Induced Transmembrane Voltage during Cell Electrofusion Using Nanosecond Electric Pulses

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Abstract—**Electrofusion is a method for facilitating fusion of cells in close contact by using microsecond-duration electric pulses. Electric pulses induce a voltage across cell membranes, which leads to membrane electroporation and brings the membranes into fusogenic state. However, electrofusion efficiency is very low when fusion partner cells considerably differ in size, since the magnitude of the induced transmembrane voltage (TMV) depends proportionally on the cell size when microsecond pulses are applied. Recently, we proposed that the problem of fusing differently sized cells could be overcome by simply reducing the pulse duration to nanoseconds (ns). Namely, during ns pulse exposure the TMV depends less on the cell size and more on the electric properties of the cells and the surrounding medium. To further investigate the possibility of fusing cells with ns pulses, we constructed a finite element model of equally and differently sized cells in contact, mimicking their arrangement during electrofusion. We calculated the time course of TMV for fusion media with two different conductivities (σ_e = 0.01 and 0.1 S/m), which are widely used in existing electrofusion protocols. Our results demonstrate that ns pulses provide the possibility to selectively electroporate the contact areas between cells (i.e. the target areas for electrofusion), regardless of the size of fusion partner cells, and for a relatively wide range of pulse durations. In medium with σ_e = 0.01 S/m, selective contact area electroporation can be achieved with pulses of up to few microsecond duration, whereas in medium with σ_e = 0.1 S/m, shorter pulses with duration below few hundred nanoseconds need to be applied. Electrofusion by means of ns pulses could, therefore, provide a method for improving fusion efficiency in cases where cells of different size need to be fused, such as in hybridoma technology for monoclonal antibody production.**

Keywords—**electrofusion, electroporation, induced transmembrane voltage, finite element model**

I. INTRODUCTION

When a cell is exposed to an electric pulse, charges build on both sides of the cell membrane causing an increase in the transmembrane voltage (TMV). If TMV reaches a sufficiently high value (~1 V), structural rearrangement of the membrane lipid bilayer occurs (a phenomenon termed electroporation), which leads to substantial increase in the membrane permeability [1]. In the highly permeable, electroporated state, cell membranes are also fusogenic; provided that electroporated cells are in close contact, they can be fused [2]. Such method of cell fusion is known as electrofusion and is for example used in hybridoma technology for monoclonal antibody production [3] and in immunotherapy for production of cell vaccines [4]. In contrast to other fusion methods, no viral or chemical additives are required.

Conventionally, electrofusion is performed with 10–100 μs duration pulses, which ensures that cell membranes become fully charged during the exposure. Under such conditions, the magnitude of TMV (and consequently the extent of electroporation) is proportional to the cell size. This presents a challenge for fusing cells, which considerable differ in size, since large cells may not recover from application of electrical pulses required for electroporation of small cells. Ultimately, this leads to extremely low fusion yields. One typical example is hybridoma technology where small B lymphocytes are fused with large myeloma cells to form antibody-producing hybridoma cell lines [5].

Recently, a numerical study by Pucihar and Miklavčič suggested that the problem of fusing differently sized cells could be overcome by using shorter, nanosecond (ns) pulses [6]. Namely, in the nanosecond range, cell membranes are still in the charging phase and their TMV depends less on the cell size. Indeed, experiments on different cell lines demonstrated that cell size and shape play little role in electroporation with ns pulses [7].

In this paper we further investigate the possibility of fusing cells with ns pulses by performing calculations of TMV on a numerical model of two equally and differently sized cells in contact, during exposure to electric pulses. Since TMV in the nanosecond range significantly depends on the extracellular medium conductivity, we performed calculations for media with conductivities of 0.01 S/m and 0.1 S/m, which correspond to conductivities of fusion media widely used in existing electrofusion protocols [2,3,5]. Our results reveal important advantages of fusing cells with ns pulses, specifically in a low conductive medium (e.g. 0.01 S/m).

II. METHODS

Two-dimensional axisymmetric finite element model of cells in contact, mimicking the arrangement of cells during electrofusion, was constructed in Comsol Multiphysics 4.3 (Comsol, Burlington, MA, USA). Two spherical cells with

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either equal or different radii were placed in a rectangle representing the extracellular medium (Fig. 1). Cell nuclei were also included in the model, since during ns pulse exposure high electric field is also present in the cell interior and could potentially affect larger cell organelles [8].

The cells were exposed to electric field by assigning an electric potential to two opposite sides of the rectangle. The right side was grounded, whereas the left side was excited by a Heaviside function (Comsol functions *flc1hs*) with 1 ns rise time. Other boundary conditions are indicated in Fig. 1.

Electric potential in each subdomain of the model (extracellular medium, cytoplasm, nucleoplasm) was determined by equation:

$$-\nabla\left(\sigma_i \nabla V\right) - \nabla \frac{\partial\left(\varepsilon_i \nabla V\right)}{\partial t} = 0\,, \tag{1}$$

where σ_i and ε_i denote the conductivity and dielectric permittivity of a given subdomain, respectively. Membranes were included in the model by assigning a current density $\boldsymbol{J}$ through boundaries, representing the membranes [9]:

$$\boldsymbol{n}\cdot\boldsymbol{J} = \frac{\sigma_m}{d_m}\left(V - V_{ref}\right) + \frac{\varepsilon_m}{d_m}\left(\frac{\partial V}{\partial t} - \frac{\partial V_{ref}}{\partial t}\right) \tag{2}$$

Here, $\boldsymbol{n}$ is the unit vector normal to the boundary surface, V is the electric potential on the interior side of the boundary, V_{ref} is the potential on the exterior side of the boundary, and σ_m, ε_m, d_m, are the membrane conductivity, membrane dielectric permittivity, and membrane thickness, respectively. The TMV was then determined as the difference between electric potentials on each side of the boundary. The values of the model parameters are given in Table 1.

Segments of cell membranes, that formed the contact areas between cells, were assigned a thickness of two lipid membranes, as they account for part of the membrane of the left cell and part of the membrane of the right cell. We assumed that the TMV distributes equally between both membranes and that twice the TMV is required for electroporation of the contact area. For this reason, we present only half of the TMV calculated over the entire contact area in Fig. 2. The nuclear envelope was considered in the same way, as it consists of two lipid membranes.

III. Results and Discussion

The model was used to calculate the time course of TMV on cells in contact after the onset of exposure to an external electric field. The TMV is presented for a time window ranging from 1 ns to 100 µs. Since the magnitude of TMV correlates with the extent of membrane electroporation, this approach can be used to identify "primary targets" for electroporation with respect to the pulse duration. For example, if the highest TMV at time 100 ns is established on

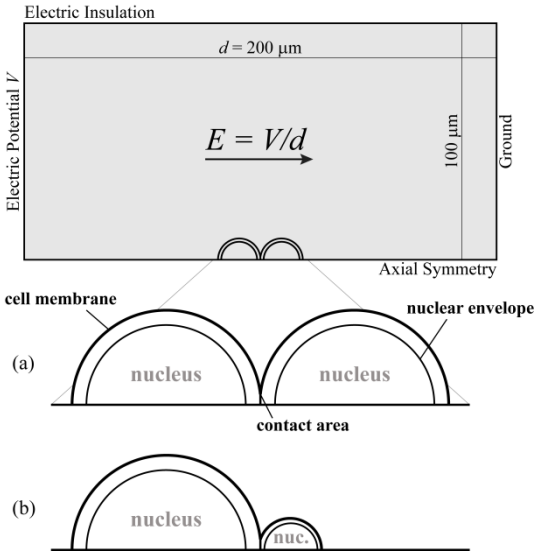

Fig. 1 Model of two cells in contact, exposed to electric field. (a) Two equally sized cells with radii of 9 µm. (b) Two differently sized cells with radii of 9 µm and 3 µm. The magnitude of the electric field was determined as the voltage difference between the left and the right side of the rectangle (the electrodes), divided by the electrode distance. The direction of the electric field is indicated with an arrow.

Table 1 Parameters of the model

Parameter	Symbol	Value
Cell radius	R_c	9 µm, 3 µm [§]
Nuclear radius	R_n	7.6 µm, 2.5 µm [‡]
Extracellular medium conductivity	σ_e	0.01 S/m, 0.1 S/m
Extracellular medium permittivity	ε_e	80 ε_0
Cytoplasmic conductivity	σ_{cp}	0.25 S/m
Cytoplasmic permittivity	ε_{cp}	70 ε_0
Cell membrane thickness	d_{cm}	5 nm
Cell membrane conductivity	σ_{cm}	$5\cdot10^{-7}$ S/m
Cell membrane permittivity	ε_{cm}	4.5 ε_0
Nucleoplasmic conductivity	σ_{np}	0.5 S/m
Nucleoplasmic permittivity	ε_{np}	70 ε_0
Nuclear envelope thickness	d_{ne}	10 nm
Nuclear envelope conductivity	σ_{ne}	$1\cdot10^{-4}$ S/m
Nuclear envelope permittivity	ε_{ne}	7 ε_0
Vacuum permittivity	ε_0	$8.85\cdot10^{-12}$ F/m

[§]Chosen to cover the size range of myeloma cells and B lymphocytes.

[‡]Nucleus occupies 60% of the cytoplasmic volume, as considered typical for lymphocyte cells [10].

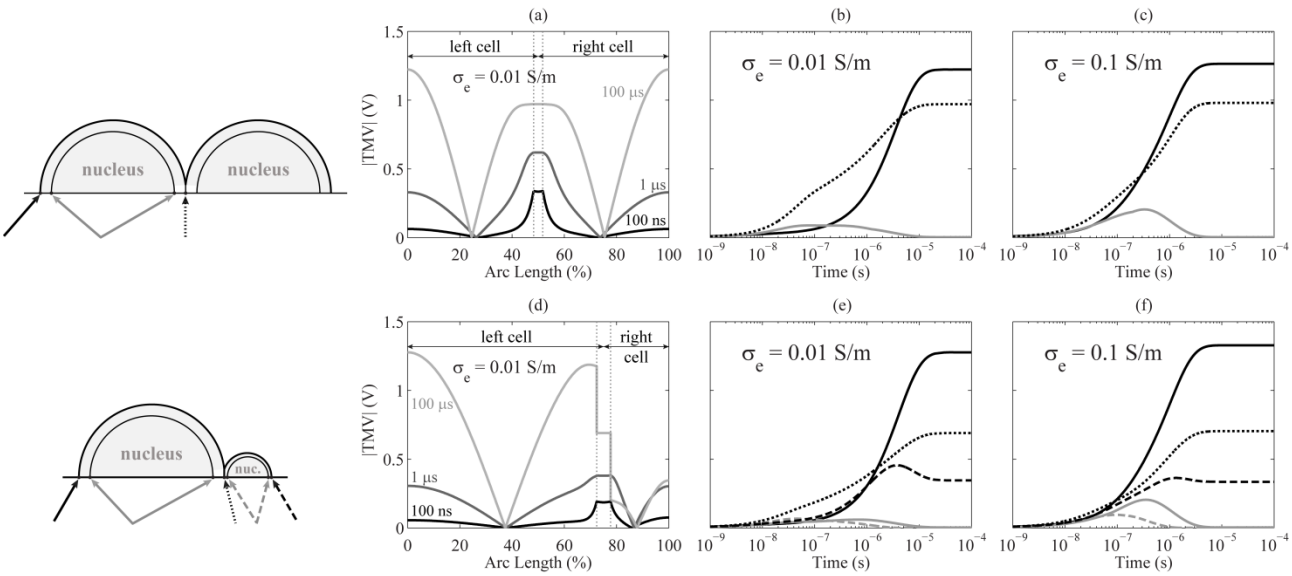

Fig. 2 Calculations of TMV on membranes of two equally sized cells with radii of 9 µm (a–c) and two differently sized cells with radii of 9 µm and 3 µm (d–f) after the onset of exposure to an electric field of 1 kV/cm. (a,d) TMV along cell membranes at times 100 ns, 1 µs, and 100 µs, in medium with conductivity 0.01 S/m. The contact areas are marked with vertical lines. (b,c) Calculated time courses of the absolute value of TMV on the pole of the left cell (black solid line), the point in the middle of the contact area (black dotted line) and the nuclear poles (grey solid line). The TMV on the nucleus was taken as the maximum TMV on either nuclear pole. These points are indicated by arrows under the image of the cell model. Calculations were performed for different extracellular medium conductivities, which are indicated in the top of graphs. (e,f) Calculated time courses of the absolute value of TMV on the pole of the large cell (black solid line), the pole of the small cell (black dashed line), the point in the middle of the contact area (black dotted line), the pole of the larger nucleus (grey solid line), and the pole of the smaller nucleus (grey dashed line). Calculations were performed in the same way as in (b,c).

the contact area between cells, this area can be selectively electroporated by applying a 100 ns pulse.

Results in Figs. 2a–c were obtained for a model of two equally sized cells with radii of 9 µm. Fig. 2a shows an example of the spatial distribution of TMV along the cell membranes, starting from the pole of the left cell and ending at the pole of the right cell, at times 100 ns, 1 µs, and 100 µs. The vertical dotted lines mark the contact area. The TMV on both cells is symmetrical (as cells are equal in size), and reaches the highest value either on the cell poles or on the contact area. The TMV along the nuclear membranes is not shown, but has a similar spatial distribution; the nuclear TMV is always the highest on the nuclear poles (the points where the membrane normal is parallel to the direction of the electric field).

Figs. 2b and c show the time course of the absolute value of TMV on the cell pole (black solid line), the point in the middle of the contact area (black dotted line), and the highest TMV on the nuclear poles (gray solid line). Note that the time is presented on a logarithmic scale as this allows one to study the TMV in the nanosecond and microsecond range simultaneously. Calculations were performed for media with two different conductivities (σ_e); 0.01 and 0.1 S/m. In medium with $\sigma_e = 0.01$ S/m (Fig. 2b) the TMV on the contact area exceeds the TMV on the cell pole during the first 4 µs. In medium with $\sigma_e = 0.1$ S/m (Fig. 2c) the TMV on the contact area still exceeds the TMV on the cell pole, however, to a lesser extent and for a shorter period of time (up to 300 ns). The TMV on the nucleus remains below the TMV on the contact area for the entire time considered.

Figs. 2d–f show similar calculations as Figs. 2a–c, however, they were performed for two differently sized cells with radii of 9 µm and 3 µm. Fig. 2d demonstrates that the TMV, which establishes in the steady state (100 µs), is considerably higher on the large cell than on the small cell and also on the contact area. This indicates the difficulty of fusing differently sized cells with "classical" microseconds pulses; namely, applying pulses that would result in electroporation of the contact area, would at the same time cause extensive electroporation and possibly death of the large cell.

Figs. 2e and f present the time course of TMV on the pole of the large cell (black solid line), pole of the small cell (black dashed line), point in the middle of the contact area (black dotted line), larger nucleus (grey solid line), and smaller nucleus (grey dashed line). In medium with $\sigma_e = 0.01$ S/m the TMV on the contact area again exceeds the TMV on all other membranes for the first 1.5 µs of the

exposure. The TMVs on both cell poles are quite similar for times below 1 μs; however, the TMV on the large cell afterwards substantially increases above the TMV on the small cell and also above the TMV on the contact area. In medium with σ_e = 0.1 S/m, the TMV on the contact area similarly exceeds the TMV on other membranes, but for a shorter time (only up to 100 ns).

The above results suggest that for a certain range of pulse durations selective electroporation of the contact areas between cells (i.e. the target areas for electrofusion) could be achieved. Most importantly, selective contact electroporation could be achieved regardless of the size of fusion partner cells. This presents the possibility for effectively fusing cells of different size without causing any damage to either large or small cells, since it is not expected that other membrane areas (apart from the contact area) would electroporate at all.

The range of pulse durations for which selective electroporation can be observed depends, however, on the extracellular medium conductivity. Our calculations demonstrate that by using medium with σ_e = 0.01 S/m, selective contact electroporation could be achieved with pulses of up to few μs duration, whereas by using medium with σ_e = 0.1 S/m, pulses would have to be approximately ten times shorter, i.e. on the order of 100 ns.

The conductivity of the extracellular medium significantly affects membrane charging dynamics, whereas its influence on the TMV becomes less important at steady state (when TMV reaches a constant value). In medium with lower conductivity, the charging of the cell membrane becomes slower. This can be used to explain why the TMV on the contact area exceeds the TMV on other membranes during the membrane charging process. The contact area is surrounded from both sides by relatively highly conductive cytoplasm (0.25 S/m), whereas the rest of the membrane is surrounded from one side with low conductive extracellular medium (0.01 or 0.1 S/m). Higher conductivity of the cytoplasm, therefore, causes charging of the contact area at a faster rate. This effect becomes more pronounced when the difference between the cytoplasmic and the extracellular conductivity is very large, i.e. in the case of medium with σ_e = 0.01 S/m.

In summary, our study demonstrates significant advantage of fusing cells with ns pulses, especially when fusion partner cells considerably differ in size. In contrast to conventionally used microseconds pulses, which primarily target large cells, nanosecond pulses provide the possibility to electroporate the contact areas between cells only. The conductivity of the fusion medium plays an important role in electrofusion with ns pulses, since it determines the range of pulse durations for which selective electroporation can be observed. In this aspect, using fusion medium with very low conductivity (0.01 S/m) could be potentially advantageous. In such medium, selective contact electroporation could be achieved even with pulses of up to few microseconds duration. Using longer pulses may additionally increase the possibility for successful fusion after electroporation, since both theoretical and experimental evidence suggest that the fusion process may already be initiated during the pulse [2], [11].

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