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Effects of cell orientation and electric field frequency on the transmembrane potential induced in ellipsoidal cells

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ABSTRACT

The transmembrane potential ($\Delta\phi$) induced by external electric fields is important both in biotech applications and in new medical therapies. We analyzed the effects of AC field frequency and cell orientation for cells of a general ellipsoidal shape. Simplified equations were derived for the membrane surface points where the maximum $\Delta\phi$ is induced. The theoretical results were confirmed in experiments with three-axial chicken red blood cells (a:b:c = 6.66 µm:4.17 µm:1.43 µm). Propidium iodide (PI) staining and cell lysis were detected after an AC electropermeabilization (EP) pulse. The critical field strength for both effects increased when the shorter axis of a cell was parallel to the field, as well as at higher field frequency and for shorter pulse durations. Nevertheless, data analysis based on our theoretical description revealed that the $\Delta\phi$ required is lower for the shorter axis, i.e. for smaller membrane curvatures. The critical $\Delta\phi$ was independent of the field frequency for a given axis, i.e. the field strength had to be increased with frequency to compensate for the membrane dispersion effect. Comparison of the critical field strengths of PI staining in a linear field aligned along semi-axis a (142 kV m^{-1}) and a field rotating in the a–b plane (115 kV m^{-1}) revealed the higher EP efficiency of rotating fields.

1. Introduction

Membrane electropermeabilization (EP, also referred to as electroporation or dielectric breakdown) is probably the most important technique for the introduction of extracellular molecules, which do not penetrate cell membranes under normal conditions. It is generally assumed that membrane re-conformation and the formation of aqueous pores play a decisive role in the process of penetration [1]. EP is observed when the transmembrane potential ($\Delta\phi$) induced by the external field reaches a "critical voltage" of approximately 1 V [2–4]. Its magnitude depends on the experimental conditions [5,6]. Nevertheless, thorough investigations have shown that the experimental observation of a "critical voltage" results from the strongly nonlinear auto-regulation processes involved in pore formation [7–9]. One important effect is the voltage drop in the external medium for the current flowing through the pore. This voltage drop increases with pore size, leading to a down-regulation of $\Delta\phi$. Nevertheless, the assumption of a "critical voltage" is feasible for practical reasons and we will use the term in the following.

EP of cells depends on several electric parameters, e.g. field strength, pulse duration, number of pulses [6,10–17] and pulse shape [18–20]. Cell size, shape and orientation, medium conductivity and temperature are also important [6,21–23]. $\Delta\phi$ is proportional to the length of the axis oriented in the field direction and a shape factor that is related to the depolarizing factors [24–25]. Chang et al. [26] described how AC-fields are more effective in EP than DC fields, and result in an increased cell viability. The frequency dependence of $\Delta\phi$ in AC-fields depends on medium conductivity [22]. Capacitive membrane bridging causes the $\Delta\phi$ to decrease with increasing field frequency [4,22,27–34]. Clearly, rotating AC-fields generated by the superposition of two perpendicularly oriented fields are even more effective than the linear AC-fields [28,35,36].

New developments focus on EP in microfluidic devices with an EP process controlled at the single cell level [37,38]. The aims are to achieve high effectiveness in the genetic manipulation of cells [39–43] and effective cell lysis prior to subcellular analysis [44].

Gimsa and Wachner [25,45] have derived analytical expressions of $\Delta\phi$ for both orientations of the symmetry axis of spheroidal cells, assuming a low conductive membrane and a highly polarizable cytoplasm. The same ansatz could be extended to arbitrarily oriented cells of the general ellipsoidal shape, including all electrical parameters [30]. Recently, we derived simplified expressions avoiding the complex depolarizing factors for spheroidal cells [46].

In this work, we analyze the $\Delta\phi$ induced by AC field pulses in arbitrarily oriented ellipsoidal cells. The theoretical description is compared to experimental results obtained from the EP of the roughly ellipsoidal chicken red blood cells (CRBCs). Experiments were conducted

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in a chip chamber with two comb-shaped electrodes. Each comb had three interdigitating fingers, allowing for five different inter-electrode distances. The EP of the cells was determined from the two criteria, propidium iodide (PI) permeabilization detected by fluorescence-staining of the cell nuclei and cell lysis, respectively. We considered the effects of field strength, pulse field frequency, pulse duration and cell orientation.

2. Theory

2.1. $\Delta\phi$ for general orientation of ellipsoidal cells

2.1.1. A finite element ansatz for $\Delta\phi$ of the oriented single shell model

Gimsa and Wachner [30] have presented a simplified finite element ansatz for the $\Delta\phi$ of a single shell ellipsoidal model. The model consists of the internal, membrane and external media, designated by the indices *i*, *m* and *e*, respectively. Each medium is presented by a prismatic element (see [30]). The impedance Z^* of each element is given by the geometry (cross-sectional area *A* and length *l*):

$$Z^* = \frac{1}{\sigma^*}\frac{l}{A} \quad \text{with } \sigma^* = \sigma + j\,\omega\,\varepsilon\,\varepsilon_0 \tag{1}$$

σ^*, ε, ε_0 and *j* stand for the complex specific conductivity of the considered medium, relative permittivity, permittivity of vacuum and $\sqrt{-1}$, respectively. Eq. (1) is equivalent to a resistor capacitor- (RC-) pair (see lump model in [30]). The length l in Eq. (1) is given by the dimensions. The cross-sectional areas A of each element are assumed to be equal and infinitely small. They are oriented perpendicular to the field. Starting from this "finite element model", for an ellipsoidal cell with semiaxis a oriented in parallel to the field direction $\Delta\phi$ at pole a can be expressed by the voltage divider properties of the lump model (see [30] for details):

$$\begin{aligned}\Delta\phi_a = -a\left(E_{\mathrm{loc},a} - E_{i,a}\right) &= -\frac{\left(Z^*_{i,a}+Z^*_m\right)\phi_a^{\max}}{Z^*_{i,a}+Z^*_m+Z^*_{e,a}} + \frac{Z^*_{i,a}\phi_a^{\max}}{Z^*_{i,a}+Z^*_m+Z^*_{e,a}}\\ &= \frac{-Z^*_m}{Z^*_{i,a}+Z^*_m+Z^*_{e,a}}\,a_{\mathrm{infl}}E_x\end{aligned} \tag{2}$$

E_x, $E_{\mathrm{loc},a}$ and $E_{i,a}$ stand for the external field in *x*-direction, the effective internal local field of the body and the cytoplasmic field, respectively. Please note that $\Delta\phi_a$, $E_{\mathrm{loc},a}$ and $E_{i,a}$ are complex terms. Nevertheless, for simplicity we only mark the impedances Z^* of the prismatic elements by asterisks. $\phi_a^{\max}$ stands for the maximum of $\phi_{\mathrm{loc},a}$ determined by the influential radius a_{infl} along axis *a*. Index *a* refers to the geometry along semiaxis *a*. No axis index is required for the membrane impedance because the membrane elements are assumed to possess the same geometry along each principal axis. Please note that the model is largely consistent with (or even superior over) the Laplace-description for reasonable cell properties [30].

2.1.2. $\Delta\phi$ for arbitrarily oriented cells of the general ellipsoidal shape

The general ellipsoidal model is described by three principal semi-axes *a*, *b*, and *c* of different length. Special cases are spheroidal models with *a* = *b* and spherical ones with *a* = *b* = *c*. The semi-axes can be used to determine a Cartesian, orthonormal coordinate system *x*, *y*, and *z* where *x*, *y*, and *z* are parallel to *a*, *b*, and *c*, respectively (Fig. 1). The homogeneous external field ***E*** is oriented arbitrarily within this coordinate system. Its orientation is determined by the angles δ and γ. The local vector ***P*** of the membrane point under consideration is determined by the angles α and β. The induced transmembrane potential ($\Delta\phi_p$) at a membrane point (index *p*) is:

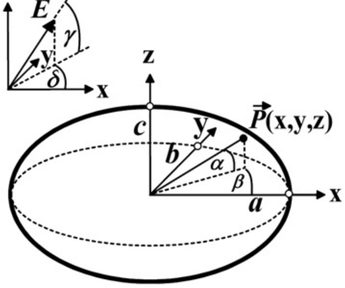

Fig. 1. Sketch of a cell of the general ellipsoidal shape. The components of *P* and *E* are given in spherical coordinates. The principal semiaxes *a*, *b* and *c* define the orthonormal coordinate system, *x*, *y* and *z*. *P* (*x*, *y*, *z*) is the local vector of a membrane surface point defined by the angles α and β. The external electric field *E* has an arbitrary orientation, determined by the angles δ and γ.

$$\Delta\phi_p = \begin{pmatrix} \dfrac{-Z^*_m}{Z^*_{i,a}+Z^*_m+Z^*_{e,a}}\dfrac{a_{\mathrm{infl}}}{a}d_xE_x \\ \dfrac{-Z^*_m}{Z^*_{i,b}+Z^*_m+Z^*_{e,b}}\dfrac{b_{\mathrm{infl}}}{b}d_yE_y \\ \dfrac{-Z^*_m}{Z^*_{i,c}+Z^*_m+Z^*_{e,c}}\dfrac{c_{\mathrm{infl}}}{c}d_zE_z \end{pmatrix} = \begin{pmatrix} \dfrac{d_x}{a}\Delta\phi^*_a \\ \dfrac{d_y}{b}\Delta\phi^*_b \\ \dfrac{d_z}{c}\Delta\phi^*_c \end{pmatrix} \tag{3}$$

for an arbitrary orientation of the inducing field (see [30] for details). a_{infl}, b_{infl} and c_{infl} stand for the influential radii of the ellipsoidal cell along the three semiaxes. Again, all $\Delta\phi$-terms are complex. The influential radius along each semiaxis refers to a certain distance from the center of the cell [45]. In the center, a symmetry plane can be defined for each semiaxis that is oriented perpendicular to the semiaxis. Field components (E_x, E_y, E_z) parallel to a semiaxis will not change the potential in the respective symmetry plane for symmetry reasons. The $\Delta\phi$-components at the three poles *a*, *b*, and *c* are solely induced by the respective field components (E_x, E_y, E_z) along the semiaxes. d_x, d_y, and d_z denote the distance of the membrane point to the three symmetry planes. At the poles $d_x = a$, $d_y = b$, and $d_z = c$. For a cytoplasmic conductivity much higher than the membrane conductivity and a very thin membrane, $\Delta\phi_p$ is given by the sum of the vector components of Eq. (3) (see [30] for a detailed consideration). Neglecting the permittivities of the cytoplasm and the external medium, from Eq. (3) we obtain [30]:

$$\begin{aligned}\Delta\phi_p = &\frac{-a_{\mathrm{infl}}E_xd_x/a}{\left(1+ag_m\left(1/\sigma_i+(a_{\mathrm{infl}}-a)/a\sigma_e\right)\right)\sqrt{1+f^2/f^2_{c,a}}}\\ &+\frac{-b_{\mathrm{infl}}E_yd_y/b}{\left(1+bg_m\left(1/\sigma_i+(b_{\mathrm{infl}}-b)/b\sigma_e\right)\right)\sqrt{1+f^2/f^2_{c,b}}}\\ &+\frac{-c_{\mathrm{infl}}E_zd_z/c}{\left(1+cg_m\left(1/\sigma_i+(c_{\mathrm{infl}}-c)/c\sigma_e\right)\right)\sqrt{1+f^2/f^2_{c,c}}}\end{aligned} \tag{4}$$

with

$$f_{c,a} = \frac{1}{2\pi C_m}\left(\frac{\sigma_e\sigma_i}{a\sigma_e+(a_{\mathrm{infl}}-a)\sigma_i}+g_m\right) \tag{5}$$

C_m and g_m stand for the area-specific membrane capacitance in Fm^{-2} and the area-specific membrane conductance in Sm^{-2}, respectively. They are given by $C_m = \varepsilon_0\,\varepsilon_m/d$ and $g_m = \sigma_m/d$ with *d* being the membrane thickness. *f*, $f_{c,a}$, σ_i, and σ_e stand for the external field frequency, the characteristic frequency of membrane polarization along semiaxis *a*, and the internal and external conductivities, respectively. Expressions analogous to Eq. (5) hold along the other two semiaxes.

2.1.3. Maximum of $\Delta\phi_p$ for semiaxis b being oriented perpendicular to the external field

The field vector is parallel to the *x*–*z* plane for semiaxis *b* being orientated perpendicular to the external field ($\delta = 0°$, Fig. 2A). A new analytical expression can be derived from Eq. (3) for the membrane surface point reaching the maximum $\Delta\phi$ if only peripheral points in the *a*–*c* plane ($\beta = 0°$) are considered. The angle $\alpha_{(\Delta\phi^{\max})}$ determining this point can be obtained considering the α-dependence of the sum of the *x*- and *z*-components of Eq. (3) (compare to Eq. (4)). The α-dependence of Eq. (3) is obtained after introduction of angular coordinates for the distances d_x, d_y, and d_z (see [30] and compare to