Enhanced Drug Uptake on Application of Electroporation in a Single-Cell Model

Nilay Mondal¹ · K. S. Yadav² · D. C. Dalal³

Received: 17 July 2022 / Accepted: 9 March 2023 / Published online: 29 March 2023
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2023

Abstract
Electroporation method is a useful tool for delivering drugs into various diseased tissues in the human body. As a result of an applied electric field, drug particles enter the intracellular compartment through the temporarily permeabilized cell membrane. Consequently, electroporation method allows better penetration of the drug into the diseased tissue and improves treatment clinically. In this study, a more generalized model of drug transport in a single cell is proposed. The model is able to capture non-homogeneous drug transport in the cell due to non-uniform cell membrane permeabilization. Several numerical experiments are conducted to understand the effects of electric field and drug permeability on drug uptake into the cell. Through investigation, the appropriate electric field and drug permeability are identified, which lead to sufficient drug uptake into the cell. This model can be used by experimentalists to get information prior to conduct any experiment, and it may help reduce the number of actual experiments that might be conducted otherwise.

Nilay Mondal, K. S. Yadav and D. C. Dalal have contributed equally to this work.

K. S. Yadav
yadav176123004@iitg.ac.in;
kuldeepsingh Yadav@gmail.com

Nilay Mondal
nilaymondal@iutripura.edu.in; nilayiitg91@gmail.com

D. C. Dalal
durga@iitg.ac.in

¹ Faculty of Science and Technology, ICFAI University, Kamalghat, Agartala, Tripura 799210, India
² Faculty of Engineering & Technology, Siksha ‘O’ Anusandhan (Deemed to be University), Bhubaneswar, Odisha 751030, India
³ Department of Mathematics, Indian Institute of Technology Guwahati, Amingaon, Guwahati, Assam 781039, India
Introduction

In order to cure a particular disease (e.g., cancer), one aims to deliver a required amount of chemotherapeutic drug to the diseased site and into the infected cells. The cell membrane is selective permeable in the sense that it does not allow all the molecules to pass through it. The drug permeability across the cell membrane depends on the properties of the drug as well as state of the cell membrane.

Over the years, several methods have been developed for drug delivery. In the process of advancement of technology, new techniques, such as electroporation, micro-injection, laser, and ultrasound are developed (Bolhassani et al. 2011). Electroporation has been used widely in in vitro and in vivo models in various applications, such as transdermal drug delivery, gene therapy, chemotherapy, to name a few (Dermol-Černe et al. 2018; Dermol-Černe and Miklavčič 2018; Kotnik et al. 2019). Electroporation-based drug delivery offers several advantages, including avoidance of erratic absorption, absence of gastric irritation, painlessness, non-invasiveness, and improvement in the patient compliance (Dermol-Černe et al. 2020). In electroporation, cell membrane is temporarily destabilized by the application of external electric field (Kotnik and Miklavčič 2000; Rems et al. 2013). This destabilization occurs due to increment in the transmembrane potential (Neumann et al. 1982). In the destabilization process, the nano-meter size pores are created in lipid bilayer (Krassowska and Filev 2007; Mahnič-Kalamiza et al. 2014; Napotnik et al. 2016; Mondal et al. 2021). Lipid peroxidation plays a vital role in the cell membrane permeabilization for the application of high voltage electric pulses (Rems et al. 2019; Bertrand and Garduño-Juárez 1863). The permeability and conductance of the membrane get increased in peroxidized bilayers. As a result, the drug transport may improve in such bilayers (Rems

Keywords Electroporation · Drug delivery · Interface method · Multiple pulses · Permeability
Enhanced Drug Uptake on Application of Electroporation in a Single-Cell Model

et al. 2019). Other components such as peptides and lipid composition also affect the pore formation, and a detailed review can be found in the article by Bertrand et al. (2021). After the withdrawal of pulse, the pores start reseal, that are opened due to external electric field, is called membrane resealing (Davalos et al. 2003; Granot and Rubinsky 2008).

It is known that the time duration of pore formation is in the order of microsecond, and on the other hand, the pore resealing occurs in minutes (Pavlin et al. 2005; Pavlin and Miklavčič 2003). The transitory and permeabilized states of the cell membrane can be exploited to transport drugs into the intracellular domain.

The physical properties of the tissue, such as cell shape, size and its distribution, as well as the electrical parameters, such as the number of pulses, pulse amplitude, pulse duration and tissue conductivity, influence drug transport into the targeted cells (Pavlin et al. 2005; Pucihar et al. 2011). Cell electroporation is commonly used with short (1 ns–1 ms) and long duration (1–100 ms) pulses in various experiments. To avoid cell death, single-cell electroporation that involves an inhomogeneous short-duration low-voltage electric field around the cell surface is generally applied. However, the application of high voltage electric field enhances cell membrane pore density (Pavlin et al. 2005; Weaver 2003).

For drug absorption in electroporated cells, it is vital to focus on the increase in permeability and control of cell death. Researchers are continuously working to improve mathematical models for these components. In the previous studies (Smith 2011; Miklavčič and Towhidi 2010; Li and Lin 2011), the electroporation techniques based on the different pulse application and its use in the molecular transport have been developed. The effects of extracellular conductivity on the molecular transport through electroporation are well explained in the study of Li et al. (2013). In their numerical model, the Smoluchowski equation to calculate membrane pore and the Nernst–Planck equations to describe ionic transport into the cell are used. Goldberg et al. (2018) proposed a multiphysics model for ion transport, which is based on the pore creation model proposed by Krassowska and Filev (2007). They presented a mass transport model using the Nernst–Planck equation for transporting various species into cells in their model. Goldberg et al. (2021) extended their previous model and described the effects of electric pulses on cisplatin transport across the plasma membrane. The model shows that an electric field induces maximum transmembrane potential at the cell poles where the electrodes are placed.

It is challenging and a difficult task to conduct actual experiments of pulse application and field strength on single cell to improve drug uptake. The detailed investigation to determine appropriate class of drugs that can easily enter the cell in a desired amount is still missing in the literature.

In the present article, a mathematical model is employed to study drug transport into a single cell by the application of electroporation. The prescribed model is more generalized in comparison to the previous models as the spatial changes in cellular drug uptake at different times are analyzed here. This study presents a rigorous analysis of drug delivery into a diseased cell emphasizing the effects of membrane resealing. Drug transport takes place due to diffusion from the extracellular space to the intracellular one.

Transport across the cell membrane takes place due to passive diffusion, which means that the diffusion takes place due to the concentration gradient and the ability of the cell membrane (permeability) to pass the drug. There are other processes such as electrodiffusion, electroosmosis, and endocytosis that also drive the mass transport across the cell membrane; however, these are not considered in this study. The drug transport equations are solved using the permeable interface method (PIM) on Cartesian mesh by treating the cell membrane as a sharp interface. Various electrode arrangements are tested to determine the locations of maximum pore formation and to analyze the mass transportation through those locations.

The numerical experiments are conducted with various electric fields and multiple pulses for a given electric field are used in electroporation to reduce the resealing effects on drug uptake. From the investigation, a suitable electric field is determined to improve the cellular uptake for a specific drug. The numerical experiments are also conducted with drugs of different permeabilities to select the appropriate drug that can be used for treatment to get the best possible efficacy. The resulted non-uniformity of the pores in the cell membrane owing to the application of electric pulses leads to non-uniform drug distribution in the intracellular space.

Model Formulation

This study investigates the drug transport through the electro-permeabilized cell membrane. A square domain ($\Omega$) of edge length $L$ is considered, and a single cell is assumed to be placed at the center of $\Omega$. Structurally, the domain can be viewed as two parts: extracellular space and intracellular space. The spaces are separated by the cell membrane, which is selective permeable and controls the mass exchange between the extracellular and intracellular domains. The schematic diagram is shown in the Fig. 1.

In order to electroporate the cell membrane, two electrodes with potential values $\phi_a$ and $\phi_e$ are placed along the vertical lines at A and B, respectively (as shown in Fig. 1). A uniform electric field $E$ is induced in the region directed from positive to negative electrode. The transmembrane potential ($V_{mm}$) increases due to the induced electric field. On increasing $V_{mm}$, cell membrane is destabilized and
nanometer-sized pores are generated in the cell membrane as a result of pulse application.

**Details About Pulse Application**

Pulses of short duration ($t_{ep} = 1 \text{ ms}$) are applied repeatedly by maintaining a fixed temporal gap between two pulses. We denote this temporal gap by $t_M$ and two different values (50 s and 100 s) are chosen in this study. Figure 2 shows the description of pulse application. It is assumed that the mass transfer takes place only when the pulse is off. The drug transport from extracellular to the intracellular domain occurs in the resealing period of the cell membrane. As $t_{ep} \ll t_M$, by neglecting the pulse time (ON TIME), the total time ($t$) for mass transport is calculated as $t = PN \times t_M$, where $PN$ is the number of pulses applied.

**Transmembrane Potential and Pore Calculations**

The transmembrane potential ($V_m$) was initially determined on a spherical cell in a uniform electrical field by Neumann et al. (1989). Later, DeBruin and Krassowska (1999) advanced their mathematical model to determine the pore density and its relation with the transmembrane potential. In the present study, we have used the model developed by DeBruin and Krassowska (1999) for the investigation of drug delivery into single cell.

We consider a single-cell electroporation in which the spherical cell with radius $a$ is immersed in a spherical shell of extracellular space with radius $3a$. A uniform electric field is induced from the boundary of the extracellular space to destabilize the cell membrane. The physical structure of the model comprising single cell is schematically portrayed through Fig. 3.

The transmembrane potential $V_m$ on the cell membrane due to the application of electric field is the difference of potentials in intracellular and extracellular domains. As both the domains are source free, the potentials are calculated using the Laplace equations as DeBruin and Krassowska (1999):

\[
\nabla^2 \Phi_i = 0 \quad \text{in intracellular space},
\]

\[
\nabla^2 \Phi_e = 0 \quad \text{in extracellular space},
\]

where $\Phi_i$ and $\Phi_e$ are the intracellular and extracellular potentials. The uniform external field $E$ is assumed at the outer boundary and the $\Phi_e$ is obtained as follows:

\[
\Phi_e = -3aE \cos \theta.
\]

The current density across the cell membrane ($S$) is given by

\[
-\hat{n} \cdot (\sigma_i \nabla \Phi_i) = -\hat{n} \cdot (\sigma_e \nabla \Phi_e) = C_m \frac{\partial V_m}{\partial t} + g_1 (V_m - E_1) + N_i_{eq},
\]

where $\hat{n}$ is the unit vector normal to the membrane’s surface. $\sigma_i$ and $\sigma_e$ are the intracellular and extracellular conductivities, respectively. $C_m$ is the specific membrane capacitance and $V_m = \Phi_i - \Phi_e$ on $S$. $g_1$ denotes the specific membrane conductance, $t$ denotes the time and $E_1$ the reversal potential.
of the ionic current. The current ($i_{ep}$) in a single pore is given as DeBruin and Krassowska (1998):

$$i_{ep} = \frac{\pi r^2_m \nu_m RT}{Fh} \frac{1}{W_{e} e^{m_{e} m_{e} - m_{e}} - W_{o} e^{m_{o} m_{o} + m_{o}}},$$

(5)

where $\nu_m = V_m \left( \frac{F}{RT} \right)$ is the non-dimensional transmembrane potential.

The pore density $N(t, \theta)$ given by Krassowska and Filev (2007) is as follows:

$$\frac{dN}{dt} = \alpha A \left[ 1 - \frac{N}{N_0} A^{-q} \right],$$

(6)

where $A = \exp \left[ \left( \frac{V_m}{V_{ep}} \right) ^2 \right]$, $t$ the time, $\alpha$ the pore creation rate coefficient, $V_m$ the transmembrane potential, $V_{ep}$ the characteristic voltage of electroporation, $N_0$ the equilibrium pore density for the membrane area at $V_m = 0$, and $q$ is an electroporation constant. The total number of pores ($N_p$) in the local area $\Delta_\theta$ of cell membrane after the application of electric pulse (duration is $t_{ep}$) is obtained as follows:

$$N_p(\theta) = \int_{\Delta_\theta} N(t_{ep}) \, d\theta,$$

(7)

where $\theta$ represents the angle in spherical coordinate system as shown in Fig. 3.

The maximum number of pores create at the poles $\theta = 0, \pi$ as transmembrane potentials are maximum at these locations, whereas negligible number of pores form at $\theta = \frac{\pi}{2}, \frac{3\pi}{2}$ as almost negligible transmembrane potential induces at these particular locations (Kotnik and Miklavčič 2000; Rems et al. 2013).

**Pore Resealing**

The pore area decreases with time after withdrawal of electroporation pulse due to the membrane resealing effect (Granot and Rubinsky 2008). The total pore area can be calculated as follows:

$$A_p(\theta, t) = \pi R_p^2 \cdot N_p(\theta) \exp \left( -\frac{t}{\tau} \right),$$

(8)

where $\tau$ is the resealing time, $\Delta_\theta$ is the local area at $\theta$, and $R_p$ is the average radius of the pores in the electroporated cell membrane.

The mass transfer coefficient is time dependent and depends on the pore density. The mathematical formula for the mass transfer coefficient ($\mu$) is given as Granot and Rubinsky (2008),

$$\mu(\theta, t) = \frac{A_p(\theta, t)}{\Delta_\theta} P,$$

(9)

where $P$ is the permeability of drug particles across the cell membrane, and $\Delta_\theta$ is the local area at $\theta$ in the cell membrane.

**Drug Transport in the Single-Cell Model**

The drug concentrations in extracellular space and in the reversibly electroporated cell (Fig. 4) are obtained using the mass transport equations given as Yadav and Dalal (2021) and (2022),

$$\frac{\partial C_E}{\partial t} = \nabla \cdot \left( D_E \nabla C_E \right),$$

(10)

$$\frac{\partial C_I}{\partial t} = \nabla \cdot \left( D_I \nabla C_I \right),$$

(11)

with the initial and boundary conditions

$$C_E(x, y, 0) = \left\{ \begin{array}{ll} C_0, & x = 0, \\ 0, & \text{otherwise}, \end{array} \right.$$  

(12)

$$C_{RE}(x, y, 0) = 0,$$

(13)

$$\frac{\partial C_E}{\partial n} = 0.$$  

(14)

Here, the subscripts $E$ and $I$ denote the variables from extracellular and intracellular spaces, respectively. $C$ is the drug concentration and $D$ drug diffusivity. $C_0$ denotes input initial drug concentration. $n$ is an outward normal vector to the domain $\Omega$ (Fig. 4).

**Interface Conditions**

The cell membrane is selective permeable and the permeability depends on the drug properties. The improved permeability of cell membrane ($\mu$) is incorporated in the drug transport as follows:

Fig. 4 A single-cell computational domain for drug transport
\[ D_E \nabla C_E = D_I \nabla C_I = \mu(\theta, t)(C_E - C_I) \mathbf{n}. \]  \hspace{1cm} (15)

**Method of Solution**

The mass transport Eqs. (11)–(15) are solved using the permeable interface method (PIM) proposed by Yadav and Dalal (2022). The PIM can be described briefly as follows. The set of equations are solved using the finite difference method. The domain is discretized using the Cartesian mesh, say the grid point is denoted by \((i, j)\), where \(i\) is index in \(x\)-direction while \(j\) is in the \(y\)-direction. The central-difference scheme used to discretize the Eq. (11) is as,

\[ \left( \delta_x (D \delta_x C) \right)_{ij} + \left( \delta_y (D \delta_y C) \right)_{ij} = 0, \]  \hspace{1cm} (16)

where

\[ \left( \delta_x (D \delta_x C) \right)_{ij} = \left\{ \frac{D_{i+1/2,j} C_{i+1,j} - (D_{i+1/2,j} + D_{i-1/2,j}) C_{i,j} + D_{i-1/2,j} C_{i-1,j}}{(\delta x)^2} \right\}, \]

and

\[ \left( \delta_y (D \delta_y C) \right)_{ij} = \left\{ \frac{D_{i,j+1/2} C_{i,j+1} - (D_{i,j+1/2} + D_{i,j-1/2}) C_{i,j} + D_{i,j-1/2} C_{i,j-1}}{(\delta y)^2} \right\}. \]

Here, the subscript \((i + 1/2, j)\) denotes the position \((x_i + \delta x/2, y_j)\) with step size \(\delta x\) in the \(x\)-direction. Similarly, other indices are defined.

However, on the grid points near the interface (as depicted in Fig. 5), central-difference scheme cannot be used directly. For this, the scheme used is as (Yadav and Dalal 2021),

\[ \left( \delta_x (D \delta_x C) \right)_{i} = \left\{ \frac{C_{i+1} - C_i}{x_{i+1/2} - x_i} - \frac{C_i - C_{i-1}}{x_i - x_{i-1}} \right\} \left( \frac{x_{i+1} - x_{i-1}}{2} \right), \]  \hspace{1cm} (17)

where \(x_{i+\theta} = x_i + \theta \delta x\) for some 0 < \(\theta < 1\). \(C_{i+\theta}\) is the limiting concentration at the point \(x_{i+\theta}\) approaching from left side (Fig. 5).

The limiting concentrations at the interface are obtained using the linear interpolation from the left and right sides, respectively, which also satisfy the interface conditions (Eq. 15) (Yadav and Dalal 2021).

The above numerical procedure is implemented in the C-programming language. The resulted system of equations is solved using the BiCGSTAB algorithm without preconditioning with maximum error between two consecutive iterative solutions falls below \(10^{-15}\). The results are plotted with the help of Tecplot.

### Results and Discussion

In this section, the effects of electroporation on drug transport in single cell are analyzed through numerical experiments. The key parameters, such as drug permeability, electric field, and pulse number are explored. In the numerical experiments, repeated pulses with a fixed voltage are applied to make the cell membrane permeabilized and retained this state for a longer period of time. Time gap between any two consecutive pulses is kept to be fixed (50 s or 100 s) for drug transport into the cell. In order to incorporate the physiological situation, the parameters are taken from the relevant literature as listed in Table 1. The values of the parameters (diffusivity and drug permeability) are generally estimated by different computational approaches, such as hybrid all-atom simulation and atomistic molecular dynamics simulation (Kniecik et al. 2016; Kar and Feig 2017). After analyzing the database for different drugs (Kyffin 2018), it is observed that the feasible values of the diffusion coefficient in the tissue medium are in the range \(10^{-4} - 10^{-3} \text{mm}^2 \text{s}^{-1}\). In this study, \(D_E = 10^{-3} \text{mm}^2 \text{s}^{-1}\) (extracellular diffusivity) and \(D_I = 10^{-4} \text{mm}^2 \text{s}^{-1}\) (intracellular diffusivity) are considered for the model simulation. For the simulation, high permeable drugs with \(\text{Log}P\) values are greater than 1.72 are considered (Papich and Martinez 2015).

The results are obtained on a mesh size \(250 \times 250\) after ensuring grid independence outcomes. The results shown in Figs. 7, 8, 9, 10, 11, 12, 13, and 14 are obtained by the vertical electrode configuration as shown in Fig. 1, whereas the result described in Fig. 15 is obtained with the horizontal electrode configuration (i.e., electrodes are placed on the top and bottom of the cell).

### Validation

The results obtained from the model are validated with that of Miyauuchi et al. (2015) and are shown in Fig. 6. For
this comparison, a square domain is considered with a cell placed at the center of it as shown in Fig. 6a. $P = 0$ is chosen, so no drug uptake is expected. The comparison validates the results for the case of molecular transport through the extracellular media. The concentration distributions along the line $AB$ are displayed in Fig. 6b. It can be seen that the results are in very good agreement. It is also observed that our results (as shown in Fig. 12) on molecular uptake into the cell follow a similar trend with the experimental data (Figs. 6, 10 of Sözer et al. (2018)).

### Drug Distribution Versus Time

Figure 7 shows the contour plots of drug transport into the intracellular space from the extracellular region through the permeabilized cell membrane. It shows drug concentrations for different time durations, as $t = 250\,\text{s}, 500\,\text{s},$ and for $1000\,\text{s}$. The drug uptake into the cell is very low due to the application of low voltage ($15\,\text{V}\,\text{mm}^{-1}$) pulses. The reason is that when low voltage pulses are applied to the tissue, fewer number of pores are created in the cell membrane, and as a result, the membrane do not get sufficiently permeabilized for drug uptake.

In order to improve membrane permeability, it is necessary to increase the electric field strength (i.e., $E > 15\,\text{V}\,\text{mm}^{-1}$) of the applied pulses in the experiments. Since a high voltage pulse enhances the number of pores and their area in the cell membrane (see Eq. 6), this gives rise to increase in mass transfer rate. The mass transfer rate may also increase for some drugs (particularly, for the small-sized pharmaceutical molecules smaller than the membrane pores) that are highly permeable to the target cell membrane. This is due to the mass transfer coefficient $\mu$, which is directly proportional to drug permeability $P$ (Eq. 9). So, several experiments are

---

**Table 1** The details of the parameters values used in the simulations

| Sym   | Value     | Definition                        | Source                  |
|-------|-----------|-----------------------------------|-------------------------|
| $r_c$ | $50\,\mu\text{m}$ | Cell radius                       | Krassowska and Filev (2007) |
| $a$   | $10^9\,\text{m}^2\text{s}$ | Pore creation coefficient         | Krassowska and Filev (2007) |
| $V_{ep}$ | $0.258\,\text{V}$ | Characteristic voltage            | Krassowska and Filev (2007) |
| $N_0$ | $1.5 \times 10^9\,\text{m}^{-2}$ | Equilibrium pore density          | Krassowska and Filev (2007) |
| $q$ | $2.46$ | Electroporation constant          | Granot and Rubinsky (2008) |
| $D_E$ | $10^{-3}\,\text{mm}^2\text{s}^{-1}$ | Extracellular diffusion coefficient | Granot and Rubinsky (2008) |
| $D_I$ | $10^{-4}\,\text{mm}^2\text{s}^{-1}$ | Intracellular diffusion coefficient | Granot and Rubinsky (2008) |
| $R_p$ | $0.8\,\text{nm}$ | Pore radius                        | Granot and Rubinsky (2008) |
| $P$ | $(0.1 - 1)\,\text{mm}\,\text{s}^{-1}$ | Permeability of drug               | Granot and Rubinsky (2008) |
| $E$ | $(15 - 40)\,\text{V}\,\text{mm}^{-1}$ | Electrical field                   | Granot and Rubinsky (2008) |
| $C_0$ | $1\,\text{M}$ | Initial drug concentration         | Fig. 1                   |
| $L$ | $3\,\text{mm}$ | Edge length of the square          | Fig. 1                   |
| $\phi_0$ | $25\,\text{V}$ | Potential at A                     | Fig. 1                   |
| $\phi_1$ | $0\,\text{V}$ | Potential at B                     | Fig. 1                   |
| $t_p$ | $1\,\text{ms}$ | Pulse length (ON TIME)             | Fig. 1                   |
| $t_M$ | $50\,\text{s}, 100\,\text{s}$ | Time for mass transfer (OFF TIME)  | Fig. 1                   |
| PN   | 5–20      | Pulse number                       | Fig. 1                   |
conducted to analyze the role of field strength of the applied pulses and drug permeability.

**Effects of Electric Field on Drug Penetration**

The experiments are conducted on the application of 20 pulses of 1 ms with three different electric fields as $E = 15$, 25, and 40 V mm$^{-1}$. Figure 8 shows the effects of electric field on drug uptake. From Figs. 8a and b, it can be noticed that the drug uptake is very less due to insufficient permeabilization of the cell membrane with low voltage pulses ($E = 15$, 25 V mm$^{-1}$). So, $E = 15$, 25 V mm$^{-1}$ are not suitable voltages for introducing sufficient drug uptake into the cell. Another experiment for $E = 40$ V mm$^{-1}$ with the same value of drug permeability ($P = 0.1$ mm s$^{-1}$) is conducted to observe the effects of high electric pulse on mass transport into the cell. The results are shown in Fig. 8c. A significant improvement in drug uptake is noticed; thus, sufficiently strong electric field is required in order to permeabilize the cell membrane appropriately. As indicated in Fig. 8c, drugs enter the cell through both the sides ($\theta = 0, \pi$), where maximum pores are created.

Figure 9 shows the concentrations against the time obtained from some selected points inside the cell. The selected points are (0.11, 0.15), (0.15, 0.15), and (0.19, 0.15). The point (0.11, 0.15) is on the left side near the pole $\theta = \pi$, (0.15, 0.15) is at the center, and (0.19, 0.15) is on the right side near the pole $\theta = 0$ of the cell. These points are chosen to calculate the amount of drug in different locations. Clearly, one can observe that the drug concentration increases with time owing to the drug uptake. Effects of several pulses can be seen. On a given pulse, drug uptake initially improves and then it gets saturated due to the resealing effect, which subsequently requires another shot of pulse for faster uptake. Clearly, the intracellular concentration increases with increasing...
Enhanced Drug Uptake on Application of Electroporation in a Single-Cell Model

1.3

The maximum intracellular concentration is achieved for \( E = 40 \text{ V mm}^{-1} \).

Figure 10 displays the concentration distribution of drug along the line \( y = 0.15 \). The result is obtained at the end of the process in which 20 pulses of 1 ms with maintaining a 50 s temporal gap between the two consecutive pulses are considered. The figure shows that the extracellular concentration of the drug is much higher than the intracellular concentration. The use of a high electric field and the application of repeated pulses improve drug uptake into the cell. Therefore, our goal is to choose the electroporation parameters based on both the properties of a drug as well as the amount of drug that needs to be introduced into the cell.

Figure 9 Concentration vs time inside the intracellular space for (a) \( E = 15 \text{ V mm}^{-1} \), (b) \( E = 25 \text{ V mm}^{-1} \), and (c) \( E = 40 \text{ V mm}^{-1} \). Here, \( P = 0.1 \text{ mm s}^{-1} \), \( PN = 20 \), \( t_{ep} = 1 \text{ ms} \), and \( t_M = 50 \text{ s} \).

Effects of Drug Permeability on Drug Penetration

In order to observe the effects of drug permeability on cellular uptake, numerical experiments are conducted with different permeability values for the voltage \( E = 25 \text{ V mm}^{-1} \). The results shown in Fig. 11 explain that the drug uptake increases with the increase in \( P \), as the mass transfer rate increases with \( P \). This shows that the drugs having higher permeability are required when low-voltage fields are employed. It is evident from Fig. 11b and c that a large amount of drug (> 0.2 M) has entered the cell, which may be sufficient to treat the infected cell. Thus, the drugs with permeability greater than 0.005 can be chosen to inject it into the cell for \( E = 25 \text{ V mm}^{-1} \).

Optimal Choice of \( E \) and \( P \) for Enhanced Drug Uptake

Figure 12 shows the drug penetration into the cell at different times on application of pulses with a suitable voltage \( (E = 25 \text{ V mm}^{-1}) \) and appropriate choice of drug permeability \( P = 0.5 \text{ mm s}^{-1} \). From Fig. 12a, it can be noticed that the drug uptake has started at 250 s from the left side of the cell when a sufficient amount of drug is diffused nearby the cell. On increasing time, the drug spreads out in the extracellular space and enters into the cell. Figure 12b illustrates that the drug enters into the cell through the pores at \( r = \pi \) once some drug reach near this location due to the molecular diffusion. At the end of the process of drug delivery \( (t = 1000 \text{ s}) \), the drug concentration inside the cell is \( > 0.25 \text{ M} \) by continuous drug uptake through both the left and right sides of the cell, which is shown in Fig. 12c. The significant increase in drug concentration into the cell is obtained due to increased mass transfer rate on a proper choice of electric field and drug permeability.

From the Fig. 13, it can be seen that the cellular drug uptake (for \( E = 40 \text{ V mm}^{-1} \) and \( P = 0.1 \text{ mm s}^{-1} \)) is almost equivalent to the drug uptake for \( E = 25 \text{ V mm}^{-1} \) and \( P = 0.5 \text{ mm s}^{-1} \).
Fig. 11 Effects of drug permeability a \( P = 0.1 \text{ mm s}^{-1} \), b \( P = 0.5 \text{ mm s}^{-1} \), and c \( P = 1 \text{ mm s}^{-1} \). Here, \( E = 25 \text{ V mm}^{-1} \), \( t = 1000 \text{ s} \), \( PN = 20 \), \( t_{ep} = 1 \text{ ms} \), and \( t_{M} = 50 \text{ s} \)

Fig. 12 Contour plots of drug distribution at various times a \( t = 250 \text{ s} (PN = 5) \), b \( t = 500 \text{ s} (PN = 10) \) and c \( t = 1000 \text{ s} (PN = 20) \) for \( P = 0.5 \text{ mm s}^{-1} \), \( E = 25 \text{ V mm}^{-1} \), \( t_{ep} = 1 \text{ ms} \), and \( t_{M} = 50 \text{ s} \)

Fig. 13 Contour plots of drug distribution at various times a \( t = 250 \text{ s} (PN = 5) \), b \( t = 500 \text{ s} (PN = 10) \) and c \( t = 1000 \text{ s} (PN = 20) \) for \( P = 0.1 \text{ mm s}^{-1} \), \( E = 40 \text{ V mm}^{-1} \), \( t_{ep} = 1 \text{ ms} \), and \( t_{M} = 50 \text{ s} \)
mm s\(^{-1}\) (see Fig. 12). One can notice from Figs. 12c and 13c that the drug concentration into the cell is in the range 0.2–0.3 M for both the cases. Therefore, these parameters' values \((E = 20 \text{ V mm}^{-1}, P = 0.5 \text{ mm s}^{-1}, E = 40 \text{ V mm}^{-1}, P = 0.1 \text{ mm s}^{-1})\) can be taken into the consideration for the treatment of diseased cells by clinical experimentalists. Also, these proposed values can guide the pharmacists to develop drugs that would be good for sufficient drug uptake. It is observed that both combination of parameters are suitable to improve the cellular uptake efficacy. For example, if the permeability of applied drug is greater than or equal to 0.5 mm s\(^{-1}\), then the choice of electric field can be less than or equal to 25 V mm\(^{-1}\) and if the permeability of the drug is less than or equal to 0.1 mm s\(^{-1}\), then the choice of electric field should be greater than or equal to 40 V mm\(^{-1}\) to get the same results.

**Effects of Number of Pulses**

In order to study the effects of pulse shots on drug uptake, numerical experiments are performed in two ways: one, where a pulse is given after each 50 s while in other, a pulse is given after each 100 s. In the latter case, the time between two consecutive pulses is large, and consequently, for a given simulation time period, the number of pulses is lesser. Concentration versus time on some intracellular points are plotted in Fig. 14. The drug concentration increases over time for the application of pulses (\(PN: 20\) if pulse gap is 50 s and 10 if pulse gap is 100 s). However, drug uptake improves on increasing the number of pulses. It is due to the resealing effects that will be pronounced if the time gap is more between two consecutive pulse shots.

**Arrangement of Electrodes**

In order to understand the effects of arrangement of electrodes, the experiments are conducted by setting electrodes on the top and bottom of the cell. In this case, the higher pore density is obtained at the top and bottom of the cell, i.e., for \(\theta = \frac{\pi}{2}\) and \(\frac{3\pi}{2}\). Figure 15 shows drug distributions for different drug permeability values. In the extracellular region, drug distribution patterns are the same as obtained in earlier cases. However, drug uptake happens from the top and bottom parts of the cell, where pore density is high owing to the particular arrangement of electrodes.

**Conclusions**

In this study, drug transport in a single-cell model has been studied. The effects of electroporation on drug uptake into the single cell are explored. The transport across the
cell membrane is incorporated using the permeable interface method available in the literature. Here, an advanced model in the area of electroporation drug delivery is analyzed as it provides the following important outcomes.

Numerical experiments are conducted for electric fields $E = 15$, $25$ and $40$ V mm$^{-1}$ and for different drug permeabilities. It is noticed that the drug uptake into the cell is almost negligible on the application of significantly low voltage ($E = 15$ V mm$^{-1}$) pulses whereas a significant improvement in drug uptake occurs for $E = 40$ V mm$^{-1}$.

A strong electric field is required to permeabilize the cell membrane properly, allowing a desired amount of drug to enter the cell. Based on the numerical experiments on the proposed model, it is learned that the suitable electric field is $25$ V mm$^{-1}$ or more, and appropriate drugs whose permeability is at least $0.5$ mm s$^{-1}$ for getting a desired amount of drug into the targeted cell. This is a new finding from the prescribed model. Thus, in practice, parameter values ($E = 25$ V mm$^{-1}$, $P = 0.5$ mm s$^{-1}$ or $E = 40$ V mm$^{-1}$, $P = 0.1$ mm s$^{-1}$) can be chosen to deliver pharmaceutical compounds into the targeted cell that needs treatment.

The drug uptake is initiated as soon as the application of pulse is completed. However, the drug uptake slows down due to the rescaling effect, another shot of pulse is required to restore the mass transport. So, multiple pulses are required to increase the drug uptake and to achieve the desired level of drug absorption into the cell. The maximum drug uptake occurs through the poles at $\theta = 0, \pi$ on setting electrodes on the left and right sides of the cell. Enhanced drug uptake is obtained for high permeable drugs, high voltage pulses, and by increasing the number of pulses.

**Funding** The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

**Data Availability** The authors declare that the results/data/figures in this manuscript have not been published elsewhere, nor are they under consideration by another publisher. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

**References**

Bertrand B, Garduño-Juárez R, Munoz-Garay C (2021) Estimation of pore dimensions in lipid membranes induced by peptides and other biomolecules: A review. Biochim Biophys Acta (BBA) 4:183551

Bolhassani A, Mohit E, Ghasemi N, Salehi M, Taghikhani M, Rafati S (2011) Enhancement of potent immune responses to HPV16 E7 antigen by using different vaccine modalities. BMC Proc 5:19

Davalos RV, Rubinsky B, Mir LM (2003) Theoretical analysis of the thermal effects during in vivo tissue electroporation. Bioelectrochemistry 61(1):99–107

DeBruin KA, Krassowska W (1998) Electroporation and shock-induced transmembrane potential in a cardiac fiber during defibrillation strength shocks. Ann Biomed Eng 26(4):584–596

DeBruin KA, Krassowska W (1999) Modeling electroporation in a single cell. I. Effects of field strength and rest potential. Biophys J 77(3):1213–1224

Dermol-Cerne J, Miklavčič D (2018) From cell to tissue properties-modeling skin electroporation with pore and local transport region formation. IEEE Trans Biomed Eng 65(2):458–468

Dermol-Cerne J, Vidmar J, Ščančar J, Urušić K, Serša G, Miklavčič D (2018) Connecting the in vitro and in vivo experiments in electrochemotherapy-a feasibility study modeling cisplatin transport in mouse melanoma using the dual-porosity model. J Control Release 286:33–45

Dermol-Černe J, Pirc E, Miklavčič D (2020) Mechanistic view of skin electroporation - models and dosimetry for successful applications: an expert review. Expert Opin Drug Deliv 17(5):689–704

Goldberg E, Suárez C, Alfonso M, Marchese J, Soba A, Marshall G (2018) Cell membrane electroporation modeling: a multiphysics approach. Bioelectrochemistry 124:28–39

Goldberg E, Soba A, Gandía D, Fernández ML, Suárez C (2021) Coupled mathematical modeling of cisplatin electroporation. Bioelectrochemistry 140:107788

Granot Y, Rubinsky B (2008) Mass transfer model for drug delivery in tissue cells with reversible electroporation. Int J Heat Mass Transf 51(23):5610–5616

Kar P, Feig M (2017) Hybrid all-atom/coarse-grained simulations of proteins by direct coupling of charm and primo force fields. J Chem Theory Comput 13(11):5753–5765

Kmieciik S, Gront D, Kolinski M, Wieteska L, Dawid AE, Kolinski A (2016) Coarse-grained protein models and their applications. Chem Rev 116(14):7898–7936

Kotnik T, Miklavčič D (2000) Analytical description of transmembrane voltage induced by electric fields on spheroidal cells. Biophys J 79(2):670–679

Kotnik T, Rems L, Tarek M, Miklavčič D (2019) Membrane electroporation and electropermeabilization: mechanisms and models. Annu Rev Biophys 48(1):63–91

Krassowska W, Filev PD (2007) Modeling electroporation in a single cell. Biophys J 92(2):404–417

Kyllin JA (2018) Establishing species-specific 3D liver microtissues for repeat dose toxicology and advancing in vitro to in vivo translation through computational modelling. Liverpool John Moores University (United Kingdom)

Li J, Lin H (2011) Numerical simulation of molecular uptake via electroporation. Bioelectrochemistry 82(1):10–21

Li J, Tan W, Yu M, Lin H (2013) The effect of extracellular conductivity on electroporation-mediated molecular delivery. Biochim Biophys Acta (BBA) 1828(2):461–470

Mahnic-Kalamiza S, Vorobiev E, Miklavčič D (2014) Electroporation in food processing and biorefinery. J Membr Biol 247:1279–1304

Miklavčič D, Towhid L (2010) Numerical study of the electroporation pulse shape effect on molecular uptake of biological cells. Radiol Oncol 44(1):34

Miyauchi S, Takeuchi S, Kajishima T (2015) A numerical method for modeling skin electroporation: an expert review. Expert Opin Drug Deliv 17(5):689–704

Miklavčič D, Towhidi L (2010) Numerical study of the electroporation pulse shape effect on molecular uptake of biological cells. Radiol Oncol 44(1):34

Mondal N, Chakravarty K, Dalal DC (2021) A mathematical model of drug dynamics in an electroporated tissue. Math Biosci Eng 18(6):8641–8660
Enhanced Drug Uptake on Application of Electroporation in a Single-Cell Model

Napotnik TB, Reberšek M, Vernier PT, Mali B, Miklavčič D (2016) Effects of high voltage nanosecond electric pulses on eukaryotic cells (in vitro): a systematic review. Bioelectrochemistry 110:1–12
Neumann E, Schaefer RM, Wang Y, Hofschneider PH (1982) Gene transfer into mouse lymphoma cells by electroporation in high electric fields. EMBO J 1(7):841–845
Neumann E, Jordan CA, Sowers AE (1989) Electroporation and electrofusion in cell biology. Springer, Cham
Papich MG, Martinez MN (2015) Applying biopharmaceutical classification system (BCS) criteria to predict oral absorption of drugs in dogs: challenges and pitfalls. AAPS J 17(4):948–964
Pavlin M, Miklavčič D (2003) Effective conductivity of a suspension of permeabilized cells: a theoretical analysis. Biophys J 85(2):719–729
Pavlin M, Kandu SM, Reberšek M, Pucihar G, Hart FX, Mag-jarevic R, Miklavčič D (2005) Effect of cell electroporation on the conductivity of a cell suspension. Biophys J 88(6):4378–4390
Pucihar G, Krmelj J, Reberšek M, Napotnik TB, Miklavčič D (2011) Equivalent pulse parameters for electroporation. IEEE Trans Biomed Eng 58(11):3279–3288
Rems L, Ušaj M, Kandušer M, Reberšek M, Miklavčič D, Pucihar G (2013) Cell electrofusion using nanosecond electric pulses. Sci Rep 3(1):1–10
Rems L, Viano M, Kasimova MA, Miklavčič D, Tarek M (2019) The contribution of lipid peroxidation to membrane permeability in electroporation: a molecular dynamics study. Bioelectrochemistry 125:46–57
Smith KC (2011) A unified model of electroporation and molecular transport. PhD thesis, Massachusetts Institute of Technology
Sözer EB, Pocetti CF, Vernier PT (2018) Asymmetric patterns of small molecule transport after nanosecond and microsecond electroporation. J Membr Biol 251(2):197–210
Weaver JC (2003) Electroporation of biological membranes from multicellular to nano scales. IEEE Trans Dielectr Electr Insul 10:754–768
Yadav KS, Dalal DC (2021) The heterogeneous multiscale method to study particle size and partitioning effects in drug delivery. Comput Math Appl 92:134–148
Yadav KS, Dalal DC (2022) Effects of cell permeability on distribution and penetration of drug in biological tissues: a multiscale approach. Appl Math Model 108:355–375

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.