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MODELING OF STRESSES AND STRAINS IN CELL MEMBRANES SUBJECTED TO MICRO-INJECTION

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Abstract. This work is concerned with the determination of stresses and strains in cell membranes subjected to micro-injections. For that purpose, a suitable variational statement of the problem is developed within a continuum mechanics approach to the analysis of cell membrane geometry and physics. In this setting, the cell membrane is regarded as an axially symmetric surface in the three-dimensional Euclidean space providing a stationary value of the bending energy functional under the constraint of fixed total area. The Euler-Lagrange equations and the natural boundary conditions associated with the foregoing variational problem are derived, analyzed and used to express the stresses and moments in the membrane. Several examples of such surfaces representing possible shapes of cell membranes subjected to micro injection are determined numerically.

1. Introduction

Nowadays micro-injection is a common procedure in genetics, drug delivering, in-vitro fertilization, etc. During the process of a micro-injection, a micro pipette pierces the cell membrane and delivers substances within the cell interior. The success of a micro-injection to a large extent depends on the mechanical properties of the injected cell membrane and on the specific way of interaction between the injection pipette and the membrane.

Observing the literature on micro-injections of cells one realizes that large cells are the most often studied, typical examples being the zebrafish and mouse embryos. The analysis is mainly experimental, but several theoretical models have also been suggested (see, e.g. [1, 6, 11, 12, 16]).

A semi-empirical model of axisymmetrical membrane deformation of zebrafish embryo is presented by Lu *et al* [6]. In this work, the stress at the injection pipette tip is obtained measuring the radius of the contact spot between the embryo membrane and the wall the cell is held to. In this model, the stretch at the border circle between the deformed and undeformed parts of the membrane is obtained approximating the observed contour of the deformed membrane by second-order polynomials.

A more sophisticated model for membrane deformation is suggested by Tan *et al* [12]. In this model, the cell membrane is supposed to be a two-dimensional Mooney-Rivlin material, its deformation being governed by a system of quasi-static equilibrium equations.

It should be underlined that from mechanical point of view, the embryos are different from the other animal cells in both, their size and coating. For instance, the zebrafish embryos are $0.6 - 1.25\,mm$ in diameter [1] whereas the size of the most eukaryotic animal cells is within $10 - 30\,\mu m$ (the red blood cells are even smaller – less than $6\,\mu m$ in size). On the other hand, that embryo's coating is a veil called chorion [1] unlike the other cells that are coated by lipid bilayer membrane with protein inclusions.

A realistic theoretical model for deformation of lipid bilayer membranes was suggested in 1973 by Helfrich [4]. This model, usually referred to as the spontaneous curvature model, is widely acknowledged and used by many authors to study stresses and strains in cell membranes (see, e.g., the exhaustive surveys [5, 8, 10, 15]). The corresponding partial differential equations determining the equilibrium shapes of closed lipid bilayer membranes (vesicles – the simplest model of cells) subjected to hydrostatic pressure is derived in 1989 by Ou-Yang and Helfrich [9]. Latter on, Capovilla *et al* [2] and Tu *et al* [13, 14] have extended the foregoing model to cell membranes with free edges.

In the present study, the deformation of cells subjected to micro-injection and the corresponding forces, stresses and strains arising in the cell membrane is examined in the line of the Helfrich spontaneous-curvature model. The cell membrane is supposed to be inextensible and to deform axisymmetrically. The evolution of the membrane shape during a micro-injection process is supposed to be a quasi-static phenomenon. Thus, our main interest is in the determination of the equilibrium shapes of an initially spherical vesicle subjected to a force applied at a contour of the surface and acting along the symmetry axis and directed inward.

The significance of these results is that the estimated stress provides a performance target for the penetration process, while the estimated strain (deflection) serves as an indicator of the deformation sustained by cell organelles prior to penetration, which may be used for the purposes of a fault diagnosis.

2. Variational Statement of the Problem

In the present study, the deformation of cells subjected to micro-injection and the corresponding forces, stresses and strains arising in the cell membrane is examined in the line of the **Helfrich spontaneous-curvature model** [4] (see also [3, 5, 8, 10, 15]).

Within the framework of this model, the cell membrane is regarded as a two-dimensional surface $\mathcal{S}$ embedded in the three-dimensional Euclidean space $\mathbb{R}^3$. The membrane is supposed to exhibit a purely elastic mechanical behaviour and to be inextensible upon deformation. The equilibrium shapes of the membrane are described in terms of its **mean** H and **Gaussian** K curvatures, which are assumed to be such that the so-called curvature (shape) energy functional

$$\mathcal{F}_c = \frac{k_c}{2}\int_{\mathcal{S}}(2H - \mathbb{h})^2 \mathrm{d}A + k_G \int_{\mathcal{S}} K \mathrm{d}A$$

has a local extremum under the constraints of fixed total area A and enclosed volume V (if a hydrostatic pressure p is applied). Here, k_c and k_G are two constants associated with the **bending rigidity** of the membrane and $\mathbb{h}$ is the so-called **spontaneous curvature**. It should be noted that the associated Euler-Lagrange equation, usually called the membrane shape equation, is a nonlinear fourth order partial differential equation with respect to the components of the position vector, see [9]. At this stage of our study, however, we assume $p = \mathbb{h} = 0$.

For an initially spherical cell membrane of radius ρ supposed to retain its axial symmetry upon deformation, as it is assumed in the present study, the curvature energy functional $\mathcal{F}_c$ takes the form

$$\mathcal{F}_{ca} = 2\pi k_c \int_0^L \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s} + \frac{\sin\varphi}{r}\right)^2 r\,\mathrm{d}s + 2\pi k_G \int_0^L \frac{\mathrm{d}\varphi}{\mathrm{d}s}\sin\varphi\,\mathrm{d}s$$

since the mean H and Gaussian K curvatures of a surface in revolution are given by the expressions

$$H = \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s} + \frac{\sin\varphi}{r}\right), \qquad K = \frac{\mathrm{d}\varphi}{\mathrm{d}s}\frac{\sin\varphi}{r}.$$

Here, s is the arclength of the profile curve of the membrane, which is assumed to lie in the ROZ-plain (see Fig. 1) and to be determined by the parametric equations $R = r(s)$, $Z = z(s)$ while $\varphi(s)$ is the slope angle defined by the relations

$$\frac{\mathrm{d}r}{\mathrm{d}s} = \cos\varphi, \qquad \frac{\mathrm{d}z}{\mathrm{d}s} = \sin\varphi. \tag{1}$$

The values $s = 0$ and $s = L$ of the arclength variable are assumed to correspond to the points at the profile curve where the injection pipette and the holding pipette,

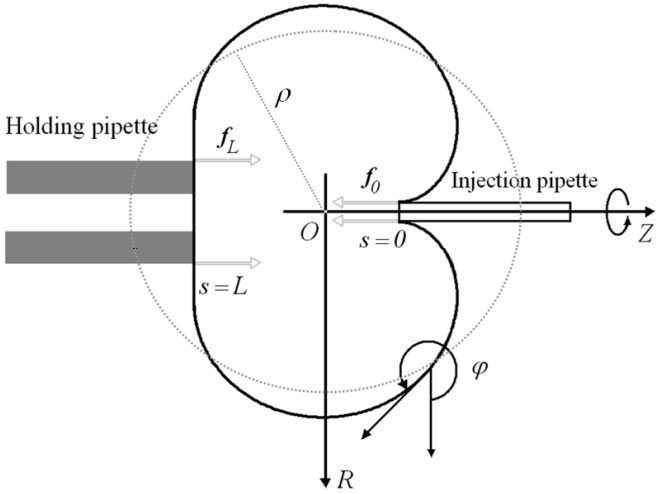

Figure 1. Sketch of an initially spherical cell membrane of radius ρ deformed axisymmetrically by two micro-pipettes in the process of a micro-injection. Here, Z-axis is the symmetry axis of the cell, φ is the slope angle of the profile curve, which is assumed to lie in the ROZ-plane while f_0 and f_L are the magnitudes of the forces (per unit contour length) exerted by the micro-pipettes at the contours $s = 0$ and $s = L$.

respectively, act on the cell membrane along the respective contours, which will be denoted by C_0 and C_L.

Taking into account the constraint of fixed total area of the membrane and the geometric relations (1) by introducing three Lagrange multipliers $\lambda(s)$, $\mu(s)$, $\eta(s)$ and an auxiliary function $\alpha(s)$ such that $\alpha(L) - \alpha(0) = A_0/2\pi$, where A_0 is a certain fixed value of the total area of the membrane, as well as accepting the additional assumption that at both ends of the membrane, i.e., at $s = 0$ and $s = L$, there are distributed forces $f_0 = k_c q_0$ and $f_L = k_c q_L$ exerted at the membrane along the Z-axis in the opposite directions and, finally, assuming that there could be line tensions $k_c\sigma_0$ and $k_c\sigma_L$ due to the membrane – injection pipette and membrane – holding pipette interactions, we arrive at the functional

$$\mathcal{A} = 2\pi k_c \left[\int_0^L \mathcal{L} \mathrm{d}s + q_0 w_0 r(0) + \sigma_0 r(0) + q_L w_L r(L) + \sigma_L r(L) \right]$$

where $w_0 = \rho - z(0)$ and $w_L = \rho + z(L)$, whose Lagrangian density $\mathcal{L}$ is given by the expression

$$\begin{aligned}\mathcal{L} =& \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s} + \frac{\sin\varphi}{r}\right)^2 r + \frac{k_G}{k_c}\frac{\mathrm{d}\varphi}{\mathrm{d}s}\sin\varphi \\ &+ \lambda\left(\frac{\mathrm{d}\alpha}{\mathrm{d}s} - r\right) + \mu\left(\frac{\mathrm{d}r}{\mathrm{d}s} - \cos\varphi\right) + \eta\left(\frac{\mathrm{d}z}{\mathrm{d}s} - \sin\varphi\right).\end{aligned} \tag{2}$$

Setting to zero the first variation of the functional $\mathcal{A}$ one obtains the following system of Euler-Lagrange equations

$$\begin{aligned}&\frac{\mathrm{d}^2\varphi}{\mathrm{d}s^2} = -\frac{\mathrm{d}\varphi}{\mathrm{d}s}\frac{\cos\varphi}{r} + \frac{\sin 2\varphi}{2r^2} + \mu\frac{\sin\varphi}{r} - \eta\frac{\cos\varphi}{r} \\ &\frac{\mathrm{d}r}{\mathrm{d}s} = \cos\varphi, \qquad \frac{\mathrm{d}z}{\mathrm{d}s} = \sin\varphi, \qquad \frac{\mathrm{d}\lambda}{\mathrm{d}s} = 0, \qquad \frac{\mathrm{d}\eta}{\mathrm{d}s} = 0 \\ &\frac{\mathrm{d}\mu}{\mathrm{d}s} = \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s}\right)^2 - \frac{1}{2}\left(\frac{\sin\varphi}{r}\right)^2 - \lambda\end{aligned} \tag{3}$$

and natural boundary conditions

$$\begin{aligned}&\left\{\left[\frac{\mathrm{d}\varphi}{\mathrm{d}s} + \left(1 + \frac{k_G}{k_c}\right)\frac{\sin\varphi}{r}\right] r\delta\varphi + \lambda\delta\alpha + \mu\delta r + \eta\delta z + \mathcal{H}\delta s\right\}_0^L \\ &\qquad + (q_0 w_0 + \sigma_0)\,\delta r(0) - q_0 r(0)\delta z(0) + Q_0\delta s(0) \\ &\qquad + (q_L w_L + \sigma_L)\,\delta r(L) + q_L r(L)\delta z(L) + Q_L\delta s(L) = 0\end{aligned} \tag{4}$$

where

$$\begin{aligned}\mathcal{H} &= \frac{1}{2}\left[\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s}\right)^2 - \left(\frac{\sin\varphi}{r}\right)^2\right] r + \lambda r + \mu\cos\varphi + \eta\sin\varphi \\ Q_0 &= [q_0 w_0 + \sigma_0 r(0)]\cos\varphi(0) - q_0 r(0)\sin\varphi(0) \\ Q_L &= [q_L w_L + \sigma_L r(L)]\cos\varphi(L) + q_L r(L)\sin\varphi(L).\end{aligned} \tag{5}$$

Actually, $\mathcal{H}$ is a conserved quantity on the smooth solutions of the Euler-Lagrange equations (3) due to the invariance of the functional $\mathcal{A}$ under the translations of the independent variable s. It should be noted also that $\delta r(0) = \delta r(L) = 0$ since the diameters of the pipettes are fixed as well as $\lambda(L)\delta\alpha(L) - \lambda(0)\delta\alpha(0) = 0$ because of the constraint of fixed total area and the fact that λ turned out to be a constant.

Observing expressions (4), one can immediately interpret

$$M = 2\pi k_c \hat{M}, \qquad \hat{M} = \left[\frac{\mathrm{d}\varphi}{\mathrm{d}s} + \left(1 + \frac{k_G}{k_c}\right)\frac{\sin\varphi}{r}\right] r \tag{6}$$

and

$$\mathbf{F} = 2\pi k_c \hat{\mathbf{F}}, \qquad \hat{\mathbf{F}} = (\mu + \mathcal{H}\cos\varphi)\mathbf{i} + (\eta + \mathcal{H}\sin\varphi)\mathbf{j} \tag{7}$$

where $\mathbf{i}$ and $\mathbf{j}$ denote the unit vectors along the coordinate axes R and Z, as the **bending moment** (couple resultant) and **force** (stress resultant), respectively, at any contour of the membrane, except for the contours C_0 and C_L at which the force suffers jump discontinuity because of the external forces

$$\mathbf{f}_0 = Q_0 \cos\varphi(0)\,\mathbf{i} + [Q_0 \sin\varphi(0) + q_0 r(0)]\,\mathbf{j} \tag{8}$$

and

$$\mathbf{f}_L = Q_L \cos\varphi(L)\,\mathbf{i} + [Q_L \sin\varphi(L) + q_L r(L)]\,\mathbf{j} \tag{9}$$

respectively, which are applied at these contours.

This means that the so loaded cell membrane is in equilibrium provided that the following jump conditions

$$[\![\hat{M}]\!]_{C_0} = [\![\hat{M}]\!]_{C_L} = 0, \qquad [\![\hat{\mathbf{F}}]\!]_{C_0} = \mathbf{f}_0, \qquad [\![\hat{\mathbf{F}}]\!]_{C_L} = \mathbf{f}_L \tag{10}$$

are satisfied. In addition, the balance of the external forces implies

$$q_0 r(0) = q_L r(L). \tag{11}$$

Thus, within the framework of the variational approach suggested here, the equilibrium states (moments, forces and profile curves) of the considered cell membranes subjected to micro-injections are determined by the solutions of the Euler-Lagrange equations (3) that meet the conditions (10) and (11).

3. Numerical Results

It is difficult to find analytical solutions to the nonlinear system (3) and, for that reason, the boundary value problem (3), (10), (11) is treated numerically using the routine `NDSolve` in `Mathematica`® (see [17, Sec. 1.6.4]) combined with a `Maple` implementation of the shooting method (package `shoot`, see [7]).

The work is still in progress and so the results presented in Fig. 2 are to be considered just as a first attempt to compare the cell membrane shapes predicted by the suggested variational approach with the experimental results presented in Fig. 3.

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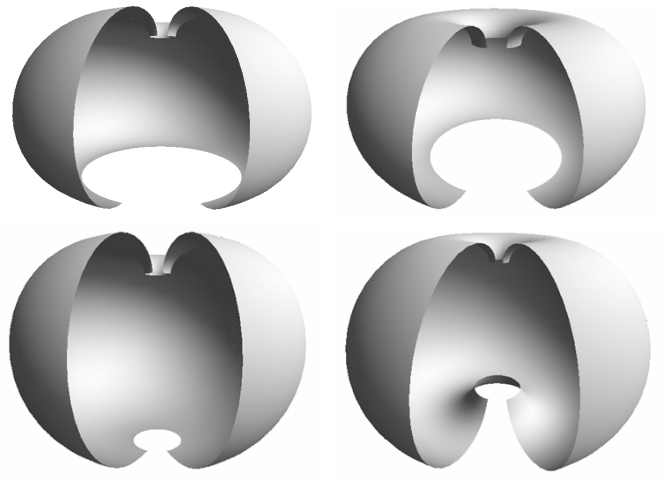

Figure 2. Shapes of axisymmetrically deformed initially spherical cell membranes subjected to micro-injections predicted by the suggested variational approach.

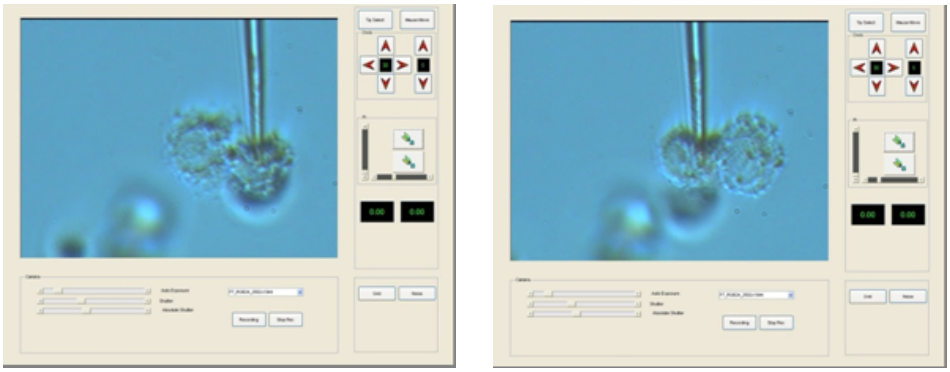

Figure 3. Screen shots of the injection process of single cells using the Hydro-MiNa robotic system.

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