Cell Membranes Under Hydrostatic Pressure Subjected to Micro-Injection

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Abstract. The work is concerned with the determination of the mechanical behaviour of cell membranes under uniform hydrostatic pressure subject to micro-injections. For that purpose, assuming that the shape of the deformed cell membrane is axisymmetric a variational statement of the problem is developed on the ground of the so-called spontaneous curvature model. In this setting, the cell membrane is regarded as an axisymmetric surface in the three-dimensional Euclidean space providing a stationary value of the shape energy functional under the constraint of fixed total area and fixed enclosed volume. The corresponding Euler-Lagrange equations and natural boundary conditions are derived, analyzed and used to express the forces and moments in the membrane. Several examples of such surfaces representing possible shapes of cell membranes under pressure subjected to micro injection are determined numerically.

Keywords: Cell membrane, micro-injection, spontaneous-curvature model, axisymmetric shapes, forces and moments, bending energy, variational statement, Euler-Lagrange equations, natural boundary conditions, jump conditions
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INTRODUCTION

The 2010 Nobel Prize in Physiology or Medicine was awarded to Robert Edwards for the development of human in-vitro fertilization in 1977 (Louise Brown, the world's first "test tube baby", was born on 25 July, 1978). On the other side, genetic engineering is a rapidly developing area of biology in the past 30 years aimed in creation of transgenic organisms with desired properties. Recently, the controlled delivery of diamond and gold nanoparticles within a single cell has being developed (see, e.g. [7]), and is expected to become a broadly applicable tool for therapy, since these nanoparticles being not toxic can be used as carriers for therapeutics, proteins, antibodies, DNA and other biological agents. Presently, these three fields of the human activity involve the intracellular delivery of substances by micro-injection. During the process of a micro-injection, a micro pipette pierces the cell membrane and releases substances within the cell interior. The success of a micro-injection depends mainly on the mechanical properties of the injected cell membrane and on the specific way of interaction between the membrane and the holding and injection pipettes.

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

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analysis is mainly experimental, but several theoretical models have also been suggested (see, e.g. [1, 18, 13, 8, 14]).

A semi-empirical model of axisymmetrical membrane deformation of zebrafish embryo is presented by Lu *et al.* [8]. 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 hold 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 is suggested by Tan *et al.* [14]. 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 general theoretical model for deformation of lipid bilayer membranes was proposed by Helfrich [5] in 1973. 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 [6, 11, 12, 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 [10]. Latter on, Capovilla *et al.* [2] and Tu *et al.* [16, 17] have extended the foregoing model to cell membranes with free edges.

In the present study, the mechanical behaviour of cells subjected to micro-injection and the corresponding forces, moments and deformed shapes of 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 quasi-static. The main tack consists in the determination of the equilibrium shapes of an initially spherical vesicle subjected to a uniform hydrostatic pressure and forces exerted by the holding and injection pipettes at two contours of the membrane. The foregoing forces are supposed to act along the symmetry axis of the injected cell membrane and to direct inward. Actually, the present model is an extension of the model proposed in [3] in which the effects due to the spontaneous curvature c_0 and hydrostatic pressure P have not been taken into account.

The results of this study is expected to provide a realistic mechanical description of the penetration process. The estimated strain (deflection) of the cell membrane may serve as an indicator of the deformation sustained by cell organelles prior to penetration, which may be used for the purposes of a fault diagnosis.

VARIATIONAL STATEMENT OF THE PROBLEM

Within the framework of the spontaneous curvature model [5] (see also [6, 11, 12, 15]), the cell membrane is regarded as a two-dimensional surface $\mathscr{S}$ 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

$$\mathscr{F}_c = \frac{k_c}{2}\int_{\mathscr{S}}(2H+c_0)^2\mathrm{d}A + k_G\int_{\mathscr{S}} K\mathrm{d}A$$

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 c_0 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 [10].

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 $\mathscr{F}_c$ takes the form

$$\mathscr{F}_{ca} = 2\pi k_c\int_0^L \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s}+\frac{\sin\varphi}{r}+c_0\right)^2 r\,\mathrm{d}s + 2\pi k_G\int_0^L \frac{\mathrm{d}\varphi}{\mathrm{d}s}\sin\varphi\,\mathrm{d}s$$

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

Taking into account the work done by the hydrostatic pressure P, 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

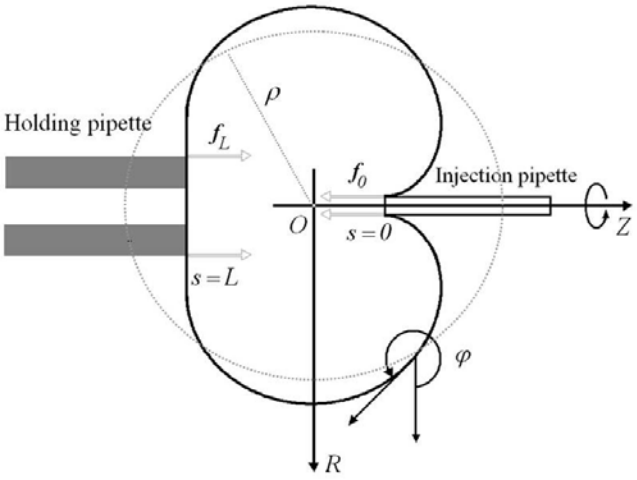


FIGURE 1. Sketch of an initially spherical cell membrane of radius ρ deformed axisymmetrically by two micro-pipettes in the process of a micro-injection. Here, Z-axis is the symmetry axis of the cell, φ is the slope angle of the profile curve, which is assumed to lie in the ROZ-plane while f_0 and f_L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

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

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

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

$$\begin{aligned} \mathscr{L} = & \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s} + \frac{\sin\varphi}{r} + c_0\right)^2 r + \frac{k_G}{k_c}\frac{d\varphi}{\mathrm{d}s}\sin\varphi + \frac{p}{3}r\left(r\frac{\mathrm{d}z}{\mathrm{d}s} - z\frac{\mathrm{d}r}{\mathrm{d}s}\right) \\ & + \lambda\left(\frac{\mathrm{d}\alpha}{\mathrm{d}s} - r\right) + \mu\left(\frac{\mathrm{d}r}{\mathrm{d}s} - \cos\varphi\right) + \eta\left(\frac{\mathrm{d}z}{\mathrm{d}s} - \sin\varphi\right) \end{aligned} \tag{2}$$

where $p = P/k_c$. Then, setting to zero the first variation of the functional $\mathscr{A}$ one obtains the following system of Euler-Lagrange equations

$$\begin{aligned} & \frac{\mathrm{d}^2\varphi}{\mathrm{d}s^2} = -\frac{\mathrm{d}\varphi}{\mathrm{d}s}\frac{\cos\varphi}{r} + \frac{\sin 2\varphi}{2r^2} + \mu\frac{\sin\varphi}{r} - \eta\frac{\cos\varphi}{r} \\ & \frac{\mathrm{d}\alpha}{\mathrm{d}s} = r, \qquad \frac{\mathrm{d}r}{\mathrm{d}s} = \cos\varphi, \qquad \frac{\mathrm{d}z}{\mathrm{d}s} = \sin\varphi, \qquad \frac{\mathrm{d}\lambda}{\mathrm{d}s} = 0, \qquad \frac{\mathrm{d}\eta}{\mathrm{d}s} = -pr\cos\varphi \\ & \frac{\mathrm{d}\mu}{\mathrm{d}s} = \frac{1}{2}\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s} + c_0\right)^2 - \frac{1}{2}\left(\frac{\sin\varphi}{r}\right)^2 - \lambda + pr\sin\varphi \end{aligned} \tag{3}$$

and natural boundary conditions

$$\begin{aligned}&\left\{\hat{M}\delta\varphi+\lambda\,\delta\alpha+\hat{\mu}\,\delta r+\hat{\eta}\,\delta z+\mathscr{H}\delta s\right\}_0^L\\&+(q_0w_0+\sigma_0)\,\delta r(0)-q_0r(0)\delta z(0)+Q_0\delta s(0)\\&+(q_Lw_L+\sigma_L)\,\delta r(L)+q_Lr(L)\delta z(L)+Q_L\delta s(L)=0\end{aligned}\tag{4}$$

where

$$\hat{M}=\left[\frac{\mathrm{d}\varphi}{\mathrm{d}s}+\left(1+\frac{k_G}{k_c}\right)\frac{\sin\varphi}{r}+c_0\right]r,\qquad\hat{\mu}=\mu-\frac{1}{3}prz,\qquad\hat{\eta}=\eta+\frac{1}{3}pr^2\tag{5}$$

$$\mathscr{H}=\frac{1}{2}\left[\left(\frac{\mathrm{d}\varphi}{\mathrm{d}s}\right)^2-\left(\frac{\sin\varphi}{r}+c_0\right)^2\right]r+\lambda r+\mu\cos\varphi+\eta\sin\varphi\tag{6}$$

and

$$\begin{aligned}Q_0&=[q_0w_0+\sigma_0r(0)]\cos\varphi(0)-q_0r(0)\sin\varphi(0)\\Q_L&=[q_Lw_L+\sigma_Lr(L)]\cos\varphi(L)+q_Lr(L)\sin\varphi(L).\end{aligned}\tag{7}$$

Actually, $\mathscr{H}$ is a conserved quantity on the smooth solutions of the Euler-Lagrange equations (3) due to the invariance of the functional $\mathscr{A}$ 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

$$M=2\pi k_c\hat{M}\tag{8}$$

and

$$\mathbf{F}=2\pi k_c\hat{\mathbf{F}},\qquad\hat{\mathbf{F}}=(\hat{\mu}+\mathscr{H}\cos\varphi)\mathbf{i}+(\hat{\eta}+\mathscr{H}\sin\varphi)\mathbf{j}\tag{9}$$

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) 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

$$\mathbf{f}_0=Q_0\cos\varphi\,(0)\,\mathbf{i}+[Q_0\sin\varphi\,(0)+q_0r\,(0)]\,\mathbf{j}\tag{10}$$

and

$$\mathbf{f}_L=Q_L\cos\varphi\,(L)\,\mathbf{i}+[Q_L\sin\varphi\,(L)+q_Lr\,(L)]\,\mathbf{j}\tag{11}$$

respectively, which are applied at these contours.

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

$$[\![\hat{M}]\!]_{C_0}=[\![\hat{M}]\!]_{C_L}=0,\qquad[\![\hat{\mathbf{F}}]\!]_{C_0}=\mathbf{f}_0,\qquad[\![\hat{\mathbf{F}}]\!]_{C_L}=\mathbf{f}_L\tag{12}$$

hold. In addition, one should be aware that the balance of the external forces implies

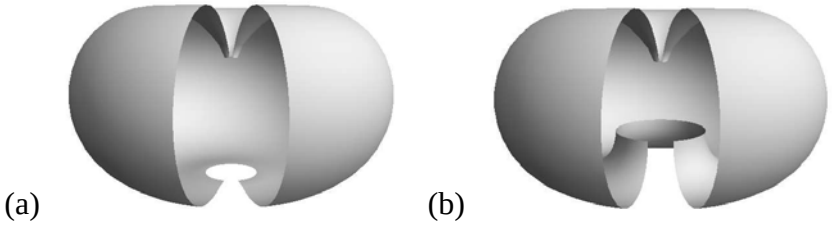
$$q_0 r(0) = q_L r(L). \tag{13}$$

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 (12) and (13).

NUMERICAL RESULTS

Explicit analytic parametrization of certain axisymmetric surfaces whose curvature is a solution of the shape equation is presented recently (see [4]). However, it is difficult to find analytical solutions of the nonlinear system (3) due to the specific form of the boundary conditions (12) and (13). For that reason, the boundary value problem (3), (12), (13) is treated here numerically using the routine `NDSolve` in `Mathematica`® (see [19, Sec. 1.6.4]) which is combined with a `Maple` implementation of the shooting method (package `shoot`, see [9]).

Typical examples of cell membrane shapes are displayed in Fig. 2. It is shown that membranes of length $L = 3$ subjected to a pressure $p = 25$ can take different equilibrium shapes near the holding pipette at a small change of the spontaneous curvature c_0. The comparison of the membrane shapes in Fig. 2 predicted by the suggested variational approach to the experimental results presented in Fig. 3 shows up their qualitative coincidence thus verifying the theoretical results presented here.



(a) (b)

FIGURE 2. Shapes of axisymmetrically deformed initially spherical cell membranes subjected to micro-injection: length $L = 3$, pressure $p = 25$ and spontaneous curvature $c_0 = 0.2$ (a), $c_0 = 0.205$ (b).

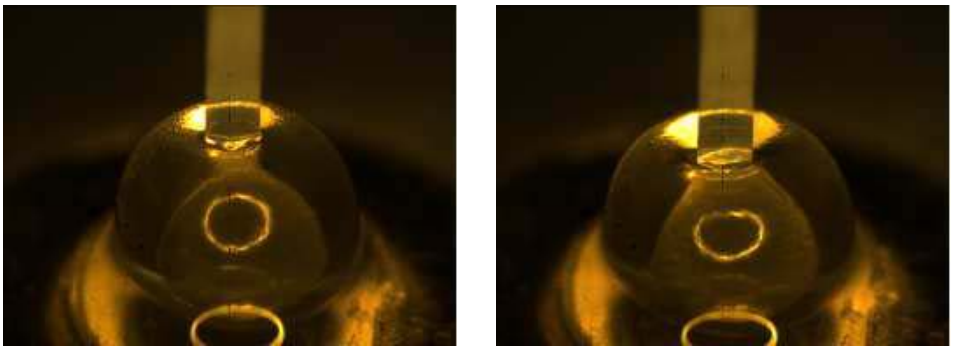


FIGURE 3. Screen shots of the injection process of a single cell using the Hydro-MiNa robotic system.

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