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Finite element analysis of microelectrotension of cell membranes

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Abstract

Electric fields can be focused by micropipette-based electrodes to induce stresses on cell membranes leading to tension and poration. To date, however, these membrane stress distributions have not been quantified. In this study, we determine membrane tension, stress, and strain distributions in the vicinity of a microelectrode using finite element analysis of a multiscale electro-mechanical model of pipette, media, membrane, actin cortex, and cytoplasm. Electric field forces are coupled to membranes using the Maxwell stress tensor and membrane electrocompression theory. Results suggest that micropipette electrodes provide a new non-contact method to deliver physiological stresses directly to membranes in a focused and controlled manner, thus providing the quantitative foundation for microelectrotension, a new technique for membrane mechanobiology.

Keywords

Electrotension; Electroporation; Transmembrane potential; Mechanotransduction; Mechanobiology; Endothelial cells

1 Introduction

Electric fields interact with lipid bilayer dielectric properties to induce compressive forces sufficient to deform membranes (Helfrich 1974; Kummrow and Helfrich 1991; Riske and Dimova 2006; Sens and Isambert 2002; Zhang et al. 2001). Because the membrane is incompressible, electrocompression leads to electrotension (ET) (Needham and Hochmuth 1989; Riske and Dimova 2005), which forms the basis of electroporation, a widely-used method by which molecules are inserted into cells through electric field-induced membrane pores (Weaver 1993, 1995). Electroporation occurs when the transmembrane potential (TP) reaches 0.2–1.0 V (Akinlaja and Sachs 1998; Kinosita Jr. and Tsong 1979; Teissie et al. 2005; Weaver 1993) resulting in membrane tensions exceeding 1 mN/m (Zhelev and Needham 1993). After pore formation, extracellular molecules enter the cell electrokinetically and diffusively. Further application of tension can lead to membrane lysis, which is the total breakdown of the phospholipid bilayer. Lysis tensions depend on membrane composition and are 10–12 mN/m (Evans et al. 1976), 9.7 mN/m for RBC lipid (Needham and Hochmuth 1989), and 5.7 mN/m for stearoyllecithin phosphatidylcholine (SOPC) vesicles (Needham and Hochmuth 1989; Riske and Dimova 2005).

Electroporation is generally applied to cell populations. However, the spatial resolution of electroporation has been dramatically improved by recent developments in microelectroporation through the use of solid carbon fiber microelectrodes (Lundqvist et al. 1998), electrolyte-filled capillaries (Nolkrantz et al. 2001), chip-based microfabricated electrode arrays (Khine et al. 2005), and micropipette electrodes (ME) (Bae and Butler 2006; Rae and Levis 2002). The ME-based single-cell electroporation method is ideal for adherent cells because the ME can indent the cell and create membrane tension that lowers the voltage needed for electroporation (Needham and Hochmuth 1989) and because the ME can be positioned onto cells precisely and automatically (Bae and Butler 2006). Single cell electroporation is a viable alternative to microinjection when dealing with very thin cell samples because it does not induce changes in cell volume.

The integration of electroporation and electrode technologies may provide the basis of a novel method of focused force application to cell membranes, here called microelectrotension (μ ET), provided electric fields and their resultant membrane stresses are accurately characterized. Thus, we quantify membrane tension at the tip of a ME using finite element analysis of a multiscale electro-mechanical model of cell culture media, cell membrane, cortical cytoskeleton, and cell cytoplasm. Electric field forces are coupled to membranes using the Maxwell stress tensor and membrane electrocompression theory. Computational results are compared to published experimental results on microelectroporation and provide the quantitative foundation for future development of μ ET methodologies.

2 Methods and materials

2.1 Background on electrocompression theory

Membrane stresses resulting from electric fields can be computed from the Maxwell stress tensor. The relation between the applied electric field and membrane tension (i.e. electrotension (ET)) is (Needham and Hochmuth 1989).

$$ET = \frac{1}{2} \text{perm} \times DC \cdot \left(\frac{TP}{h_e} \right)^2 \cdot h, \quad (1)$$

where TP is transmembrane potential, perm is vacuum permittivity, DC is the relative dielectric constant for the membrane (~ 2.2), and h is cell membrane thickness (~ 4 nm). The membrane is assumed incompressible and comprised of three homogeneous layers: two headgroup layers and dielectric layer with thickness (h_e) of 2.8 nm (Simon and McIntosh 1986).

When the membrane undergoes tension in the membrane plane, its area is increased to A from A_0 . Since the cell membrane is assumed incompressible,

$$A_0 \cdot h_0 = A \cdot h. \quad (2)$$

As in Needham et al. (Needham and Hochmuth 1989; Riske and Dimova 2005), the ratio of stress-induced changes in membrane thickness ($h - h_0$) to initial thickness, h_0 , is the z -strain (ϵ_z) given by:

$$\epsilon_z = \frac{h - h_0}{h_0} = \frac{A_0/A \cdot h_0 - h_0}{h_0} = \frac{A_0}{A} - 1 = \frac{-\alpha}{1+\alpha}, \quad (3)$$

where $\alpha = (A - A_0)/A_0$ is the relative increase in area and z is the coordinate defining the normal to the lipid bilayer. Lysis-induced α are 0.023 and 0.03 for RBC lipids and SOPC corresponding to ϵ_z values of -2.2 and -2.9% , respectively. Negative strains are considered compressive and indicate membrane thinning.

Similarly, lateral strain or r -strain ($\epsilon_r = (r - r_0)/r_0$) is related to areal changes according to:

$$\alpha = \frac{A - A_0}{A_0} = \frac{\pi r^2}{\pi r_0^2} - 1 = (\epsilon_r + 1)^2 - 1, \quad (4)$$

where r_0 is the radius of a representative unstressed disk of membrane, and r is the radius of the stressed disk. Evans et al. (1976) and Needham (Needham and Hochmuth 1989; Riske and Dimova 2005) found that a 3% increase in membrane area (or ϵ_r of $\sim 1.4\%$) resulted in membrane lysis. These lysis-inducing ϵ_z and ϵ_r values were used in the present study as experimental verification of the computational analysis of membrane tension.

2.2 Model development and analysis

ME model geometry was obtained from measurements of pipettes fabricated as described previously (Bae and Butler 2006). The pipette dimensions were 2 mm taper length, 0.3 μm inner (r_{in}), and 0.5 μm outer (r_{out}) tip radii. The distance between the ME tip and the surface of the cell membrane was defined as the cleft size (d_c) and the distance from the ME axis was denoted as d_p . As shown Fig. 1, a cell was positioned under the ME within given d_c . The 4 μm high hemisphere-shaped cell consisted of three parts: the cytoplasm, actin cortex (0.1 μm , Charras et al. 2004), and a 4 nm thick cell membrane. The radius of the cell was 15 μm . The cell and ME was modeled with 2D axial symmetry (Figs. 1 and 2) with space dimensions of 1.5 mm $\times$ 2 mm. A second geometry included the ME tip and membrane with space dimensions of 30 μm $\times$ 4 μm plus d_c . The dimensions of ME and model are described in Table 1.

A multiscale model was constructed from two interconnected geometries to account for the wide disparities in dimensions between the whole cell model and the membrane. The first geometry model (G1) was used to evaluate the electric potential distribution at the inner ME region and in the media. The second geometry model (G2) was used to evaluate TP, ET, and membrane stress and strain. Electrical potential values from corresponding planes in G1 and G2 were coupled using extrusion coupling variables.

Simulations were carried out using commercial finite element analysis software, COMSOL Multiphysics version 3.3 on a Dell 64-Bit computer with 16GB RAM, using time-independent 2D axial symmetry and electromagnetic conductive media DC mode (E-mode) and structural mechanics stress-strain mode (S-mode)

2.3 Coupled electromagnetics and membrane mechanics

Steady-state electric field distribution was computed from Gauss' law and the equation of continuity according to:

$$-\Delta \cdot (\text{Cond} \times \Delta V) = 0, \quad (5)$$

where Cond is electrical conductivity of the media, and V is voltage. Boundary conditions for E-mode are described in Fig. 1b. The axis of the pipette was the symmetry axis and all the other boundaries in the figure were designated as electrical insulators. The simulated

voltages in the plane of pipette tip in G1 were extruded onto the top boundary of G2 as boundary conditions of input voltage (Fig. 1c, d).

The mechanical equilibrium equations for axial symmetry used for S-mode were

$$\frac{\partial \sigma_r}{\partial r} + \frac{\partial \tau_{rz}}{\partial z} + \frac{\sigma_r - \sigma_\theta}{r} + F_r = 0 \quad (6)$$

and

$$\frac{\partial \tau_{rz}}{\partial r} + \frac{\partial \sigma_z}{\partial z} + \frac{\tau_{rz}}{r} + F_z = 0, \quad (7)$$

where σ and τ are normal and shear stresses, respectively. In the S-mode for G2, normal force (compression) per unit area (N/m^2) was applied on the inner (F_n) and outer ($-F_n$) surfaces of the cell membrane as boundary conditions, where F_n was determined from the 2D axial symmetry form of the Maxwell stress tensor:

$$F_n = \frac{ET}{h} = \frac{1}{2} \text{perm} \times \text{DC} \cdot \left(\frac{h}{h_e}\right)^2 \cdot (\text{TP})^2. \quad (8)$$

F_n will have components in the r (F_r) and z (F_z) coordinate directions depending on the membrane orientation. The four mechanical and electrical parameters are described in Table 2 and were used as input as material properties in the subdomain settings for both E- and S-modes.

2.4 Computation protocols

Electric potentials were first solved in E-mode of G1 and stored. The stored solution provided input parameters to solve for the S-mode electro-mechanical variables. TPs were obtained by using voltages of the outer surface of the membrane. Using these TPs, ETs on the cell membrane were calculated on the basis of Eq. (1). In order to quantify dependence on cleft size and applied voltage on membrane TPs, ETs, stress, and strain, a series of simulations were performed with cleft sizes of 0.1, 0.2, 0.5, 1.0, and 2.0 μm and pipette dc voltages ranging from 0.65 to 25 V. Membrane stress and strain values were obtained using post-processing features of COMSOL. The number of elements was on the order of 1,800 and 85,700 for G1 and G2, respectively. Results were insensitive to changes in mesh density (not shown).

3 Results and discussion

Electric potential distributions, membrane tensions, and the associated stresses and strains induced by local external electric fields were quantified for a computational model comprised of measured pipette geometries and realistic mechanical and electrical properties of the media, cell membrane, cell cortex, and cytoplasm. Predictions of effects of cleft size and applied voltages on membrane tension were quantified to facilitate the development of microelectrotension methodologies.

3.1 Electrical potential distribution

Voltage distributions in the vicinity of the ME and around the cell membrane due to applied dc voltages at the pipette entrance were first simulated for G1 (Fig. 2a). Subsequently,

voltages in the plane of the pipette tip were extruded onto the corresponding plane of G2 as boundary conditions. Figure 2a and b illustrate the voltage distribution in the pipette tip vicinity at an input voltage of 20 V and a 0.1 μm cleft size. The calculated resistance of the ME from the simulation was 10.3 M Ω , which is consistent with our previous experimental findings (Bae and Butler 2006). The tip voltage was 7% of the input voltage with most of the voltage drop occurring near the pipette tip. Maximum voltage on the cell was 1.37 V and was localized in a narrow region under the tip. Figure 2c is a zoomed image indicated as a box in Fig. 2b, and is a quantitative representation of the voltage distribution under the pipette tip. Due to low conductivity (high resistance) of the membrane (4×10^{-7} S/m), the voltage drop occurred primarily across the membrane, and additional voltage drops for the cortex and cytoplasm region were negligible.

3.2 Transmembrane potential is dependent on cleft size and input voltage

TPs were obtained either by division of simulated electric field in the domain of the membrane by membrane thickness or by comparing the inner and outer surface membrane voltages. TPs obtained by these methods differed by <1%. The peak values of TP (i.e. the TP at $d_p = 0$) were obtained from simulations with parameters of cleft sizes of 0.1, 0.2, 0.5, 1.0, and 2.0 μm and input voltages of 5, 10, 15, 20, and 25 V. TPs increased linearly with input voltage at a given cleft size (Fig. 3 (inset)). For example, the relationship between peak TP and input voltage at 0.1 μm cleft size was $\text{TP} = 0.0685 V_i - 3 \times 10^{-8}$. The effect of cleft size (d_c) on the TP was normalized to the maximum value at $d_c = 0.1 \mu\text{m}$ and plotted against d_c (Fig. 3). TPs were highly dependent on d_c with highest TPs occurring when pipette was within 0.1 μm of the membrane. This value of cleft size (0.1 μm) was used in further analysis to determine electrically-induced membrane tension, stress, and strain.

3.3 Pore-inducing transmembrane potential and electrotension are focused by the pipette

To determine the degree to which membrane tension was restricted to the areas immediately under the pipette, TP/ET spatial distribution in the cell membrane was simulated (Fig. 4a, b). The TP and ET corresponding to the inner pipette lumen (< 0.3 μm) increased as the input dc voltage amplitude increased, but dropped sharply at the outer radius of the pipette tip (0.5 μm) for all voltage amplitudes evaluated. The simulation suggests that that ETs corresponding to tension, pore formation, or membrane lysis are localized to the membrane area corresponding to the ME lumen. These results suggest that pore formation in ME-based single-cell electroporation studies are confined to a small portion of cell surface, a finding consistent with high cell viability demonstrated in single-cell electroporation studies (Bae and Butler 2006; Rae and Levis 2002). From Fig. 5a–c, therefore, we conclude that initial positioning of ME is important not only for controlling the degree of tension, but also for focusing tension on precise portions of the cell membrane. Prescribed positioning of a ME near a cell was demonstrated in our recent previous study (Bae and Butler 2006).

These focused TPs resulted in focused ϵ_z and ϵ_r (Fig. 5a–c) and stresses (Fig. 6a–c). Computations of ϵ_z (Fig. 5a), predicted that the cell membrane was compressed (thinned) by the external electric field, and the degree of thinning was a function of the square of the input voltage amplitude, which is in agreement with Crowley (1973). Membrane thinning occurred maximally at the center of the ME tip and sharply dropped at the edge of the ME lumen. Computations of ϵ_r (Fig. 5b, c) showed that membrane lateral expansion occurred under the ME lumen and dropped sharply at the outer radius of the ME tip, but membrane lateral compression was generated outside of the ME tip and decreased with increasing distance from the pipette. In Fig. 6a, the normal z -stress is shown as a function of the square of the input voltage amplitude and was maximum at the center of the ME tip, and σ_z and σ_r both sharply decayed with increasing distance from the edge of ME lumen. Collectively,

these results suggest that μ ET can be used for focused application of membrane tensions, to induce localized membrane stresses and strains.

3.4 Microelectrotension, strain, and stress values range from physiological to lysis-inducing levels

A potential advantage of μ ET is that the lowest membrane tension one could apply is limited only by the noise of the voltage source. Shear stress-induced tension calculated from the theoretical analysis of Fung is ~ 0.01 mN/m (Fung and Liu 1993). According to our computations, this value can be obtained by applying 0.65 V at the pipette entrance with 0.1 μ m cleft size. Furthermore, voltage could be applied with any temporal pattern desired, provided the time constant of application is greater than the membrane charging time ($\sim \mu$ s, Kinoshita Jr. et al. 1988; Riske and Dimova 2005). Normal membrane tensions for renal epithelial cells and human melanoma cells measured by laser tweezers are 0.003 and 0.01 mN/m, respectively (Dai and Sheetz 1999). This level of tension corresponds to a value of tension computed from Eq. (1) using a steady state TP of the cell (~ 70 mV). Thus, there is a reciprocal relationship between ET and TP in which applied TP alters ET and applied tensions alter TP. This interrelationship demonstrates an equivalence of tension induced by voltage application and that induced by external forces. Furthermore, membrane tensions on the order of 0.5 mN/m lead to opening of 50% of pressure-sensitive non-selective cation channels (Charras et al. 2004). These combined experimental and computational observations suggest that μ ET could be an effective method for focused opening of pressure sensitive channels.

Regarding electrically-induced membrane breakdown, it is known that electroporation occurs when $ET > 1$ mN/m and lysis is induced by tensions exceeding about 5.7 mN/m. As shown in Figs. 3 and 4a, lysis-inducing TP (indicated as horizontal dashed line at 1.1 V where ET equals 5.7 mN/m (Riske and Dimova 2005; Zhelev and Needham 1993) resulted from an applied voltage of 20 V and 0.1 μ m cleft size. In Fig. 5a, maximal ϵ_z with 20 V input voltage was -1.8% , which is of the same order of magnitude as lysis-induced ϵ_z studied by Needham et al. (-2.2% and -2.9% for RBC lipids and SOPC, respectively).

3.5 Model limitations

It is reported that membrane surface tension decreases simultaneously with pore formation (Isambert 1998). One of limitations of our study is that we have not accounted for the effects of membrane pores on changes in the tension, stress, and strain of membrane. Thus, the present simulation of μ ET applies most directly to sub-pore forming membrane stresses. However, because the area of pore formation is small, the effects of pores on overall membrane tension is likely to be small as well.

It is known that cytoskeleton-generated membrane tension is in the range of μ N/m. To understand the role of the actin cortex in modulating electric field-induced membrane tension we carried out simulations in which the cortex elastic modulus was equal to 100–1,000 Pa (Tracqui and Ohayon 2004). We found that the cortex elastic modulus has a negligible influence on membrane tension induced by electric fields (data not shown) likely due to the fact that membrane deformations were very small.

The simulation neglected the effect of small membrane curvatures which orient the membrane away from its flat configuration leading to altered electrically-induced forces (Sens and Isambert 2002). However, there exists evidence that the membrane is relatively flat in cultured endothelial cells (Satcher et al. 1997) thus justifying the neglect of surface curvatures in the present simulation.

3.6 Comparison with other micromechanical techniques

Currently, micromechanical techniques involve contact of micro or nano-scale probes whose force or displacement is tightly controlled. For example, micropipette aspiration (Sato et al. 1987) enables determination of bulk viscoelastic properties of cells by quantifying the temporal relationship between aspiration pressure and entrance length of cell into the pipette. Similarly, magnetic twisting (Gosse and Croquette 2002) and optical trap rheometry (Ashkin et al. 1987; Svoboda and Block 1994) apply forces to beads which interact with cell surface receptors such as integrins (Bausch et al. 1998), and the force displacement curves yield mechanical constants of elasticity and viscoelasticity. Atomic force microscopy provides surface elasticity measurements by monitoring the relationship between probe indentation and force (Charras et al. 2004). To complement these techniques, μ ET enables a non-invasive method to apply physiological tensions exclusively on the membrane. Such methodology would be great advance in elucidating the role of membrane mechanics in cell biology.

4 Summary

We quantified membrane tensions at the tip of a microelectrode using multiscale finite element analysis of electrostatics and mechanics, measured pipette geometries and realistic mechanical and electrical properties of the media, cell membrane, cell cortex, and cytoplasm. This study provides quantitative interpretation of microelectroporation and forms the foundation for microelectrotension (μ ET) a new non-contact method to apply prescribed focused tensions on cell membranes.

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Fig. 1. Microelectrode and 2-D axial symmetry simulation model. **a** The microelectrode and cell. **b** Meshed G1. **c** Meshed G2. **d** Zoomed-in image of meshed G2 indicated as *black box* in (c). Position illustrates ME tip area. r_{in} and r_{out} : inner ($0.3 \mu\text{m}$) and outer ($0.5 \mu\text{m}$) radii of ME tip, d_c : cleft size (μm), d_p : distance from pipette axis (μm)

Fig. 2. Simulated surface electric potential. **a** Simulated results of G1: ME and cell in the media. **b** Simulated results of G2: simulated voltage on the plane of ME tip of G1 was extruded into top surface of G2 as boundary voltage. **c** Zoomed-in image under the ME tip indicated as *red box* in (b): voltage drop occurred predominantly across cell membrane. Color-scale refers to electric potential (V)

Fig. 3.

a The quantitative relationship between peak value of TP (i.e. TP at $d_p = 0$) and cleft size. The normalized peak TPs were calculated by dividing the peak TP values for each input voltage by the corresponding peak TP values for 0.1 μm cleft size. (inset) Peak TPs as a function of cleft size

Fig. 4.

The quantitative relationships between **a** TP and **b** ET and input voltages for 0.1 μm cleft size. The two vertical *dashed lines* represent the glass pipette wall. The horizontal *dashed lines* represent the lysis-inducing threshold TP (~1.1 V) and tension (~5.7 mN/m)

Fig. 5.

The quantitative relationships between membrane strain and distance from pipette axis at $0.1 \mu\text{m}$ cleft size. **a** ϵ_z normalized to input voltage squared (V_i^2). **b**, **c** ϵ_r for physiological level of tension (0.65–3 V) and pore- and lysis-inducing level of tension (5–20 V), respectively. The two vertical *dashed lines* represent the glass pipette wall

Fig. 6.

The quantitative relationships between membrane stress and distance from pipette axis at 0.1 μm cleft size. **a** Normalized σ_z was a function of square of input voltage. **b**, **c** σ_r for physiological level of tension (0.65–3 V) and pore-inducing level of tension (5–20 V), respectively. The two vertical *dashed lines* represent the glass pipette wall

Table 1

The dimensions used for finite element analysis

Cell height	4 μm
Cell radius	15 μm
Membrane thickness (h)	4 nm
Dielectric thickness (h_e)	2.8 nm
Pipette inner tip radius (r_i)	0.3 μm
Pipette outer tip radius (r_o)	0.5 μm
2D axial symmetry G1	1.5 $\times$ 2.0 mm
2D axial symmetry G2	30 $\times$ (4 + d_c) μm

Table 2

The mechanical and electrical parameters used for finite element analysis

	Conductivity (S/m)	Young's modulus (Pa)	Density (kg/m ³)	Poisson's ratio
Cell membrane	4×10^{-7a}	100×10^6b	1,150	0.3 ^c
Cytoplasm	0.5 ^a	75 ^c	1,000	0.3 ^c
Actin cortex	0.5	300 ^c	1,100	0.3 ^c
Media	1.0 ^d			

^aKo et al. (2004); Ermolina et al. (2000)^bTang et al. (2006); Rand and Burton (1964)^cCharras et al. (2004)^dConductivities were measured using EC400 ExStik Conductivity meter (ExTech, Long Branch, NJ)