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A simulation study of the electrical model of a biological cell

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Abstract

This paper presents the results of a simulation study of an electrical model (using resistors and capacitors) for a biological cell. The Cadence Spectre tool, a versatile mixed-signal simulator, used extensively in the semiconductor industry to perform in-depth ac, dc, and transient analyses, was used for this purpose. The response of a cell model at various frequencies, and the effect of cell parameters, such as cell membrane resistance and capacitance, were studied. The results correlate very well with previous research results, which show that at low frequencies—the plasma membrane can be electroporated—while at high frequencies, the induced plasma membrane potential can be much lower than that at low frequencies for the same applied voltage or electric field.

Keywords: Biological cells; Simulation; Electroporation; Cytoplasm; Nucleoplasm; Frequency response

1. Introduction

Charges accumulate at the plasma membrane of a biological cell when a voltage pulse is applied to it, and the induced potential across the membrane is increased [1–3]. Electric field strengths of suitable magnitudes and durations cause molecules

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and molecular organizations such as membranes to undergo structural rearrangements [1]. Depending upon the magnitude and the duration of the voltage, and hence the field applied, the cell membranes can temporarily breakdown creating pores that can be resealed eventually. The membrane potential V_m is given as [1]:

$$V_m = 1.5ER\cos\delta/[(1 + RG_m(\rho_i + 0.5\rho_a))(1/(1 + j\omega T))], \quad (1)$$

where ρ_i and ρ_a are resistivities inside and outside the cell, R is the cell radius, and δ is the angle between the electric field E and the radius vector, ω is the radian frequency $= 2\pi f$, where f is the frequency, T is the time constant, and G_m is the membrane conductance. If we assume that the cytoplasm and medium are purely resistive, then the influence of their dielectric constants can be neglected. If the conductance is also neglected, the membrane potential is given as:

$$V_m = 1.5ER\cos\delta/(1 + j\omega T). \quad (2)$$

The magnitude of Eq. (2) can be expressed as

$$V_m = 1.5ER\cos\delta/(1 + (\omega T)^2)^{1/2}. \quad (3)$$

Eq. (2) can be considered as the general expression for the induced plasma membrane potential at all frequencies. When $\omega T \ll 1$ or $f = 0$, as in the dc case, Eq. (2) becomes the well-known frequency-independent expression [3]

$$V_m = 1.5ER\cos\delta. \quad (4)$$

Whether the applied input is ac or dc, when the applied electric fields are of such a magnitude so as to induce a membrane voltage of about 0.5–1 V, the membrane becomes permeable to macro- and xeno- molecules to which it would otherwise be impermeable [1–4]. Although it is possible to manipulate biological cells with the application of electric fields for practical use, their behavior under electrical conditions is not completely understood. This might be due to their extremely complex structure [1].

With the advent of latest powerful simulation software tools, it is possible to explore the cell behavior in detail without conducting tedious experiments. The purpose of this research is to investigate the behavior of the cells by varying the electrical parameters of the model using an industry-standard simulation tool. This knowledge, which can be used as a complement to experimental analysis, is essential for effective manipulation of cells for practical, real life applications, such as electroporation-mediated gene therapy and enhancement of drug delivery (electrochemotherapy [5]).

2. Simulation tool

For fast and accurate simulation results, we chose the semiconductor, rf, and analog-industry preferred Cadence Spectre Circuit Simulator, product version 4.4.6, released in June 2000 [6]. Spectre is commonly used for complex analog and

mixed-signal circuits [7–13]. In an application, Spectre was used to study the circuit design of an analog/digital spiking neuron with controllable spike delay by a Japanese research group [9]. The design topology of a phase splitter, which was used as an rf building block for the third Industrial Scientific and Medical (ISM) band (5.1–5.9 GHz) wireless local area network, was studied with the use of the Spectre rf Simulator [10]. Similarly, the software was used to investigate the use of on-chip high-precision resistors to terminate computer USB cables [11]. The concept of leakage power reduction using self-biased transistors in VLSI circuits was also studied using the Spectre Circuit Simulator [12].

Spectre can handle high-capacity, SPICE-level simulations at radio frequencies. Accurate time step and convergence specifications reduce the ''time step too small'' error that occurs in most SPICE simulators. The Spectre Simulator incorporates successive convergence methods, hence, fast dc convergence is possible. Spectre has built-in integrated Monte-Carlo analysis, parametric statistical analysis, and dc mismatch analysis. The curve-tracer analysis capability is very helpful for fast model development and also for error correcting. It provides for comprehensive circuit analysis including dc, ac, transient, noise, transfer-function, and sensitivity analyses. The tool can be used in Sun/Solaris, HP-UX, IBM and Linux machines. In this research, the Spectre tool was installed on a Solaris computer and was accessed remotely on a PC using Exceed.

3. Electrical model

For this simulation study, a slightly modified version of the more detailed electrical model of a cell reported by Schoenbach et al. was used [3]. Fig. 1 illustrates the cell model used in this study. Here, the cell was modeled as a homogenous conductive medium (cytoplasm) surrounded by a leaky dielectric membrane. The plasma membrane was modeled as resistors R_{c1} and R_{c3}, together with capacitors C_{m1} and C_{m2}. The interior of the cell substructure, i.e., the nucleus, was modeled as resistor R_n and membrane capacitors C_{n1} and C_{n2}. These nucleus model elements were connected in parallel with the R_{c2}, which represents the conductive cytoplasm.

The values of the resistances and capacitances chosen for cytoplasm and nucleoplasm were estimated using a cubic cell of dimension 10 μm and a nucleus of dimension 5 μm [3]. The resistivities of the cytoplasm and nucleoplasm were assumed to be the same at, 100 Ω cm. The specific capacitance of the outer membrane was 1 $\mu F/cm^2$. The capacitance of the nuclear membrane was assumed to be half that of the outer membrane because two lipid membranes comprise the nuclear envelope, whereas the outer membrane consists of only one [1].

Input voltages of magnitudes between 0.8 and 2 V and 0.5 μs duration were used in the numerical simulation study of electroporation dynamics [14]. An electric field strength of 1300 V/cm was used by earlier researchers for electroporation to occur [4,5]. This value is also used in clinical trials. Consequently, an input voltage of 1 V ac was used to test and simulate the characteristics of a single cell. Assuming the cell

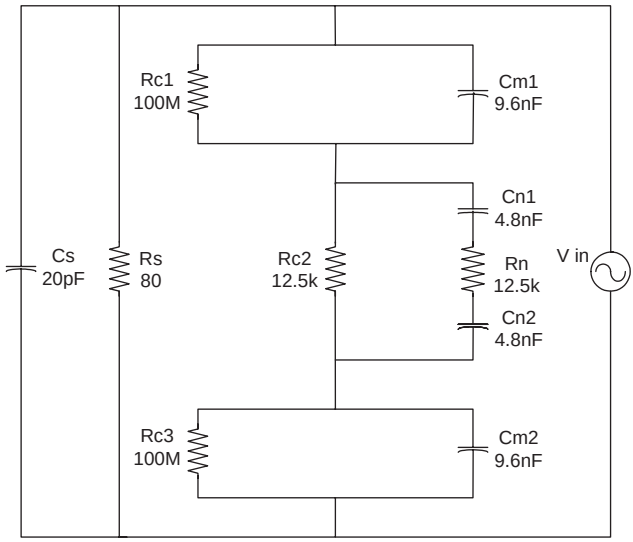

Fig. 1. Electrical model of a cell with nucleus [3]. The suspension medium is represented by a capacitor and a resistor (left). The nucleus is represented by the membrane capacitance C_{n1} and C_{n2} and a resistance R_n (right). The cytoplasm is represented by R_{c2} and the leaky dielectric outer membrane is represented by resistances R_{c1} and R_{c3} and capacitances C_{m1} and C_{m2}.

dimension to be 10 μm, the resulting field strength becomes 1 kV/cm, which is in close correlation with the value of 1300 V/cm used in practice.

An ac analysis was performed on the circuit of Fig. 1. The output response for various input frequencies, and the effect of changing the medium resistances and capacitances were studied.

4. Results and discussion

The transmembrane potential induced in a biological cell depends upon the magnitude, and duration (frequency) of the applied electric field [3]. We studied this phenomenon by varying the frequency of the applied voltage and by performing an ac analysis. Figs. 2–5 show the potentials induced across the outer membrane and the interior of the cell as functions of frequency.

The frequency response of the voltage across the outer cell membrane is shown in Fig. 2. This result correlates well with that reported by a previous research [3]. It exhibits low-pass filter-like characteristics with the cutoff frequency occurring at about 10 kHz. As the frequency was increased, the voltage output at the cell membrane decreased approaching a very small value around 1 MHz. At low frequencies of applied voltage, the outer membrane, which has a large capacitance, is affected. At these frequencies, the induced voltage for a 1 V input was about 0.5 V, which is the threshold for electroporation [2,3].

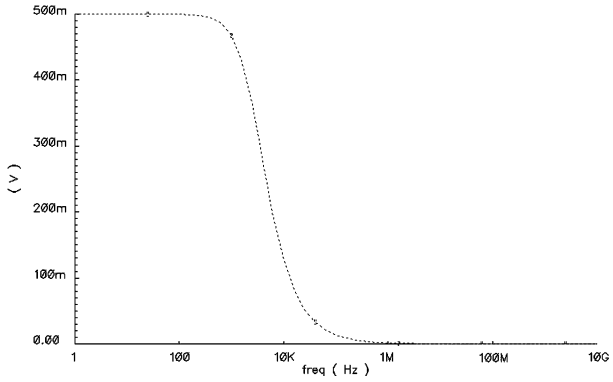

Fig. 2. Frequency response across the outer cell membrane. At low frequencies, the outer plasma membrane with large capacitance is affected. At high-frequency electric field, the outer membrane is shorted and the input voltage is applied cross the inner membrane.

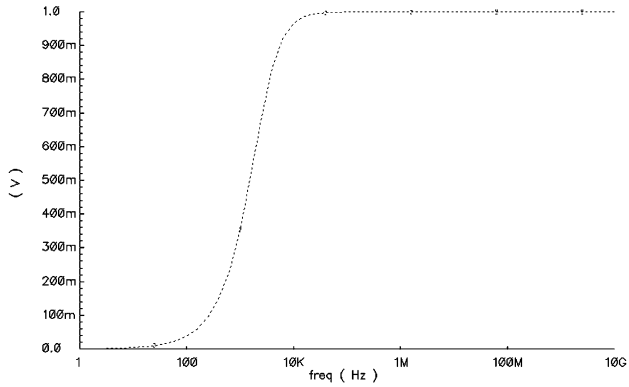

Fig. 3. Frequency response across the interior of the cell. At low frequencies there is no voltage across the nucleus. As the frequency is increased to megahertz range, we can gain control over the nucleus.

The frequency response across the interior of the cell, the nucleus is shown in Fig. 3. It can be seen that at low frequencies, the potential across the interior is very small. As the frequency increases, the outer membrane begins to behave like a short circuit, and the applied input voltage appears mainly across the interior of the cell. Thus, at frequencies in the rf range, the input voltage is applied across the nucleus and other cell components.

Generally, the electric pulses do not affect the intracellular organelles, such as the nucleus, at the low-to-medium frequencies traditionally used for electroporation [1–3]. At low frequencies, the internal field strength is very low. The cell interior is shielded by the capacitive plasma membrane (leaky dielectric). Thus, the outer

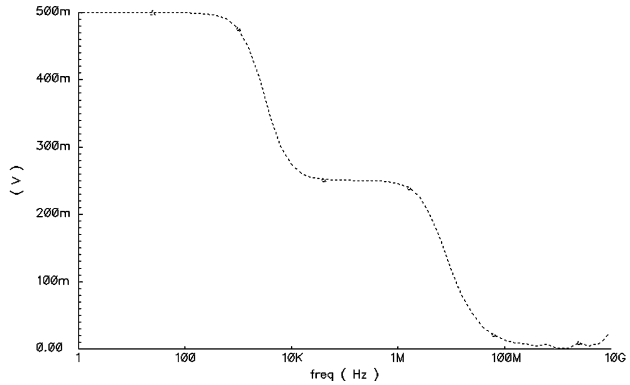

Fig. 4. Frequency response of the cell for an ac input. It is evident that more control of the nucleus and other interior parts of the cell is possible at radio frequencies.

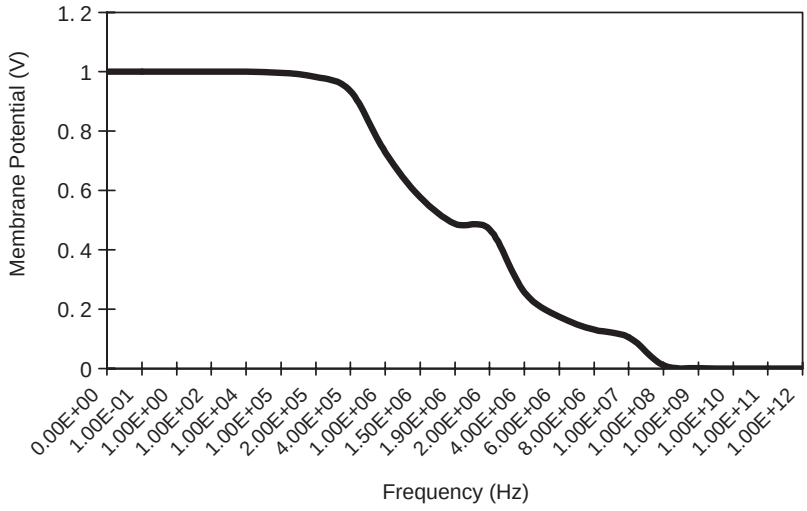

Fig. 5. Frequency response across the outer plasma membrane calculated analytically. At low frequencies, the outer membrane is affected.

plasma membrane receives the total potential applied to the cell, inducing voltage across the membrane and causing electroporation of the outer membrane. As the frequency is increased, however, the voltage appearing across the nucleus increases, and less voltage appears across the outer membrane. Membrane potentials at microwave frequencies are therefore much smaller than at lower frequencies. Hence, membrane interactions are less likely to occur at such high frequencies. At very high frequencies, the membrane capacitance sustains almost no voltage drop, and the

total voltage applied to the cell is available to the cytoplasm [2]. This phenomenon was simulated using the present model. Fig. 3 shows the voltage across the nucleus. The results correlate very well with the experimental results of Beebe et al. [15]. Using high fields of the order of tens of kilovolts per cm and duration in the range of 10–300 ns, they observed that, as the pulse durations decrease, the effect on the external plasma membrane decreases and effects on intracellular signal transduction mechanisms increase.

Fig. 4 represents the combined frequency response of the outer plasma membrane and the interior nucleus of the cell. This result matches the beta and gamma dispersions reported by Schwan [1,16]. The beta dispersion, first analyzed by Fricke and Cole, normally occurs over a frequency range of 10^4–10^7 Hz [16–18]. Electrical dispersion occurs when the applied field is ac, and the movement and orientation of the dipoles of the membranes do not vary in accordance with the changes in the field. The cellular structure of tissues having poorly conducting membranes that separate the cytoplasm from the extra-cellular space causes the beta dispersion. The finite time constant of the cell membrane associated with charging the membranes through the conducting phases inside and outside the cell membranes, as determined by cell membrane capacitance, cell radius, and the fluid resistivities, causes the delay. The beta dispersion of the frequency spectrum is caused by the changes in the surface charges and recharging processes occurring in the membrane. The cell membrane potential decreases above the characteristic frequency of the beta dispersion, which characterizes the rf behavior of cell suspensions. The Gamma effect occurs above 1 GHz. Water relaxes near 10 GHz, and tissues and cells contain a significant amount of water.

This theory also correlates closely with the frequency response obtained using the analytical expression provided by Eq. (3). This result is shown in Fig. 5. The software package MATLAB, Version 5.3 was used for this purpose.

More simulations were run to study other effects. A radio frequency electric field (in the GHz range) has several advantages over the conventional electroporation

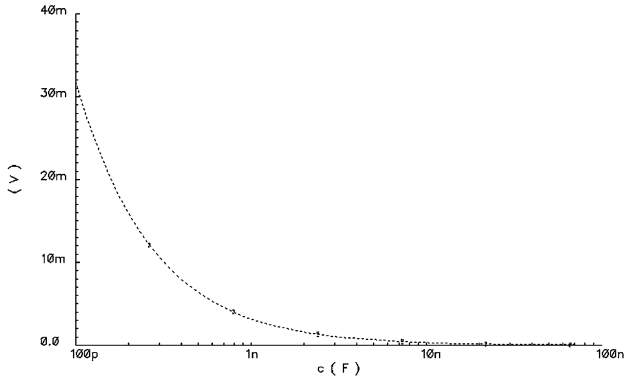

Fig. 6. Variation in the output voltage when the medium capacitance is changed from 100 pF to 100 nF at a very high frequency of 1 GHz.

which uses a short duration dc field [19]. Realizing that a high-frequency alternating electric field has useful effects that could be utilized for rf electroporation, we decided to study the effect of changing the medium resistance and capacitance at very high (1 GHz) and very low (50 Hz) frequencies. Figs. 6–9 show the results obtained. Fig. 6

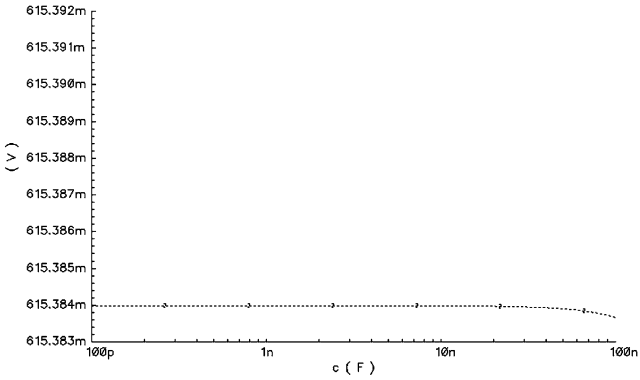

Fig. 7. Variation in the output voltage when the medium capacitance is changed from 100 pF to 100 nF at a very low frequency of 50 Hz.

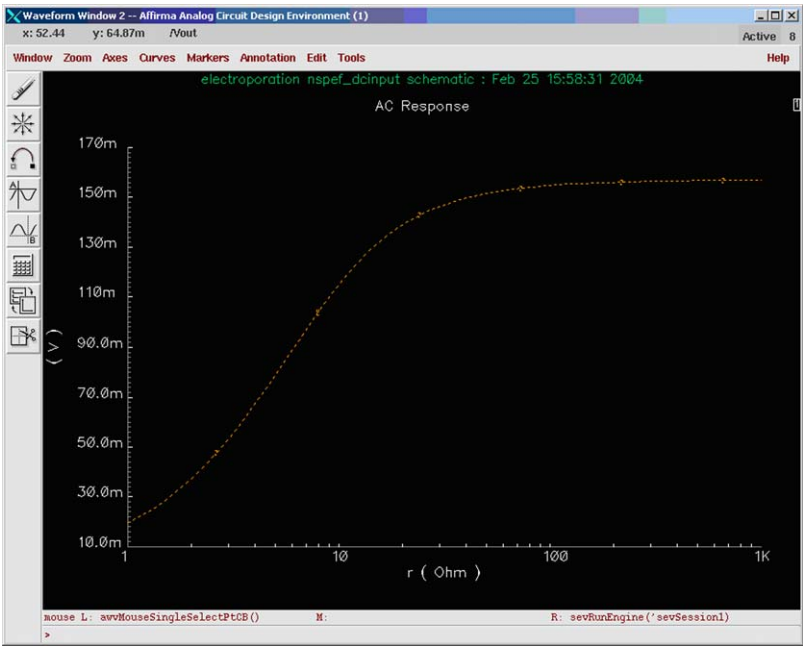

Fig. 8. Variation in the output voltage when the media resistance is changed from 1 Ω to 1 kΩ at a very high frequency of 1 GHz.

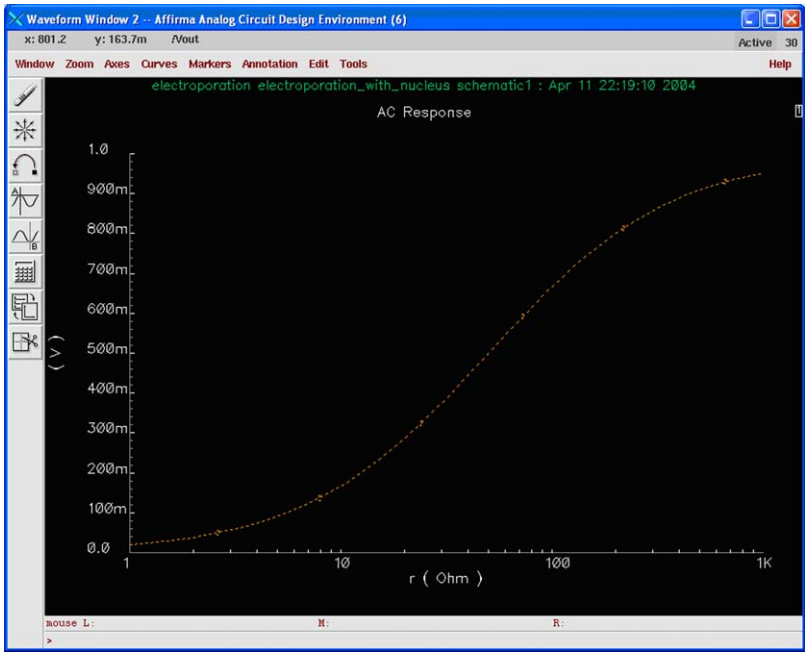

Fig. 9. Variation in the output voltage when the media resistance is changed from 1 Ω to 1 kΩ at a very low frequency of 50 Hz.

shows the change in the induced membrane potential when the medium capacitance was changed from 100 pF to 100 nF. There is an exponential decay of voltage induced across the membrane. There is not a very significant effect in this case because the membrane voltage is very small at high frequencies. Fig. 7 shows the noticeable effect obtained in the case of a low frequency of 50 Hz when the medium capacitance was changed from 100 pF to 100 nF. Note the significant difference in membrane potential values in each case (about 600 mV at 50 Hz, and less than 30 mV at 1 GHz).

Fig. 8 shows the results when the medium resistance was varied from 1 Ω to 1 kΩ at 1 GHz. An exponential increase occurs in the voltage measured at the output, with the membrane potential in the order of 20–160 mV. Compare these results with those obtained at a low frequency of 50 Hz (Fig. 9) for the same medium resistance change as above. Again, the membrane potentials are high (up to 900 mV) at 50 Hz and low (170 mV max) at 1 GHz. This result corresponds with the experimental observations reported in [20], where, with decreasing medium conductivity (or increasing medium resistivity) increased propidium iodide (PI) uptake was observed with the application of electric pulses. This result might be due to the increased voltage causing a dielectric breakdown to occur more easily at higher resistivities than at lower resistivities.

5. Summary

The electrical properties of tissues and cells are extremely complex. They behave differently at various frequencies. Electrical modeling can be effective in studying the ac frequency response of biological cells. The electrical model serves as a great aid to understanding the behavior of cells under over a wide frequency range. Simulation results that compare well with published data validate the model used and encourage the use of this simulation tool. The results confirm that a high frequency, alternating electric field might also be used for electroporation. The dielectric properties of the model can also aid in understanding the basic physiological difference between normal and cancerous cells on a molecular level [21]. More work has to be done to explore these possibilities.

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