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ELECTRIC FIELD-INDUCED TRANSMEMBRANE POTENTIAL DEPENDS ON CELL DENSITY AND ORGANIZATION

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ABSTRACT

Electrochemotherapy is a novel technique to enhance the delivery of chemotherapeutic drugs into tumor cells. In this procedure, electric pulses are delivered to cancerous cells, which induce membrane permeabilization, to facilitate the passage of cytotoxic drugs through the cell membrane. This study examines how electric fields interact with and polarize a system of cells. Specifically, we consider how cell density and organization impact on induced cell transmembrane potential due to an external electric field. First, in an infinite volume of spherical cells, we examined how cell packing density impacts on induced transmembrane potential. With high cell density, we found that maximum induced transmembrane potential is suppressed and that the transmembrane potential distribution is altered. Second, we considered how orientation of cell sheets and strands, relative to the applied field, affects induced transmembrane potential. Cells that are parallel to the field direction suppress induced transmembrane potential, and those that lie perpendicular to the applied field potentiate its effect. Generally, we found that both cell density and cell organization are very important in determining the induced transmembrane potential resulting from an applied electric field.

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INTRODUCTION

When an electric field is applied to a cell or cell system, a nonuniform transmembrane potential is induced in the exposed cells. Typically, under the assumption that cell membrane conductivity is much lower than cytosolic and extracellular conductivity, the anodal end of the cell becomes hyperpolarized and the cathodal end becomes depolarized (1). If the transmembrane potential induced by the field is large enough, i.e., above the threshold value, the cell membrane can break down in a reversible process called electropermeabilization (2), thus allowing entrance of molecules that otherwise cannot easily cross the cell membrane.

Because many chemotherapeutic drugs have a limited ability to cross the cell membrane under normal conditions, electropermeabilization has been applied in conjunction with chemotherapy to potentiate the cytotoxic effects of anticancer drugs (3–5). This process is known as electrochemotherapy.

In developing electrochemotherapy procedures, and in a variety of other areas of physiology as well (6), many questions have surfaced concerning how electric fields interact with cells and tissue. In particular, it has been noted that changing electrode orientation during treatment significantly increases the effectiveness of electrochemotherapy. Protocols that deliver eight pulses in one orientation have been shown to be less effective than protocols that deliver four pulses in one orientation, followed by four pulses delivered with a 90-degree electrode shift (7). To explain and understand this phenomenon, several ideas have been put forth. First, the role of nonuniform cell shape has been implicated (7). Second, the anisotropic conductive properties of the underlying muscle layer have also been established as a contributing factor (8).

Here, we will establish that nonuniform cell packing can be another contributing factor in this orientation-dependent behavior. Specifically, we will establish that cell-to-cell interactions are very significant in determining the response of a cell to an electric field. The orientation and organization of these neighboring cells relative to the direction of the field is very significant.

METHODS

A finite element model of an infinite volume of spherical cells was created for the study. The model was formulated as an infinite volume to take advantage of several model symmetries. In the first section of the study, a uniform infinite array of spherical cells was modeled. These cells were organized into a regular cubic lattice (Fig. 1). When exposed to a uniform field in one direction, this full infinite system can be modeled by formulating one quadrant of one cell and applying the appropriate boundary conditions. By examining the symmetries of the system, the proper boundary conditions are readily apparent. Isopotential conditions were applied at the two opposing faces in the direction of the applied field. The potential at these faces were set in accordance with a normalized 1-V/cm field. No flux boundary conditions were assigned to any other faces. Therefore, all transmembrane potential results are given in relative units of mV/(V/cm), that is, results are given in millivolts of transmembrane potential that will develop as a result of each V/cm of applied field strength. This is referred to as the relative transmembrane potential. In the first section of the study, the distance between adjacent cells, the packing ratio, was modulated to determine the impact of nearby cells

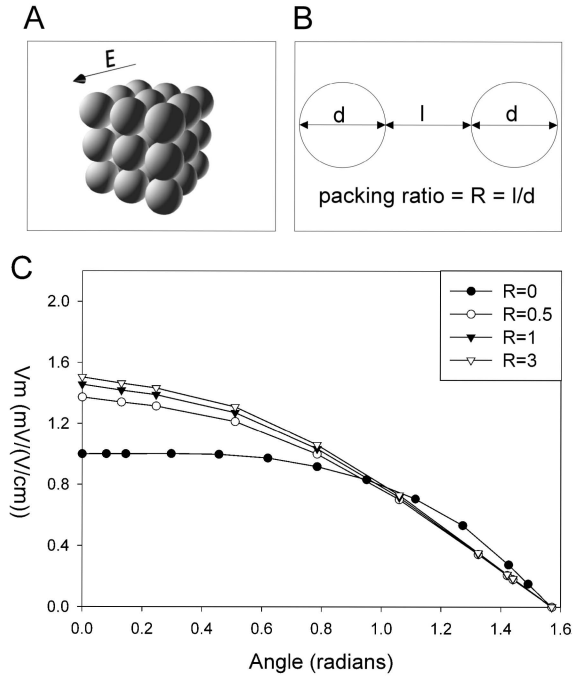

FIGURE 1. Transmembrane potential response of a uniformly packed infinite cell volume. Sample of cell packing lattice (A). Definition of a cell density ratio (B). Transmembrane potential for a single cell as a function of polar angle, with respect to applied field direction (C). Several packing densities are shown.

on induced transmembrane potential response. The packing ratio is defined as the ratio of cell distance to cell diameter (Fig. 1).

To simplify the modeling of this system, steady-state conditions were assumed, based on the fact that the length of the electric pulse is very long in comparison with the time constant of the cell membrane. The cells are assumed to be spherical, with radius of 10 μm, and nonconductive. Because the cell membrane is far less conductive than the extracellular space, this is a reasonable assumption. Certainly there are second-order effects associated with the nonlinear conductive properties of the cell membrane, which are not discussed here.

For the second part of the study, infinite sheets of spherical cells were modeled. The rationale for assembling the model in this section was exactly the same as in the first section. To model an infinite sheet of cells, one dimension is expanded such that the cells are far apart in this dimension. A packing ratio of 3.0 was chosen, based on the results of the first section. The other two dimensions were assumed to be fully packed (i.e., R = 0.0). Thus, the system is modeled as an infinite volume fille with sheets of cells. With a separation factor of 3.0 between sheets, there is negligible interaction between cell layers. The direction of the electric field, relative to the cell sheet orientation, was changed by 90 degrees between trials to determine the impact of sheet orientation on induced transmembrane potential response.

In the third part of the study, infinite strands of spherical cells were modeled. This model formulation follows the same rules as the infinite sheet formulation, except with R = 3.0 in two dimensions and 0.0 in a third dimension. To determine the impact of strand orientation on induced transmembrane potential, the field direction was changed by 90 degrees between trials.

The finite element models were built using the MSC/EMAS (ElectroMagnetic Analysis System) software package (MacNeal-Schwendler Corporation, USA) (9).

RESULTS

Uniform Cell Packing

Four trials were run to determine the effects of cell packing density on induced transmembrane potential due to a uniform applied field. Cells were packed with density ratios of 0.0, 0.5, 1.0, and 3.0 (Fig. 1). With low-density cell packing (R = 3), relative transmembrane potential followed a sinusoidal distribution by polar angle. Maximum relative potential was 1.5 mV/(V/cm), as predicted analytically (see Discussion). With higher cell packing densities (R = 1.0, R = 0.5, and R = 0.0), maximum relative transmembrane potential decreased, reaching a value of 1.0 mV/(V/cm) at a packing ratio of 0.0 (complete cell packing).

With changing cell density, the curvature of the potential profile plot also changed (Fig. 1). At high cell density, the plot approached maximum potential very rapidly. Compared with 28.7% for an isolated single cell, 52.2% of the membrane was within 10% of the maximum potential (Table 1).

Table 1. Summary of Results[a]

Cell system structure	Maximum relative transmembrane potential (mV/(V/cm)	% of Curve within 10% of maximum relative transmembrane potential
Single cell	1.50	28.7
Uniform cubic lattice		
R = 0.0	1.00	52.2
R = 0.5	1.37	28.8
R = 1.0	1.46	26.9
R = 3.0	1.50	26.4
Planar sheets		
Parallel to field	1.00	45.8
Perpendicular to field	2.12	36.1
Linear strands		
Parallel to field	1.00	45.3
Perpendicular to field	1.65	26.3

[a]R, the cell packing density, is the ratio of the cell radius (10 μm) to cell separation distance. Single cell data were found using the analytical formula for a single nonconductive cell in an infinite volume (Eq. 1).

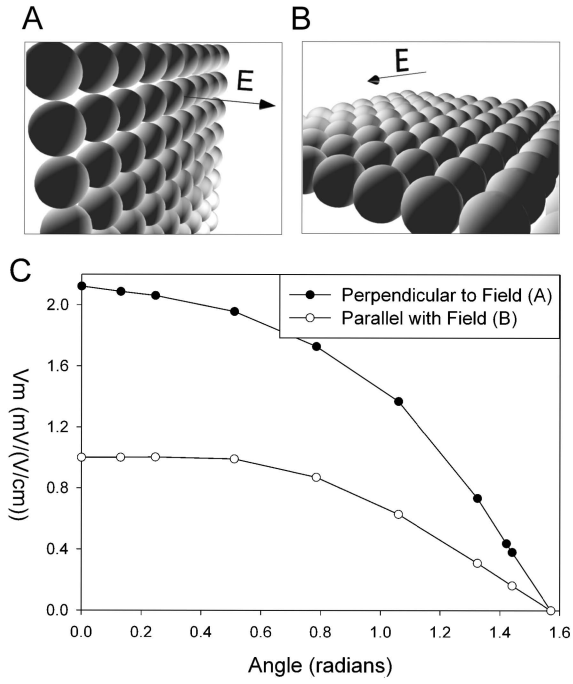

FIGURE 2. Transmembrane potential response of a fully packed (R = 0.0) sheet of spherical cells with a packing ratio of 3.0 between sheets. Cell sheet perpendicular to field (A). Cell sheet parallel to field (B). Transmembrane potential for a single cell as a function of polar angle, with respect to applied field direction (C). Parallel and perpendicular orientations are shown.

Cell Sheets

Two trials were run to determine the impact of orientation on the induced transmembrane potential in a planar sheet of cells (Fig. 2). First, the field was applied parallel to the plane. The induced relative transmembrane potential change reached a maximum of 1.00 mV/(V/cm), and 45.8% of the profile curve was within 10% of this maximum. Second, the field was applied perpendicular to the sheet. The induced relative potential reached a maximum of 2.12 mV/(V/cm) with 36.1% of the profile within 10% of this maximum.

Note that when the field is applied parallel to the cell sheet, the potential along the two principle planes of the cells will not be exactly the same (because one plane is perpendicular to the sheet whereas the other is parallel to the sheet; Fig. 2,B). However, the two profiles agree very well in shape and magnitude. For clarity, only the profile from the plane perpendicular to the sheet is presented.

Cell Strands

Two trials were run to determine the impact of orientation on the induced transmembrane potential in a linear strand of cells (Fig. 3). First, the field was applied

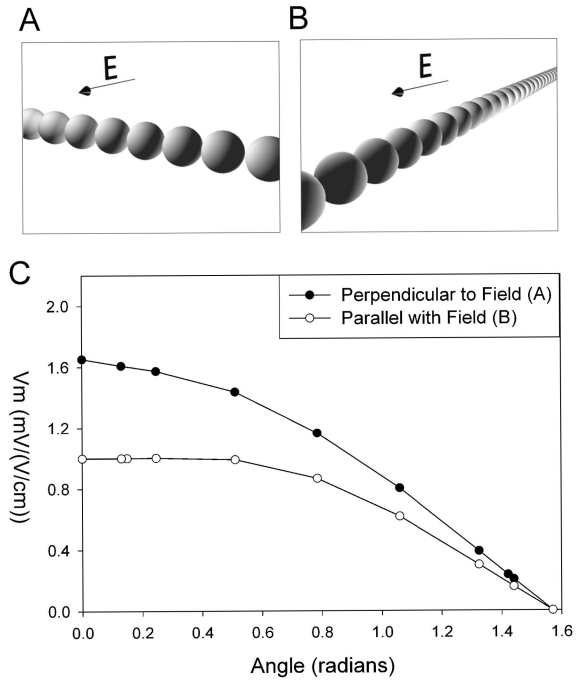

FIGURE 3. Transmembrane potential response for a fully packed (R = 0.0) strand of spherical cells with a packing ratio of 3.0 between strands. Cell strand perpendicular to field (A). Cell strand parallel to field (B). Transmembrane potential for a single cell as a function of polar angle, with respect to applied field direction (C). Parallel and perpendicular orientations are shown.

parallel to the axis of the strand. The induced relative potential reached a maximum of 1.00 mV/(V/cm) with 45.3% of the profile within 10% of this maximum. Second, the field was applied perpendicular to the axis of the strand. The induced relative transmembrane potential change reached a maximum of 1.65 mV/(V/cm), and 26.3% of the profile curve was within 10% of this maximum.

Note that when the field is applied perpendicular to the strand, the potential along the two principle planes of the cells will not be exactly the same (because one plane is perpendicular to the strand axis and the other is parallel to the strand axis; Fig. 3A). Again, as in the case of the cell sheets, the two profiles are very similar in both magnitude and shape. For clarity, only the profile from the plane perpendicular to the strand axis is presented.

DISCUSSION

We find that cell density and organization significantly impact induced transmembrane potential due to an external electric field. Full cell packing in a three-dimensional volume decreases transmembrane potential with increasing cell density. Furthermore, adjacent cells lying perpendicular to the field increase maximum trans-

membrane potential, and cells lying parallel to the field reduce transmembrane potential magnitude.

Cell Packing Perpendicular to the Field Direction

From the results, it is clear that a high degree of cell packing perpendicular to the applied field direction increases induced transmembrane potential. Maximal relative potential rose to 2.12 mV/(V/cm) for a sheet of cells and to 1.65 mV/(V/cm) for a strand of cells, compared with 1.50 mV/(V/cm) for a single isolated cell.

This result can be understood by considering the effect that neighboring cells have on extracellular current density. Nearby cells, which lie in the same plane with a cell of interest, will decrease the available extracellular space for the passage of the current. As a result, the current density in the remaining space will be increased. Increased current density corresponds to an increased potential drop around the cell. Therefore, maximul induced transmembrane potential, both at the anodal and cathodal ends of the cell, will be increased, as seen in the results.

Cell Packing in the Field Direction

In contrast to cells lying perpendicular to the field direction, adjacent cells lying in the field direction hinder the development of transmembrane potential. In all cases of maximal packing density in the direction of the field (including strands, sheets, and uniform volume packing), relative induced transmembrane potential rises to only 1.0 mV/(V/cm).

In the case of a single nonconductive spherical cell in an infinite volume conductor, steady-state induced transmembrane potential due to an applied field is given by the well-known expression (1):

$$\Delta\Phi_m = \frac{3}{2} ER\cos\theta \tag{1}$$

where E is field magnitude, R is the radius of the cell, and θ is polar angle measured with respect to the field. The 3/2 term is a form factor that reflects the impact of the nonconductive cell on the extracellular field distribution.

We can explain the influence of adjacent parallel cells by examining the form factor. In the case of a single cell, the extracellular potential distribution is perturbed by the presence of the nonconductive cell space. Current density near the cell is increased, as current must flow around the cell, resulting in the 3/2 factor. However, in the case of multiple adjacent cells oriented parallel to the field, this 3/2 form factor disappears [maximum relative potential was 1.0 mV/(V/cm)]. By examining the extracellular potential distribution, we find that nearby cells aligned with the direction of the field, reduce the degree to which the field forms around each individual cell. The field has already been reformed by the other cells in the direction of the field, so there is little redistribution around a given individual cell. In the case of a maximally packed three-dimensional volume, sheet, or strand of cells, the potential drop across every cell due to the symmetry of the system, must be equal, explaining the exact 1.0 mV/(V/cm) relative transmembrane potential.

Effects of Adjacent Cells on Transmembrane Potential Distribution

In addition to changes in the magnitude of induced transmembrane potential, cell packing and orientation also impacts the distribution of transmembrane potential. With a high degree of cell packing (i.e., R = 0), the percentage of the transmembrane potential profile curve within 10% of the maximal potential increased from 26% to 52% for cells packed in all three dimensions. Also, for cells closely packed in just two and one dimension, 45.76% and 45.27% of the profile curve fell within 10% of the maximum, respectively. However, these large changes are only seen when cells are closely packed (i.e., density ratio near 0.0) in the dimension of the applied field.

As discussed previously, with close cell packing along the field, the degree to which each cell perturbs the extracellular potential is reduced. Essentially, Φ_e becomes fixed between adjacent cells. This is apparent from an examination of the symmetries of the system. This virtual fixation of potential keeps more of the membrane at a high degree of polarization. As a result, whereas maximal potentials are lower with close packing in the field dimension (i.e., there is no 3/2 form factor), the percentage of the membrane that is strongly polarized is increased. There is significant deviation from the cosine dependence for a single isolated cell given in Eq. (1).

Implications for Electrochemotherapy

Recently, it has been noted that the efficacy of electrochemotherapy treatment is a function of electrode orientation (7). A variety of explanations have been put forward to explain this result. These include the influence of nonuniform cell shape (7), nonuniform field distribution in the tumor (7), and the anisotropic properties of the underlying muscle layer (8).

From these results, we find that nonuniform cell packing may be another factor that contributes to this electrode orientation dependence. If the tumor cell packing is not completely uniform, then there will necessarily be a preferential direction for generating maximum induced transmembrane potential. Therefore, by applying the field in two or more directions, there is a greater chance of achieving a transmembrane potential above the threshold for electropermeabilization in a large portion of the tumor. This results in increased drug delivery and increased effectiveness of electrochemotherapy treatment.

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