Electromagnetic radiofrequency fields and red blood cell aggregation

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Abstract

A radiofrequency electromagnetic field over red blood cells introduces an additional negative polarization energy component that may promote the *rouleau* formation. For studying this effect, a computational model has been developed, considering the cell as a uniformly shelled dielectric ellipsoid. The induced polarization charges on the *rouleau* under an electric field applied along the column axis are computed using BEM. The energy of the arrangement is calculated as that of the induced dipole moments of cells plus the interaction energy between dipoles. The energy decrease depends quadratically on the field intensity and could affect RBC aggregation at high exposure fields.

1. Introduction

An important aspect in the rheology of blood circulation is the well-known aggregation of red blood cell (RBC) and the formation of linear *rouleaux*. Several studies about *rouleau* formation analyse the general properties of membrane-membrane interactions [1] or the mechanism of formation leading to stable *rouleau* from an energetic point of view [2]. In fact, the equilibrium state of erythrocytes in the *rouleau* corresponds to the minimum of the total energy, including the adhesion and elastic energy of the membrane and the basic electric energy due to the resting transmembrane potential. Therefore the *rouleau* formation can be facilitated when the total energy of the erythrocyte cell system is reduced. This variation of energy can arise from both internal (structural, metabolic or temporary changes) and external causes, such as aggregating agents addition -e.g. dextran polymers in the solution- or the presence of an electromagnetic field, on which we will focus our attention.

Besides the various physiological alterations that have been reported even at relatively low intensity levels, the electric field produces a change in the normal transmembrane potential and an overall polarization of the cell resulting from the induced superficial charges on the cell membrane. These parameters are of importance for the physiological state of the cell as well as for practical applications, such as electropermeabilization. Cells immersed in the field attract each other because of the favourable dipole-dipole interaction energy. This type of electrical interaction is frequently used for the alignment of biological cells as a step prior to electrofusion.

2. Electromagnetic field-RBC interaction model

In order to gain a better understanding of the interaction between the EM field and the bioparticles, the electrical response of the cell is analyzed through the induced surface charges and the induced transmembrane voltage. From them, we perform a quantitative estimation of the electric energy decrease involved in the formation of a RBC *rouleau* immersed in a linearly polarized electromagnetic field. We assume $\boldsymbol{E} = \boldsymbol{E_0}\, e^{-i\omega t}\, \boldsymbol{u_x}$ with a frequency of 1.8 GHz – extensively used as the carrier frequency by cellular phones– and of amplitude 1V/m.

The equilibrium shape of human erythrocyte is a non nucleated flattened biconcave disk-shape cell with around 8 μm in diameter. In a first approximation to the cells stacked in a *rouleau* (Figure 1a), we will model the cells as layered oblate ellipsoids (Figure 1b). When the electric field is applied, the cells stack together with their minor axis parallel to the field (Figure 1c). The compartments representing an homogeneous inner medium or cytoplasm, the membrane and the external medium are characterized by a complex permittivity, $\bar{\varepsilon} = \varepsilon - i\sigma/\omega$, where ε is the permittivity, σ the conductivity and ω the angular frequency. Cell dimensions are a = 1.1 μm, b = 3.9 μm, the membrane thickness, δ = 8 nm and the electrical parameters [3]: ε_1 = 80, σ_1 = 0.12 S/m, ε_m = 9.04, $\sigma_m = 10^{-6}$ S/m, ε_2 = 50, σ_2 = 0.53 S/m, for the extracellular fluid, the membrane and the cytoplasm, respectively. As cell dimensions are much smaller than the wavelength at the working frequency (≈ 2 cm), a quasi-static approximation can be used. The ellipsoidal model normally used for the analytical solution of the Laplace's equation in ellipsoidal coordinates, assumes the surface of the membrane to be confocal with the core, producing a shell of non uniform thickness. However, several works have demonstrated the importance of an adequate representation of the cell geometry in order to avoid errors in calculating the polarizability of confocal ellipsoids with large excentricity [4,5], as it is the case of erythrocytes. Therefore, we have turned to numerical methods to obtain a more precise estimation of polarizability values. The different size scales involved in the problem, i.e., microns for the cell radius and nanometers for the membrane thickness, make a boundary element method (BEM) probably the best suited: it avoids the use of very dense grids and the handling of a very large number of equations, needed in traditional finite difference (FDM) or finite element methods (FEM). In BEM the discretization is restricted to the boundaries, reducing the memory required and making this approach especially appropriate for treating open problems, as it is the case of a bioparticle subjected to an electromagnetic field. Our special version of BEM has been checked in cases analytically solvable with excellent accuracy and numerical stability [6], and has also been applied for the assessment of the electrical behaviour of several bioparticles [7].

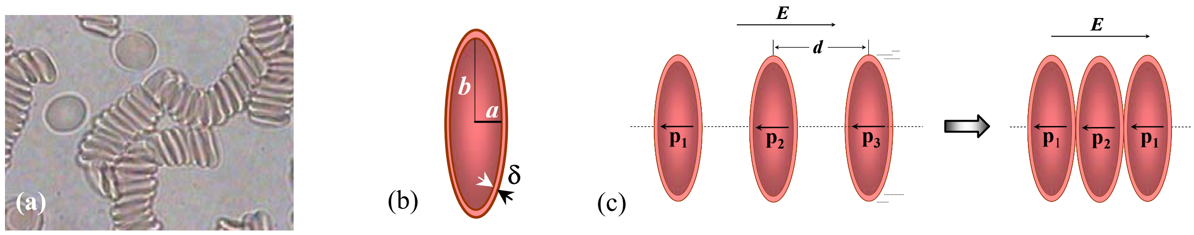

Figure 1 (a). Microscopic image (100x) of erythrocytes in a sample of blood forming *rouleaux*. (b) Transversal view of the oblate ellipsoidal model used for the RBC. (c) RBCs immersed in the field attract each other because of the favourable dipole-dipole interaction energy, leading to the *rouleau* formation

3. Numerical method for the surface charge density computation

Consider the RBC immersed in a conductive medium, and subjected to an external electromagnetic field of frequency ω. The total complex charge density $\tilde{\tau}(\boldsymbol{r})$ at a point defined by $\boldsymbol{r}$ at the interface between two different media i and j -pointing from medium i to medium j-, is given by the following Fredholm integral equation [6]

$$\tilde{\tau}(\boldsymbol{r}) = 2\,\tilde{w}_{ij}\,\tilde{\tau}^{0}(\boldsymbol{r}) - \frac{1}{2\pi}\tilde{w}_{ij}\sum_{S_k}\int_{S_k}\tilde{\tau}(\boldsymbol{r}')\frac{\partial G}{\partial n}(\boldsymbol{r},\boldsymbol{r}')\,dS',\tag{1}$$

where $\tilde{w}_{ij} = (\tilde{\varepsilon}_i - \tilde{\varepsilon}_j)/(\tilde{\varepsilon}_i + \tilde{\varepsilon}_j)$, $\tilde{\tau}^0 = \varepsilon_0\,\tilde{\boldsymbol{E}}^0_{ij}(\boldsymbol{r})$, being $\tilde{\boldsymbol{E}}^0_{ij}$ the normal component of the applied field at $\boldsymbol{r}$, and $\tilde{\tau}(\boldsymbol{r}')$ the charge density located on the interface S_k, that can be a non-connex surface as it is the case for a layered particle. $G(\boldsymbol{r}, \boldsymbol{r}')$ is the electrostatic Green's function; for a 3D geometry, $G(\boldsymbol{r}, \boldsymbol{r}') = 1/|\boldsymbol{r} - \boldsymbol{r}'|$. A convenient discretization of the surfaces is obtained by slicing the cell geometry perpendicularly to the x-axis into N small elements. On each of these surface elements the charge density is assumed to be approximately constant. Thus, the integral equation is converted into a set of linear equations easily solvable. The approach has demonstrate to give accurate results, even for a value of N as small as ~ 100, and is free of numerical instabilities. Having solved the linear system and computed the polarization charges and $\tilde{\tau}(\boldsymbol{r})$ the dipole moment induced by the external field is calculated as

$$\tilde{\boldsymbol{p}} = \sum_{i=1}^{N}\tilde{\tau}_i\,\Delta_i\,\boldsymbol{r}_i\tag{2}$$

Once the dipole moment has been calculated, an analytical estimation of the interaction energy between two of them can be obtained using the expression

$$U = \frac{\mathrm{Re}\left(p_1 p_2^*\right)}{8\pi\varepsilon_1 d^3}\left(\cos\alpha - 3\cos\theta_1\cos\theta_2\right)\tag{3}$$

where α is the azimuthal orientation angle of the dipole moment $\boldsymbol{p}_2$ in reference to $\boldsymbol{p}_1$, and θ_1 and θ_2 are the polar orientation angles of $\boldsymbol{p}_1$ and $\boldsymbol{p}_2$, respectively, and $d = |\boldsymbol{r}_2 - \boldsymbol{r}_1|$ the distance between their centers.

4. RBC mutual interactions and *rouleau* electric energy

Polarization cell properties have been the subject of extensive research in recent years, but the study of cell electric interactions has received much less attention. In our study, RBCs are modelled as oblate uniformly shelled ellipsoidal particles, separated a distance *d*. The distortion of the electric field in the vicinity of a cell affects the neighbouring ones and modifies their polarization, as it is shown in the following analysis.

First, we have studied the surface charge distribution along the cytoplasm-membrane and the membrane-external medium interfaces for a *rouleau* with $n = 3$ cells. Next, the transmembrane induced potential can be calculated from the relation between the induced charge density and the normal component of the electric field at the cytoplasm-membrane interface, as a function of the corresponding charge density that has already been obtained. Figure 2 shows these distributions for the cases of distant and stacked cells, when the applied field amplitude is 1 V/m. It has been considered an isolated RBC (cells separated a distance $d \gg a_0$), a central cell in the *rouleau* (cells in contact, $d=2a$), and an external cell in the *rouleau*. The mutual interactions affect significantly the charge distribution, especially along the minor axis direction. The induced transmembrane potential curves mimic the corresponding charge density distribution on the membrane-external medium interface. For cells in contact along the direction of the external electric field, the depolarizing fields lead to a lower induced voltage across the membrane compared to that of an isolated cell. This effect is physically related to the negative polarizability of cells in this range of frequencies: the field produced by the induced dipole in a RBC counteracts the effect of the external field on the neighbour cells. The transmembrane potential values at 1.8 GHz are lower than those found at low frequencies [8], because at high frequency the dominant factor is the ratio of the permittivity of the external medium to that of the membrane, whereas at low frequency the dominant effect is the ratio of the corresponding conductivities.

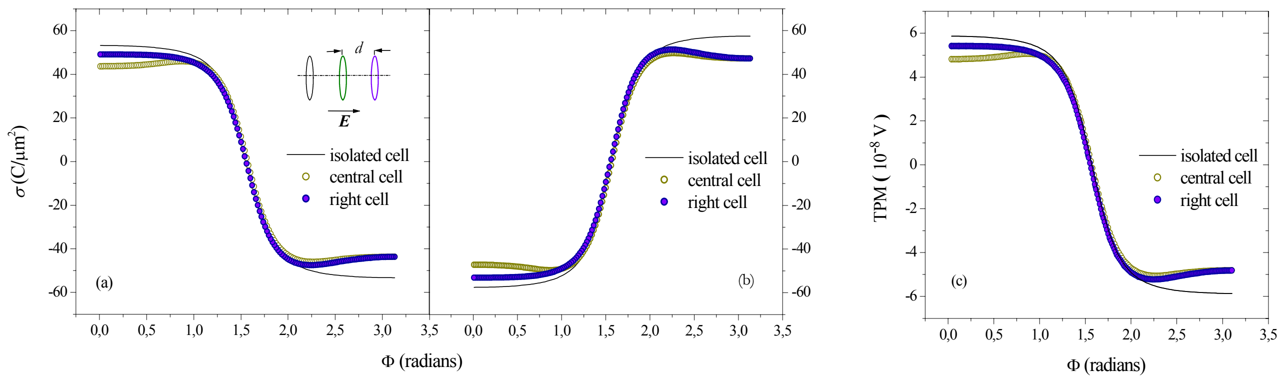

Figure 2. (a) Induced charge density distribution along the membrane-external medium interface; (b) along the cytoplasm-membrane interface; and (c) induced transmembrane potential along the cell surface. Solid line represents the case of an isolated cell in the external field, empty circles correspond to the central cell in the triplet, and solid circles, to the right cell. Φ is the angular coordinate of a surface point with respect to the field direction. The applied field amplitude is 1 V/m.

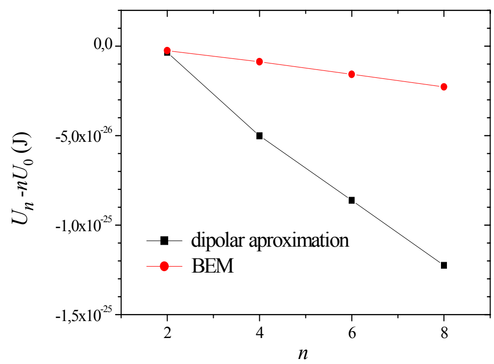

Figure 3. Energy decrease of a rouleau of n cells in an external field, calculated as the difference between the energy of isolated cells ($d \to \infty$) and that of the cells in the rouleaux ($d = 2a$).

Finally, the energy of a RBC *rouleau* has been calculated as the energy of the induced charges on each cell interface in the external field plus the interaction electrical energy between charges. This calculation includes not only dipole-dipole interactions but multipole effects at all orders. Results for the energy decrease when n RBCs aggregate to form a *rouleaux* are shown in figure 3, compared with the interaction energy of cells using dipole-dipole interactions. It can be observed that the dipole approximation overestimates the energy decrease in almost one order of magnitude for a *rouleau* with $n \geq 8$.

5. Conclusions

In the present work we have calculated the effect of a radiofrequency electric field on the polarization properties, the transmembrane potential and the resulting electric energy of a set of red blood cells in the process of forming a *rouleau*. The RBC has been modelled as shelled oblate spheroid. The membrane thickness in this geometry is uniform in contrast to analytical models that deal with nonuniform shells defined by confocal ellipsoids. The numerical results are in qualitative agreement with analytical calculations, using the confocal model and dipole-dipole interaction. Our approach has allowed studying the influence of neighbouring cells on important electrical magnitudes: the induced charge densities on the cytoplasm-membrane and membrane-external medium, the corresponding net dipole moment and the induced transmembrane voltage in a given erythrocyte during *rouleau* formation.

The comparison of the energies of free erythrocytes and aggregated erythrocytes reveals that the *rouleau* formation is energetically favourable when cells are immersed in the electromagnetic field. The energy differences found in this work for an external field of 1 V/m (a value in body fluids within the present exposure standards at the working frequency) are much smaller that the adhesion and mechanical energies involved in normal aggregated erythrocytes, but it has to be taken into account that the electric energy increases as the square of the field intensity and therefore the influence of exposure to high level electric fields on the aggregation properties of RBC can not be discarded. Besides, the existence of inhomogeneities in the membrane, not considered in our model, may produce high field local regions and therefore modify the aggregation properties. Our model gives insight into the cell interactions leading to *rouleau* or "*pearl-chain*", and is of potential value in the related fields of dielectrophoresis for cell handling and alignment.

7. References

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