Electric Field-Mediated Inactivation of Tumor Cells

V. Gowri Sree, K. Udayakumar and R. Sundararajan

***Abstract*— Electroporation or electropermeabilization, the bio-physical effect on cells exposed to an external electrical field is gaining applications in medical treatments, especially for chemotherapy and gene therapy. The efficacy of this treatment depends on the magnitude and the distribution of electric field applied, in addition to the physiological parameters, such as the conductivities and relative permittivities of the cell membranes and cytoplasm. In addition, physical parameters, such as the thickness and size of the cell also influence the efficiency of the electroporation technique. In this research, the electric field distribution of normal and tumor cells were studied using Finite Element Analysis (FEA), to elucidate the difference in responses of the normal and tumor cells for a given input voltage. For this purpose, Maxwell's FEM Software (ANSOFT, USA) was used. A comparison of the induced transmembrane potential (TMP) of the tumor cells against normal cells shows that nearly 37.5% increased TMP was observed in the tumor cell compared to the normal cell for an applied field of 10kV/cm to 1.15µm thick cell membranes.**

***Index Terms*—Electric field, Tumor, Cells, Electroporation, Cancer, Finite Element Method.**

I. Introduction

Cancer is a group of diseases in which cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues) and metastatic (spread to other locations in the body) [1]. These malignant properties of cancer differentiate the benign tumors, which are self-limited in the growth and do not invade or metastasize. Some of the methods used for treating cancer are surgery, chemotherapy, radiation therapy, immunotherapy, and monoclonal antibody therapy. Choice of therapy depends upon location and grade of tumor and stage of disease and state of patient [1]. However, these conventional therapies have major side effects, and are costly too. Hence attractive alternative, affordable, effective treatments are sought and one of the upcoming treatments is the use of electrical pulse-mediated chemotherapy using the electroporation concept, known as Electrochemotherapy (ECT) [2-8].

There are many potential advantages of ECT over conventional treatments. It may be best suitable for cancers near critical organs where surgery and/or radiation therapy have failed or could not be performed without damaging other normal parts. Furthermore, this treatment modality has the potential of overcoming the multi-drug resistance problem and it has been reported to reduce the number of metastatic nodules when primary tumor was treated.

ECT can be performed at relatively low, non-toxic drug concentration as a single treatment with minimal side effects. Therefore, it may be suitable for nonresectable tumors and can save functional tissues. This is one of the reasons for moving to electric field method for treating cancer [2-4].

Exposing tumor cells to electric field causes build up of charges in the cell membrane and consequently a change in the transmembrane potential of the cell. For low electric fields, this causes voltage gating of the protein channels in the cell membrane. With increasing electric field, induced transmembrane voltages on the order of 1V form electropores on tumour cell membranes. It is believed that the pore formation generates large openings in membrane accounting for transfer of large molecules across the tumor cell membrane. The most important parameters for effective electroporation are the electrical field strength and length of time the field is applied [2]. A large variety of other parameters can influence the efficacy of electroporation, such as the shape of the electrical pulse, polarity, size of target cells, and thermal conditions during and after the pulse, as well as other cellular and environmental factors. The uptake of molecules depends also on

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their molecular size, charge, and other physico-chemical properties. [6]

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 [9]. Electric field strengths of suitable magnitudes and durations cause molecules and molecular organizations such as biological cell membranes to undergo structural rearrangements. So it is necessary to study the electric field distribution of different electrode configurations [5]. Whether the applied input is ac or dc, when the applied electric field is of such a magnitude to induce a membrane voltage of about 0.5 - 1V, the membrane becomes permeable to macro and xeno molecules to which it would otherwise be impermeable [7].

II. Methods and Materials

A. Electrical Model of a Cell

Fig. 1 shows a cross section of a mammalian cell. Modeling allows us to determine the response of electric fields on the entire cell, including plasma and intracellular membrane [10]. A simple analytical, passive and linear approach that could describe the transmembrane potential and pore formation on cell is described in this section. In these models the cells are represented as micro resistor-capacitor (RC) elements. The equivalent circuit of a cell with one substructure, the nucleus is shown in Fig. 2.

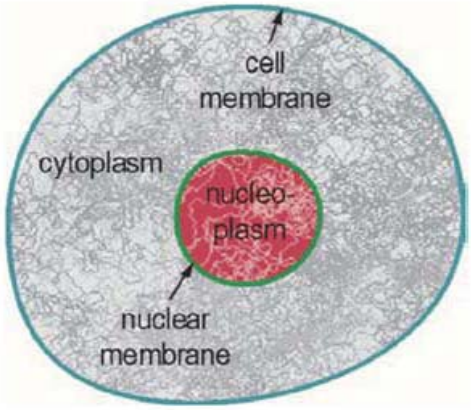


Fig.1. Cross-section of a biological cell with nucleus.

B. An Analytical Approach

In this analytic approach, substructures of the cell were neglected (the cytoplasm was treated as a homogeneous medium which fills the entire cell). The electric field in the membrane at the poles of the cell can be estimated from the condition that the current density at the interface of cytoplasm and membrane is continuous, as,

$$\sigma_M E_M = \sigma_C E_C \qquad \text{(i)}$$

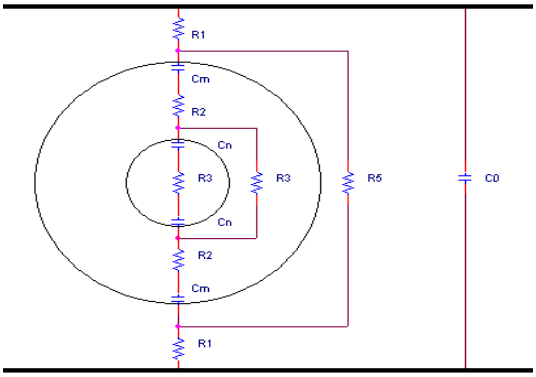


Fig. 2. Electrical equivalent circuit of cell between electrodes.

Assuming the electric field along the axis of a cell with diameter D, and membrane thickness, d, is constant in the cytoplasm and in the membrane, respectively, gives us:

$$E_C D + 2\, E_M d = V_C \qquad \text{(ii)}$$

Combining the two equations allows us to calculate the electric field in the membrane, depending on the applied voltage, V:

$$E_M = V_C/((\sigma_M/\sigma_C)D + 2d) \qquad \text{(iii)}$$

The energy density deposited in the membrane:

$$W = \sigma_M E_M 2\tau \qquad \text{(iv)}$$

is then on the order of 10^{-5} J/cm^3.

C. 2D Electric Field Analysis of Normal and Tumor Cells

The tumor cell was modeled as shown in Fig. 3, using the simulation package, MAXWELL 2D simulator. The MAXWELL simulator is an interactive package that uses finite element analysis (FEA) to solve two dimensional electrostatic problems. The normal and the tumor cell parameters used for the model are shown in Table I [11].

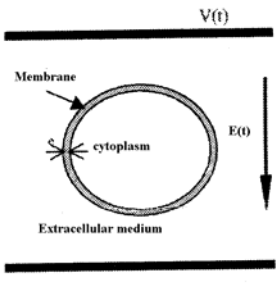


Fig. 3. Tumor cell model used in this study.

Two electrodes are placed on both the side of the cell. High voltage of the order of 10kV was applied and electric field distribution is studied for plate and needle electrode. The electrode gap spacing was about 10mm.

TABLE I
SIMULATION PARAMETERS FOR NORMAL AND CANCER CELL

	NORMAL CELL	CANCER CELL
Conductivities (S/m)		
Environment	0.6	0.6
Cell membrane	5.6e-5	9.1e-6
Cytoplasm	1.31	0.48
Nuclear Envelope	1.11e-2	4.4e-3
Nucleoplasm	2.04	1.07
Relative Permittivity		
Environment	80.0	80.0
Cell membrane	12.8	9.8
Cytoplasm	60.0	60.0
Nuclear Envelope	106	60.3
Nucleoplasm	120	120
Geometry Parameters (μm)		
Radius of simulated region	10.0	10.0
Radius of cell	3.3	5.2
Thickness of cell membrane	0.007	0.007
Radius of nucleus	2.8	4.4
Thickness of nuclear envelope	0.04	0.04

D. 3D Electric Field Analysis of Normal and Tumor Cells

In order to get more accurate insight of electric field distribution inside cells with complex geometries, 3D modeling was studied. The tumor and the normal cells were modeled using the MAXWELL 3D simulator. The electric field along the cell membrane was studied and compared with analytical results. The same parameters given in Table I were used for 3D study also.

III. RESULTS AND DISCUSSION

A. Equipotential Plots of Normal and Tumor Cells with Plane Electrodes

The equipotential plots for the normal cell and the cancer cells using plane electrodes along with the stress plots are shown in Figs. 4 and 5. Fig. 4 shows the equipotential distribution of the normal and the tumor cells, while Fig. 5 shows the stress plots for the same.

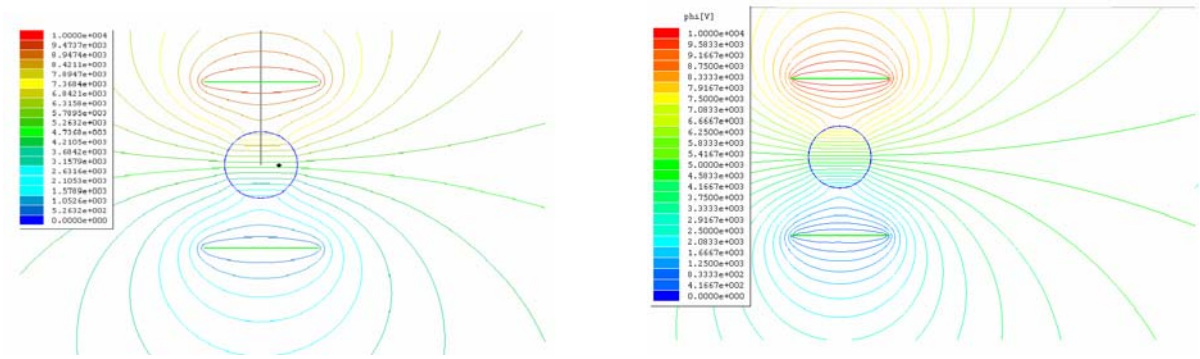


Fig. 4. Equipotential Contours of the normal cell (left) and the tumor cell (right) using Plane electrodes.

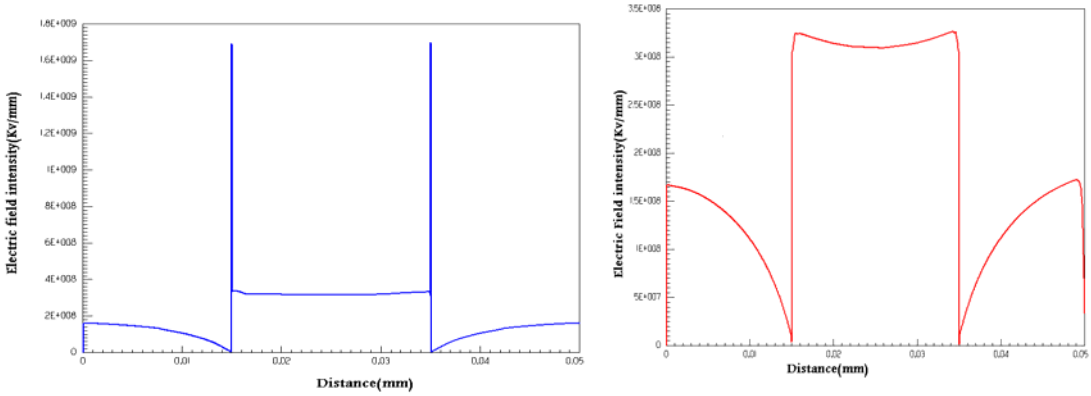


Fig. 5. Stress plot for biological cell (left) and tumor cell (right).

The distribution of the equipotential contours is slightly different between the tumor and normal cells; tumor cells having more contour plots than the normal cell. A close look at the stress plots shows (Fig. 5) a significant difference in the magnitudes of the electric field intensities. The tumor cell has an electric field intensity of up to 3.5 x 10^8kV/mm while the normal cell has the maximum intensity of about 1.7x 10^8 kV/mm. The applied input voltage was the same (10kV) in both cases while the cell geometry and other physical parameters for the normal and tumor cells vary as shown in Table I. The higher value of electric field intensity of the tumor cell indicates the more susceptibility or vulnerability of the malignant cells towards electroporation, compared to the normal cells. These results clearly indicate the distinct difference between the responses of the same applied voltage of the tumor cells and the normal cells, indicating the significant effect of the difference in their physical properties, such as the conductivities of the cell membranes, cytoplasm, nuclear envelope and the nucleoplasm and the variations in the relative permittivity of the cell membranes, nuclear envelope and radius of cells and nucleuses (Table I). These results demonstrate that normal cells remain relatively robust compared to the malignant cells under electroporation conditions. Similar results have also been obtained by other researchers [11].

B. Equipotential Plot of Tumor Cell with needle Electrodes

Fig. 6 shows the equipotential contour using needle electrodes for the tumor cell. A comparison of the electric field intensities of the tumor cell with plane and needle electrodes shows a slight variation in the intensities of the electric field intensities for the configuration shown. Normally, needle electrodes have highly non-homogeneous fields around their tips due to the sharp geometry. In general, from clinical applications point of view, normally non invasive plate electrodes are better suited for tumors on the surface of the skin, whereas needle electrodes with appropriate depth of insertion are more suitable for treating subcutaneous tumor seeded deeper in the skin.

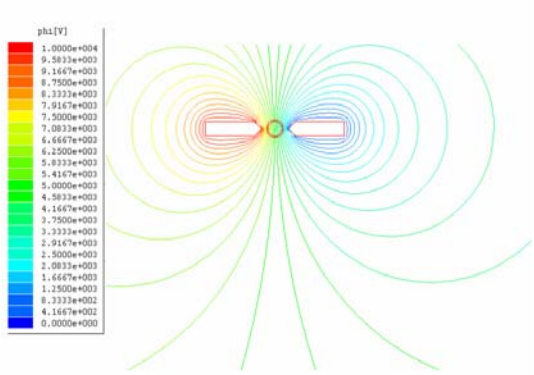
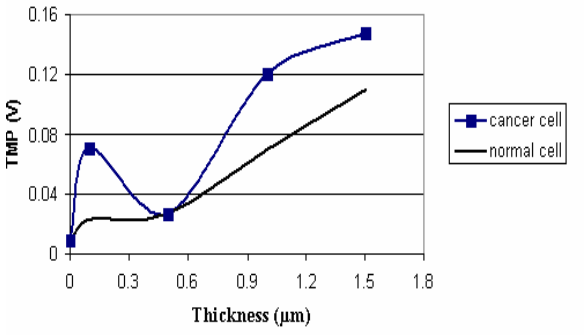


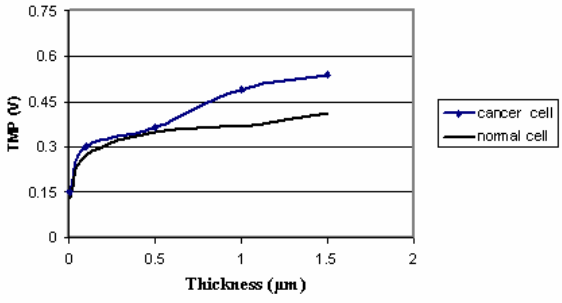
Fig. 6. Equipotential plot of tumor cell under needle electrodes.

C. Comparison of Normal and Tumor cells based on Thickness of Cell membranes

Fig. 7 shows the influence of the cell membrane thickness on the Trans Membrane Potential (TMP). The thickness of the membrane was varied from 0.007µm to 1.5µm, and the TMP was evaluated by varying the applied voltage from 1 to 10 kV between the electrodes. It was inferred that, as the thickness of the cell membrane increases the TMP also increases. Further the TMP of the tumor cell was 21% more than that of the normal cell. The variation of normal and tumor cell TMP values at 1kV and 10 kV are shown in Figs. 7a and b.



(a)



(b)

Fig. 7. Variation of TMP with Thickness; a) for 1kV, and b) for 10kV.

D. Comparison of Normal and Tumor cells based on Applied Voltage Magnitude

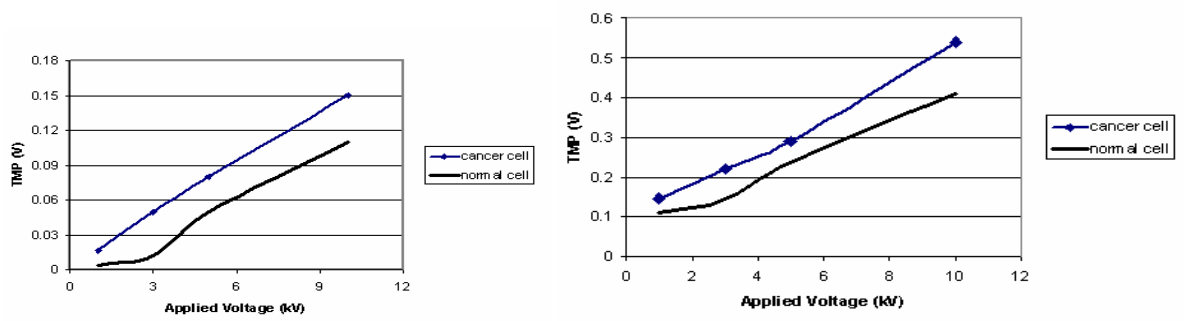
By varying the applied voltage between the electrodes for different thickness of cell membranes, the TMP was evaluated and compared for the normal and tumor cells. The TMP increases with the applied voltage and the TMP of the tumor cell is nearly 24% more than the normal cell. The variation of the TMP with applied voltage at 0.007µm and 1.5µm is given in Figs. 8a and b.

E. Comparison of Normal and Tumor cells based on Distance between Electrodes

The distance between two electrodes was varied from 8 to 20µm and its effect on TMP was studied. Evaluation of the TMP values revealed a decrease in TMP as the distance increases and further that the TMP of the tumor cell is nearly 13% more than the normal cell.

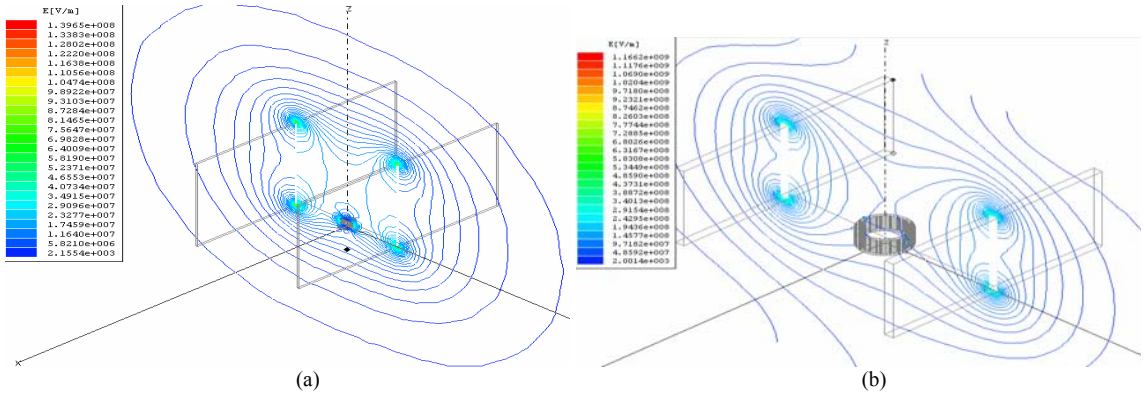
F. Comparison of Equipotential Plots of Normal and Tumor cells using 3D modeling

Figs. 9 and 10 show the results obtained using 3D model. Figs. 9a and b show the electric potential contour and the electric field intensity for the normal and tumor cells. In this case also, like 2D modeling, the electric field intensity was higher for the tumor cell than the normal cell, differentiating the behavior of the malignant and benign cells under the same applied voltage conditions. Figs. 10a and b show the electric field distribution of the normal and tumor cells. The profile of these distributions along the distance looks different. Even though the electric field intensity was higher in the tumor cells (Fig. 9b) than the normal cell (Fig. 9a), the electric field magnitudes in Fig. 10 are slightly lower for the tumor cell compared to the normal cell. This needs further investigation.



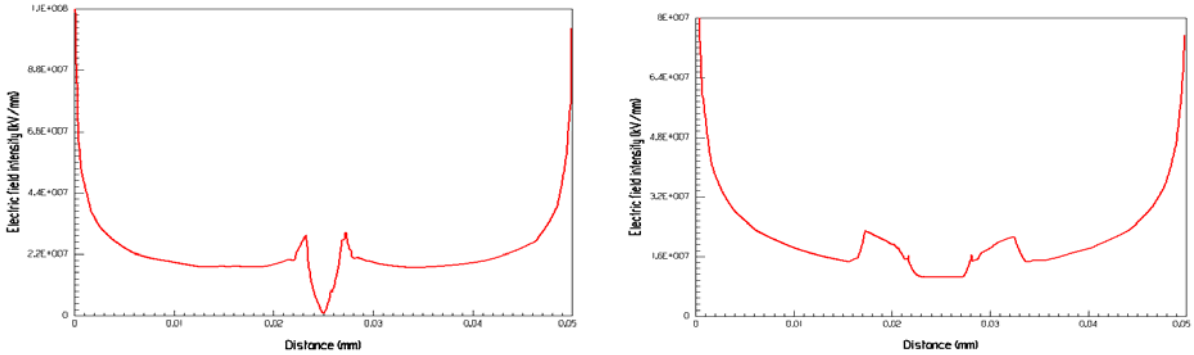
(a) (b)

Fig. 8. Variation of TMP with Applied Voltage for a) 0.007µm thickness, and b) 1.5µm thickness.



(a) (b)

Fig. 9. Equipotential plots for a) Normal and b) tumor cell



(a) (b)

Fig. 10. Electric field distribution plots for a) Normal and b) tumor cell

G. *Comparison of Simulation Results with*

The simulated results are compared with the analytical solution obtained from equation (iii). Table II shows a comparison of the electric field of the cell membranes for various thicknesses. In general, there is good correlation between the analytical and the modeling results, validating the modeling parameters and the techniques used.

TABLE II
Electric Field Values for Normal and Cancer Cells

Applied Voltage (kV)	Thickness (μm)	Electric Field on the cell membrane (kV/mm)			
		Cancer cell		Normal cell	
		Simulated value (*e8)	Numerical value (*e8)	Simulated value (*e6)	Numerical value (*e6)
1	0.007	0.4	0.7	1.4	1.6
3		1.2	1.5	4.4	4.7
5		2.0	2.2	7.4	7.7
7		2.8	3.1	10.4	10.9
10		4.0	4.3	14.9	15.3

IV. CONCLUSIONS

The electric field distribution in normal and tumor cells were investigated using 2D and 3D Finite Element Analysis for plane and needle electrode geometries. The tumor cells showed higher intensities of electric field compared to the normal cell. These results demonstrate the susceptibility of malignant cells to the electric field application and the relative robustness of the normal cells, illustrating the enhanced efficacy of the electrochemotherapy using lower drug doses. The field analysis results can be used for assessing effective treatment parameters of tumor cells.

The electrical characteristics of the membrane and the cytoplasm, such as the conductivity and permittivity of the membrane and the cytoplasm as well as membrane thickness also govern the response due to electroporation in addition to the intensity and distribution of the electric field applied.

It is well known that normal and tumor cells have different electric and geometrical parameters, which enable electrically-mediated treatments to be more efficient for a given dose.

A comparison of the analytical solution with the numerical modeling solution shows close correlation indicating the viability of the modeling, the model parameters and the geometry chosen. This indicates that electric field analyses could be used for selecting suitable parameters for effective treatment of tumor cells.

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