Chapter 3

Electro-active response of living cell

The chapter undertakes the preliminary study of the interaction of electric field with the living cells. The study is based on the inherent characteristic of the living cell as an electrically active unit to facilitate its various metabolic activities which can be altered / controlled under the externally applied electric field (E-field). Towards this end, four different electrical equivalent circuits for living cell have been developed, based on various different ionic pathways under an applied electrical potential. The evaluation of time constant as response characteristic and the influence of cell size, cytoplasmic and nucleoplasmic resistances as well as cell and nuclear membrane capacitances on time constant have been analytically computed. The computational analysis follows two fold assumptions i.e., by considering a) cell membrane as leaky dielectric and b) both, the cell and nuclear membranes as leaky dielectrics. Further, for the validation of the proposed electric equivalent models, an analytical study for well-known electroporation phenomenon has been carried out using similar electrical equivalent circuits. Using MATLAB simulations, models have also been analysed with various pulsed voltage input signals of different durations.

3.1. Introduction

The investigation of biophysical properties of a living cell such as electrical, optical, mechanical, thermal characterization, etc. allow clinicians, pharmacists and medical researchers to diagnose the physiological state of the cell such as detecting cancers, bacterial infections, toxicity, etc.[1,2] Single cell analyses have attracted scientific community to develop in-depth understanding of the cell differentiation/proliferation which are present in the individual molecular machinery of the single cell.[3] This is due to the fact that single cell carries specific gene expression levels which can be different from other cell types in a

particular group because of their heterogeneous behaviour.[4] Out of these biophysical properties, the electrical properties of the cell indicate the electro-physiological state of the single cell and any irregularity such as bacterial infections alters the conductivity of the ionic channels as well as cytoplasm.[5] The electro-physiological state of the cell almost controls every aspect of the metabolism in the human body such as heart beat, neuronal functioning, muscle contraction, renal functioning, digestive controls to name a few. Therefore, in view of the electrical properties of the living cell, the applications of the electrical field (E-field) in the cell culture medium as well as in clinical trials have gained much prominence because of their effectiveness on biophysical as well as biochemical properties.

The effectiveness of E-field on the cellular functionalities paves a way to a more fundamental question of how E-field affects the single living cell which can ultimately be drawn into a much larger picture (i.e. multiple cells). In addition, the external electric field stimulation has been recently utilized in cell culture medium along with the biomaterial substrates *in-vitro* to analyse the influence of E-field parameters (strength, duration, no. of pulses applied) on the interaction between the cells and biomaterial substrates.[6,7] In this view, electric field influences various cell fate processes such as proliferation and differentiation which would have profound effect on the cell-material interaction. Therefore, it becomes necessary to first understand the response characteristics of single cell under external electric field. In this consideration, analytical models of cell membrane have been designed and theoretical studies are conducted based on the transport kinetics of ionic flow as well as molecules across it.[8] Deng et al.[9] designed a simple electrical equivalent of a single cell to study the effect of electric field on the intracellular substructures (e.g., nucleus) when the duration of E-field is reduced from microseconds to nanoseconds. In this study, Jurkat cells, suspended in the physiological buffer of propidium iodide were interacted with E-field of various intensities (3-150 kV/cm) and durations (100 μs - 60 ns). It was reported that the large duration E-field

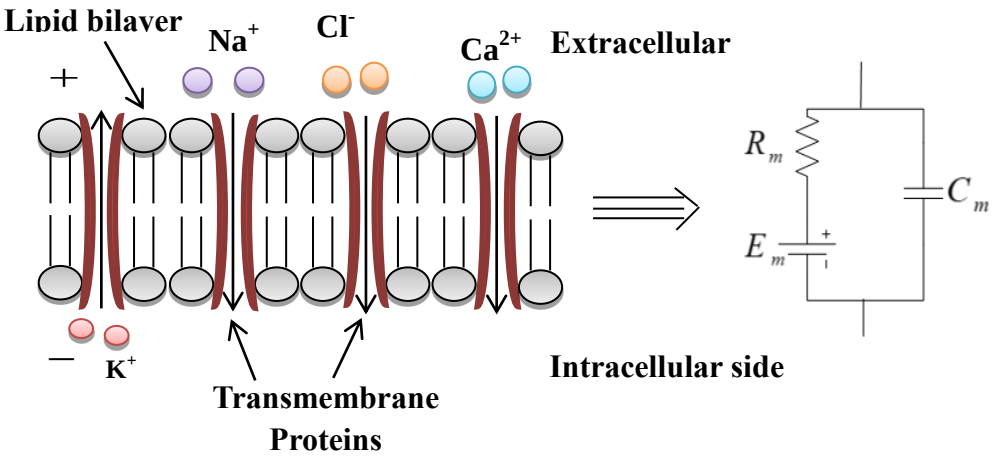
pulses (100 μs - 300 ns) affects the cellular membrane instantaneously while the short duration pulses (60 ns) have delayed response on the cell membrane. The proposed electrical equivalent of the single living cell in this study contains nucleus as the only organelle. Considering the proposed analytical model of a single living cell by Deng et al.[9], various studies have been conducted on its electrical properties.[10,11,12] Deng et al.[9] assumed the membranes (cell and nuclear) to be nonconducting. However, both cell and nuclear are semiconducting in nature because cell membrane consists of ionic channels, pumps, transporters and other channels which mediate the flow of ions across it, while nuclear membrane consists of pores which passes selective molecules to be swapped across cytoplasm and nucleoplasm. Thereafter, Ellappan and Sundararajan[13] assumed an electrical equivalent of the cell with cell membrane to be a leaky dielectric and nuclear membrane to be perfect dielectric. The study further attempted to understand the interaction mechanism of E-field with single living cell with a more profound as well as realistic model. The study reported that the low frequency E-field pulses have substantial effect on the cell membrane as compared with that of high frequency E-field pulses for the similar intensity of E-field.

These studies have assumed both the membranes to be of capacitive nature (perfect dielectric) rather than that of RC type. However, as mentioned, cell and nuclear membrane comprises of various ionic conducting channels as well as pores. These channels and pores facilitate the transport of ions and molecules across the membrane resulting in currents. These currents further can influence various cellular functionalities. Therefore, present study considers the simple electrical equivalent of living cell with cell and nuclear membrane acting as leaky dielectrics. These are represented as the parallel combination of resistor and capacitor. Resistor in the cell membrane is suggested to mimic the behaviour of voltage gated channels, while in nuclear membrane, it is a symbolized behaviour of pores. Under the application of E-field, ions traverse the least resistance path, which is represented as two

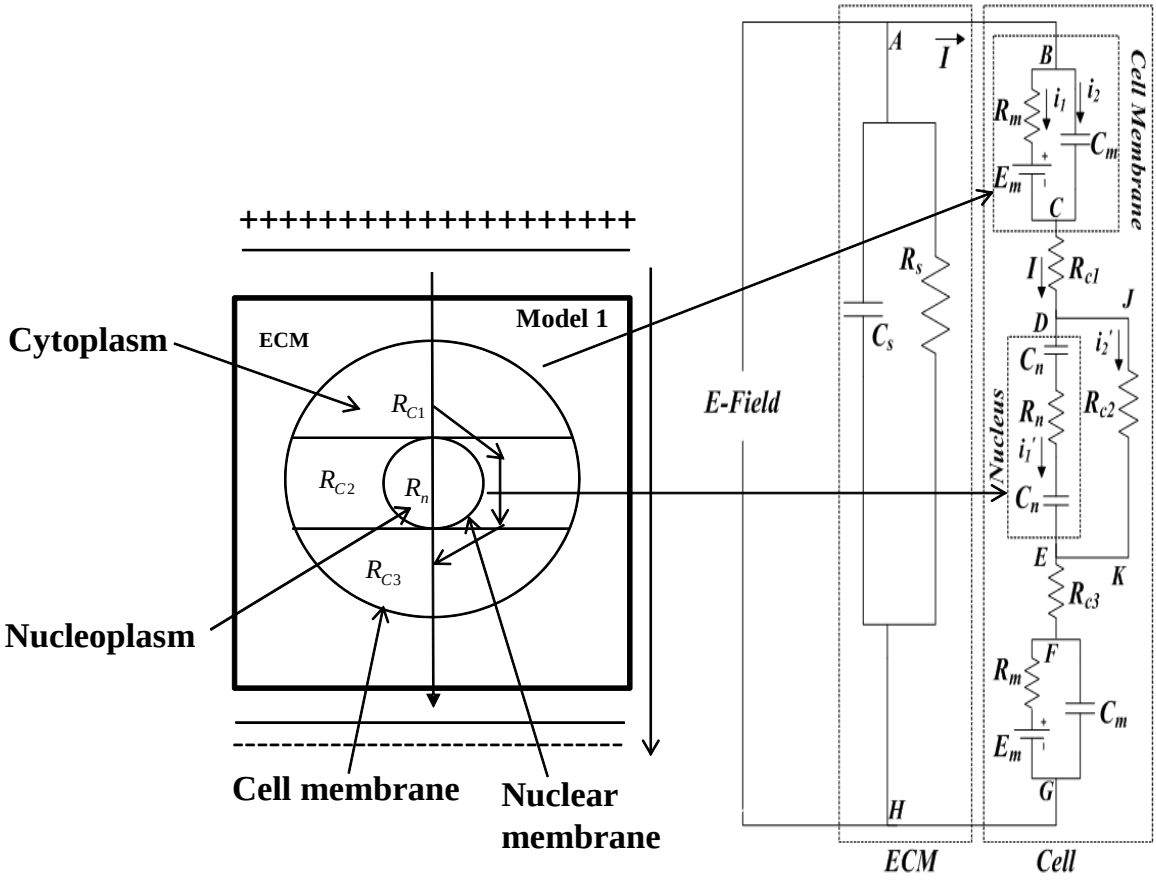
electrical equivalent circuits. The time constants of the circuits have been evaluated and their variations with various cellular adaptation processes are analysed. The E-field strength, required to electroporate the cells is also been calculated, assuming a various critical transmembrane potentials (TMPs). Using MATLAB simulations, models were also analysed with different pulsed voltage input signals of various durations (milli to nanoseconds).

3.2. Model description

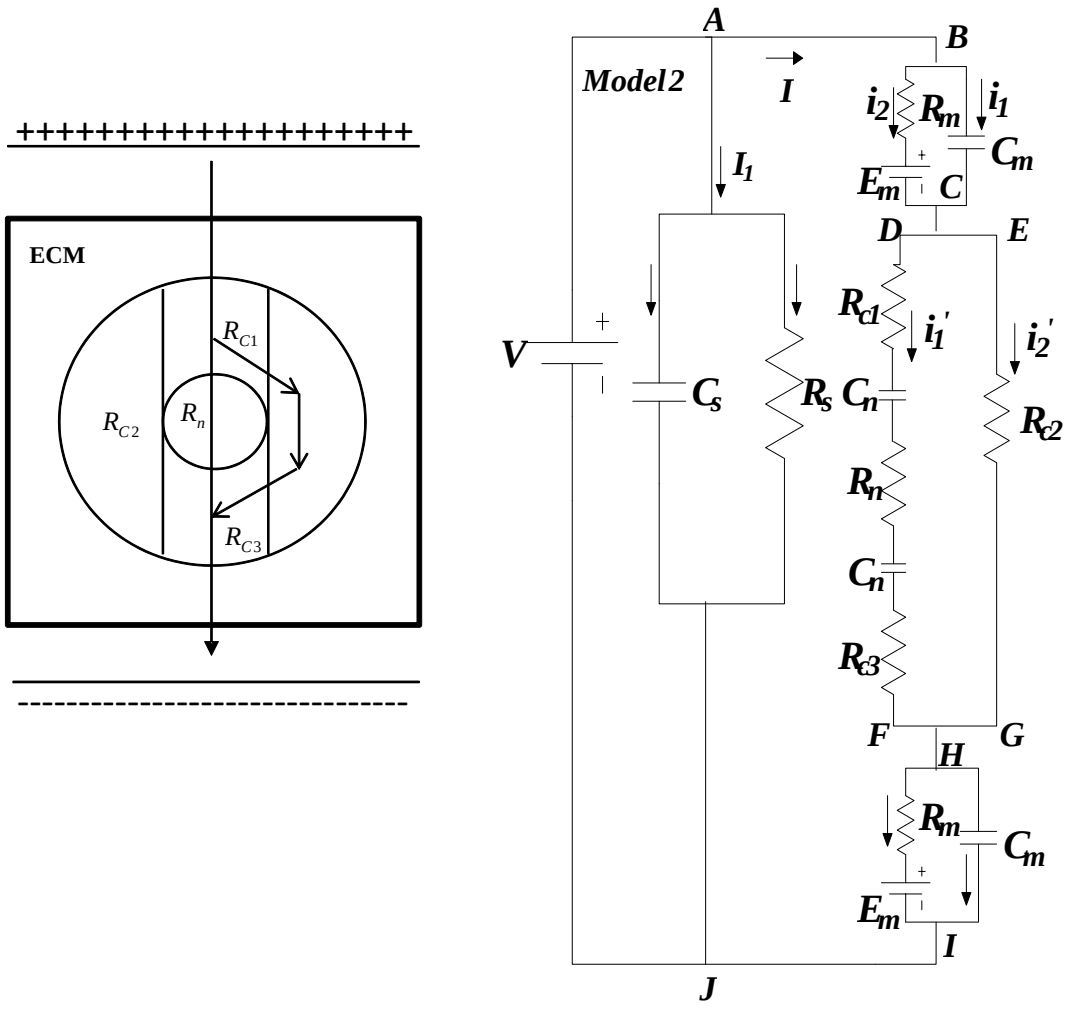
Cell, as the smallest unit of any living organism, behaves as a complete unit with proper external environment (extracellular matrix [ECM]). The cytoplasm and other internal organelles are externally protected by cell membrane. It is semipermeable in nature due to the presence of lipids and proteins. The hydrophobic ends of lipid bilayers insulate the membrane from various inorganic ions (Na^+, K^+, Ca^{2+}, etc.) while proteins provide different pathways in the form ionic channels (gated and non-gated), pumps, transporters, aquaporins, etc.[14] Depending on the energy utilization mechanisms, channels are categorized into active and passive. To facilitate the exchange of ions, pumps and transporters consume energy from the hydrolysis of adenosine triphosphate (ATP) and ionic gradients (electrochemical gradient) and therefore, are called as active channels. While, gated and non-gated channels are passive in nature as they require external stimulation (mechanical, electrical and chemical) to conduct ions.[15] On the other hand, aquaporins are open channels which allow passage of water molecules only. Apart from it, there are various other channels present in the cell membrane.


Lipid bilayer
Na+
Cl-
Ca2+
Extracellular
+
−
K+
Transmembrane
Proteins
Intracellular side
Rm
Cm
Em

a)


Cytoplasm
Nucleoplasm
Cell membrane
Nuclear
membrane
ECM
Model 1
RC1
RC2
Rn
RC3
E-Field
A
I
H
Rs
Cs
ECM
B
i1
i2
Rm
Cm
Em
C
I
Rc1
J
D
Cn
Rn
i1′
i2′
Rc2
Cn
Nucleus
E
K
Rc3
F
Rm
Cm
Em
G
Cell
Cell Membrane

b)



c)

Fig. 3.1: a) Schematic illustrating the electrical equivalent circuit of cell membrane. b) A generalized electrical equivalent circuit for a single living cell with cell membrane as a leaky dielectric, each section of a living cell is represented by a corresponding electrical component in a circuit (model 1). c) Model 2 represents the other ionic pathway (left hand side) under the applied electric stimulation. R_s and C_s represent the resistance and capacitance of extracellular matrix (ECM), respectively. E_m represents the membrane potential, C_m and C_n represent the cell and nuclear membrane capacitances, R_{c1}, R_{c2} and R_{c3} represent the cytoplasmic resistances and R_n is the nucleoplasmic resistance.

Due to the gradient of inorganic ions, present in the intracellular as well as extracellular fluids, an electric potential is developed across the cell membrane with negative potential inside the cell and a positive potential outside. Therefore, the polarized nature of the cell membranes gives rise to the endogenous E-field to cell. On the other hand, nuclear membrane consists of pores, which provides pathway to specifically selected molecules to be exchanged across the cytoplasm and nucleoplasm. Though, with the presence of channels and pores in both the membranes, the impedance of nuclear membrane is comparatively higher (~ 5-10 times) than the cell membrane. Accordingly, cell membrane can be represented as a combination of resistor and capacitor. Moreover, in the complete electrical equivalent circuit of the living cell, presence of extracellular matrix is also depicted with the presence resistor and capacitor.[16] In addition, the cytoplasm and nucleoplasm are depicted as resistors. Therefore, when the E-field is applied across the cell, it stimulates numerous voltage gated channels which result in depolarization of the cell. Thereby, transmembrane potential (TMP) increases to the critical value (0.7 V - 1 V). On reaching to the critical value, structural changes with pore formation takes place in the cell membrane. This phenomenon is generally known as electroporation.[17] Initially, on the application of E-field, conductive currents flow across the various channels, pores and fluids (cytoplasm and nucleoplasm) while displacement currents flow across the dielectric portion (lipids in the membrane) of the membranes. The stability of the pores formed in the cell membrane depends upon the E-field parameters as well as electro-physical state of the cell.[8,18] The pores can be resealed and overall structure of the cell remains unaffected or it can lead to permanent damage to the structure which results in cell death. The former process is known as reversible electroporation while latter is irreversible electroporation. E-field also causes other cellular processes such as apoptosis, proliferation and differentiation.[19, 20]

Fig. 3.1 represents the electrical equivalent circuit of the cell by considering the cell membrane as leaky dielectric in earlier proposed model.[10] In this study, voltage gated channels are taken into account which equivalently can be represented as combination of resistance R_m and a voltage source E_m. R_S and C_S are resistance and capacitance of extracellular matrix (ECM), respectively. C_m and C_n the cell and nuclear membrane capacitances, respectively. R_{C1}, R_{C2}, R_{C3} are cytoplasmic resistances and R_n is the nucleoplasmic resistance.

3.3. Analytical computation and discussion

Generally, the mechanism of E-field interaction with the biological cells is evaluated using Laplace equation ($\nabla^2 V = 0$) under certain assumptions (cell size and shape) and boundary conditions.[21] The E-field induced transmembrane potential (V) controls cellular processes such as, electroporation, apoptosis, differentiation and proliferation.[19,20] However, if any of the intracellular structures are considered, the analysis becomes quite complex. On the other hand, the electrical equivalent circuit is naturally simpler and predicts the dynamics of interaction mechanism more closely.

The solution of the circuit for model 1 [Fig. 3.1(b)] is evaluated using basic physical laws of current and voltage i.e., Kirchoff's current and voltage law. The solution of which is as follows,

By Kirchoff's current law,

$$I = i_1 + i_2 = i_1^{'} + i_2^{'} \Rightarrow \frac{dI}{dt} = \frac{di_1}{dt} + \frac{di_2}{dt} = \frac{di_1^{'}}{dt} + \frac{di_2^{'}}{dt} \tag{3.1}$$

Similarly, considering closed loop BCB, using Kirchoff's voltage law, one can obtain loop equation as,

$$i_i R_m + E_m = \frac{1}{C_m}\int i_2 dt \Rightarrow \frac{di_1}{dt} = \frac{i_2}{R_m C_m} \tag{3.2}$$

Considering loop DJKED, one can obtain,

$$\frac{2}{C_n}\int i_1^{'}dt + i_1^{'}R_n = i_2^{'}R_{C_2} \Rightarrow \frac{2i_1^{'}}{C_n} + R_n\frac{di_1^{'}}{dt} = R_{C_2}\frac{di_2^{'}}{dt} \tag{3.3}$$

Considering loop ABCDEFGHA, one can obtain,

$$\frac{2}{C_m}\int i_2 dt + \frac{2}{C_n}\int i_1^{'}dt + I(R_{C_1} + R_{C_3}) + i_1^{'}R_n = V_{E-Field} \Rightarrow \frac{2i_2}{C_m} + \frac{2i_1^{'}}{C_n} + \left(R_{C_1} + R_{C_3}\right)\frac{dI}{dt} + R_n\frac{di_1^{'}}{dt} = 0 \tag{3.4}$$

Considering loop ABCDJKEFGHA, one can obtain,

$$2E_m + 2i_1R_m + I(R_{C_1} + R_{C_3}) + i_2^{'}R_{C_2} = V_{E-Field} \Rightarrow \left(R_{C_1} + R_{C_2}\right)\frac{dI}{dt} + 2R_m\frac{di_1}{dt} + R_{C_2}\frac{di_2^{'}}{dt} = 0 \tag{3.5}$$

The eqs. (3.1) - (3.5) are linearly dependent equations of model 1, therefore, by substitution and using eq. 3.1, a second order differential equation can be obtained in $i_1^{'}$ as,

$$\left(\frac{C_m}{2}(R_{C_1} + R_{C_3}) + \frac{C_mR_n}{2R_{C_2}}(R_{C_1} + R_{C_3}) + \frac{C_mR_n}{2}\right)\frac{d^2i_1^{'}}{dt^2} + \left(\begin{array}{l}\dfrac{(R_{C_1} + R_{C_3})}{2R_m} + \dfrac{R_n(R_{C_1} + R_{C_3})}{2R_mR_{C_2}} + \dfrac{R_n}{2R_m} \\ + \dfrac{C_m(R_{C_1} + R_{C_3})}{C_nR_{C_2}} + \dfrac{C_m}{C_n} + \dfrac{R_n}{R_{C_2}} + 1\end{array}\right)\frac{di_1^{'}}{dt}$$
$$+\left(\frac{(R_{C_1} + R_{C_3})}{R_mC_nR_{C_2}} + \frac{1}{R_mC_n} + \frac{2}{C_nR_{C_2}}\right)i_1^{'} = 0 \tag{3.6}$$

The general solution of the second order differential eq. (3.6) is,

$$i_1^{'} = A_1e^{-k_1t} + A_2e^{-k_2t} \tag{3.7}$$

Where, A_1 and A_2 are arbitrary constants and k_1 and k_2 are the roots of the characteristics equation of (3.6). The reciprocal of the roots of the characteristic equation gives the time constant of the model 1, i.e.,

$$\tau_1 = \frac{1}{k_1}, \tau_2 = \frac{1}{k_2} \tag{3.8}$$

The specific capacitance per unit area of the cell and nuclear membranes is taken as 10^{-2} F/m^2.[9,10] The resistivity of both the fluids (cytoplasm and nucleoplasm) is taken as 100 Ωcm.[9] The resistivity of the cell membrane has been reported to vary between 0.01 to 1 Ωm^2 [(22)]

which depends on the cell type and its physiological state. The present study considered the membrane resistivity to be 1 Ωm^2. Further, the resistances of various parts of cytoplasm and nucleoplasm have been taken from earlier report.[11]

The value of time constants for model 1 using eqs. (3.6), (3.7) and (3.8) are evaluated to be,

$\tau_1 = 3.26\times10^{-6}s, \tau_2 = 1.535\times10^{-6}s$.

Similarly, for model 2 [Fig. 3.1(c)] eq. (3.1) would be the same while the rest linearly independent equations can be obtained as,

Considering loop BCB, one can obtain,

$$i_2R_m + E_m = \frac{1}{C_m}\int i_1 dt \Rightarrow \frac{di_2}{dt} = \frac{i_1}{R_mC_m} \tag{3.9}$$

Considering loop DEFGD, one can obtain,

$$i_1^{'}\left(R_{C_1} + R_n + R_{C_3}\right) + \frac{2}{C_n}\int i_1^{'}dt = i_2^{'}R_{C_2} \Rightarrow \left(R_{C_1} + R_n + R_{C_3}\right)\frac{di_1^{'}}{dt} + \frac{2i_1^{'}}{C_n} = R_{C_2}\frac{di_2^{'}}{dt} \tag{3.10}$$

Considering loop ABCDFHIJA, one can obtain,

$$\frac{2}{C_m}\int i_1 dt + i_1^{'}\left(R_{C_1} + R_n + R_{C_3}\right) + \frac{2}{C_n}\int i_1^{'}dt = V \Rightarrow \frac{2}{C_m}i_1 + \left(R_{C_1} + R_n + R_{C_2}\right)\frac{di_1^{'}}{dt} + \frac{2i_1^{'}}{C_n} = 0 \tag{3.11}$$

Considering loop ABCEGHIJA, one can obtain,

$$2i_2R_m + 2E_m + i_2^{'}R_{C_2} = V \Rightarrow 2R_m\frac{di_2}{dt} + R_{C_2}\frac{di_2^{'}}{dt} = 0 \tag{3.12}$$

Considering these linearly independent equations and using eq. (3.1), through substitution, a linear homogeneous second order differential equation can be obtained in $i_1^{'}$ as,

$$\left[\frac{C_m(R_{C_1} + R_n + R_{C_3})}{2}\right]\frac{d^2i_1^{'}}{dt^2} + \left[1 + \frac{(R_{C_1} + R_n + R_{C_3})}{R_{C_2}} + \frac{(R_{C_1} + R_n + R_{C_3})}{2R_m} + \frac{C_m}{C_n}\right]\frac{di_1^{'}}{dt}$$
$$+\left[\frac{2}{C_nR_{C_2}} + \frac{1}{C_mR_m}\right]i_1^{'} = 0 \tag{3.13}$$

A similar analysis yields the value of time constant for model 2 [Fig. 3.1(c)] as,

$\tau_1 = 4.801 \times 10^{-6} s, \tau_2 = 1.815 \times 10^{-6} s$

From the above equations, it can be suggested that the time constant values are dependent upon various cellular parameters such as radius of cell, resistance of cytoplasm and nucleoplasm, resistance and capacitance of cell and nuclear membranes. Using MATLAB simulations, the variation of time constant has been studied as a function of various cellular parameters and its effect on cellular adaptation processes. On evaluating the values of time constants, it can be said that cells promptly respond to the applied external field.

3.3.1. Model implications

Fig. 3.2 illustrates the variation of time constant with cell size for different radius ratios of nucleus to cell. In all the cases, the time constant is observed to vary linearly with cell size. In addition, it is observed that as the ratio of nucleus to cell size increases, the steepness of the curve increases. It can also be observed that in case of model 2, as ratio increases there is comparatively larger increase in the steepness of the curves with that of model 1 [Fig. 3.2(a) & (b)]. However, the curves designate that cells with different shape and size of the nucleus responds differently under the applied electrical stimulus. Cells under internal/external stimulus change their size (atrophy and hypertrophy) as well as other electrophysical parameters (such as induced transmembrane potential, nuclear size, etc.), which are generally called as cellular adaptation processes. Cells also undergo size change during the interaction with the biomaterial surfaces.[23] Therefore, the variation of time constant with cell size indicates that there would be different responses of cell as their size varies under the applied stimulus.

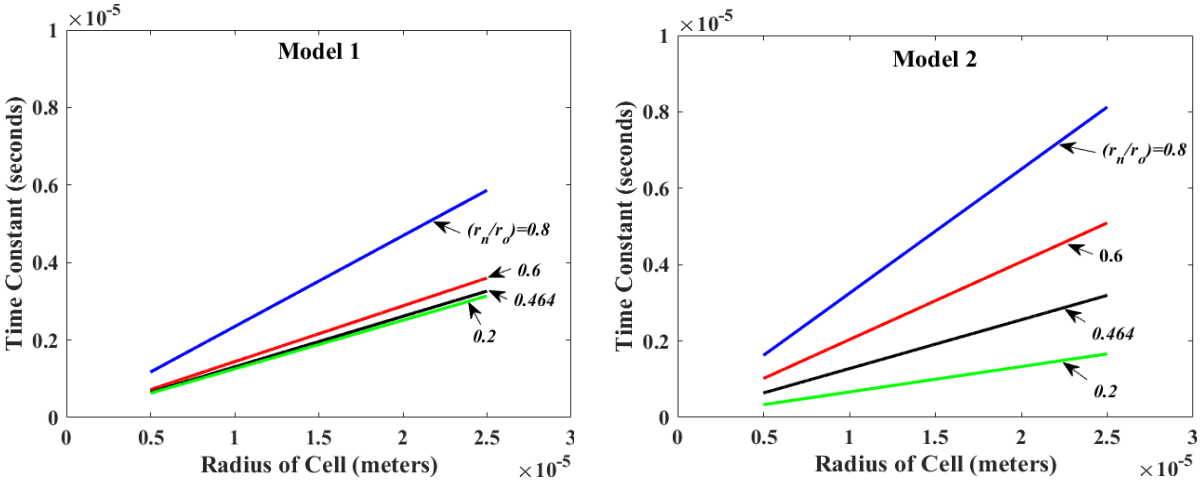


Fig. 3.2: Variation of time constant with cell size at a few selected nucleus to cell size (r_n/r_o) ratios.

The variation of time constant with cell and nuclear membrane capacitances is as shown in Fig. 3.3. The variations of both the parameters are linear with time constant, however, the nuclear membrane capacitance is observed to vary more steeply than cell membrane capacitance. This is indicative of the charging behaviour of both the membranes i.e., nuclear membrane charges more promptly than cell membrane. In addition, large sized cells would have larger capacitance and therefore, their response is slow under the applied electrical stimulus. Physically, time constant is the duration for which the entire cell (interior and exterior) is exposed to the external E-field. It can also be suggested that the time constant is the duration of the pulse, required to observe the measurable effect on the cell such as pore formation, increased conductance through membrane etc. This time is similar to the charging of cell and nuclear membrane capacitances as a whole. Therefore, nucleus with small size as compared to entire cell, charges more rapidly than cell membrane.

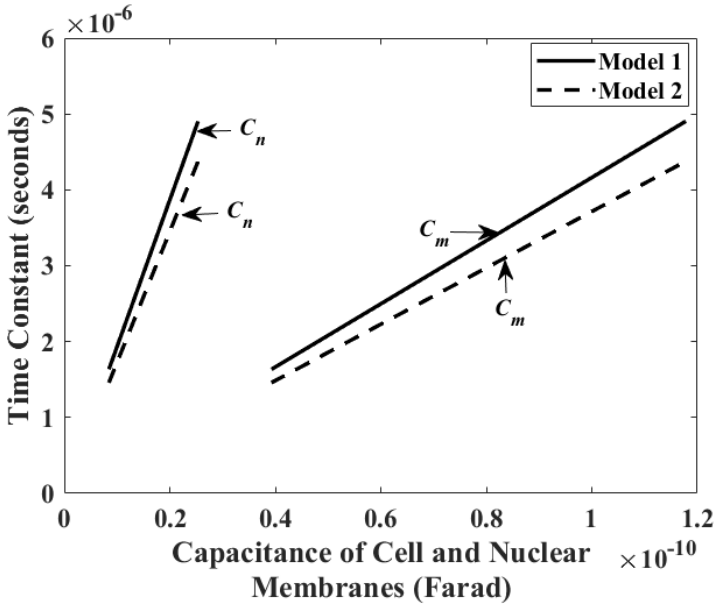
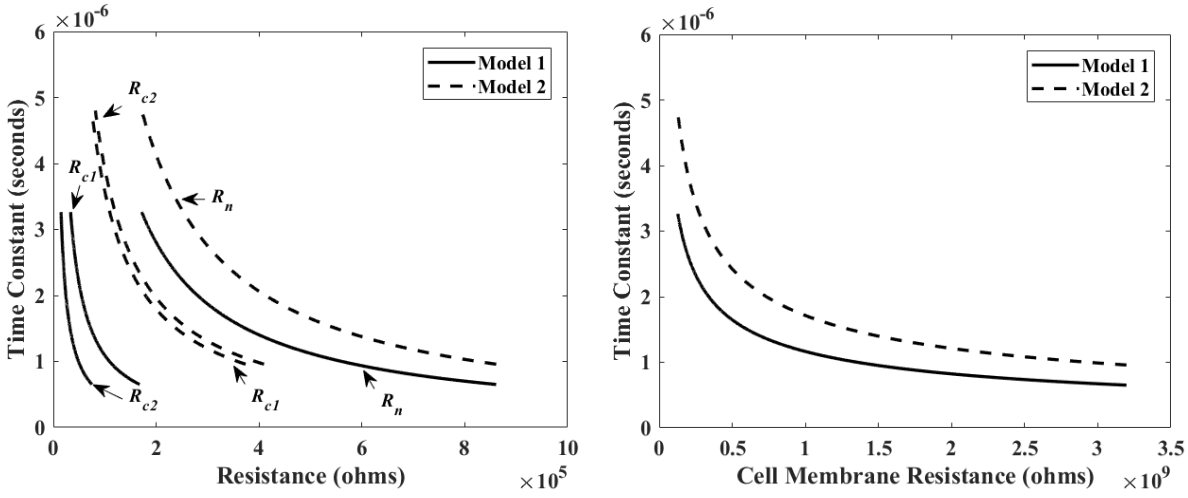


Fig. 3.3: Variation of time constant with capacitance of cell and nuclear membranes for models 1 and 2.

Fig. 3.3 is plotted by considering the radius of the cell to be constant and varying the capacitance per unit area. The capacitance per unit area is directly proportional to the induced transmembrane potential. Therefore, different cell types with varying capacitance per unit area can have different induced transmembrane potential due to the applied external electrical stimulus. The transmembrane potential of cancerous cells differ from that of normal cells, therefore, E-field response is expected to be different for both the cell types.[24] It has also been reported that, based on the difference in transmembrane potentials between cancerous cells and normal cells, E-field can normalise cell proliferation.[25] In addition, properly tuned E-field parameters can kill the cancer cells selectively without affecting the normal cells within the same tissue.[12]



a) **b)**

Fig. 3.4: Variation of time constant with a) resistances of cytoplasm and nucleoplasm and b) cell membrane resistance.

Fig. 3.4(a) depicts the variation of time constant with resistances of cytoplasm and nucleoplasm, which is illustrated to be nonlinear in nature. The hyperbolic nature of the curve indicates that as the resistances of various volumetric regions of the cytoplasm and nucleoplasm increase, time constant decreases. The cytoplasmic resistances depict steeper behaviour as compared to nucleoplasmic resistance. This indicates the influence of cytoplasmic and nucleoplasmic resistances on response of the cells under the applied E-field. The variation of time constant with cell membrane resistance is illustrated in Fig. 3.4(b). The non-linearity of the curve indicates that cells with higher membrane resistance respond rapidly under the applied electrical stimulus. Higher membrane resistance is analogous to the superior dielectric characteristics. The time constant is analysed with the variation of different parameters of the cell based on the simple electrical equivalent circuit. The passive electrical components are used to represent the characteristics of the different parts of the cell. The combination of resistance and capacitance represents the heterogeneous electrical properties of the cell membrane while resistance represents the conductive nature of the fluids, present inside the cell. The electrical equivalent model is validated using well

established electroporation technique. The E-field required to electroporate a living cell is computed using the predicted models and the results have been compared with the experimental values.

3.3.2. Evaluation of the E-field, necessary to electroporate a cell

Under the applied E-field, structural changes in the membrane take place resulting in increased permeability (pore formation) of the cell membrane. This phenomenon is generally known as electroporation, as mentioned above. Various non-linear phenomena take place under the applied E-field but overall the gradual depolarization of the membrane creates pores. These pores are created at the instant when E-field is applied, however, they are observable when the potential of the membrane reaches to the critical value. The exact threshold potential of cell membrane is hard to determine, therefore, the approximate range of the critical potential is specified in literature. Electroporation of any cell depends on its size, orientation with respect to the field and E-field parameters such as strength, pulse duration and number of applied pulses.[12] However, the cell response is dominantly dependent on the strength of the field and its duration.[26] In addition, parameters such as, capacitance per unit area of cell and nuclear membranes as well as impedance of conductive fluid and membranes play a crucial role in deciding the E-field parameters for electroporation to occur. The E-field strength, required to electroporate the cell, has been computed using models 1and 2 [Figs.3.1 (b) & (c)] as, discussed below,

For Model 1

Using eq. 3.7, one can obtain,

$i_1^{'} = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}$, assuming the initial condition as, $i_1^{'}(0) = 0$, one can get A_1 = -A_2 and by substitution in eq. 3.7, one can obtain,

$$i_1^{'} = A_1 e^{-k_1 t} - A_1 e^{-k_2 t} \tag{3.14}$$

From the Fig. 3.1(b), we have, $I = i_1^{'} + i_2^{'}$, by substituting $i_1^{'}$ and $i_2^{'}$, one can obtain,

As *I* is a time dependent function, i.e., *I = i(t)*,

$$i(t) = \left(\frac{R_n}{R_{C_2}} - \frac{2}{C_n R_{C_2} k_1} + 1 \right) A_1 e^{-k_1 t} + \left(\frac{2}{C_n R_{C_2} k_2} - \frac{R_n}{R_{C_2}} - 1 \right) A_1 e^{-k_2 t} \tag{3.15}$$

Applying a constant voltage, *V* for particular time duration t_o (pulse duration), therefore from loop eq. (3.4) one can get,

$$V_{E-Field} = \frac{2}{C_m} \int_0^{t_o} i_2(t)dt + \frac{2}{C_n} \int_0^{t_o} i_1^{'}(t)dt + i(t_o)\left(R_{C_1} + R_{C_3}\right) + i_1^{'}(t_o) R_n \tag{3.16}$$

Considering loop ABCDJKEFGHA, expression for i_2 can be written as,

$$i_2 = -\frac{C_m}{2}\left(R_{C_1} + R_{C_3}\right)\frac{di}{dt} - \frac{C_m R_{C_1}}{2}\frac{di_2^{'}}{dt} \tag{3.17}$$

Using eq. (3.14), (3.15), (3.16) and (3.17), one can obtain,

$$A_1 = \frac{V_{E-Field}}{C_o} \tag{3.18}$$

Where C_o is,

$$\begin{aligned} C_0 = & \left[k_1(R_{C_1} + R_{C_3}) + \frac{R_n k_1 (R_{C_1} + R_{C_3})}{R_{C_2}} - \frac{2(R_{C_1} + R_{C_3})}{C_n R_{C_2}} - \frac{2}{C_n} + R_n k_1 \right] \left[\frac{e^{-k_1 t_0}}{-k_1} - \frac{1}{k_1} \right] + \\ & \left[\frac{2(R_{C_1} + R_{C_3})}{C_n R_{C_2}} - \frac{R_n (R_{C_1} + R_{C_3})}{R_{C_2}} - k_2 (R_{C_1} + R_{C_3}) + \frac{2}{C_n} - R_n k_2 \right] \left[\frac{e^{-k_2 t_0}}{-k_2} + \frac{1}{k_2} \right] \\ & + \frac{2}{C_n} \left[\frac{e^{-k_1 t_0}}{-k_1} + \frac{e^{-k_2 t_0}}{k_2} + \frac{1}{k_1} - \frac{1}{k_1} \right] + (R_{C_1} + R_{C_3}) \left[\left[1 + \frac{R_n}{R_{C_2}} - \frac{2}{C_n R_{C_2} k_1} \right] e^{-k_1 t_0} + \right. \\ & \left. \left[\frac{2}{C_n R_{C_2} k_2} - \frac{R_n}{R_{C_2}} - 1 \right] e^{-k_2 t_0} \right] e^{-k_2 t_0} \end{aligned} \tag{3.19}$$

Where, $\tau_1 = \frac{1}{k_1}, \tau_2 = \frac{1}{k_2}$

From model 1 [Fig. 3.1(b)], the induced transmembrane potential can be written as,

$$V_m = \frac{1}{C_m} \int_0^{t_o} i_2 dt \tag{3.20}$$

Using expression for V_m, one can get,

$$V_m = \frac{V_{E-Field}}{C_o}\left(\overbrace{\left(\left[\frac{k_1(R_{C_1}+R_{C_3})}{2}+\frac{R_n k_1(R_{C_1}+R_{C_3})}{2R_{C_2}}-\frac{(R_{C_1}+R_{C_3})}{C_n R_{C_2}}-\frac{1}{C_n}+\frac{R_n k_1}{2}\right]\right)}^{\alpha}\left[\frac{e^{-k_1 t}}{-k_1}\right]_0^{t_o}+\underbrace{\left(\left[\frac{(R_{C_1}+R_{C_3})}{C_n R_{C_2}}-\frac{R_n(R_{C_1}+R_{C_3})}{2R_{C_2}}-\frac{k_2(R_{C_1}+R_{C_3})}{2}+\frac{1}{C_n}-\frac{R_n k_2}{2}\right]\right)}_{\beta}\left[\frac{e^{-k_2 t}}{-k_2}\right]_0^{t_0}\right) \quad (3.21)$$

Electric field can be evaluated using, $E = \frac{V_{E-Field}}{2R_{cell}}$, where R_{cell} is the radius of the cell, therefore using eq. (3.21) one can obtain,

$$E = \frac{V_m C_o}{2R_{cell}(\alpha\tau_1 + \beta\tau_2 - \alpha\tau_1 e^{-t_0/\tau_1} - \beta\tau_2 e^{-t_0/\tau_2})} \quad (3.22)$$

For Model 2,

Similar calculation for model 2 yields the expression of E-field, required to electroporate the cells as,

$$E = \frac{V_m C_0}{R_{cell}\left[\left[(R_{C1}+R_n+R_{C3})+\frac{2\tau_1}{C_n}\right]\left[-\tau_1 e^{-t_0/\tau_1}+\tau_1\right]-\left[(R_{C1}+R_n+R_{C3})-\frac{2\tau_2}{C_n}\right]\left[-\tau_2 e^{-t_0/\tau_2}+\tau_2\right]\right]} \quad (3.23)$$

Where C_0 is**,**

$$\begin{aligned} C_0 = {} & \left[(R_{C1}+R_n+R_{C3})-\frac{2\tau_1}{C_n}\right]\left[\tau_1-\tau_1 e^{-t_0/\tau_1}\right]-\left[(R_{C1}+R_n+R_{C3})-\frac{2\tau_2}{C_n}\right]\left[\tau_2 e^{-t_0/\tau_2}-\tau_2\right] \\ & +\left[(R_{C1}+R_n+R_{C3})-\frac{2\tau_1}{C_n}\right]e^{-t_0/\tau_1}+\left[\frac{2\tau_2}{C_n}-(R_{C1}+R_n+R_{C3})\right]e^{-t_0/\tau_2} \end{aligned} \quad (3.24)$$

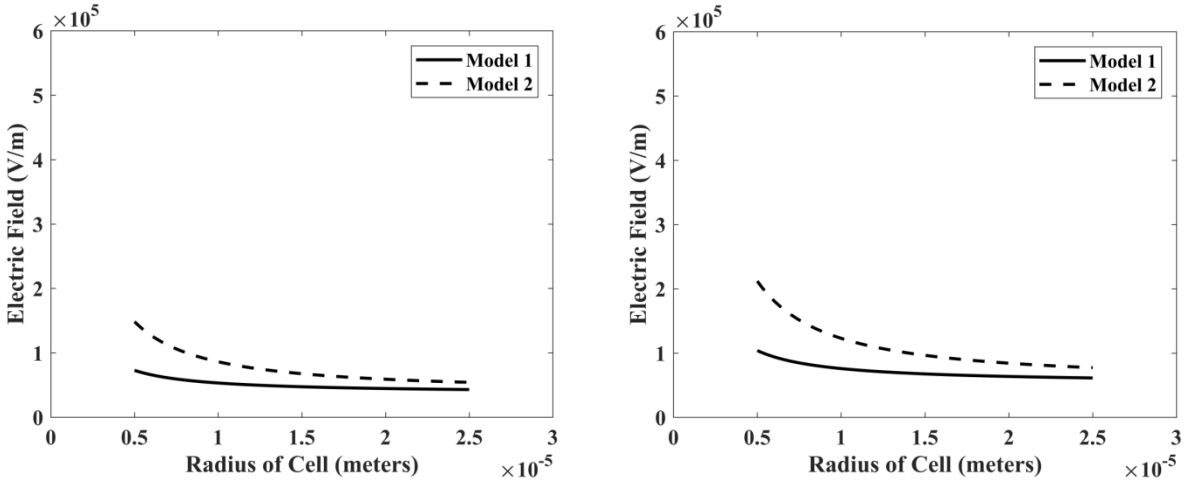
The critical transmembrane potential of the cell varies from 0.7 to 1.5 V.[26,27,28] However, in the present study, 1 V is considered to evaluate the E-field, required for electroporation to take place. The calculated values of E-field strength by considering the cell size to be 25 μm for our model has similar order of magnitude to those of the experimental values (Table 3.2) are given in Table 3.1.

Table 3.1: Strength of E-field, required for onset of electroporation for various pulse durations (cell size: 25 µm).

Model 1		Model 2	
Pulse Duration	**E-field**	**Pulse Duration**	**E-field**
1 ms	0.16 kV/cm	1 ms	0.4 kV/cm
1 µs	0.7 kV/cm	1 µs	0.77 kV/cm
10 ns	62.5 kV/cm	10 ns	52.76 kV/cm
1 ns	624.34 kV/cm	1 ns	525.3 kV/cm

From Fig. 3.5, it is clear that the E-field strength required to electroporate the cell decreases with increase in cell size. It has been experimentally verified that E-field, required to electroporate the cell is inversely proportional to the cell size ($V_m = 1.5\ ER\ cos\theta$, where R and θ are the cell radius and angle between E-field vector and surface area vector, respectively).[29] Schwan's equation suggests[30] that the application of external E-field induces a potential across a simple spherical cell as,

$$V_m = fER\cos\varphi(1-e^{\frac{t}{\tau}}) \tag{3.25}$$



a) **b)**

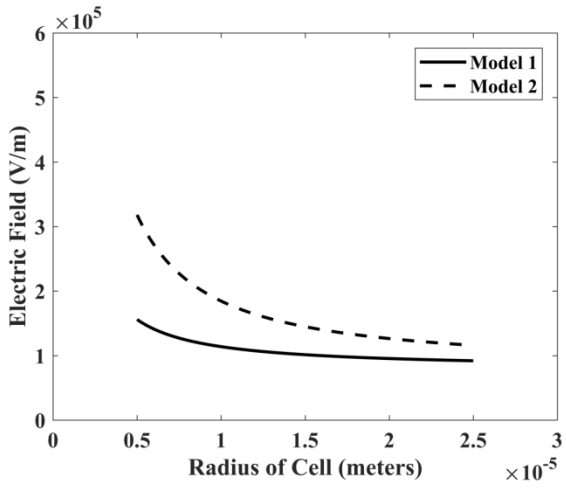


Fig. 3.5: Variation of electric field, required for electroporation, with various critical membrane potentials: a) 0.7 V, b) 1 V and c) 1.5 V.

This equation also reflects the inverse relationship between cell size and E-field, where, *f*, *t* and τ are the shape factor, time elapsed for the onset of E-field as well as time constant for membrane charging, respectively. The cellular adaptation processes such as, atrophy and hypertrophy provide major relevance to the present set of results. It is observed that when the biomaterial is placed in the cell culture medium, proteins get adhered on the surface and therefore, cells first interact to the adhered proteins on the surface and in the process, cells change their shape and size. The cells, adhered on the biomaterial surface would have different electrical response with that of non-adhered cells under the similar electrical stimulus. Therefore, it can be expected that the cell viability on the biomaterial surface can be altered using external electrical stimulation. Fig. 3.5 suggests that for a particular cell size and electrophysical condition, a narrow window of electrical stimulation is required to electroporate the cell. These variations are plotted for the microsecond duration pulses and can be reiterated for the nanosecond pulses as well.

Weaver et al.[26] proposed the E-field strength duration map, which suggests that for conventional (reversible) electroporation, field strength of 0.1 to 100 kV/cm with pulse duration of 1 μs to 1 s is required. Earlier, Neumann et al.[31] reported the absorption of DNA

molecules into mouse lyoma cells under the application of E-field with strength of 8 kV/cm and duration of 5 μs, which is first reported *in vivo* application of electroporation. It is consistent with the suggested E-field strength duration map. In electrochemotherapy (reversible electroporation) as well as necrosis (irreversible electroporation), E-field strength of 1.5 kV/cm with duration of 100 μs are utilized, while for cellular apoptosis (in the case of tumour treatment) large strength E-field (40 kV/cm) of short duration pulses (~ 10 ns) are used.[32] Therefore, considering the E-field strength - duration map, pulse duration in the range of 100 μs to 1 s is indicated for reversible electroporation, applications of which include gene therapy (0.1 kV/cm to 1 kV/cm), electro-chemotherapy (0.1 kV/cm to 1 kV/cm), etc. On the other hand, for irreversible electroporation, E-field duration of 1 ms to 1 s is desirable with the strength ranging from 1 kV/cm to 10 kV/cm. Tissue ablation (1 kV/cm to 10 kV/cm), bacterial decontamination, extraction of biomolecules and necrosis (1 kV/cm to 10 kV/cm) are few of the irreversible electroporation processes.[26] Accordingly, it can be used for various applications of irreversible and reversible electroporation. Consequently, it has been reported that the E-field strength in the range of 4.5 kV/cm to 8.1 kV/cm with pulse duration of 40 μs, leads to apoptosis in Jurkat T-lymphoblasts and HL-60 cells.[33]

Table 3.2 summarizes various experimental (*in vitro* and *in vivo*) reports for the E-field parameters (field strength, pulse duration) utilized to electroporate various type of cells for number of applications. A comparison of the above set of results along with data reported in Table 3.1 for the E-field parameters, required for electroporation to occur with those, calculated from using our electrical equivalent circuits suggest the validity of our models.

Table 3.2: Summary of experimental (*in vitro* and *in vivo*) reports for the E-field parameters (field strength, pulse duration) utilised to electroporate various type of cells for number of applications.

S.No.	E-field	Pulse duration	Type of cells	Application	Reference
1	5-10 kV/cm	5 – 10 µs	Mouse muscle cells	Gene transfer (in vivo)	T-K Wong et al.[34] (1982)
2	5 kV/cm	2 ms	Mouse tumor cells	Administration of blomycein drug to cancerous cells (in vivo)	M.Okino et al.[35] (1987)
3	0.3 kV/cm	1 ms	Rat Skeletal muscle cells	Electrotransfer of DNA (in vivo gene therapy)	L. M. Mir et al.[36] (1999)
4	5.3 MV/m	60 ns	Human eosinophils cells	Intracellular effects [apoptosis induction, gene delivery, etc.] (in vitro)	K.H. Schoenbach et al.[37] (2001)
5	≤ 300 kV/cm	10 – 300 ns	Human Jurkat cells and fibrosarcoma in C57B1/6 mice	Controlled cell death and apoptosis induction (in vitro)	S.J. Beebe et al.[38] (2003)
6	0.8 - 1.3 kV/cm	100 µs	Melanomas, breast, head and neck cancerous cells	Electrochemotherapy (ex-vivo)	A. Gothelf et al.[39] (2003)
7	80 - 240 kV/cm	10 ns	1,2-di-oleoyl-sn-glycero-3-phosphocholine phospholipid vesicles, COS-7 cells	Permeabilization of intracellular vacuoles (in vitro)	E. Tekle et al.[40] (2005)
8	1 kV/cm	20 ms	Liver cells of male Sprague-Dawley rats	Tissue ablation (in vivo)	J.F. Edd et al.[41] (2006)
9	≥ 20 kV/cm	360 ns	Murine melanoma B16-F10 cells	Tumour growth inhibition (in vitro)	R. Nuccitelli et al.[42]
10	320 V- 550 V with electrode gap of 5.7 mm	100 µs	Rat skeletal muscle cells and liver cells	Electrotransfer of DNA (In vivo gene therapy)	D. Cukjati et. al.[43]

	and 4.4 mm				(2007)
11	2.5 kV/cm	100 µs	Tumor cells in mice	Tissue ablation (in vivo)	B A-Sakere et al.[44] (2007)
12	60 kV/cm	60 ns / 300 ns	$HCT116p53^{+/+}$, $HCT116p53^{-/-}$ colon carcinoma cells	Apoptosis (in vitro)	E.H. Hall et al.[45] (2007)
13	80 kV/cm	1 µs	Bacteria found in wastewater (pseudomonas aeruginosa, enterococcus faecium)	Bacterial Inactivation (in vivo)	A. Rieder et al.[46] (2008)
14	25 - 100 kV/cm	60 ns	Jurkat cells	Regulation of intracellular calcium ions (in vitro)	S.S. Scarlett et al.[47] (2009)
15	0 - 60 kV/cm	300 ns	E4 squamous carcinoma cells	Apoptosis induction (in vitro)	W.Ren et al.[48] (2011)
16	Combination of high and low voltage pulses (500 V and 45 V with 0.7 cm electrode gap)	500 µs and 250 ms	Dermatomed pig cells	Transdermal Drug delivery (in vivo)	B. Zorec et al.[49] (2013)
17	1.3 kV/cm	50 – 50 µs (biphasic pulse)	Human alveolar basal epithelial carcinoma cell line A549	Electrochemotherapy (in vitro)	EP Spugnini et al.[50] (2014)
18	3 kV with 2.5 cm electrode gap	70 - 80 µs	Pancreatic adenocarcinoma cancerous cells	Tumour ablation (in vivo)	J.-P. Tasu et al.[51] (2016)
19	0.5 kV/cm, 1.2 kV/cm and 35 kV/cm	100 µs, 240 µs and 60 ns	MDA-MB-231 human breast cancer cells	Cancer cells viability tests, electroporation and electrochemotherapy (in vitro)	L.Mittal et al.[52] (2017)
20	0.1 - 0.9 kV/cm	100 µs	Human Mesenchymal stem cells	Control of Differentiation and proliferation due to Ca^{2+} oscillations (in vitro)	H. Hanna et al.[53] (2017)
21	32 - 64 V/mm	50 ms	Human T	Transfection	D.J.Im et al.[54]

			lymphoma cell line derived from T cell leukaemia (Jurkat cells)	(in vitro)	(2017)
22	400 V with linear array of needle electrodes	0.1 ms (8 pulses with 5 kHz frequency)	Cutaneous metastases from breast cancer and malignant melanoma	Calcium electroporation with electrochemotherapy (in vivo)	H. Falk et al.[55] (2018)
23	10 kV Electrode gap of 3mm - 6mm	60 ns	Osteoblast like cells (MG63) and cardiomyocytes	Calcium mobilization leading to new bone formation (in vitro)	P Zhou et al.[56] (2018)
24	1.5 kV/cm	90 µs	Hepato-pancreatic obiliary tumour cells	Tumour ablation (in vivo)	A.H. Ruarus et al.[57] (2018)
25	0.4 - 1.2 kV/cm	100 µs	Human malignant melanoma cell lines (Me45 and MeWo)	Inhibition of Tumour growth (in vivo)	A. Choromanska et al.[58] (2018)

The above results are computed based on the consideration of only cell membrane acting as leaky dielectric and nuclear membrane as perfect dielectric. However, nuclear membrane consists of pores along with bilipid layer which allows selective molecules to pass through it. Therefore, additional equivalent circuits have been designed by considering the nuclear membrane as leaky dielectric. Further, the time constant for the electrical equivalent circuits have been analytically computed and the implication of these models in terms various cellular adaptation processes have been discussed.

3.3.3. Evaluation of time constant by considering nuclear membrane as leaky dielectric

From Fig. 3.6(a) [Model 3], one can obtain the linearly independent loop equations as,

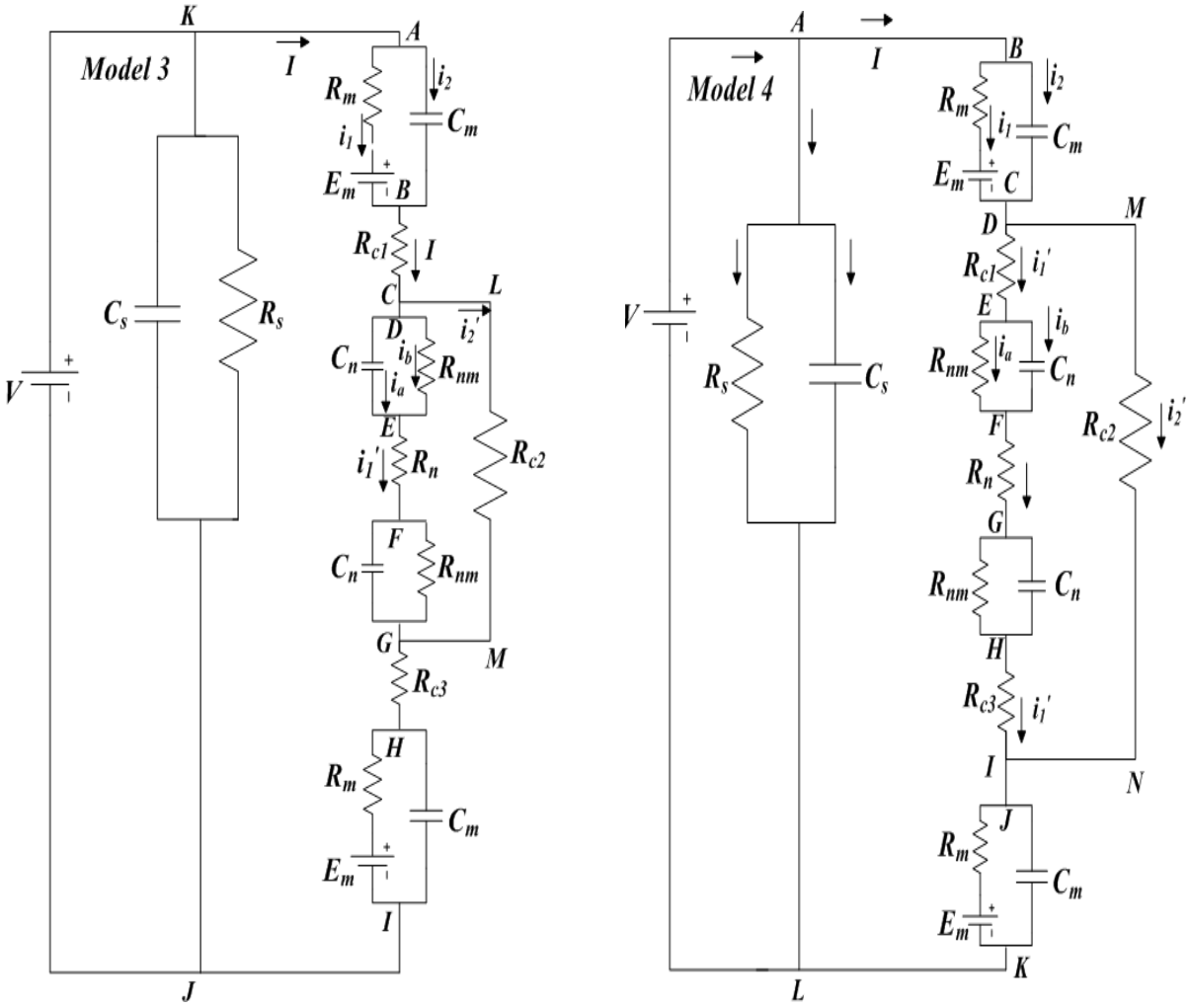


Fig. 3.6: Electrical equivalent circuit of single living cell by considering cell and nuclear membranes as leaky dielectrics. (a) and (b) refer to different ionic paths.

$$I = i_1 + i_2 = i_1^{'} + i_2^{'} \Rightarrow \frac{dI}{dt} = \frac{di_1}{dt} + \frac{di_2}{dt} = \frac{di_1^{'}}{dt} + \frac{di_2^{'}}{dt} \tag{3.26}$$

$$i_1^{'} = i_a + i_b \Rightarrow \frac{di_1^{'}}{dt} = \frac{di_a}{dt} + \frac{di_b}{dt} \tag{3.27}$$

Considering loop ABA, one can obtain,

$$i_1 R_m + E_m = \frac{1}{C_m}\int i_2 dt \Rightarrow \frac{di_1}{dt} = \frac{i_2}{R_m C_m} \tag{3.28}$$

Considering loop DED, one can obtain,

$$\frac{1}{C_n}\int i_a dt = i_b R_{nm} \Rightarrow \frac{di_b}{dt} = \frac{i_a}{C_n R_{nm}} \tag{3.29}$$

Considering loop CLMGFEDC, one can obtain,

$$2i_b R_{nm} + i_1^{'} R_n = i_2^{'} R_{C_2} \Rightarrow \frac{di_2^{'}}{dt} = \frac{2R_{nm}}{R_{C_2}}\frac{di_b}{dt} + \frac{R_n}{R_{C_2}}\frac{di_1^{'}}{dt} \tag{3.30}$$

Considering loop KABCLMGHIJK, one can obtain,

$$V = 2i_1 R_m + 2E_m + I\left(R_{C_1} + R_{C_3}\right) + i_2^{'} R_{C_2} \Rightarrow 2R_m \frac{di_1}{dt} + \left(R_{C_1} + R_{C_2}\right)\frac{dI}{dt} + R_{C_2}\frac{di_2^{'}}{dt} = 0 \tag{3.31}$$

Considering loop KABCDEFGHIJK, one can obtain,

$$V = \frac{2}{C_m}\int i_2 dt + I\left(R_{C_1} + R_{C_3}\right) + \frac{2}{C_n}\int i_a dt + i_1^{'} R_n \Rightarrow \frac{2i_2}{C_m} + \left(R_{C_1} + R_{C_3}\right)\frac{dI}{dt} + \frac{2i_a}{C_n} + R_n\frac{di_1^{'}}{dt} = 0 \tag{3.32}$$

Using eq. (3.26) - (3.32) third order linear homogeneous differential equation in i_b is obtained as,

$$\Rightarrow \left(\frac{C_m R_{nm} C_n (R_{C1} + R_{C3})}{2} + \frac{C_m C_n R_{nm} R_n (R_{C1} + R_{C3})}{2R_{C2}} + \frac{R_n C_m R_{nm} C_n}{2}\right)\frac{d^3 i_b}{dt^3} +$$

$$\left(\begin{array}{l} \frac{R_{nm} C_n (R_{C1} + R_{C3})}{2R_m} + \frac{R_n R_{nm} C_n (R_{C1} + R_{C3})}{2R_m R_{C2}} + \frac{R_n R_{nm} C_n}{2R_m} + \frac{C_m (R_{C1} + R_{C3})}{2} + \frac{C_m R_{nm} (R_{C1} + R_{C3})}{R_{C2}} \\ + \frac{C_m R_n (R_{C1} + R_{C3})}{2R_{C2}} + C_m R_{nm} + \frac{R_n C_m}{2} + R_{nm} C_n + \frac{R_{nm} R_n C_n}{R_{C2}} \end{array}\right)\frac{d^2 i_b}{dt^2}$$

$$+\left(\frac{(R_{C1} + R_{C3})}{2R_n} + \frac{R_{nm}(R_{C1} + R_{C3})}{R_m R_{C2}} + \frac{R_n (R_{C1} + R_{C3})}{2R_m R_{C2}} + \frac{R_{nm}}{R_m} + \frac{R_n}{2R_m} + 1 + \frac{2R_{nm}}{R_{C2}} + \frac{R_n}{R_{C2}}\right)\frac{di_b}{dt} = 0 \tag{3.33}$$

The solution of eq. (3.33) is of the form as,

$$i_b = A_1 + A_2 e^{-k_1 t} + A_3 e^{-k_2 t} \tag{3.34}$$

The nuclear membrane resistance is evaluated using similar membrane resistivity value as that of the cell membrane and it is,

$R_{nm} = 591390.25\,\text{k}\Omega$

Using the numerical values of various parts of cytoplasmic and nucleoplasmic resistances as well as cell and nuclear membrane capacitances and resistances of cell and nuclear membranes, the numerical value of the time constant for model 3 can be obtained as,

$\tau_1 = 3.26\times10^{-6}s, \tau_2 = 1.535\times10^{-6}s$

Similarly, for model 4 [Fig. 3.6(b)], third order linear homogeneous differential equation in i_a is analytically computed as,

$$\Rightarrow\left[\frac{C_m C_n R_{nm}(R_{C1}+R_n+R_{C3})}{2}\right]\frac{d^3 i_a}{dt^3}+\left[\begin{array}{l}\frac{R_{nm}C_n(R_{C1}+R_n+R_{C3})}{2R_m}+\frac{C_m(R_{C1}+R_n+R_{C3})}{2}+C_m R_{nm}+C_n R_{nm}\\ +\frac{R_{nm}C_n(R_{C1}+R_n+R_{C3})}{R_{C2}}\end{array}\right]\frac{d^2 i_a}{dt^2}$$
$$+\left[\frac{R_{nm}}{R_m}+\frac{(R_{C1}+R_n+R_{C3})}{2R_m}+1+\frac{(R_{C1}+R_n+R_{C3})}{R_{C2}}+\frac{2R_{nm}}{R_{C2}}\right]\frac{di_a}{dt}=0 \quad (3.35)$$

Finally, the time constant values are obtained as,

$\tau_1 = 4.801\times10^{-6}s, \tau_2 = 1.815\times10^{-6}s$

The values of time constants are similar to those for the models 1 and 2 [Fig. 3.1 (b) and (c)]. It is because the resistance of nuclear membrane is very high as compared to cell membrane resistance, therefore, nuclear membrane can be considered as a good dielectric. The variation of various cellular adaptation processes for model 3 and 4 are plotted as Fig. 3.7. It is observed that the characteristics of the plot are similar with that of the models 1 and 2 [Figs. 3.2-3.4]. Therefore, it is suggested that the nuclear membrane resistance does not have significant influence on these adaptation processes.

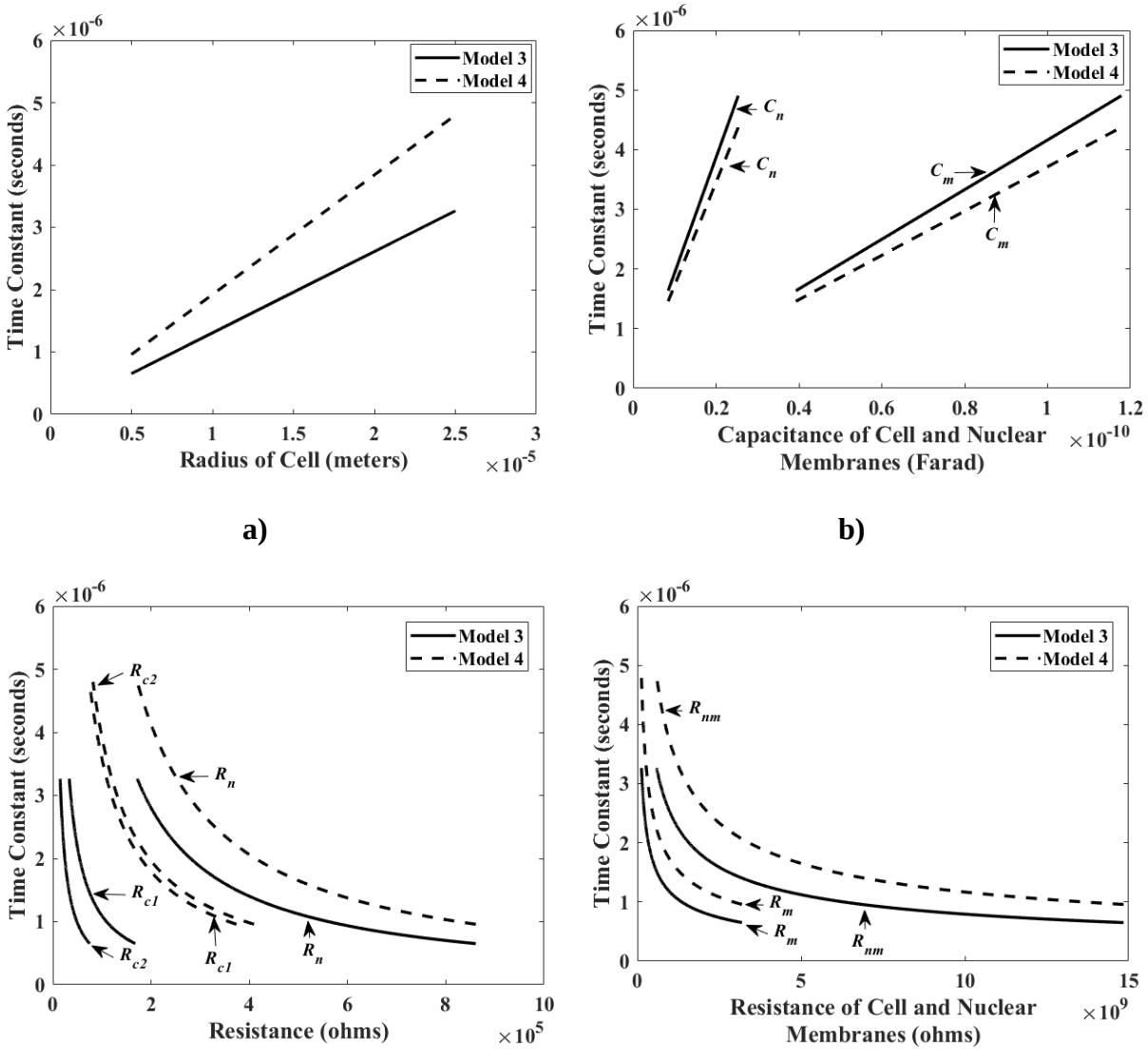
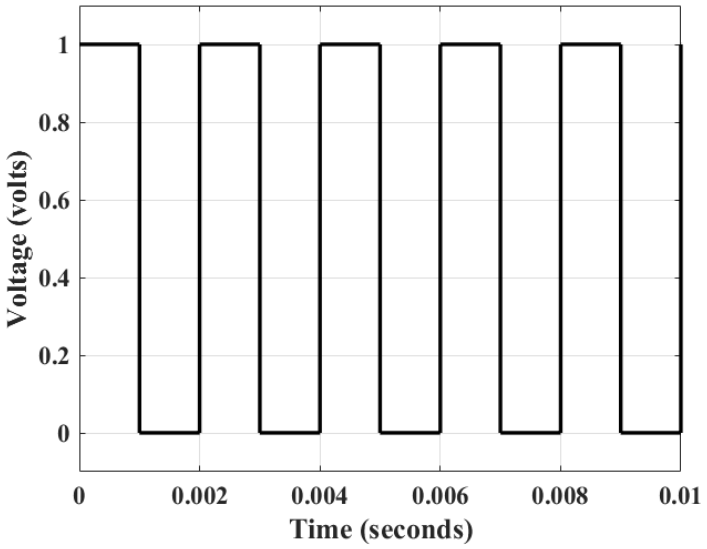


Fig. 3.7: Variation of time constant with a) cell size, b) capacitances of cell and nuclear membranes, c) cytoplasmic and nucleoplasmic resistances and d) resistances of cell and nuclear membranes

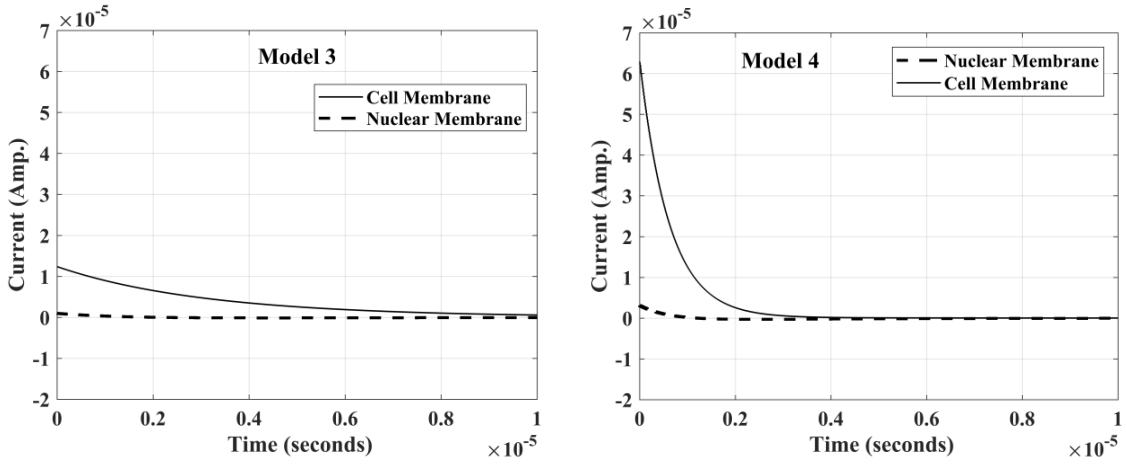
3.3.4. Current and voltage, induced across the cell and nuclear membranes

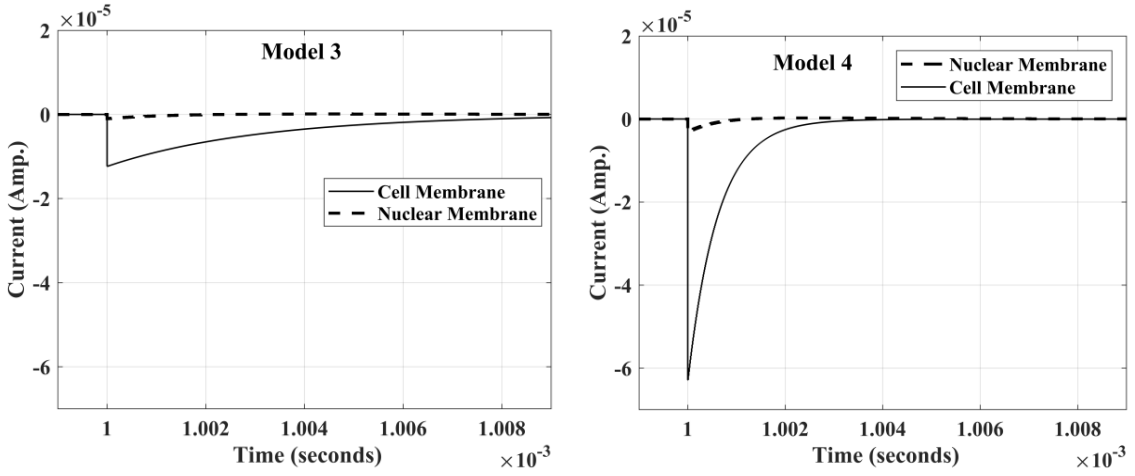
Fig. 3.8 illustrates the current and voltage, induced across cell and nuclear membranes due to application of external stimulus with strength and duration of 1 V and 1 ms, respectively, for models 3 and 4. The flow of current through both the membranes is analysed to be in microampere range. The sharp rise and gradual fall in the current across both the membranes is obtained due to the step response of the current through RC network. On the other hand,

gradual rise and fall in voltage across both the membranes suggest a simple capacitive behaviour. Higher nuclear membrane resistance offers lower current across it as compared to that in cell membrane. Fig. 3.9 depicts the current and voltage across cell and nuclear membranes for models 3 and 4 after the application of pulsed voltage (1 V) with 1 µs duration which is of the similar order of magnitude to that of the time constant. The current and voltage, induced across the cell membrane shows a more profound effect as compared to nuclear membrane. Further, Fig. 3.10 demonstrates similar response with nanosecond duration pulses for models 3 and 4. The gradual variation of voltage across both the membranes is due to the bypassing of the capacitive portion of the membranes by nanosecond pulses.

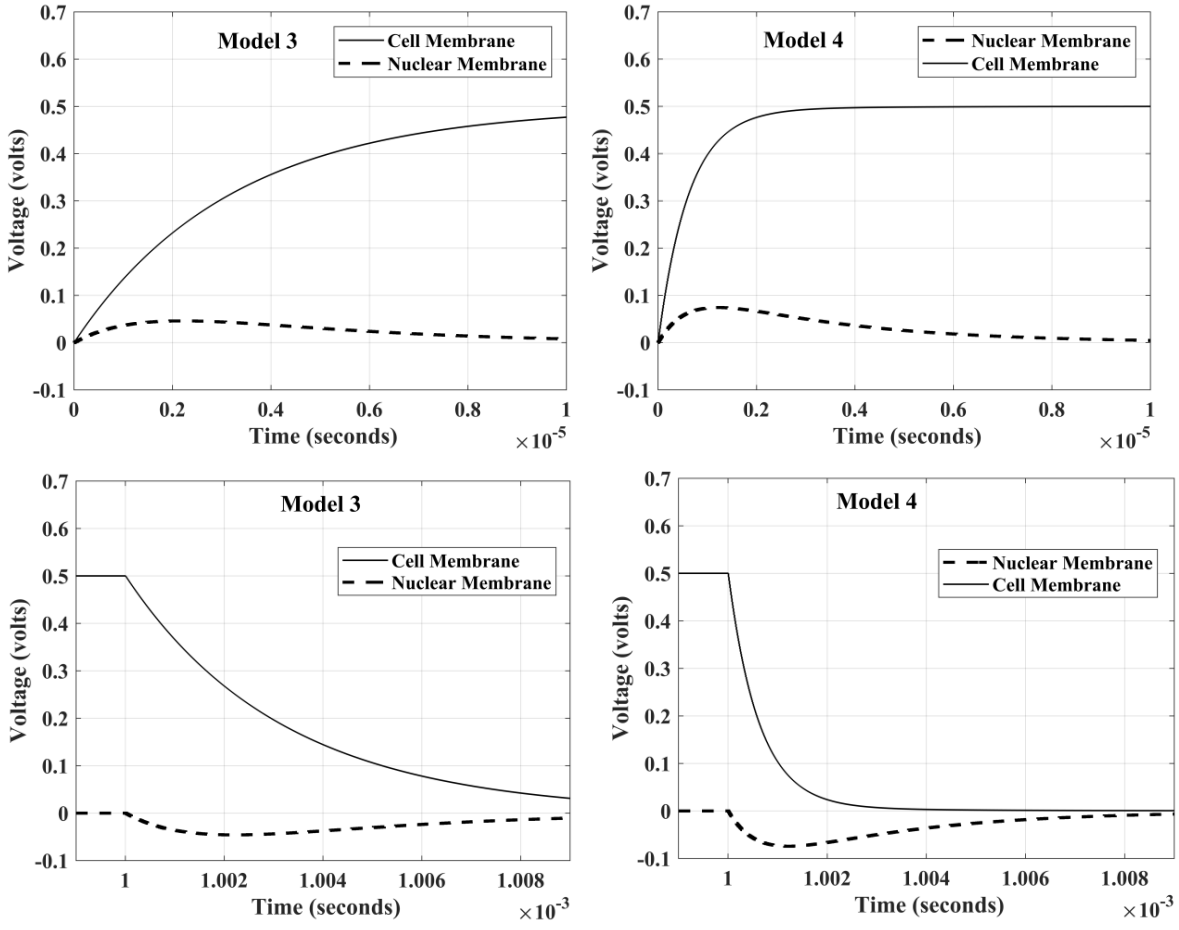


a)



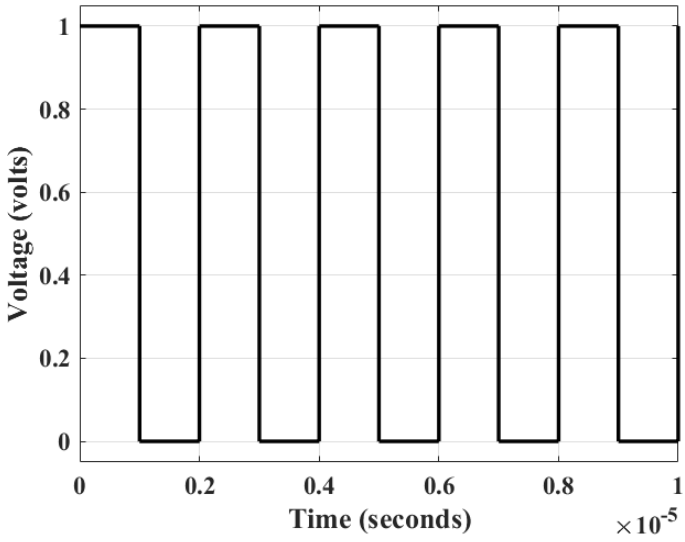


b)

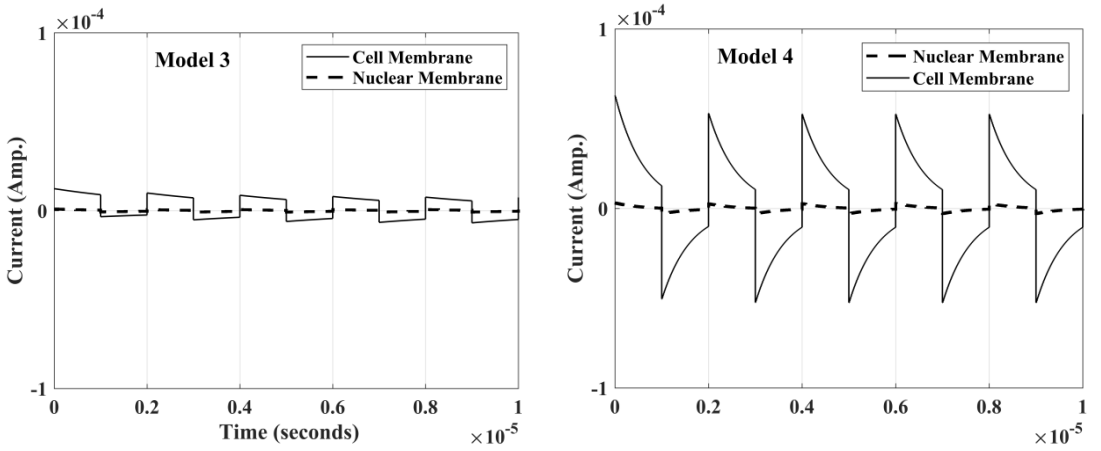


c)

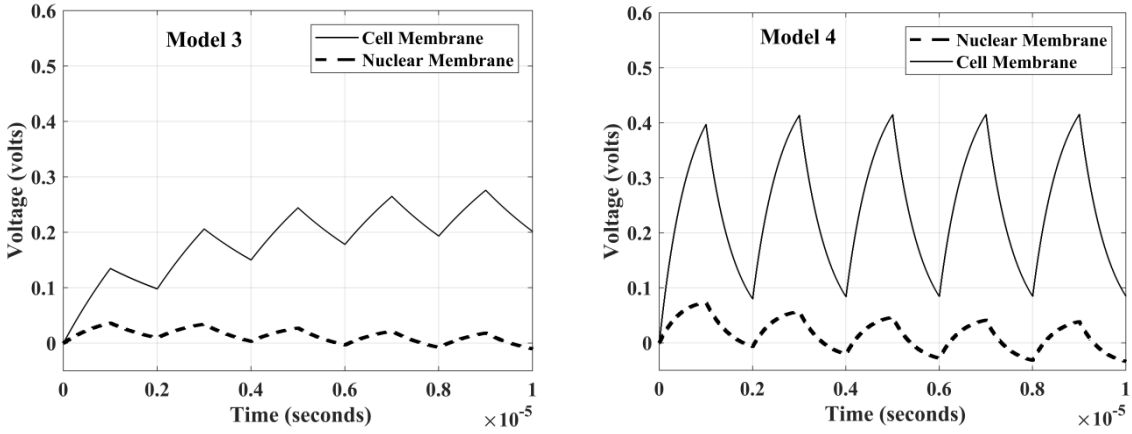
Fig. 3.8: Variation in current (b) and voltage (c) across the cell and nuclear membranes due to application of pulsed stimulation of strength and duration of 1 V and 1 ms (a), respectively.



a)

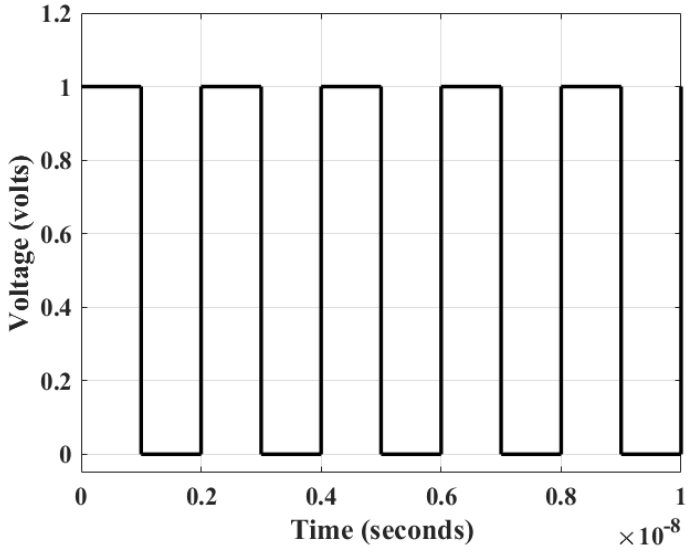


b)

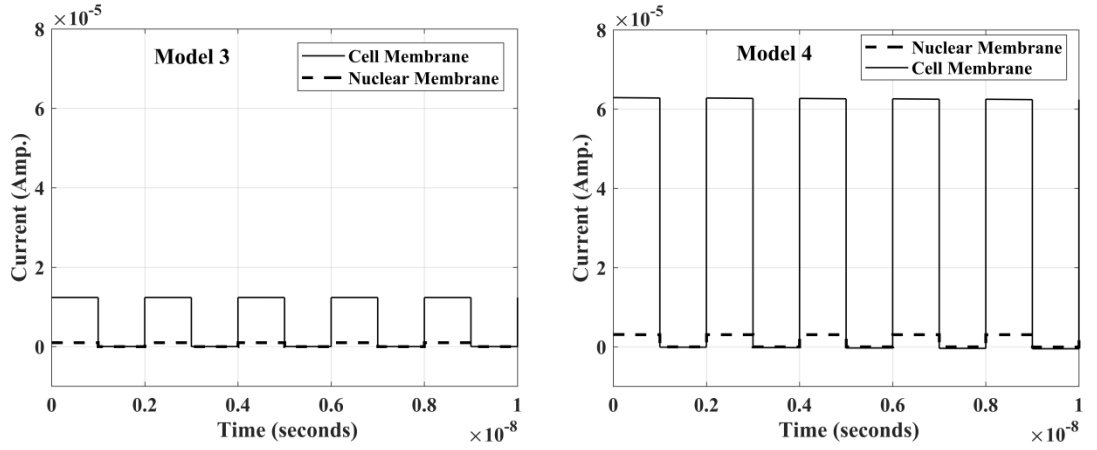


c)

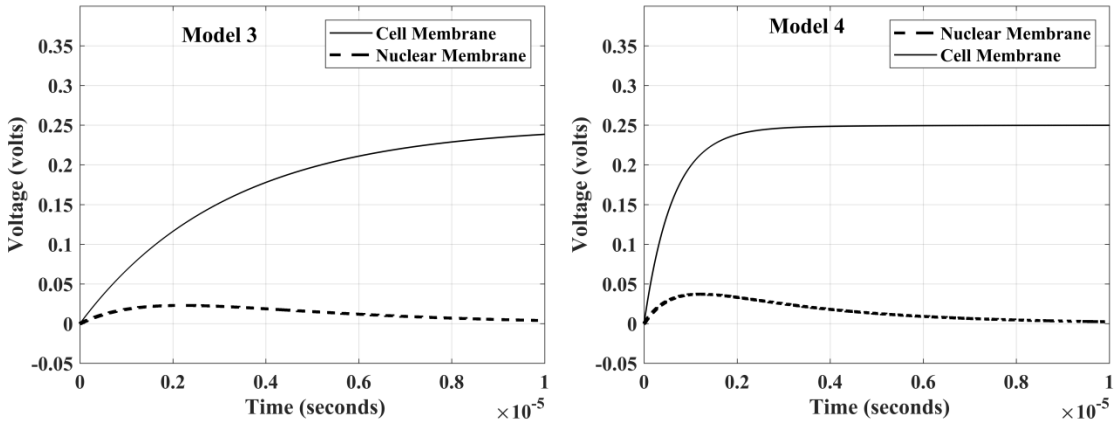
Figure 3.9: Variation in current (b) and voltage (c) across the cell and nuclear membranes due to application of pulsed stimulation of strength and duration of 1 V and 1 μs (a), respectively.



a)



b)



c)

Figure 3.10: Variation in current (b) and voltage (c) across the cell and nuclear membranes due to application of pulsed stimulation of strength and duration of 1 V and 1 ns (a), respectively.

The pulsed voltage being considered here has different rise and fall times. The decrease in the duration of the pulses (1 ms to 1 ns) would subsequently decrease the rise and fall times of the pulses, therefore, the current during these phases would be higher as compared to the constant phase. This is because the current through the capacitive portion of the membranes is directly dependent on the time derivative of the pulsed voltage. As duration of the pulse decreases, the time derivative of the voltage would increase therefore, the current would increase.

When the membranes are charged above the critical voltage (1 V), the present analysis will fail as there would be a nonlinear increase in the conductivity of the membranes. However, this analysis would be valid for transmembrane potential below the critical voltage. The voltage [Fig. 3.8(b), Fig. 3.9(b) and Fig. 3.10(b)] across the cell and nuclear membranes depicts different characteristics for different duration of the applied pulses. It is seen that the voltage induced on the membranes is increasing gradually. Overall, when the varying electric field is applied across the membrane bound structures (cell and nuclear membranes as well as membranes of other organelles), charging of these membranes takes place. This type of capacitive coupling depends on the charging time constant of the membrane. If the characteristic rise time of the E-field pulses is less than the charging time of the membrane, increase in its transmembrane potential takes place. Therefore, if very short duration pulses (ns) are applied to the cell culture medium it would directly affect the subcellular structures.

3.4. Summary

The electrical behaviour of single cell under the applied electrical stimulus is extremely complex. However, the electrical equivalent circuit of the living cell, based on the reasonable characteristics of its structures, provide fundamental insight to the interaction mechanism. In the present study, four electrical equivalent circuits for a single living cell are considered based on the cell and nuclear membrane's conductive properties and an analytical solution of

time constant has been provided. The computed time constant value suggests that the response of the cells to electrical field is in the microsecond range.

The time constant varies linearly with cell size and capacitance of cell and nuclear membranes. However, nonlinear variation with resistances of cytoplasm and nucleoplasm as well as cell and nuclear membranes resistance is observed. The E-field response to living cells is computed to be highly dependent on the electrophysical state of the cell.

The electrical equivalent circuits have been validated using well established electroporation phenomenon. The strength of E-field, required to electroporate a cell, has been evaluated for these circuits which are observed to be consistent with the experimentally reported values. The flow of current and induced voltage across the cell as well as nuclear membranes is strongly dependent on the duration of applied pulses for similar voltage. Overall, the considered models predict the fundamental behaviour of single living cell under the applied electrical stimulus.

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