.7 \times 10^{-14}$ kg/L (approximately 5% decrease). For cells with $R_{\text{cell}} = 6$ µm (THP1 cells), the increase in membrane cholesterol content from 15% to 29% the total area available for diffusion decreases from $5.3 \times 10^{-11}$ m$^2$ to $5.1 \times 10^{-11}$ m$^2$ resulting in a decrease in intracellular DOX...
concentration from $3.63 \times 10^{-14}$ kg/L to $3.50 \times 10^{-14}$ kg/L (approximately 3.5% decrease). Thus, for each cell type increase in mole-fraction of membrane cholesterol content reduced the pore dimensions and the total area available for diffusion. This may be caused by cholesterol induced denser packing of the lipids in the bilayer.

**Discussion**

In the present study, simulations have been performed to assess the effects of cell membrane composition in terms of cholesterol content on electroporation efficiency (pore formation and distribution) and drug uptake.

For pore dynamics simulations, our model predicts the surface distribution of pores. For any given cell the pores are formed in the regions S1 and S2, that is, for $\theta= 0$ to $\pi/4$. There is no poration in the regions of the cell with $\theta >\pi/4$ (regions S3 and S4). Pores formed in the Segment S1 ($\theta=0$ to $\theta=\pi/8$) are smaller in radii but more in number than those in the Segment S2 ($\theta=\pi/8$ to $\theta=\pi/4$). This prediction of the surface distribution of pores is similar to the experimental reports by Golzio et al.\(^\text{39}\), who recorded fluorescent images of cells during electroporation. Golzio et al. showed that pores were formed in the polar regions of the cell that extends approximately from $\theta=0$ to $\theta=\pi/4$, whereas no pores were formed for $\pi/4 \geq \theta \geq \pi/2$, this being true for both halves of a cell.

It was observed that: 1) For a fixed cell radius (geometry) the pore radii decrease with increase in membrane cholesterol content. This results in a reduction in the total available area for molecular diffusion. Hence, our model predicts that for a particular cell (fixed cell geometry) with the increase in cholesterol content, there is a decrease in the molecular uptake of Doxorubicin. This prediction is similar to trends reported from experiments in membranes\(^\text{40-42}\). Membrane cholesterol content affects the physical properties of the bilayer membrane by altering the line tension $\zeta$, which alters the energy of the pore perimeter (Equations 1.4, 1.5). The values of $\zeta$ for membranes with 15%, 20% and 29% mole-fraction cholesterol contents are $2.84 \times 10^{-11}$, $3.22 \times 10^{-11}$ and $3.9 \times 10^{-11}$ SI units respectively. The increase in line tension $\zeta$ increases the edge energy of the pore perimeter $2\pi r_j \zeta$, the second term of the equation 1.4 that describes the bilayer energy $W$ (Eqn 1.3). It follows from Eqn 1.3 that increase in line tension results in a decrease of pore radius ($r_j$). Hence, for a particular cell geometry (i.e., fixed cell radius) increase in membrane cholesterol content results in a decrease of pore size (pore radii). In our results, this phenomenon was observed for both large cells (PS cells, $R_{cell} \approx 9 \mu m$) as well as small cells (THP1 cells, $R_{cell}=6 \mu m$). Experimental evidence shows that different cell-lines having comparable cell geometry (cell radius) but different cholesterol content differ in electroporation response\(^\text{43}\). Puech et al.\(^\text{44}\) in different experimental construct determined that bilayer membranes with lower line tension $\zeta$ are
easier to electroporate. Karatekin et al.\textsuperscript{45} have shown by experiments that membrane cholesterol increased line tension and decreased electroporation efficacy. Numerical solutions from our model showed similar trends indicating the reliability of the model.

2) The smaller cells are difficult to electroporate is a well-established fact from experimental observations over decades\textsuperscript{46}, which means that smaller the cell radii, higher the voltage of electroporation (applied electric field) required. Numerical simulations (of pore dynamics) based on our model revealed that effective poration occurs in THP1 cells (radii ~6 µm) at higher applied voltages (with generated electric field, $E_e \geq 1.45$ kV/cm) compared to PS cells (radii ~9 µm) which could be electroporated easily at $E_e = 1$ kV/cm.

Since our model accounts for the surface distribution of pores as well as incorporates variation in membrane cholesterol content, results are more in agreement with trends in experimental observations than the earlier models which assumed uniform poration of the whole membrane and ignored the presence of cholesterol\textsuperscript{27}. Assuming cholesterol free membrane with uniform DPPC was an approximation followed in earlier models\textsuperscript{27} with an effect that predictions were not always in tune with experimental observations and often predicted catastrophic scenario (breakdown) at $E_e \geq 1$ kV/cm (electrochemotherapy applications range) in mammalian cells.

However, no model is free from limitations of assumptions. It is necessary to look at the assumptions we made in the model. Firstly, for computing the Doxorubicin uptake, we assumed an average radius for all pores covering the total permeable region of the cell membrane (S1 and S2 regions of the cell). This was done to avoid complications in programming and computer-based simulations. Secondly, as we assumed diffusion of Doxorubicin as in dilute solution through pores, it was necessary to assume the same dimension for all pores and also consider a uniform value of partition coefficient for DOX. We assumed an effective partition coefficient for diffusion of DOX through all pores in the permeable area (S1 and S2 together) as a weighted average to account for the two populations of pores with different diameters (one type in S1 and another in S2 segments).

Conclusions

In this paper, we present numerical formulation to study the dynamics of electroporation-mediated doxorubicin uptake in a biological cell incorporating the effects of changes in membrane cholesterol content.

This model can be a useful tool for predicting a more realistic nature of pore dynamics in the electroporation of mammalian cells and study the effect of variation in membrane cholesterol content on pore distribution and dynamics. It can also help researchers in estimating theoretically the nature of electroporation-mediated uptake of various drugs in mammalian cells.

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