

The effect of resting transmembrane voltage on cell electropermeabilization: a numerical analysis

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Abstract

The transmembrane voltage induced due to applied electric field superimposes to the resting transmembrane voltage of the cell. On the part of the cell membrane, where the transmembrane voltage exceeds the threshold transmembrane voltage, changes in the membrane occur, leading to increase in membrane permeability known as electropermeabilization. This part of the cell membrane represents the permeabilized area through which the transport of molecules occurs. In this paper we calculated numerically the permeabilized area for different electric field strength, resting transmembrane voltage, cell shape and cell orientation with respect to the applied electric field.

Results show that when the transmembrane voltage is near the threshold transmembrane voltage, the permeabilized area of the cell is increased on the anodic side and decreased on the cathodic side due to the resting transmembrane voltage. In some cases, only anodic side of the cell is permeabilized. Therefore, by using bipolar pulses, the permeabilized area can be significantly increased and consequentially also the efficiency of electropermeabilization. However, when the induced transmembrane voltage is far above the threshold, the effect of the resting transmembrane voltage is negligible. These observations are valid for different cell shapes and orientations.

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1. Introduction

One of commonly used techniques for achieving transmembrane transport of molecules which otherwise cannot cross membrane is electropermeabilization, a method where the applied electric field is used to permeabilize cell membrane [1–6]. Under normal physiological conditions and when no electric field is applied, only resting transmembrane voltage exists. When the electric field is applied, the induced transmembrane voltage is superimposed to the resting transmembrane voltage [2,7,8], thus, the resulting transmembrane voltage is a sum of both the resting and the induced transmembrane voltage. When the threshold value of the transmembrane voltage is exceeded, biochemical and physiological changes occur in the cell membrane, which consequently lead to the increased permeability for ions and molecules [5,9]. Further increase in applied electric field intensity may cause irreversible membrane permeabilization and cell death.

The transmembrane voltage ($\Delta\phi$) is therefore a critical parameter for cell permeabilization [10]. The absolute value of the threshold transmembrane voltage ($\Delta\phi_c$) is in the range of 200–1000 mV [8,11–14]. Since $\Delta\phi_c$ can in many cases be considered much larger than the resting transmembrane voltage ($\Delta\phi_r$) (which is between -20 and -200 mV [15]), in most cases $\Delta\phi_r$ is not taken into account. To verify if such a simplification is justified, we evaluated the importance of $\Delta\phi_r$ on the cell permeabilization. As a parameter that determines the extent of permeabilization, we chose the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ since permeabilization occurs only on the part of the cell membrane where $\Delta\phi$ exceeds the threshold value, i.e. the area which represents the pathway for transport of molecules through the membrane [16–19].

The asymmetry of permeabilization due to $\Delta\phi_r$ was observed in several experiments [7,8,20–23] where in some cases only permeabilization on the anodic side of the cell was observed. We therefore analyzed also the difference in permeabilized area on the anodic and cathodic side of the cell membrane and evaluated this effect for different parameters.

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2. Materials and methods

The induced transmembrane voltage ($\Delta\phi_i$) was calculated numerically for a sphere and for prolate and oblate spheroids having different ratios of radii $\rho = R_1/R_2$ at various angles of cell orientation α with respect to the applied electric field (Fig. 1). For cells that slightly differ from spherical shape ratio $\rho = 10/8$ was used for prolate spheroid ($\rho = 8/10$ for oblate spheroid). In the case of cells that deviate more from spherical shape, the ratios $\rho = 10/5$ (5/10) and 10/2 (2/10) were used (like plated CHO cells, which are approximately 40 μm long and 20 μm wide; bacillus, which are 4 μm long and 0.8 μm wide; and erythrocytes, which are similar to 2 μm long and 8 μm wide oblate spheroid).

All calculations were performed for only one size of the cells, however, due to the linearity of the problem, the results for all other cell sizes can be obtained by rescaling the results obtained for our specific case. If we have a cell, which is two times larger, then the same $\Delta\phi_i$ and consequentially the percentage of the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ will be achieved at two times lower electric field strength, since $\Delta\phi$ is proportional to $E \times R$, where R is the larger of both radii. In all our models we set R to be 10 μm (for prolate spheroid $R = R_1$, for oblate spheroid $R = R_2$).

A cell was modeled as a non-conductive spheroid placed in a conductive medium. By applying positive and negative potential of the same absolute value on the two opposite sides of the box we generated homogenous electric field. More detailed description of the model is given in Valič et al. [24].

If no $\Delta\phi_r$ is included in the model, the centre of the cell has potential zero volts due to the symmetry of the geometry. $\Delta\phi_r$ was superimposed by setting the centre of the cell on the preferred value of $\Delta\phi_r$, 0 or -100 mV.

The object of the analysis was the area of the cell membrane which is permeabilized, i.e. where membrane permeability is increased due to the exposure to the applied electric field. Therefore, we calculated $\Delta\phi$ for the above mentioned cell geometries and calculated the

area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ for different angles of cell orientation α (0° , 15° , 30° , 45° , 60° , 75° and 90°). We used a static model, where $\Delta\phi$ is calculated at the moment after the transient and before the permeabilization takes place therefore membrane is assumed to be non-conductive.

A finite-elements model of a cell in a conducting medium was built using a program package of Ansoft (Pittsburgh, PA, USA), Maxwell. Additional drawings and comparison between results were performed with Matlab (The Math-Works, Massachusetts, USA).

3. Results

The induced transmembrane voltage ($\Delta\phi_i$) was calculated numerically for different cell orientations and applied electric field strengths from 100 to 700 V/cm in steps of 100 V/cm. By superimposing $\Delta\phi_i$ to the resting transmembrane voltage ($\Delta\phi_r$) we obtained transmembrane voltage ($\Delta\phi$). As the area which will be permeabilized we defined the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ for different $\Delta\phi_c = 200, 300, 400, 500$ mV.

In Fig. 2, the percentage of the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ for a sphere and for prolate spheroids having ratios $\rho = 10/5$ and 10/2 is presented for different angles of cell orientation α . On the left side (a, c, e), data for applied electric field $E = 100, 200$ and 300 V/cm are shown for $\Delta\phi_c = 200$ mV, whereas on the right side (b, d, f) data for applied electric field $E = 300, 500$ and 700 V/cm are shown for $\Delta\phi_c = 500$ mV. For each applied electric field strength two cases were examined. One, when $\Delta\phi_r$ is zero, and the other, when $\Delta\phi_r$ is -100 mV (denoted with index r). In Fig. 2, a and b data for a sphere are presented. As expected the angle of cell orientation α due to the spherical symmetry has no effect on the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$. Altogether, the value of $\Delta\phi_r$ is not important when $\Delta\phi_i \gg \Delta\phi_c$. However, when $\Delta\phi_i$ is close to $\Delta\phi_c$ we can see that $\Delta\phi_r$ reduces the applied electric field strength needed for permeabilization. For an ideal non-conductive membrane and steady-state conditions, the transmembrane voltage is described by:

$$\Delta\phi_i = fER\cos\vartheta. \quad (1)$$

From the above equation, $\Delta\phi_i$ is calculated for the applied electric field and added to $\Delta\phi_r$. Therefore the $\max |\Delta\phi_{\text{anodic}}| = \max |\Delta\phi_i| \pm |\Delta\phi_r|$ will give for a sphere ($f = 1.5$) in the case of $\Delta\phi_r = 100$ mV 550 mV on anodic side and 350 mV on cathodic side. Therefore if $\Delta\phi_c = 500$ mV a part of the cell membrane is permeabilized, whereas in the case of $\Delta\phi_r = 0$ mV $\max \Delta\phi_{\text{anodic}} = \Delta\phi_{\text{cathodic}} = 450$ mV and no permeabilization occurs. For comparison with $\Delta\phi_c$ the corresponding $\Delta\phi_i$ for the applied electric field is presented in legend of Fig. 2, calculated from the depolarizing factors given in Valič et al. [24].

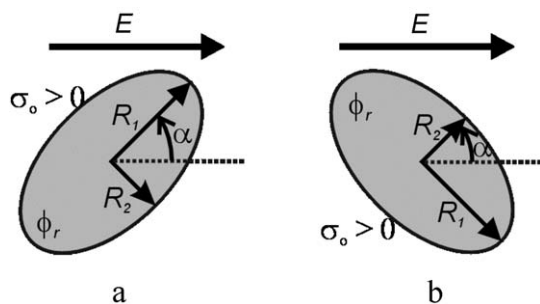


Fig. 1. Cross-section of a prolate (a) and oblate (b) spheroid exposed to the applied electric field. α is the angle of cell orientation between the applied electric field E and the axis of symmetry, which is in the case of a prolate spheroid parallel to R_1 and in the case of oblate spheroid parallel to R_2 .

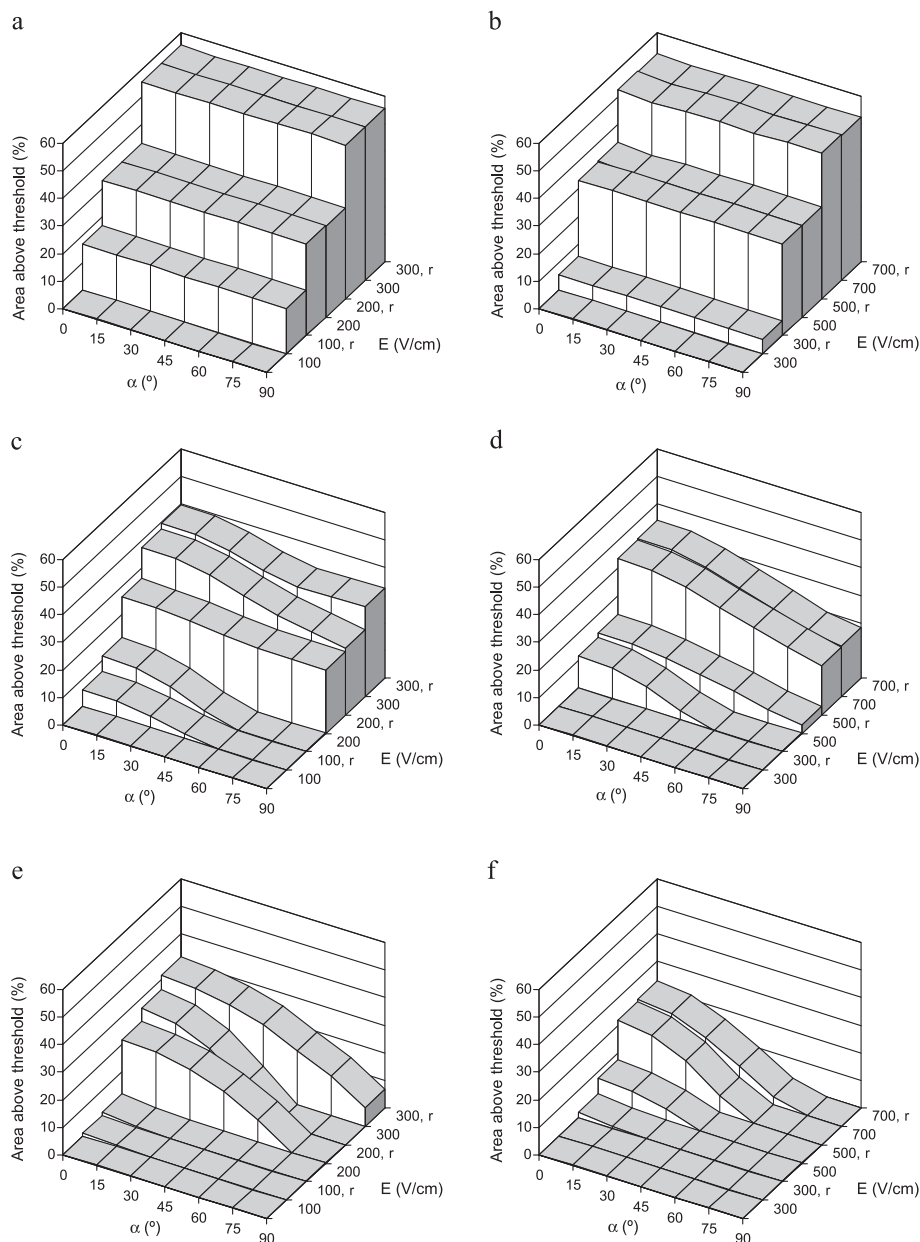


Fig. 2. The percentage of the area of the cell membrane having the absolute value of the transmembrane voltage higher than the threshold value of the transmembrane voltage ($|\Delta\phi| > \Delta\phi_c$) for a sphere (a and b), for prolate spheroid with ratio $\rho = 10/5$ (c and d) and for prolate spheroid with ratio $\rho = 10/2$ (e and f) is presented respectively for different cell orientation α . The non-zero resting transmembrane voltage ($\Delta\phi_r = -100$ mV) is denoted by r , else $\Delta\phi_r = -0$ mV. On the left side (a, c, e) $\Delta\phi_c$ is 200 mV and on the right side (b, d, f) 500 mV. Applied electric field strength [100, 200, 300, 500, 700] V/cm corresponds to maximum induced transmembrane potential $\max \Delta\phi_i = [150, 300, 450, 750, 1050]$ mV on a sphere, to [120, 240, 360, 600, 840] mV on a prolate spheroid with ratio $\rho = 10/5$ and to [120, 240, 360, 600, 840] mV on a prolate spheroid with ratio $\rho = 10/2$.

By comparing Fig. 2a to 2b, we can see that $\Delta\phi_r$ is more important in the cases where $|\Delta\phi|$ is only a little higher than $\Delta\phi_c$ and when $\Delta\phi_r$ is close to the $\Delta\phi_c$.

Results for spheroidal cell (shown in Fig. 2c,d,e,f) for $\rho = 10/5$ and $10/2$ are similar to the results for the sphere with some differences: (i) lower average area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$, since $\Delta\phi_i$ depends also on geometric factor f ; (ii) for spheroidal cells the orientation angle α significantly influences the area of

the cell membrane having $|\Delta\phi| > \Delta\phi_c$. This effect is more pronounced for cells that deviate more from spherical shape.

Another important effect of the $\Delta\phi_r$ is the difference between the permeabilized area of the cathodic and anodic side. When $\Delta\phi_r$ is zero, both areas are equal. However, if $\Delta\phi_r = -100$ mV, then the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ on the anodic side is larger than the permeabilized area on the cathodic side (Fig. 3). This is

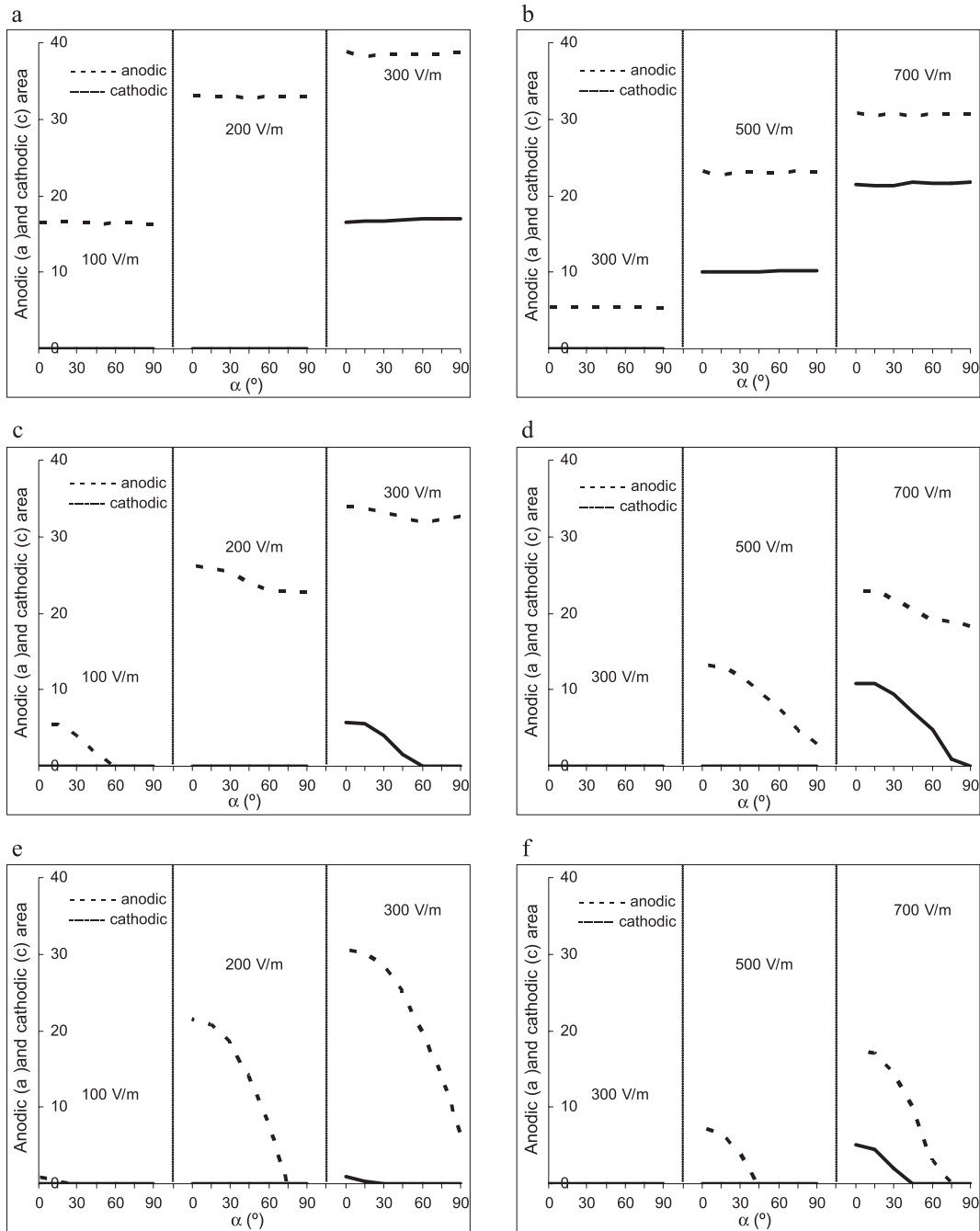


Fig. 3. Comparison of anodic (solid line) and cathodic (dashed line) area of the cell membrane having the absolute value of the transmembrane voltage higher than the threshold value of the transmembrane voltage ($\Delta\phi > \Delta\phi_c$) for resting transmembrane voltage $\Delta\phi_r = -100$ mV. Data for a sphere (a and b), for prolate spheroid with $\rho = 10/5$ (c and d) and $10/2$ (e and f) is presented for different angles of cell orientation α . On the left side (a, c, e) $\Delta\phi_c$ is 200 mV and on the right side (b, d, f) $\Delta\phi_c$ is 500 mV.

due to the fact that $\Delta\phi_r$ is negative and when it is superimposed to the $\Delta\phi_i$, which is also negative on the part of the cell membrane facing the anode, there is higher $|\Delta\phi|$ on the anodic side than on the cathodic side. Thus, permeabilization occurs first at the anodic side, as was demonstrated experimentally in a number of experiments [7,8,16,20–22].

4. Discussion and conclusion

The area of the cell membrane where the transmembrane voltage ($\Delta\phi$) exceeds the threshold value ($\Delta\phi_c$) represents the pathway for transport of molecules in electropereabilized cells [16–19]. In the presented study we theoretically analyzed the effect of cell geometry and orientation, resting

transmembrane voltage ($\Delta\phi_r$) and applied electric field strength, on the area having $|\Delta\phi| > \Delta\phi_c$. The values of $\Delta\phi_r$ and $\Delta\phi_c$ were varied in a range given in literature; however the data for different cells exhibit large differences. Therefore, for specific experimental conditions realistic values of the $\Delta\phi_r$, $\Delta\phi_c$ and cell shape have to be taken into account.

Based on the results which we obtained we can conclude that the major factor which determines the importance of $\Delta\phi_r$ is the ratio between $\Delta\phi_i$ and $\Delta\phi_c$. If $\Delta\phi_i \gg \Delta\phi_c$ the $\Delta\phi_r$ has negligible effect on the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$, i.e. on the permeabilized area. However, in most of the experimental conditions $\Delta\phi_i$ has to be near the $\Delta\phi_c$ to preserve cell viability therefore in this case the influence of $\Delta\phi_r$ is significant. In Fig. 2, it can be seen that the orientation of the spheroidal cell in applied electric field affects the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$, the effect being larger for cells that deviate more from spherical shape as already shown in our previous paper [24]. In general, however, the effect of resting transmembrane potential is similar for different orientations.

Furthermore, we evaluated for different parameters the effect of $\Delta\phi_r$ on the difference between the cathodic and anodic area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$. Again, the asymmetry is more pronounced when $\Delta\phi_i$ is close to $\Delta\phi_c$, however, the asymmetry is not significantly affected by the angle of cell orientation α .

The calculated asymmetry of the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ agrees with the observed asymmetric $\Delta\phi$ distribution and uptake of molecules [7,8,16,20–23].

It was already suggested previously by Kotnik et al. [25] that by using bipolar pulses instead of unipolar pulses, the area of the cell membrane having $|\Delta\phi| > \Delta\phi_c$ and by this also electropermeabilization can be increased for the same applied electric field while preserving the viability of cells. Our calculations support this suggestion that for $\Delta\phi_i$ in the range of $\Delta\phi_c$ or relatively high $\Delta\phi_r$ compared to $\Delta\phi_c$, bipolar pulses can significantly increase the efficiency of permeabilization.

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