Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioelectrochemistry 68 (2006) 213 – 217
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Numerical study of the electrical conductivity and polarization in a suspension of spherical cells

A. Ramos [a,*], D.O.H. Suzuki [b], J.L.B. Marques [b]

[a] Electrical Engineering Department, Centre of Technological Sciences, State University of Santa Catarina (UDESC), Joinville, Santa Catarina, Brazil
[b] Institute of Biomedical Engineering, Department of Electrical Engineering, Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil

Received 3 September 2004; received in revised form 2 May 2005; accepted 16 August 2005
Available online 26 October 2005

Abstract

The spatial distribution of electrical potential and current in a suspension of spherical cells under an applied electric field was numerically obtained using the equivalent circuit method (ECM). The effect of the proximity of the cells was studied in a set of simulations where the volumetric fraction varied from 0.24 to 0.66. The results show that the transmembrane potential for cells in the suspension is lower than the theoretically predicted value for a single dielectric membrane under a uniform electric field. It was also observed that as the volumetric fraction is increased, the transmembrane potential on the pole of the cells decreases linearly. Furthermore, the conductivity of the suspension was also observed to be a function of the volumetric fraction and this result is in a good agreement with the Maxwell's model for spherical particles suspended in a volume conductor.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Numerical simulation; Electrical polarization; Spherical cells polarization; Electromagnetic modeling

1. Introduction

The biological cell exposed to an electric field produce transiently cell membranes porous and permeable to otherwise ions, DNA, proteins, drugs and impermeable substances [1–3]. This phenomenon is named membrane electropermeabilization or electroporation, this electrical stimulation of the plasmatic membrane may have important effects on the morphology and physiology of the cell. The study how electric fields interact with the cell suspension presents significant theoretical aspects and it can help us to understand the basis of the electroporation phenomenon [4–6].

Cell membranes are very thin structures containing a bi-layer of lipidic molecules. Due to the low permittivity of the lipidic matrix, there is a high energy barrier hindering the ionic movement across the membrane (Bohr energy) [7]. Then, the electrical conductance of the cell membrane is usually very low.

When a biological tissue or suspension of cells is excited by an electric field, ionic currents are established and ions from the electrolyte accumulate on both sides of the cell membranes. Therefore, membranes are polarized and the transmembrane potential depends, mainly, on the strength of the applied field, geometry of the cells, density of the suspension, cells organization and extracellular medium conductivity [4,5,8,9].

For a single spherical membrane of radius a and thickness h immersed in a conductor liquid under an uniform electric field of strength E_o applied in $t=0$, the transmembrane potential is given by Eq. (1), extracted from [10]:

$$V_m = -F\ a\ E_o \cos\theta \left(1 - e^{-t/\tau}\right) \quad (1)$$

where, $V_m = V_i - V_o$, V_i and V_o is the inside and outside membrane potential, respectively. θ is the angle between the direction of the field and the position vector of the point on the membrane, relative to the centre of the cell. Newmann et al. [11] present F like a function of the conductances of the external solution (σ_o), of the cell interior (σ_i), of the membrane (σ_m) and of the ratio h/a. When $a >> h$, $\sigma_m << \sigma_i$, $\sigma_i >> \sigma_o$ (low conductivity media), but still $\sigma_o >> \sigma_m$ such that $F = 1.5[1 + 0.5(a\ \sigma_m)/(h\ \sigma_o)]^{-1}$. Kotnik et al. [8] calculated V_m by solving the Laplace equation and the $F = 1.5\sigma_o[3ha^2\sigma_i + (3h^2a - h^3)(\sigma_m - \sigma_i)]/$

* Corresponding author.
E-mail address: dee2ar@joinville.udesc.br (A. Ramos).

1567-5394/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2005.08.001

$[a^3(\sigma_m+2\sigma_o)(\sigma_m+0.5\sigma_i)-2(a-h)^3(\sigma_o-\sigma_m)(\sigma_i-\sigma_m)]$, and the time constant is $\tau=aC_m/\{[2\sigma_o\sigma_i/(2\sigma_o+\sigma_i)]+(\sigma_m a/h)\}$, C_m is the capacitance per unit area of the membrane. This analytical calculation is used where cells are surrounded by a medium of low conductivity ($\sigma_o<10^{-2}$ S m^{-1}), but it does not apply in case of a charged membrane surface. In case of a single nonconductive spherical cell in an infinite volume conductor, $a>h$ and $\sigma_m=0$, $F=1.5$. The time constant is reduced to (2), from [12]:

$$\tau = aC_m\left(\frac{1}{\sigma_i}+\frac{1}{2\sigma_o}\right). \tag{2}$$

For cells suspended in an electrolytic solution, the transmembrane potential is different from the cosine distribution given by Eq. (1) because the proximity between cells distorts the current distribution in the interstitial space close to the membranes [4,5]. The transmembrane potential is also a function of the volumetric fraction occupied by the cells and it is quite hard to obtain it by analytical methods. Numerical approaches are better to do it. Miklavcic et al. [13] present a numerical model validated through experimental observation determined the induced transmembrane voltages. They estimated the transmembrane potential difference in a single cell of the rabbit liver to be 394±75 and 694±136 mV for reversible and irreversible electroporation threshold. Schemeer et al. [6] show experimental data of reduction in the conductivity of cell suspension, before the electroporative field pulse, when the volume fraction increases.

In this work, we present a numerical study using the equivalent circuit method (ECM) [14,15] for analysis of the induced membrane voltage on spherical cells in dense suspension.

2. Method

The ECM is a numerical approach suitable for field calculation in inhomogeneous and anisotropic media. It is based on the modelling of the transport properties of the media by means of an electric circuit whose elements are associated to a discrete mesh of regular blocks that fill the analyzed space (Fig. 1a). Each block is modelled as a node of the equivalent circuit. In the cell scale model (Fig. 1b), which is suitable for analysis around the cells, the total current between two adjacent blocks in the mesh is described as having three components: conduction, diffusion and shift currents, and it is given by Eq. (3), from [15]:

$$I_{ox} = \sum_n [g_{nox}(V_o - V_x) + k_{nox}(\rho_{no} - \rho_{nx})] + \sum_x c_{ox}\frac{\delta}{\delta t}(V_o - V_x) \tag{3}$$

where the nodes are identified by 'o' and 'x' and n indicates summation over all types of ions in the media surrounding the cells. V and ρ are the voltage and charge density in the node, respectively, while g, k and c are the conductance, diffusion

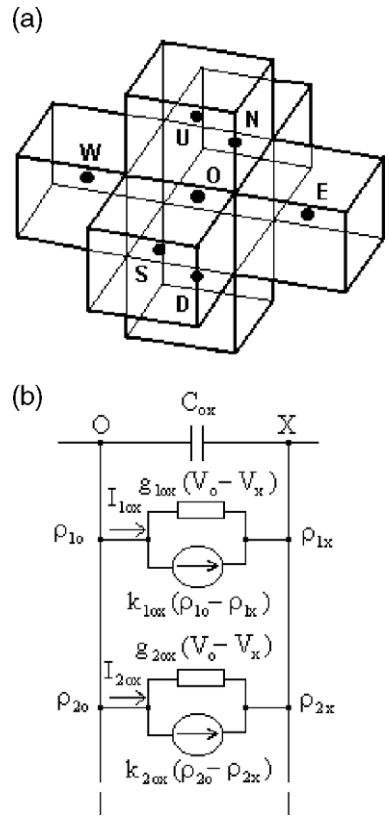

Fig. 1. (a) Discretization scheme used in the MCE. Each block is represented by a node in the equivalent circuit. (b) Equivalent circuit with the lumped elements modelling the transport process in the media.

coefficient and capacitance of the connection, respectively. These parameters are given by Eq. (4), extracted from [15]:

$$g_n = \mu_n\rho_n A/L,\ k_n = f_n D_n A/L,\ c = \varepsilon A/L \tag{4}$$

where μ_n and D_n are the mobility and diffusion coefficient of the ion 'n', respectively, and ε is the electrical permittivity of the media. A and L are the area and length of the connection between two adjacent nodes, respectively. f_n is a function of the voltage difference between the nodes [15]. It is obtained from the spatial averaging of the Nernst–Planck equation, supposing that the current density is uniform in the area A and the voltage varies linearly along the length L. Its value is given by:

$$f_n = \frac{\Delta V}{2v_n}\frac{\exp(\Delta V/v_n)+1}{\exp(\Delta V/v_n)-1} \tag{5}$$

where $v_n=KT/ez_n$ is a constant for each type of ion in the media and $\Delta V=V_o-V_x$.

The ECM consists in obtaining and analyzing the equivalent circuit of the media aiming to obtain the electric potential and charge density distribution in the space with known initial and boundary conditions. We obtained the potential distribution by computing the node equations system and the charge distribution by finite integration of the continuity equation for each block of the mesh.

The body centred cubic (BCC) diagram of the cells suspended into the electrolytic solution is shown in Fig. 2. We built the discretization mesh for two levels of resolution. A

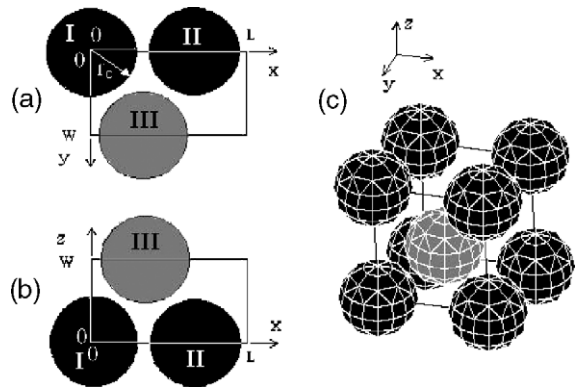

Fig. 2. Body centred cubic (BCC) structure for simulation. In the (a) and (b) I, II and III are three spherical cells, it is the partial structure of BCC. Other cells are not shown due to the fact that the symmetry allows to limit the analyzed space into a volume defined by the rectangles. (a) is on the plane $z=0$ and (b) is on the plane $y=0$. (c) The complete 3D structure of BCC. A potential difference is applied between the faces $x=0$ and $x=L$.

regular mesh was built by using a cubic block with edges equal to $r_c/12$, where r_c is the cell radius. Around the membranes, we built a high-resolution mesh by dividing each big block in eight small ones. This procedure is important when a refined geometry of the membranes and the interstitial spaces are required. We varied the distance between the neighbour cells along 10 simulations in order to obtain the values of the volumetric fraction ranging from 0.24 to 0.66. The volumetric fraction is the ratio between the internal volume of the cell and the specific volume occupied by the cell in the suspension. The final number of blocks varied from 23,740 to 35,570. Table 1 summarizes the geometry, composition and the electrical properties of the media.

The electrolyte is an aqueous solution composed of five types of ions: Na^+, Cl^-, K^+, HCO_3^+ and proteins. The concentrations are in Table 1. The proteins are assumed to have mobility zero and they are only considered for equilibrating the total charge of the electrolyte.

Table 1
Geometrical characteristics and electrical properties of the media

Property	Value	
Cell radius (μm)	7	
Membrane thickness (nm)	7	
Ionic concentration inside the cells ($\times 10^{-3}$ M)	Na^+	10
	Cl^-	4
	K^+	140
	HCO_3^-	10
Ionic concentration outside the cells ($\times 10^{-3}$ M)	Na^+	143
	Cl^-	123
	K^+	5
	HCO_3^-	25
Ionic mobility[a] ($\times 10^{-4}$ cm^2 V^{-1} s^{-1})	Na^+	5.19
	Cl^-	7.91
	K^+	7.62
	HCO_3^-	7.40
Dielectric constant of water	81	
Membrane capacitance[b] (μFcm^{-2})	1	

[a] Values for 298K from [16].
[b] [17].

We assumed zero initial charge and potential values in all nodes. The boundary conditions are considered to have 0 V for all nodes on the face $x=0$ and E_oL volts for all nodes on the face $x=L$. E_o is the applied electric field in the x direction with a value of 100 V/cm. Because of symmetry, the normal components of field and current vanish on the other faces ($y=0$, $z=0$, $y=W$, $z=W$).

We specified the time step of the iterative process in 0.3 ns according to the convergence rule given in Ref. [15]. The simulation time was 1.2 μs, which was divided into 4000 steps. This time interval is much higher than 78 ns obtained with Eq. (2). Therefore, the transmembrane potential distribution can be considered as reaching the steady state.

As the cell I shown in Fig. 2(a) and (b) has the highest number of neighbouring cells, this cell was chosen to be the target cell for the calculations of the transmembrane potential and conductivity. Although the Fig. 2(a) and (b) shows the two neighbouring cells, the Fig. 2(c) shows the total arrangement of BCC.

3. Results

We use the ECM to calculate the transmembrane potential distribution and the conductivity in a suspension of spherical cells.

Fig. 3 shows the transmembrane potential distribution on the target cell as a function of the angle between the direction of the field and the position on the membrane, assuming three values of the volumetric fraction. It also shows the theoretical curve for a single spherical membrane according to Eq. (1). The curves are normalized with respect to the highest transmembrane potential on the pole of the cell ($V_{mo}=-1.5\,aE_o$). Fig. 4 shows the normalized transmembrane potential on the pole of the target cell as a function of the volumetric fraction of the suspension. By using the spatial distributions of charge and potential, it was calculated the averaged value of the current density and the electric field in the specific volume of

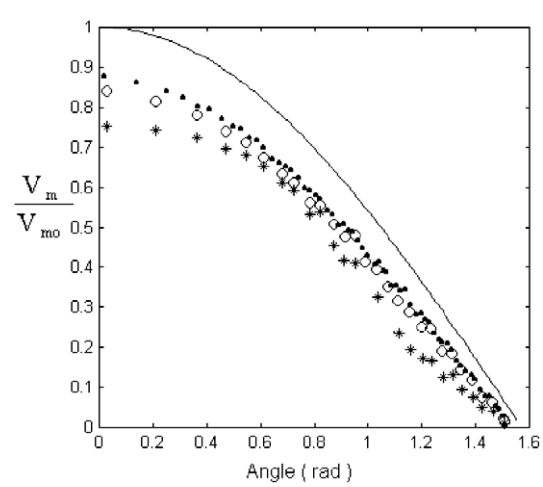

Fig. 3. Normalized transmembrane potential distribution on the target cell as a function of the angle between the field direction and the position on the membrane. V_{mo} is the potential on the pole of a single cell ($V_{mo}=-1.5aE_o$). (*) $p=0.66$; (O) $p=0.44$; (•) $p=0.31$; (–) Eq. (1).

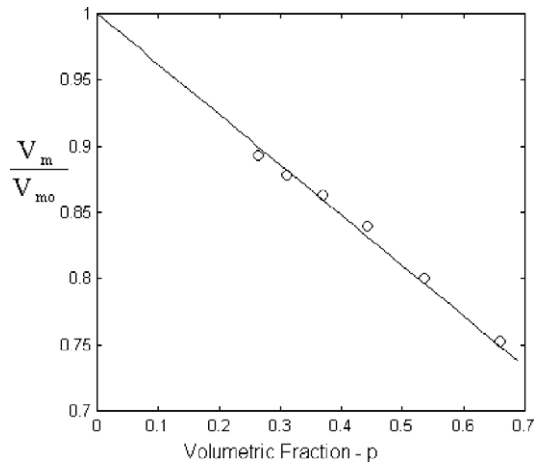

Fig. 4. Normalized transmembrane potential on the pole of the target cell as a function of the volumetric fraction of the suspension. V_{mo} is the potential on the pole of a single cell ($V_{mo} = -1.5aE_o$). The circles are the numerical results. The solid line is the trend of the results.

the target cell. By using these values, the conductivity of the suspension was obtained. Fig. 5 shows the dependence of the conductivity to the volume fraction.

4. Discussion

It can be seen in the Fig. 3 that as the suspension becomes more concentrated, the transmembrane potential is reduced on the pole and distorted from the cosine distribution. This happens because the ionic currents arriving to the pole of the cells are reduced as they are closer to each other. This study agrees with other numerical models using the finite-element method (FEM) presented by Susil et al. [5]. The authors examined how the density of cells change the cosine induced transmembrane potential for a single cell, they implemented different cell packing densities and organization.

Fig. 4 shows a linear decrease of the potential as the volumetric fraction is increased. This result can not be taken into account analytically, especially when a volumetric fraction of 0.50 or more is considered. The relation between V_m and p is important to obtain the threshold of electroporation in suspension of different concentrations of cells. According to Fig. 4, the potential on the pole of the cell can be given by:

$$V_m = -1.5aE_o(1 - 0.38\,p). \tag{6}$$

If the threshold of the electric field E_{p1} for electroporation is measured for a known volumetric fraction (p_1), then the threshold of E_{p2} for another value (p_2) can be given by:

$$\frac{E_{p2}}{E_{p1}} = \frac{1 - 0.38\,p_1}{1 - 0.38\,p_2}. \tag{7}$$

The solid line in Fig. 5 is the theoretical model from Maxwell [17] for the dielectric spherical particles suspended in a volume conductor and is given by:

$$\frac{\sigma}{\sigma_o} = \frac{1-p}{1+p/2}. \tag{8}$$

This equation can be used to estimate the volumetric fraction of the suspension from a measured conductivity.

Pavlin et al. [4] calculated the effective conductivity of cell suspension for different volume fractions and membrane conductivity. The authors utilized the finite-element method to compare different arrangements of cells and reported this effective conductivity for complex and dense structures. Comparing the BCC FEM [4] and the BCC ECM the results are similar.

The Fig. 5 results agree with the experimental data presented by Schemeer et al. [6], where the conductivity of the cell suspension, before the electroporative field pulse, decreases when the volume fraction increases.

The ECM results are closer to those of the analytical model, the experimental results [6] and the result of the different numerical methods [4,5], showing the reliability of the numerical approach.

5. Conclusion

The ECM technique was applied to solve the complex problem of the polarization for the spherical membranes suspended in an electrolyte solution. Numerical results showed an important and simple relation between the transmembrane potential and the volumetric fraction. This relationship can be used for obtaining a precise estimation of the transmembrane potential, which is the case used in electroporation studies of suspended cells. Furthermore, the agreement between the numerically calculated conductivity and that one in Eq. (8) from Maxwell's model demonstrates the reliability of the method. The similarity of results with [4] and [5] contribute to the feasibility of the ECM.

Future work will focus on the electroporation modelling by using the equivalent circuit method and its application in experimental studies.

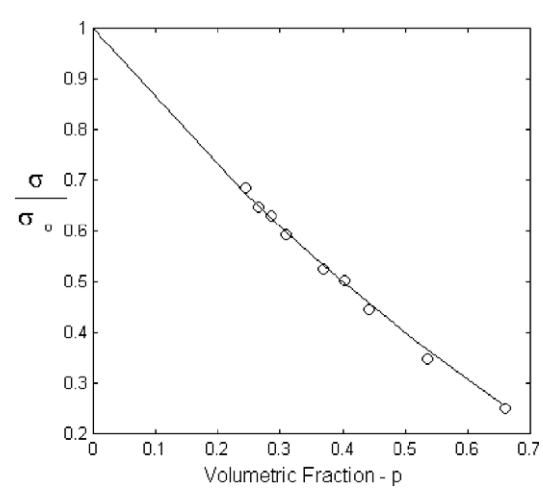

Fig. 5. Dependence of the conductivity to the volumetric fraction of the suspension. σ_o is the conductivity of the electrolyte outside the cells. The circles are the numerical results. The solid line is related to the model proposed by Maxwell for dielectric spherical particles suspended in a conductor liquid [17].

References

[1] M.J. Jaroszeski, R. Heller, R. Gilbert, Electrochemotherapy, Electrogenetherapy and Transdermal Drug Delivery: Electrically Mediated Delivery of Molecules to Cells, Humana Press, Totowa, 1999.

[2] S.D. Dev, D.P. Rabussay, G. Widera, G.A. Hofmann, Medical applications of electroporation, IEEE Trans. Plasma Sci. 28 (2000) 206–223.

[3] K. Kinosita Jr., eT.Y. Tsong, Voltage-induced pore formation and hemolysis of human erythrocytes, Biochim. Biophys. Acta 471 (1977) 227–242.

[4] M. Pavlin, T. Slivnik, D. Miklavcic, Effective conductivity of cell suspensions, IEEE Trans. Biomed. Eng. 49 (2002) 77–80.

[5] R. Susil, D. Semrov, D. Miklavcic, Electric field-induced transmembrane potential depends on cell density and organization, Electro-Magnetobiol. 17 (1998) 391–399.

[6] M. Schmeer, T. Seipp, S. Kakorin, E. Neumann, Mechanism for the conductivity changes caused by membrane electroporation of CHO cell-pellets, Phys. Chem. Chem. Phys. 6 (2004) 5564–5574.

[7] J.C. Weaver, Y. Chizmadzev, Electroporation, in: C. Polk, E. Postow (Eds.), Handbook of Biological Effects of Electromagnetic Fields, CRC, New York, 1996, pp. 247–274.

[8] T. Kotnik, F. Bobanovic, D. Miklavcic, Sensitivity of transmembrane voltage induced by applied electric fields – a theoretical analysis, Bioelectrochem. Bioenerg. 43 (1997) 285–291.

[9] B. Valic, M. Golzio, M. Pavlin, A. Schatz, C. Faurie, B. Gabriel, J. Teissié, M.P. Rols, D. Miklavcic, Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment, Eur. Biophys. J 32 (2003) 519–528.

[10] H. Fricked, The electric permittivity of a dilute suspension of membrane-covered ellipsoids, J. Appl. Phys. 24 (1953) 644–646.

[11] E. Neumann, A. Sprafke, E. Boldt, H. Wolf, Biophysical considerations of membrane electroporation, in: D.C. Chang, B.M. Chassy, J.A. Saunders, A.E. Sowers (Eds.), Guide to Electroporation and Electrofusion, Academic Press, San Diego, 1992, pp. 77–90.

[12] H.P. Schwan, Electrical properties in tissue and cell suspensions, Adv. Biol. Med. Phys. 5 (1957) 147–209.

[13] D. Miklavcic, D. Semrov, H. Mekid, L.M. Mir, A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy, BBA 1523 (2000) 73–83.

[14] A. Ramos, A. Raizer, J.L.B. Marques, A new method for field calculation using the equivalent electric circuit, Proceedings of the 13th COMPUMAG, Evian, 2001, pp. 232–233.

[15] A. Ramos, A. Raizer, J.L.B. Marques, A new computational approach for electrical analysis of biological tissues, Bioelectrochemistry 59 (2003) 73–84.

[16] P.H. Barry, J.W. Lynch, Liquid junction potential and small cells effects in patch-clamp analysis, J. Membr. Biol. 121 (1991) 101–117.

[17] K.R. Foster, H.P. Schwan, Dielectric properties of tissues, in: C. Polk, E. Postow (Eds.), Handbook of Biological Effects of Electromagnetic Fields, CRC, New York, 1996, pp. 25–102.