Modeling of Stresses and Strains in Cell Membranes Subjected to Micro-Injection[1]

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Abstract

The 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. Each such surface is supposed to provide a stationary value of the bending energy functional under the constraint of fixed total area, which reflects the inextensibility of the structure, two more purely geometric constraints being taken into account too. The Euler-Lagrange equations as well as the natural boundary conditions associated with the foregoing variational problem are derived and analyzed. A typical example of such a surface representing a possible shape of a cell membrane subjected to micro injection is determined numerically.

Key words: Cell membrane, micro-injection, spontaneous-curvature model, forces and moments, stresses and strains, bending energy, variational statement, Euler-Lagrange equations, natural boundary conditions.

1 Introduction

Nowadays micro-injection is a common procedure in genetics, in-vitro fertilization, drug release, etc. During the process of micro-injection, a micropipette 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 interaction between the micro-pipette and the membrane.

Observing the literature on micro-injection of cells one realizes that large cells are the most often studied, typical examples being the zebrafish and mouse embryos (see, e.g., the recent dissertation [1]). The analysis is mainly experimental, but several theoretical models are also suggested. An empirical model of axisymmetrical membrane deformation of zebrafish embryo is presented by Lu *et al.* [2]. The stress at the micro-pipette tip is obtained measuring the radius of the contact spot between the embryo and the wall the cell is attached to. The stretch at the border circle between the deformed and undeformed parts of the membrane in this model is obtained approximating the observed contour of the deformed membrane by second-order polynomials.

[1]A report to be presented at the XII-th International Conference on "Geometry, Integrability and Quantization", 4–9 June 2010, Varna, Bulgaria.

A more sophisticated model for membrane deformation is suggested by Tan *et al.* [3]. In this model, the membrane is supposed to be a two-dimensional Mooney-Rivlin material and its deformation is governed by 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 is suggested in 1973 by Helfrich [4]. This model, currently 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, 6, 7, 8]). 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.* [10] and Tu *et al.* [11, 12] 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 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 micro-injection process is supposed to be a quasi-static phenomenon. Thus, our main interest is in the determination of the equilibrium shape of a spherical vesicle subjected to a force applied at a point of the surface and acting along the radius 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

Within the framework of the Helfrich spontaneous-curvature model [4], a cell membrane is regarded as a two-dimensional surface $\mathcal{S}$ embedded in the three-dimensional Euclidean space $\mathbb{R}^3$. It is observed that in a large scale the membrane of a living cell exhibits a purely elastic mechanical behaviour (see, e.g., [5, 6, 7, 8]). Its equilibrium shapes 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

$$\mathcal{F}_c = 2k_c \int_{\mathcal{S}} H^2 dA + k_G \int_{\mathcal{S}} K dA$$

has a local extremum under the constraint of fixed enclosed volume V total area A. Here, k_c and k_G are two constants associated with the bending rigidity of the membrane. 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].

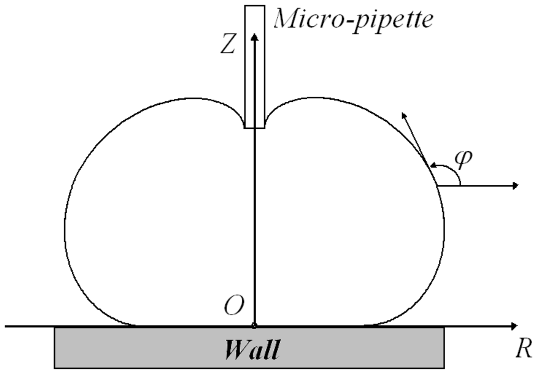

Figure 1: Sketch of an initially spherical cell membrane deformed axisymmetrically by a micro-pipette in the process of a micro-injection procedure. Here Z-axis is the symmetry axes of the cell and φ is the slope angel of the profile curve, which is assumed to lie in the ROZ-plain.

For an initially axially symmetric cell membrane, which retain its axial symmetry upon deformation as it is assumed in the present study, the curvature energy functional takes the form

$$\mathcal{F}_c = 2\pi k_c \int_0^L \frac{1}{2}\left(\frac{d\varphi}{ds} + \frac{\sin\varphi}{r}\right)^2 r ds + 2\pi k_G \int_0^L \frac{d\varphi}{ds}\sin\varphi ds$$

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

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

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

$$\frac{dr}{ds} = \cos\varphi, \qquad \frac{dz}{ds} = \sin\varphi. \tag{1}$$

The values $s = 0$ and $s = L$ of the arclength variable are assumed to correspond to the point at the profile curve where the micro-pipette is attached to the cell membrane and to the point in which the latter get in touch with the supporting wall, respectively.

Taking into account the constraint of fixed total area of the cell 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 $\alpha(L) - \alpha(0) = A/2\pi$, where A is a certain fixed value of the foregoing total area of the membrane, we arrive at the action functional

$$\mathcal{A} = 2\pi k_c \int_0^L \mathcal{L} ds$$

whose Lagrangian density $\mathcal{L}$ is given by the expression

$$\mathcal{L} = \frac{1}{2}\left(\frac{d\varphi}{ds} + \frac{\sin\varphi}{r}\right)^2 r + \frac{k_G}{k_c}\frac{d\varphi}{ds}\sin\varphi$$

$$+\lambda\left(\frac{d\alpha}{ds} - r\right) + \mu\left(\frac{dr}{ds} - \cos\varphi\right) + \eta\left(\frac{dz}{ds} - \sin\varphi\right). \tag{2}$$

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

$$\begin{aligned}
&\frac{d^2\varphi}{ds^2} = -\frac{d\varphi}{ds}\frac{\cos\varphi}{r} + \frac{\sin 2\varphi}{2r^2} + \mu\frac{\sin\varphi}{r} - \eta\frac{\cos\varphi}{r},\\
&\frac{dr}{ds} = \cos\varphi, \quad \frac{dz}{ds} = \sin\varphi, \quad \frac{d\alpha}{ds} = r, \quad \frac{d\lambda}{ds} = 0, \quad \frac{d\eta}{ds} = 0,\\
&\frac{d\mu}{ds} = \frac{1}{2}\left(\frac{d\varphi}{ds}\right)^2 - \frac{1}{2}\left(\frac{\sin\varphi}{r}\right)^2 - \lambda,
\end{aligned} \tag{3}$$

and natural boundary conditions

$$\left[\left(\frac{d\varphi}{ds}r + \left(1 + \frac{k_G}{k_c}\right)\sin\varphi\right)\delta\varphi + \lambda\delta\alpha + \mu\delta r + \eta\delta z + \mathcal{H}\delta s\right]_0^L = 0, \tag{4}$$

where

$$\mathcal{H} = \frac{1}{2}\left(\left(\frac{d\varphi}{ds}\right)^2 - \left(\frac{\sin\varphi}{r}\right)^2\right)r + \lambda r + \mu\cos\varphi + \eta\sin\varphi. \tag{5}$$

Now, taking into account that: $\delta\alpha(L) - \delta\alpha(0) = 0$ due to constraint of fixed total area and $\lambda = \mathrm{const}$; $\delta\varphi(L) = \delta\varphi(0) = 0$ because of the specific boundary conditions

$$\varphi(0) = -\frac{\pi}{2}, \quad \varphi(L) = -\pi, \tag{6}$$

inherent to our problem and the fact that $\mathcal{H}$ is a conserved quantity on the smooth solutions of the Euler-Lagrange equations (3) due to the translational invariance of the functional $\mathcal{A}$, we arrive at the conclusion that the natural boundary conditions (4) are satisfied provided that the components $f_R(0)$ and $f_Z(0)$ of the applied force along the R and Z axes, respectively, meet the conditions

$$f_R(0) = \mu = 0, \qquad f_Z(0) = \eta - \mathcal{H}. \tag{7}$$

Thus, within the framework of the variational approach suggested here, it is assumed that the profile curves of the considered types of membrane deformations are determined by the functions that satisfy the Euler-Lagrange equations (3) and meet the boundary conditions (6) and (7).

3 Numerical results

It is difficult to find analytical solutions to the nonlinear system (3) and, for that reason, the boundary value problem (3), (6), (7) is treated numerically using the routine *NDSolve* in *Mathematica* (see [13], Sec. 1.6.4) combined with a *Maple* implementation of the shooting method (package *shoot*, see [14]).

The work is still in progress and so the result presented in Fig. 2 below is 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.

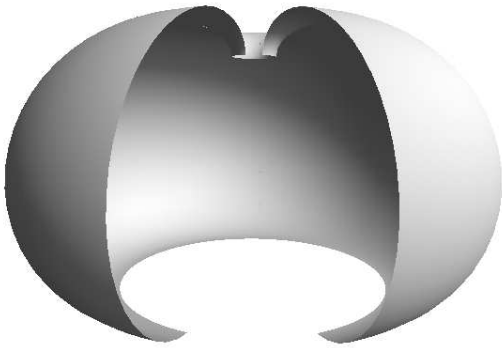

Figure 2: Shape of an axisymmetrically deformed cell membrane predicted by the suggested variational approach.

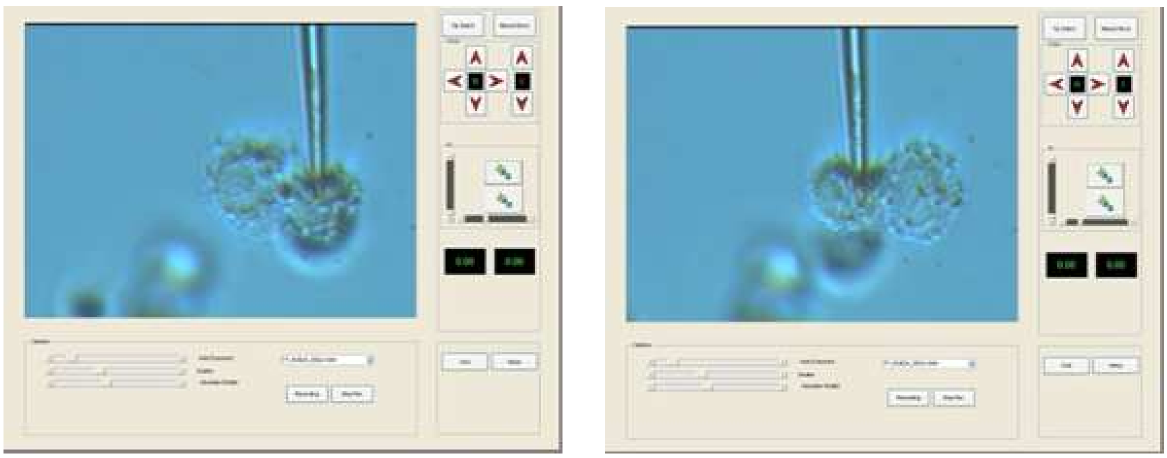

Figure 3: Screen shots of the injection process of single cells using the Hydro-MiNa robotic system.

Acknowledgements

This research is supported by the contract FP6 EC Hydromel. "Hybrid Ultra Precision Manufacturing Process Based on Positional- and Self-assembly for Complex Micro-Products".

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