Study of Transmembrane Potentials of Inner and Outer Membranes Induced by Pulsed-Electric-Field Model and Simulation

Chenguo Yao, Dengbin Mo, Chengxiang Li, Caixin Sun, and Yan Mi

Abstract—A more proper and realistic multilayer dielectric model of spherical biological cell, in which nuclear was taken into consideration, was proposed based on the classic dielectric model in this paper. The general analytical method was also deduced and analyzed in detail calculating the time courses of transmembrane potentials of both inner and outer membranes induced by constant and time-varying electric field. The time course of transmembrane potential of the outer membrane for multilayer dielectric model was compared to that of the classical model. It is shown that the latter is larger than the former, particularly for a cell with larger nucleus. The time courses of transmembrane potentials of both inner and outer membranes induced by pulsed electric fields (PEFs) with different durations were also studied based on the multilayer dielectric model. Long PEF targets outer membrane mainly, and there is little influence to cell nucleus, mitochondrion, and other organelles; thus, it causes electroporation to the outer membrane. As the pulse duration decreases, the electroporation effect changes gradually from the outer membrane to intracellular organelle membrane. Ultrashort PEF (tens of nanoseconds) induces larger voltage across the inner membrane and acts mostly on intracellular substructures. However, submicrosecond PEF (several hundreds of nanoseconds) can induce significant voltages across both the inner and outer membranes, therefore, causing damage to both the inner and outer membranes. This property of submicrosecond PEF has much practical value for tumor treatment.

Index Terms—Inner and outer membranes, multilayer dielectric model, pulsed electric field (PEF), simulation, transmembrane potential.

I. INTRODUCTION

IN RECENT years, the biological and medical effects of pulsed electric field (PEF) have attracted more and more attention [1]–[4]. Weaver et al. [5]–[7] found that the intact cell membrane (outer membrane) is to be a good barrier from ions and hydrophilic molecules in normal physiological conditions. But when exposed to long PEF, many aqueous channels are temporarily formed in the outer membrane, often called pores. After the application of PEF, the pores in the outer membrane will reseal. This transient reversible membrane alteration is termed as reversible electroporation (RE). Later, Yao et al. [8], [9] and Rubinsky et al. [10], [11] found that pores induced in the outer membrane do not reseal if the pulse parameters are chosen above that of RE; they called the phenomenon irreversible electrical breakdown (IREB) or irreversible electroporation. Traditionally, most electroporation studies have focused on relatively low external electric field (less than a kilovolt per centimeter) applied over time periods ranging from several tens of microseconds to milliseconds. In a very recent development, researchers have applied PEF with strengths as high as 100 kV/cm and pulse durations in the nanosecond range to cell suspension. Beebe et al. [12]–[14] observed pores in organelle membranes (inner membrane) after human cells were exposed to high-intensity (26–300 kV/cm) short (10–300 ns) PEF without chemotherapeutic drug. The opening of transient aqueous channels in the inner and outer membranes will make the membrane more permeable to a large variety of hydrophilic molecules, which cannot enter the cell or nuclear in normal conditions; therefore, it can be used to introduce large molecules, such as genetic materials, medical molecules, protein, lipid, and others, into cells. Electroporation of the inner and outer membranes, which alter cellular-structure function, has been applied for tumor treatment, such as electrochemotherapy [14], gene therapy [15], and the other therapies.

Although the mechanism of membrane electroporation is not well understood, much theoretical and experimental research suggests that electroporation of the inner and outer membranes accounts for transmembrane potentials induced by external PEF. Furthermore, the pulse duration, amplitude, rise time, and other parameters of PEF relate remarkably to perforation efficiency. In order to get in-depth understanding of the electroporation mechanism and guide it to treat tumor, it is necessary to develop a proper model and study the discipline of the transmembrane potential induced by external PEF.

Schwan et al. [16]–[18] developed a classical dielectric model of singular spherical biological cell (extracellular medium, outer membrane, and cytoplasm) and derived the Schwan equation of transmembrane potential of the outer membrane induced by constant electric field. Afterwards, Kontik et al. [19], [20] extended the Schwan theory and presented a general analytical method for analysis of time course of the transmembrane potential induced by time-varying electric fields. However, these methods could not consider the influence of subcellular organelles (such as nuclear, mitochondria, etc.) on field distribution, so it could not be used to calculate the transmembrane potential of the inner membrane. In fact, the
size of the nucleus is on the same order with that of the whole cell, particularly for cancer cell, and the organelles should not be ignored [21]. Foster [22] brought forward simplified methods to calculate the potential of subcellular organelles in complex-frequency domain: 1) The cell will be regarded as classical three-layer dielectric model (includes only the extracellular medium, outer membrane, and cytoplasm) to get the field strength in the cytoplasm with the nuclear ignored and 2) the cytoplasm, nuclear membrane, and nucleoplasm will compose the other three-layer dielectric model to acquire the potential of the inner membrane. But the first step in this method neglected the influence of the nuclear on the field distributions, so there may be some errors of calculating results. As viewed from electric circuit, Schoenbach et al. [23], [24] introduced an equivalent model-coupling PEF with a biological cell in suspension. Each section of the biological cell (such as extracellular medium, outer membrane, cytoplasm, nuclear membrane, and nucleoplasm) is described as a resistor or capacitor. Since the equivalent parameters are used to describe a cell with distributing property, which may introduce more errors, and it is difficult to obtain the exact equivalent resistances or capacitances, the equivalent circuit mode can only be used to analyze qualitatively. Joshi et al. [25], [26] developed a distributed network model and proposed a numerical method for calculating the transmembrane potentials of both the inner and outer membranes. Undoubtedly, the model includes several improvements to the pore-formation physics, but the calculation of the transmembrane potential seems more complex.

In this paper, we developed a new multilayer dielectric model of the singular spherical biological cell, taking into account the influence of intracellular nuclear on field distribution. According to the electromagnetic theory, we derived general analytical expressions for the transmembrane potentials of both the inner and outer membranes based on the multilayer dielectric model. The time courses of the transmembrane potentials of both the inner and outer membranes under rectangular PEF, which is widely used in electroporation study, were analyzed.

II. MULTILAYER DIELECTRIC MODEL OF BIOLOGICAL CELL

A cell consists of cell membrane, cytoplasm, nucleus, and other organelles [27], [28]. The cytoplasm, which fills up much of the cell, contains dissolved proteins, electrolytes, and glucose and is moderately conductive. So are the nucleoplasm and the cytoplasm in other organelles. On the other hand, the membranes, which surround the cell and subcellular structures, provide channels for ions, demonstrating a little conductivity. Therefore, we can describe the cell as a conductor surrounded by an approximately insulating envelope and containing substructures with similar properties [23]. Inevitably, every region also demonstrates some dielectric permittivity and conductivity.

![Fig. 1. Classical dielectric model in calculating the transmembrane potential.](image)

The cell is a sphere with radius of \( R_c \), enclosed by the outer membrane of uniform thickness \( d_m \). External electric field is homogeneous and retains its orientation represented by the arrow, although its strength \( E \) changes with time. Specific conductivities and permittivities are attributed to regions occupied by the cytoplasm (\( \lambda_c, \varepsilon_c \)), membrane (\( \lambda_m, \varepsilon_m \)), and extracellular medium (\( \lambda_o, \varepsilon_o \)). Moreover, \( \theta \) is the polar angle measured with respect to the direction of the field.

In this section, the classical dielectric model was reviewed before the development of multilayer dielectric model of the spherical biological cell.

A. Classical Dielectric Model of Spherical Biological Cell

Without consideration of the influence of intracellular organelles on field distribution, Schwan et al. [16]–[18] developed a classical dielectric model of the singular spherical biological cell, as shown in Fig. 1. The model incorporates three concentric layers (cytoplasm, cell membrane, and extracellular medium). The electrical property of each region is described by conductivity and permittivity. Schwan proposed the calculations of the transmembrane-potential inducement based on the model. In this theory, both the cytoplasm and the extracellular medium are described as purely conductive (having zero dielectric permittivity), while the membrane is treated as a lossy dielectric (having nonzero conductivity and dielectric permittivity).

Afterwards, Kontik et al. [19], [20] improved the theory with consideration of the permittivities of the cytoplasm and the extracellular medium. The transmembrane potential of the outer membrane in the complex-frequency domain can be expressed as

\[
\Delta \varphi_m'(s) = F'_m(s)E(s) \cos \theta. \tag{1}
\]

![Equation 1](image)

\[
F'_m(s) = \frac{3 \lambda_o \left[ 3d_m R_c^2 \lambda_c + (3d_m^2 R_c - d_m^2) \left( \lambda_m - \lambda_c \right) \right]}{2 R_c^3 \left( \lambda_m + 2 \lambda_o \right) \left( \lambda_m + \frac{1}{2} \lambda_c - \frac{1}{2} \lambda_o \right) - 2 (R_c - d_m) \lambda_o \left( \lambda_m - \lambda_m \right) (\lambda_c - \lambda_m) \left( \lambda_m - \lambda_m \right)}. \tag{2}
\]
membranes are deduced strictly according to electromagnetic theory, the method proposed should be correct in principle. According to the theoretical analysis, two improvements of the model can be summarized in comparison to the classical model. One is that the calculation of the transmembrane potential of the outer membrane is more precise because the influence of the nuclear to field distribution is taken into consideration in the multilayer model, while it is not in the classical model. The other is that the model can be used to calculate the transmembrane potential of the inner membrane, while the classical model is helpless. The two improvements will be illustrated through simulations and discussions in Sections IV-B and IV-C, respectively.

III. General Method in Calculating the Transmembrane Potentials of Both the Inner and Outer Membranes

A. Calculation of the Transmembrane Potentials Induced by Constant Electric Field

If a spherical biological cell is exposed to a uniform constant electric field $E$ and the space-charge effects are neglected, the steady-state spatial distribution of electric potential (denoted by $\varphi$) can be governed by the Laplace equation [28] in spherical coordinates

$$\nabla^2 \varphi = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial \varphi}{\partial r} \right) + \frac{1}{r^2 \sin \theta} \frac{\partial}{\partial \theta} \left( \sin \theta \frac{\partial \varphi}{\partial \theta} \right) + \frac{1}{r^2 \sin^2 \theta} \frac{\partial^2 \varphi}{\partial \alpha^2} = 0. \quad (4)$$

Supposed that $\varphi_{nc}$, $\varphi_{nm}$, $\varphi_{c}$, $\varphi_{m}$, and $\varphi_{o}$ denote the electric potentials in the nucleoplasm, inner membrane, cytoplasm, cell membrane, and extracellular medium, respectively. The spatial electric potential is obtained by solving (4) with separation of variables. In each of the five regions of the model, it has the general form [28]

$$\varphi_k = (A_k r + B_k r^{-2}) \cos \theta \quad (5)$$

where constants $A_k$ and $B_k$ are specific for each region. $B_{nc} = 0$ and $A_o = -E$ are resulted from finiteness of the electric potential at $r = 0$ and uniformity of the field at $r \rightarrow \infty$.

The remaining eight constants ($A_{nc}$, $A_{nm}$, $A_{c}$, $A_{m}$, $B_{nm}$, $B_{c}$, $B_{m}$, and $B_o$) are determined by boundary conditions, which incorporate continuity of the potential and continuity of the normal component of the electric-current density at each of the four boundaries between the regions. Because the dielectric property may be ignored in constant electric field, the boundary conditions can be expressed as follows:

$$\varphi_{nc}(R_n - d_n) = \varphi_{nm}(R_n - d_n) \quad (6)$$

$$\varphi_{nm}(R_n) = \varphi_{c}(R_n) \quad (7)$$

$$\varphi_{c}(R_c - d_c) = \varphi_{m}(R_c - d_c) \quad (8)$$

$$\varphi_{m}(R_c) = \varphi_{o}(R_c) \quad (9)$$

$$\gamma_{nc} \frac{\partial \varphi_{nc}}{\partial r} \bigg|_{R_n-d_n} = \gamma_{nm} \frac{\partial \varphi_{nm}}{\partial r} \bigg|_{R_n-d_n} \quad (10)$$

Fig. 2. Multilayer dielectric model in calculating for transmembrane potential. In the center of cell, there is a spherical organelle (nucleus) with radius of $d_m$ enclosed by an inner membrane of uniform thickness $d_n$. The nucleoplasm and inner membrane are characterized by conductivities ($\Lambda_{nc}$, $\Lambda_{nm}$) and dielectric permittivities ($\varepsilon_{nc}$, $\varepsilon_{nm}$). The other denotations have the same meanings as that in the classical model, as shown in Fig. 1.

$$F_m'(s)$$ is the transfer function of the transmembrane potential, and it also reflects the electrical and geometrical properties of the cell. Thus (2), shown at the bottom of the page, where $\Lambda_o = \lambda_o + \varepsilon_o s$, $\Lambda_m = \lambda_m + \varepsilon_m s$, $\Lambda_c = \lambda_c + \varepsilon_c s$, and $s$ represents the complex frequency.

The time course of the transmembrane potential can be obtained by the inverse Laplace transform of (1)

$$\Delta \varphi_m'(t) = L^{-1} \left( \Delta \varphi_m'(s) \right). \quad (3)$$

As was stated in Section I, the model could be used to approximately calculate the time course of the transmembrane potential of the outer membrane.

B. Multilayer Dielectric Model of Spherical Biological Cell

As the organelles with different conductivities and dielectric permittivities cause heterogeneity to the interior of a cell, the electric field will be, in fact, distorted by the organelles. In order to calculate the transmembrane potentials of both the inner and outer membranes more accurately, the influence of organelles, such as nuclear, on the electric-field distribution should be taken into consideration. Therefore, a multilayer dielectric model of the spherical cell was developed in this paper, as shown in Fig. 2. The model, with a concentric spherical organelle (the nucleus), may be more proper and realistic in modeling the biological cell than the classical dielectric model. Obviously, the model is composed of five concentric layers (nucleoplasm, inner membrane, cytoplasm, outer membrane, and extracellular medium). In the same way as the classical dielectric model, the conductance and permittivity is used to represent the electrical property of each region in the model.

As the multilayer model, shown in Fig. 2, is abstracted from a biological cell in suspension and the general calculation expressions of the transmembrane potentials of the inner and outer membranes are deduced strictly according to electromagnetic theory, the method proposed should be correct in principle.
we introduce the general conduction operators [which is given to the time derivative of electric-field strength \(30\)]. Therefore, material is proportional to the electric-field strength and consists of two components: one (due to the conductivity of the material) is \(\gamma\), and another (due to the dielectric property) is \(\varepsilon\). However, according to the dielectric theory, when exposed to an external field, the material’s permittivity in each part of cell and neglect its dielectric property.

\[
\text{transmembrane potentials}
\]

\[
\Delta \varphi = \varphi_c(R_n) - \varphi_c(R_m - d_n)
\]

\[
= f_{nm}(\gamma_c, \gamma_m, \gamma_c, \gamma_m, \gamma_o)E \cos \theta
\]

\[
\Delta \varphi_m = \varphi_o(R_c) - \varphi_c(R_m - d_c)
\]

\[
= f_m(\gamma_c, \gamma_m, \gamma_c, \gamma_m, \gamma_o)E \cos \theta
\]

where the coefficients \(f_{nm}\) and \(f_m\), which are functions of \(\gamma_{nc}, \gamma_{nm}, \gamma_c, \gamma_m, \text{and } \gamma_o\), represent the transmembrane potentials of the inner and outer membranes at the pole \((\theta = 0)\) when the external field is 1 V/m.

**B. Calculation of the Transmembrane Potentials Induced by Time-Varying Electric Field**

For the cases where external electric-field strength is constant, (14) and (15) can yield the transmembrane potentials induced at any given moment if we consider the conductivity in each part of cell and neglect its dielectric property. However, according to the dielectric theory, when exposed to time-varying electric field, the current through any material consists of two components: one (due to the conductivity of the material) is proportional to the electric-field strength and the other (due to the permittivity of the material) is proportional to the time derivative of electric-field strength [30]. Therefore, we introduce the general conduction operators [which is given by (16)] in order to account for both the permittivities and conductivities of a cell

\[
\Lambda = \gamma + \varepsilon \frac{d}{dt}
\]

where \(d/dt\) is a differential operator that represents the time derivative \((dy/\delta t)\) of a differentiable function \(y(t)\).

If \(\Lambda_{nc}, \Lambda_{nm}, \Lambda_c, \Lambda_m, \text{and } \Lambda_o\) substitute for \(\lambda_{nc}, \lambda_{nm}, \lambda_c, \lambda_m, \text{and } \lambda_o\) in (14) and (15), respectively, the analytical expressions of the transmembrane potentials under time-varying electric field can be obtained. It is well known that the calculations of the differential operators in the time domain are very complicated, while they become multiples by the complex frequency \(s\) in the complex-frequency domain, so we transform the analysis from the time domain into the complex-frequency domain. Here, the new operator is formulated as

\[
\Lambda = \lambda + \varepsilon s
\]

where \(\Lambda\) and \(\lambda\) are transfer functions of the transmembrane potentials in the complex-frequency domain can be expressed as

\[
\Delta \varphi_{nm}(s) = F_{nm}(\Lambda_{nc}, \Lambda_{nm}, \Lambda_c, \Lambda_m, \Lambda_o)E(s) \cos \theta
\]

\[
= F_{nm}(s)E(s) \cos \theta
\]

\[
\Delta \varphi_m(s) = F_m(\Lambda_{nc}, \Lambda_{nm}, \Lambda_c, \Lambda_m, \Lambda_o)E(s) \cos \theta
\]

\[
= F_m(s)E(s) \cos \theta
\]

where \(\Delta \varphi_{nm}(s), \Delta \varphi_m(s), \text{and } E(s)\) are the Laplace transforms of \(\Delta \varphi_{nm}(t), \Delta \varphi_m(t), \text{and } E(t)\), respectively; \(F_{nm}(s)\) and \(F_m(s)\) are transfer functions of the transmembrane potentials of the inner and outer membranes, respectively, which are expressed as (20) and (21), shown at the bottom of this page, where \(D(s)\) is in both case given by (22), shown at the bottom of the page.

\[
F_{nm}(s) = \frac{27R_nR_n^2d_n(R_c - d_m)^3 + 3R_m^3\Lambda_{nc} - d_n(3R_n - d_n)(\Lambda_{nc} - \Lambda_{nm})\Lambda_c\Lambda_m\Lambda_o}{D(s)}
\]

\[
F_m(s) = \frac{3R_c d_m}{D(s)} \left\{ \begin{array}{l}
(R_n - d_m)^3(\Lambda_{nc} - \Lambda_{nm}) \left[ 2(3R_n^2 - 3R_c d_m + d_m^2)(R_c - d_m)(\Lambda_{nc} - \Lambda_{nm}) + (2R_m^3)(\Lambda_{nc} - \Lambda_{nm}) + (2R_m^2)(\Lambda_{nc} - \Lambda_{nm}) \right] \\
+ R_m^3(\Lambda_{nc} + 2\Lambda_{nm}) \left[ (R_c - d_m)^3(\Lambda_{nc} + 2\Lambda_{nc}) + (2R_m^3)(\Lambda_{nc} - \Lambda_{nm}) \right] \end{array} \right\} \Lambda_o
\]

\[
D(s) = 2(R_n - d_n)^3(\Lambda_{nc} - \Lambda_{nm}) \left\{ \begin{array}{l}
(R_c - d_m)^3(\Lambda_{nc} - \Lambda_{nm}) \left[ (R_c - d_m)^3(\Lambda_{nc} - \Lambda_{nm}) + (2R_m^3)(\Lambda_{nc} + 2\Lambda_{nc}) \right] \\
+ (R_m^2)(2\Lambda_{nc} + \Lambda_{nm}) \left[ (R_c - d_m)^3(\Lambda_{nc} + 2\Lambda_{nc}) + (2R_m^3)(\Lambda_{nc} - \Lambda_{nm}) \right] \end{array} \right\}
\]

\[
+ R_m^3(\Lambda_{nc} + 2\Lambda_{nm}) \left\{ (R_c - d_m)^3(\Lambda_{nc} + 2\Lambda_{nc}) + (2R_m^3)(\Lambda_{nc} - \Lambda_{nm}) \right\}
\]

\[
+ R_m^2(2\Lambda_{nc} + \Lambda_{nm}) \left\{ (R_c - d_m)^3(\Lambda_{nc} + 2\Lambda_{nc}) + (2R_m^3)(\Lambda_{nc} - \Lambda_{nm}) \right\}
\]
By expanding both the numerators and denominators, (20) and (21) can be expressed as rational functions

\[
F_{mn}(s) = \frac{b_{nm4} s^4 + b_{nm3} s^3 + b_{nm2} s^2 + b_{nm1} s + b_{nm0}}{a_5 s^5 + a_4 s^4 + a_3 s^3 + a_2 s^2 + a_1 s + a_0}
\]

(23)

\[
F_m(s) = \frac{b_{m4} s^4 + b_{m3} s^3 + b_{m2} s^2 + b_{m1} s + b_{m0}}{a_5 s^5 + a_4 s^4 + a_3 s^3 + a_2 s^2 + a_1 s + a_0}
\]

(24)

where the coefficients \(a_i, b_{mi},\) and \(b_m\) are relatively lengthy expressions, which incorporate the geometric and electric parameters of all five regions of the model. For brevity, they are not given here.

It is shown that the numerators and denominators of both (23) and (24) are fourth- and fifth-order polynomials of complex frequency \(s\), respectively. Therefore, (23) and (24) can also be used to analyze the frequency response in the frequency domain, which is not included in this paper for length limitation and will be given in another paper.

For any time course \(E(t)\) that can be transformed into the complex-frequency domain (i.e., its Laplace transform \(E(s)\) exists), the induced transmembrane potentials can be calculated according to the above method. By the inverse Laplace transform, the time courses of the transmembrane potentials, which are induced by time-varying electric fields, are available

\[
\Delta \phi_{mn}(t) = L^{-1} \left[ (\Delta \phi_{mn}(s)) \right]
\]

(25)

\[
\Delta \phi_m(t) = L^{-1} \left[ (\Delta \phi_m(s)) \right].
\]

(26)

The basic principle of the above method can also be illustrated by a block diagram, as shown in Fig. 3. From the point of view of the system, the external electric field acts as the “input” or the “excitation” of the system. \(F_{mn}(s)\) and \(F_m(s)\) play the roles of transfer functions, while the induced transmembrane potentials are the “outputs” or the “responses.”

For any given time course of the external electric field, this method gives the time course of the transmembrane potentials of both the inner and outer membranes at any point at any time. For simplicity, the transmembrane potentials of the inner and outer membranes, just at \(\theta = 0\), were simulated in this paper.

C. Calculation of the Transmembrane Potentials Induced by Rectangular PEF

The described method can be used directly for simple mathematical functions \(E(t)\), for which both the Laplace transform and the inverse transform are easily calculated. However, for the more complex functions \(E(t)\), the calculation of the transmembrane potentials is much simpler, if \(E(t)\) is treated as a linear combination of simple functions

\[
E(t) = K_1 E_1(t) + K_2 E_2(t) + \cdots + K_n E_n(t).
\]

(27)

The linearity of both the Laplace transform and the inverse Laplace transform implies that the responses \(\Delta \phi_m(t)\) and \(\Delta \phi_{mn}(t)\), induced by \(E(t)\) which conforms to (27), can be expressed as a sum of partial responses

\[
\Delta \phi_{mn}(t) = K_{nm1} \Delta \phi_{m1}(t) + q K_{nm2} \Delta \phi_{m2}(t) + \cdots + K_{nmm} \Delta \phi_{mnm}(t)
\]

(28)

\[
\Delta \phi_m(t) = K_{m1} \Delta \phi_{m1}(t) + K_{m2} \Delta \phi_{m2}(t) + \cdots + K_{nmn} \Delta \phi_{mn}(t)
\]

(29)

where \(\Delta \phi_{mnm}(t)\) and \(\Delta \phi_{m1}(t)\) denote the responses to \(E_i(t)\) alone.

For a rectangular pulse of amplitude \(E_0\) and duration \(T\), it can be regarded as a sum of two opposite step functions with amplitudes \(+E_0\) and \(-E_0\), the second step response delayed for \(T\) with respect to the first one, as is sketched in Fig. 4. According to the superposition principle, the induced responses \(\Delta \phi_{mn}(t)\) and \(\Delta \phi_m(t)\) are obtained by a sum of two-step responses bearing opposite signs, which can be expressed as

\[
\Delta \phi_{mn}(t) = \Delta \phi_{m1}(t) \cdot \varepsilon(t) - \Delta \phi_{m1}(t-T) \cdot \varepsilon(t-T)
\]

(30)

\[
\Delta \phi_m(t) = \Delta \phi_{m1}(t) \cdot \varepsilon(t) - \Delta \phi_{m1}(t-T) \cdot \varepsilon(t-T)
\]

(31)

where \(\varepsilon(t)\) is a unit step function. \(\Delta \phi_{m1}(t)\) and \(\Delta \phi_{m1}(t)\) are responses of the step function with amplitude \(+E_0\).

IV. RESULTS AND DISCUSSION

A. Determination of Cell Parameters

Cell properties, such as conductivity, permittivity, and geometric parameters, play an important role in electroporation. Each part of a cell has complex chemical composition and its conductivity and permittivity is very difficult to be determined. It is well known that different cells have different dielectric and geometric properties. Even for the same cell, different simulative parameters are used by different researchers. However, this paper aims at presenting a general method in calculating the transmembrane potentials of both the inner and outer membranes, not the cell parameters themselves. Therefore, the typical parameters are used for theoretical calculation in this paper, which are cited from [19], [20], [27], and [28] and listed in Table I.
TABLE I

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular medium conductivity</td>
<td>0.3 S/m</td>
</tr>
<tr>
<td>Outer membrane conductivity</td>
<td>3x10^{-7} S/m</td>
</tr>
<tr>
<td>Cytoplasm conductivity</td>
<td>0.3 S/m</td>
</tr>
<tr>
<td>Inner membrane conductivity</td>
<td>3x10^{-7} S/m</td>
</tr>
<tr>
<td>Nucleoplasm conductivity</td>
<td>0.3 S/m</td>
</tr>
<tr>
<td>Extracellular medium permittivity</td>
<td>7.1x10^{-10} As/Vm</td>
</tr>
<tr>
<td>Outer membrane permittivity</td>
<td>4.4x10^{-11} As/Vm</td>
</tr>
<tr>
<td>Cytoplasm permittivity</td>
<td>7.1x10^{-10} As/Vm</td>
</tr>
<tr>
<td>Inner membrane permittivity</td>
<td>4.4x10^{-11} As/Vm</td>
</tr>
<tr>
<td>Nucleoplasm permittivity</td>
<td>7.1x10^{-10} As/Vm</td>
</tr>
</tbody>
</table>

| Radius of cell                      | 10µm     |
| Thickness of outer membrane        | 7nm      |
| Radius of nuclear                   | 5µm, 8µm |
| Thickness of inner membrane         | 40nm     |

- From references [19, 20].
- Set at values as conductivity and permittivity of outer membrane.
- Typical geometrical parameters of cell from references [27, 28].
- For some cells with large nuclear.

B. Transmembrane Potentials of the Outer Membrane for Classical and Multilayer Dielectric Model

Fig. 5 shows the time courses of the transmembrane potentials of the outer membrane induced by the rectangular PEF for classical and multilayer models. After the pulse is turned ON, the external electric field charges the outer membrane and the transmembrane potential is induced. However, field charges the outer membrane for classical dielectric model more rapidly than that for the multilayer one; consequently, the transmembrane potential for the classical dielectric model is larger than that for the multilayer one. Generally, nuclear volume take up about 10% of the whole cell; thus, we set the nuclear radius 5 µm when cellular radius is 10 µm. As for 5-µm nuclear, the difference of the transmembrane potentials between the classical and multilayer model is very little, so the improvement of the modified multilayer model does not seem significant. However, for some cells, the nuclear size is comparable to the cell itself, taking normal oncellar B-cell as example, the nuclear occupies 60% in the whole cell [25], or for most cancer cells, cancentration will cause the nuclear size to increase [21]. Therefore, we also simulated the transmembrane potentials when the nuclear radius is 8 µm (about 50% of the cell), as shown in Fig. 5. It is shown that the transmembrane potential for the multilayer model is much lower than that for the classical one, which demonstrate that the improvement of the multilayer model is remarkable for large nuclear.

Fig. 6 indicates the difference of the maximum transmembrane potentials between the two models under PEF with different durations. The absolute values shown in Fig. 6 may not express the difference well, thus we also plot the ratio of potentials for the classical to the multilayer model, as shown in Fig. 7. From the two figures, we can see that there is little difference between the two models for long pulse (several microseconds to milliseconds). However, when pulse duration is less than one or two microseconds, the potential for multilayer model is lower than that of the classical one, particularly for large nuclear. For 8-µm nuclear, the maximal difference is much considerable and reaches 53%. Therefore, we can conclude that the multilayer model is more exact in calculating the transmembrane potential induced by PEF, particularly short PEF and for larger nuclear. It is well known that the application of short PEF to biological cells has been the research focus in recent years. Therefore, we believe that the multilayer model can play an important role in studying the interaction between PEF, particularly short PEF, and the biological cell.

The difference above can be explained qualitatively based on cellular-circuit model [23]. When PEF is turned ON, it charges the membrane through external extracellular medium
Fig. 8. Ratio of maximal transmembrane potentials induced by rectangular PEF with different durations for classical to multilayer model.

Fig. 7. Ratio of maximal transmembrane potentials induced by rectangular PEF with different durations for classical to multilayer model.

and cellular interior. As the conductivity of the inner membrane is much smaller than that of nucleoplasm or cytoplasm, when the interior of a cell is regarded as a whole for multilayer dielectric model, its equivalent conductivity is smaller than the conductivity of the uniform cytoplasm for the classical model. According to circuit principle, smaller conductivity implies larger charging resistor, which also implies a larger charging-time constant. In other words, PEF charges membrane for multilayer model more slowly than for the classical one, as a result, the transmembrane potential increases more slowly. Therefore, the transmembrane potential for multilayer model is smaller than that of the classical model when the pulse duration is less than the charging time. However, the transmembrane potential is determined by cellular geometrical parameters and external field when the pulse duration is larger than the charging time; consequently, the transmembrane potentials for two models have little difference.

C. Time Courses of the Transmembrane Potentials of Both the Inner and Outer Membranes Based on Multilayer Dielectric Model

In recent years, the interaction between external PEF and the intracellular organelles has become a research hotspot, so it is significant and necessary in studying the intracellular effects to exactly calculate the transmembrane potential of the inner membrane. It is a pity that the classical model is helpless in calculating the transmembrane potential of the inner membrane. However, the calculation can be achieved by multilayer dielectric model, which is also the other important improvement of the model.

Fig. 8 shows the time courses of the transmembrane potentials of both the inner and outer membranes induced by rectangular PEF with duration of 1 µs based on the multilayer dielectric model. As the outer membrane is a leaky dielectric and not an absolute insulator, it cannot shield external PEF from acting on the interior of a cell completely. Therefore, the external PEF can penetrate the outer membrane; thus, it also charges the inner membrane with smaller charging time constant when charging the outer membrane. During the charging time of the inner membrane, the transmembrane potential of the inner membrane increased more rapidly than that of the outer membrane, thus the former is larger than the latter. Afterwards, the latter is increased continually to a value above the former. When the pulse duration is larger than the charging time of the inner membrane, the maximum transmembrane potentials of the inner membranes are also determined by cellular geometrical parameters and external field.

Moreover, the negative transmembrane potential shown in Fig. 8 is a peculiar property of the inner membrane; in other words, the voltage across the inner membrane is bipolar. The positive voltage is well understood. PEF charges the outer and inner membranes when pulse is turned on, which induces positive voltage across the inner membrane. While the charges stored in the outer membrane discharges at the fall edge of PEF, which forms a discharge current. The discharge current will charge the inner membrane again. As the discharge current is in the direction opposite to the charged one, as a result, the transmembrane potential induced by discharge current is negative.

Fig. 9 shows the maximum transmembrane potentials of the inner and outer membranes induced by PEF with different durations. It can be concluded that PEF with different durations induce transmembrane potentials of the inner and outer membranes with different relative values. Ultrasound PEF (tens of nanoseconds) affects the inner membrane more and probably induces apoptosis or modifies cell function because it can induce larger voltage across inner membrane than across outer membrane. As pulse duration increases, the maximal transmembrane potentials of both the inner and outer membranes gradually increase. When pulse duration is in the range of submicrosecond (several hundred nanoseconds), the maximal transmembrane potential of the inner membrane reaches a plateau, while that of the outer membrane still increases with...
duration. As a result, the two curves will intersect, and voltages across both the inner and outer membranes will have comparable large values in the vicinity of the point of intersection. Therefore, external PEF has strong effect on both the inner and outer membranes at the same time. If submicrosecond PEF is applied to kill cells, the efficiency can be improved greatly. The property of submicrosecond PEF has much practice significance for tumor treatment.

The above statement has been validated by Beebe et al. [12], [13]. Human HL-60 cell suspension was exposed to rectangular PEF with 60 ns–60 kV/cm and 300 ns–26 kV/cm, respectively, which provided similar energy densities. As a result, 60-ns 60-kV/cm PEF-induced apoptosis of cells without obvious effect on the outer membrane. While both apoptosis and obvious electroporation of the outer membrane are observed for cells treated by 300 ns–26 kV/cm PEF.

It can also be drawn from Fig. 9 that, when pulse duration is in the range from microsecond to millisecond (PEF of this kind is usually used to study electroporation of the outer membrane), the maximal transmembrane potentials of both the inner and outer membranes reach plateaus. Although both of the inner and outer membranes have significant transmembrane potentials, the transmembrane potential of the outer membrane keeps the same value during the lasting time of the pulse, while that of the inner membrane lasts several hundreds of nanoseconds only. As a result, the external PEF affects the outer membrane more and opens transient pores in the outer membrane. It is in keeping with many experimental observations of applying PEF with duration ranging from microsecond to millisecond to induce electroporation of the outer membrane [5]–[7].

V. CONCLUSION

A more realistic multilayer dielectric model of a singular spherical physiological cell was developed in this paper based on the classical one, in which the influence of nuclear on the transmembrane potential was taken into consideration. According to the electromagnetic theory, the general analytical method was presented in calculating the transmembrane potentials of both the inner and outer membranes induced by constant and time-varying electric field. The method can be used to determine the pulse parameters that would provide a specific value of the induced transmembrane potential and (or) retain this value for a specific duration.

The transmembrane potentials induced by PEF were simulated for classical and multi-layer dielectric model. The results suggest that there are two improvements for the multilayer dielectric model. One is that the transmembrane potential of the outer membrane is more precise than that of the classical one, particularly for a cell with large organelles and short duration (less than the charging time of the outer membrane). The other is that the model can be used in exactly calculating the transmembrane potential of the inner membrane. The two improvements have much significance in studying the interaction between the short PEF and the cell with large nucleus.

It has also been shown that PEF with different durations induce different time courses of the transmembrane potentials of both the inner and outer membranes based on the multilayer dielectric model, thus result in different cell responses. Ultrashort PEF (tens of nanoseconds) can penetrate the outer membrane to target on intracellular substructures and induce apoptosis or modify cell function. While long PEF, with duration ranging from microsecond to millisecond, mainly acts on the outer membrane, they are usually used to induce electroporation of the outer membrane. However, submicrosecond PEF (several hundreds of nanoseconds) generates significant effect on both the inner and outer membranes. If submicrosecond PEF is applied to treat tumor, the treatment efficiency can be improved greatly. Therefore, application of submicrosecond PEF to treat tumor may be a new and promising tumor therapy.

REFERENCES


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