

Journal of
Mechanics of
Materials and Structures

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Volume 2, N° 6

June 2007



mathematical sciences publishers

QUANTITATIVE EVALUATION OF MECHANICAL PROPERTIES OF CELL MEMBRANES: AN EXACT SOLUTION

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Properties of cell membranes are determined by means of a single mechanical experiment involving probing of the membrane with an atomic force microscope. The membrane is modeled as a two-dimensional medium with bending resistance whose energy has three contributions: from the strain energy of the membrane itself, from the internal pressure, and from the point load applied by the microscope. The problem is cast into a variational form and the emerging Euler–Lagrange equations are linearized and solved for the shape of the deformed cell, which can be readily compared with experimental data on the deformed shape to yield the mechanical properties of the membrane.

1. Preliminaries

Compliance of cell membranes has been observed to be correlated to the state of health of a cell; or more generally, mechanical properties of cell membranes can be important markers for diseases. For example, a correlation has been established between red blood cell membrane properties and diseases, such as sickle cell anemia, that result in diminished cell function [Evans and Mohandas 1987]. There is also evidence that a change in cell membrane compliance in a malignant tumor indicates the onset of metastasis, and the same change in compliance of neurons can indicate the presence of multiple sclerosis, long before the patient feels any symptoms. Therefore, there is a need to establish envelopes of normalcy for mechanical parameters of various cell membranes. An accurate quantitative understanding of cell membrane mechanical properties is key to attaining this goal.

The typical membrane of a mammalian cell consists of at least one bilayer composed primarily of lipid molecules, with hydrophilic and hydrophobic ends [Brock et al. 1994; Garrett and Grisham 1999; Alberts et al. 2002; Boal 2002]. In general, the membrane is connected to a protein network scaffold called the cytoskeleton. The cellular membrane has been modeled in the literature mostly as a solid thin shell, starting with the pioneering work of Fung [1966]. However, there is experimental evidence to suggest that the bilayers are fluid-like in nature, as the molecules do not oppose resistance when *shuffled* past each other—that is, there is no resistance to shear [Engelhardt and Sackmann 1988; Discher et al. 1994; Strey et al. 1995]. Thus, we propose to model the membrane as a *fluid* thin film endowed with bending resistance. We employ Noll’s definition of a fluid: a material with a symmetry group (a set of deformations undetectable by subsequent mechanical experiments) coinciding with the unimodular group (tensors with the determinant equal to plus or minus one). In contrast, solids have as a symmetry group the orthogonal group (that is, with determinant equal to plus one). Further details about this important point may be found in [Steigmann 1999; Truesdell and Noll 1965; Naghdi 2002] and references cited

Keywords: cell membrane, energy minimization, bilayer.

The authors gratefully acknowledge the support of the National Science Foundation through Grant #0311833.

therein. Also, the molecules of the bilayer offer resistance to being taken apart due to the chemical interaction of the constituent molecules and to the hydrophobicity of the *inner heads*. As a result, we model the membrane as a fluid elastic medium endowed with bending resistance (characterized by an elastic constitutive equation satisfying invariance with respect to the unimodular group). The concept of a fluid elastic crystal medium is also treated in Ericksen's work on liquid crystals [Ericksen 1974] and by Jenkins [1977a; 1977b]. It is well known that cells undergo large deformations during their lifetime: for example, red blood cells with diameters of about 8 microns squeeze through capillaries only 3–4 microns in diameter. In the biomechanics literature, with few exceptions, models of red blood cells, as well as other cells, are primarily linear elastic. Extracting mechanical properties from global experiments on cells, helpful though they are in obtaining a general idea of membrane compliance, are not relevant for obtaining accurate mechanical parameters which would be useful as disease markers [Hochmuth et al. 1973; 1990; Skalak et al. 1973; Evans 1974; Evans and Hochmuth 1977; Evans and Skalak 1980; Evans 1983; 1992; Svetina et al. 1985; Chasis et al. 1988; Lim et al. 2002; Kuzman et al. 2004]. In the context of nonlinear theories, classical mechanical moduli such as Young's modulus and shear modulus have no meaning as they are not unique. New parameters, derived in the context of a robust mathematical treatment, are necessary for modeling this complicated medium.

From the experimental point of view, there is a plethora of chemical methods used to explore the constituency of cellular membranes. However, mechanical experiments (especially nondestructive ones) offer significant advantages for a program of *in vitro* and *in vivo* testing since soft, living matter has a higher degree of robustness with respect to mechanical forces compared to chemical manipulations.

In experimental techniques involving (nano)indentation of a solid material, the Hertzian theory [Johnson 1987; Morris et al. 1999] is conventionally employed to extract elastic constants of the material from force versus penetration depth plots. This technique has been employed in experiments involving cells as well (for example, [Hassan et al. 1998]), but it has proven problematic and has been plagued by irreproducibility, primarily due to the presence of large deformations. Further, the finite size of the cell violates the mathematical assumptions of the Hertzian theory. Hertzian theory (see, for example, [Yin and Costa 1999]), assumes that the system undergoing indentation is a half-space, that is, a semifinite system bounded by a surface (before indentation). This is, of course, not a good approximation for cells. In fact, the failure of the basic assumptions of the Hertzian theory renders the traditional output of an atomic force microscope (AFM) experiment—the force versus displacement at the tip of the cantilever—insufficient for characterizing the deformed shape of the cell. As such, new experiments are needed to determine the mechanical properties of cellular membranes. Indeed, we can have two very different shapes that coincide only in one point: the point of contact with the cantilever. This problem is not encountered in the testing of metals or generally hard materials that do not undergo large deformations and are nearly linear in their response.

New modeling of the contact mechanics, as well as a sequence of models for the membrane itself, is proposed in [Steigmann et al. 2003; Steigmann 2003] and [Baesu et al. 2004], which start from the most general constitutive equation for the strain energy of a two-dimensional fluid membrane with bending resistance. As linearization of this model is the subject of the current paper, we mention briefly the main features of the model. In [Baesu et al. 2004], an asymptotic analysis of this most general form yields as a first-order approximation a form of strain energy, which is traditionally referred to in the physics literature as the Canham–Helfrich strain energy [Helfrich 1973; Deuling and Helfrich 1976]. Moreover,

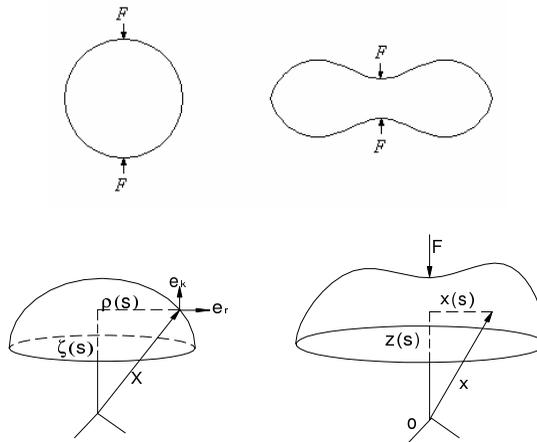


Figure 1. The geometry of deformation.

the framework presented in [Baesu et al. 2004] is more general and allows refinement of the model in ways compatible with mathematical requirements leading to the most general form. Variational methods are employed in [Baesu et al. 2004] to extract the deformed shape of a cell under an AFM experiment as a solution to the Euler–Lagrange equation. The interior of the cell is modeled as an incompressible material—a reasonable assumption as the cell is known to be made up of 50–55% water. Three new material parameters emerge as relevant for such a fluid medium: surface tension, and two bending moduli. The *shape equations* that describe the deformation of the cell under an AFM probe are highly nonlinear.

In this paper, we linearize the Canham–Helfrich model about small curvature and obtain an exact solution of the *poking problem* that describes an AFM experiment. We emphasize however that we use it only because it is the simplest model (first approximation) that satisfies the requirements of the most general model derived in [Baesu et al. 2004]. This general context is important especially for defining the theoretical envelope within which this simpler model can be further refined. For the sake of clarity, the general model, kinematics and constitutive aspects, including discussions concerning fluidity in the context of symmetry groups of a material, are briefly sketched in Section 2 and Section 3. Section 4 is concerned with Euler–Lagrange’s equations and Section 5 with the linearized model and the exact solution.

2. Kinematics

We treat the case of an axisymmetric shape of the cell membrane, as illustrated in Figure 1, where the symbol F stands for the force applied by the AFM tip, and \mathbf{x} and \mathbf{X} are the position vectors of an arbitrary particle on the membrane in the present and in the deformed configurations, respectively.

For clarification and more background on continuum mechanics we refer to the classic texts [Truesdell and Noll 1965; Green and Zerna 1968; Chandrasekharaiah and Debnath 1994; Chadwick 1999; Naghdi 2002]. For background on geometric methods in mechanics, especially membrane theory, see [Nitsche 1993; Ou-Yang et al. 1999].

The cylindrical coordinates of a point on the surface in the deformed configuration are given by

$$\mathbf{x} = r\mathbf{e}_r + z(r)\mathbf{k}. \tag{1}$$

The components of the surface metric tensor for the given coordinates are

$$\begin{aligned} a_{11} &= \mathbf{a}_1 \cdot \mathbf{a}_1 = x_{,1} \cdot x_{,1} = (\mathbf{e}_r + z'(r)\mathbf{k}) \cdot (\mathbf{e}_r + z'(r)\mathbf{k}) = 1 + (z')^2, \\ a_{22} &= \mathbf{a}_2 \cdot \mathbf{a}_2 = x_{,2} \cdot x_{,2} = r\mathbf{e}_\theta \cdot r\mathbf{e}_\theta = r^2. \end{aligned} \tag{2}$$

Also, note that $a_{12} = a_{21} = 0$. Further, the normal to the deformed surface and the components of the curvature tensor are given by

$$\begin{aligned} \mathbf{n} &= \frac{\mathbf{a}_1 \times \mathbf{a}_2}{\sqrt{a}} = \frac{1}{\sqrt{1 - (z')^2}}(\mathbf{k} - z'\mathbf{e}_r), \\ b_{11} &= \mathbf{n} \cdot \mathbf{a}_{1,1} = \frac{z''}{\sqrt{1 - (z')^2}}, \\ b_{22} &= \mathbf{n} \cdot \mathbf{a}_{2,2} = \frac{rz'}{\sqrt{1 - (z')^2}}, \\ b_{12} &= b_{21} = 0, \end{aligned} \tag{3}$$

where the second-order curvature tensor \mathbf{b} is given by $\mathbf{b} = b_{11}\mathbf{a}^1 \otimes \mathbf{a}^1 + b_{22}\mathbf{a}^2 \otimes \mathbf{a}^2 + b_{12}\mathbf{a}^1 \otimes \mathbf{a}^2$. Consequently, the mean and Gaussian curvature, which appear in our constitutive equation, are calculated from (3) to be

$$\begin{aligned} 2H &= \text{tr } \mathbf{b} = \text{tr}[b_{11}\mathbf{a}^1 \otimes \mathbf{a}^1 + b_{22}\mathbf{a}^2 \otimes \mathbf{a}^2] = \frac{z''}{(1 + (z')^2)^{\frac{3}{2}}} + \frac{z'}{(1 + (z')^2)^{\frac{1}{2}}}, \\ K &= \det \mathbf{b} = \frac{z'rz''}{1 - (z')^2}. \end{aligned} \tag{4}$$

We linearize the model about small curvatures by taking $z' \ll 1$. Then, the linearization of the mean and Gaussian curvature, from (4) becomes

$$\begin{aligned} 2H &\approx z'' + \frac{z'}{r} = \frac{1}{r}(rz')', \\ K &\approx 0. \end{aligned} \tag{5}$$

3. Constitutive equation

The mechanical response of the cell membrane is described by a strain energy per unit area of the membrane surface dependent on H , the mean curvature K , the Gaussian curvature, and J , the local area stretch, in the present (deformed) configuration ω . The most general form of the strain energy function for fluids, as shown in [Steigmann 1999; 2003; Steigmann et al. 2003], is represented by

$$w(H, K, J), \tag{6}$$

where $2H = \text{tr}(\mathbf{b})$, $K = \det(\mathbf{b})$, and $J = \lambda\mu$, and where λ and μ are the two principal stretches. An asymptotic expansion presented in [Baesu et al. 2004] in terms of a parameter representing the thickness/radius of curvature, indicates that the first approximation of (6) is

$$w = TJ + kH^2 + k_1K, \quad (7)$$

where T , k , k_1 are the material moduli in this formulation. We emphasize that there is no connection whatsoever between these moduli and Young's modulus or Poisson's ratio, as this is a nonlinear theory employing completely different moduli.

4. Euler–Lagrange equations

The total potential energy of membrane described by (6), or its approximation (7), has three parts: one part due to the strain energy of the membrane itself, one from the applied point load, and one from the internal pressure. As we consider the material incompressible, the internal pressure is calculated as a Lagrange multiplier after the boundary conditions are enforced. For the sake of simplicity, we employ constant area and volume constraints. The energy functional for this problem was discussed in [Baesu et al. 2004] and [McElfresh et al. 2002], and for brevity we reproduce here only the final form:

$$E = \int_{\omega} w(H, K)da + \lambda S(\omega) - pV(\omega), \quad (8)$$

where $S(\gamma)$ is the total surface of the deformed configuration γ , $V(\gamma)$ is its volume, w is the strain energy of the membrane, and p and λ are the two Lagrange multipliers associated with the two constraints present in the problem (constant area and constant volume). The latter constraint is of particular interest as it allows us to calculate the pressure as a Lagrange multiplier without the need to know the pressure inside the cell a priori. In other words, a nondestructive experiment would suffice as there is no need to learn the pressure at some point inside the cell. It is shown in [Steigmann et al. 2003; Steigmann 2003] that the Euler–Lagrange equations for the energy (8) lead to the following shape equation:

$$\Delta\left(\frac{1}{2}w_H\right) + (w_K)_{;\alpha\beta}b^{\alpha\beta} + w_H(2H^2 - K) + 2H(Kw_K - w) - 2HT = p. \quad (9)$$

Employing the area constraint, the first approximation of the strain energy $w(H, K)$ (7) becomes

$$w = kH^2 + k_1K, \quad (10)$$

where the coefficients are material constants to be determined. We remark that this approximation of $w(H, K)$ (see [Ou-Yang et al. 1999]) can be obtained through an asymptotic analysis conducted in terms of a parameter representing the ratio of the membrane thickness (t) to the infimum over the minimum of the radii of curvature over the whole deformed surface. Consequently, for the strain energy (10), the associated shape Equation (9) becomes

$$k[\Delta H + 2H(H^2 - K)] - 2\lambda H - p = 0, \quad (11)$$

where λ is the Lagrange multiplier associated with the area constraint. We will be concerned next with the linearization of (11).

5. Linearized model

Using results from Section 2, the linearization of the shape Equation (11) leads to a new system of ordinary differential equations (ODE's) for the shape of the deformed surface. In what follows we consider only small curvatures; that is, we linearize the model about small curvatures. Once again, with the assumption that

$$(z')^2 \ll 1 \Rightarrow \sqrt{1 - (z')^2} \approx 1,$$

the expression for H is given by Equation (5). Similarly, the Laplacian of H is obtained in the following fashion

$$\Delta H = \frac{1}{\sqrt{a}}(\sqrt{a}a^{\alpha\beta}H_{,\beta})_{,\alpha} = \frac{1}{r\sqrt{1 - (z')^2}}(r\sqrt{1 - (z')^2}(1 + (z')^2)^{-1}H')'. \tag{12}$$

The linearized expression for ΔH is given by

$$\Delta H \approx \frac{1}{r}(rH')'. \tag{13}$$

Consequently, the linearized form of the shape equation (11) is given by

$$H'' + \frac{1}{r}H' - \frac{2\lambda}{k}H = \frac{p}{k}, \tag{14}$$

where λ, p, k are the constants as defined earlier. The net pressure across the membrane is very small and due to the linearization, the model is valid only for small curvatures. For what follows, we neglect p . Therefore, the shape equation takes the following form of a homogeneous equation:

$$H'' + \frac{1}{r}H' - \frac{2\lambda}{k}H = 0. \tag{15}$$

This equation is a modified Bessel equation of order zero whose general solution is given by

$$H(r) = BK_0(Ar) + CI_0(Ar), \tag{16}$$

where $I_0(Ar)$ and $K_0(Ar)$ are the Bessel functions of the first and second kind of order zero, respectively [Abramowitz and Stegun 1968], and $A = \sqrt{2\lambda/k}$. The integration constants B and C are to be found from the boundary conditions. The first boundary condition, is given by

$$\lim_{r \rightarrow 0} rH'(r) = \frac{-F}{2\pi k}, \tag{17}$$

where, again, F is the value of the point load applied on the membrane. This boundary condition is obtained by integrating tractions along a small circle around the point of application of the point load and then taking the limit as the radius goes to zero. From (16) and (17) we obtain

$$rH' = -BArK_1(Ar) + CArI_1(Ar), \tag{18}$$

$$\lim_{r \rightarrow 0} ArI_1(Ar) = 0, \tag{19}$$

$$\lim_{r \rightarrow 0} ArK_1(Ar) = 1. \tag{20}$$

Since for $I'_0 = I_1$ and $K'_0 = -K_1$, $\lim_{r \rightarrow 0} I_1(Ar) = 0$, and $\lim_{r \rightarrow 0} Ar K_1(Ar) = 1$, it follows that (18) is satisfied if and only if $B = \frac{F}{2\pi k}$. The expression for $H(r)$ becomes

$$H(r) = \frac{F}{2\pi k} K_0(Ar) + C I_0(Ar). \quad (21)$$

Next, from (16) we get the ODE for $z(r)$:

$$\frac{1}{2r} (rz')' = \frac{F}{2\pi k} K_0(Ar) + C I_0(Ar), \quad (22)$$

with the solution

$$z(r) = \frac{F}{2\pi\lambda} K_0(Ar) + \frac{Ck}{\lambda} I_0(Ar) + D \ln(Ar) + E. \quad (23)$$

This equation is solved in a bounded domain with r ranging from 0 to $5 \mu\text{m}$ (approximately half the length of the cell measured from its pole, owing to axisymmetry). The boundary conditions for this equation, used to determine the constants C , D and E , are given from geometrical conditions by

$$z'(0) = 0, \quad z'(5) = 0, \quad z(5) = 0, \quad (24)$$

where $z = z(r)$. These boundary conditions arise from the fact that at the point of application of the load, the slope of the membrane is zero due to the bending resistance. At the outer bound, the membrane is unaffected by the point load. As r approaches zero, x also approaches zero. The condition (24)₁ can be rewritten (with $x = Ar$) as

$$\lim_{x \rightarrow 0} \left(D - \frac{F}{2\pi\lambda} \right) \frac{1}{x} = 0. \quad (25)$$

From the properties of Bessel functions indicated above it is clear that a necessary and sufficient condition for (25) is:

$$D = \frac{F}{2\pi\lambda}.$$

Therefore, the equation for the slope is given by

$$\frac{dz}{dx} = \frac{F}{2\pi\lambda} \left(\frac{1}{x} - K_1(x) \right) + \frac{Ck}{\lambda} I_1(x). \quad (26)$$

Applying the second boundary condition, the constant C is given by

$$C = \frac{-F}{20\pi k} \frac{\left(\sqrt{\frac{2k}{\lambda}} - 10K_1(5A) \right)}{I_1(5A)}. \quad (27)$$

Substituting the final boundary condition in Equation (23), the constant E is given by

$$E = \frac{F}{20\pi\lambda} \left[\frac{I_0(5A)}{I_1(5A)} \left(\sqrt{\frac{2k}{\lambda}} - 10K_1(5A) \right) - 10(K_0(5A) + \ln(5A)) \right]. \quad (28)$$

Therefore, after substituting all the constants into Equation (23), the final solution is

$$z(r) = \frac{F}{2\pi\lambda} (K_0(Ar) + \ln(Ar)) - \frac{F}{20\pi\lambda} \left[\frac{I_0(Ar) - I_0(5A)}{I_1(5A)} \left(\sqrt{\frac{2k}{\lambda}} - 10K_1(5A) \right) + 10(K_0(5A) + \ln(5A)) \right], \quad (29)$$

where, again, A is expressed as $A = \sqrt{2\lambda/k}$. This equation can be used to find the values of the bending (k) and stretching (λ) moduli for a given point load and deflection of the membrane. The results of AFM imaging experiments performed by McElfresh et al. [2002] give the deflection of a bovine sperm cell membrane under the point of application of the load. The results for two different loads were used. However the values of the material constants k and λ cannot be uniquely obtained as the shape is not unique. The values of coordinates at two different points are required for solving the equations, and there is no experimental result available for this purpose. Since there are no experimental data to get an actual deformed shape under an AFM experiment, assumed values of bending and stretching moduli based on known values for other fluids are used to illustrate what the deformed shape might look like, using Equation (7). In particular, using material constants available in the literature for lipid bilayers (like surfactants), we were able to plot the deformed shape of the membrane (see Figure 2). Bending modulus (k) for surfactants is of the order 10^{-12} ergs ($1 \text{ erg} = 0.1 \mu\text{J}$) [Ou-Yang et al. 1999; McElfresh et al. 2002] and the stretching or dilatation modulus (λ) for phospholipid bilayers is approximately equal to 10^{-1} N/m [Castro-Roman and Ligoure 2001]. The cell membrane is expected to offer a greater resistance to bending, hence it is expected to have a greater value for k . Assuming that the value of the bending modulus is of the order 10^{-7} ergs and an arbitrary value of the point load, $F = 6.6 \times 10^{-9}$ N [McElfresh et al. 2002], the deformed shape in Figure 2 was obtained where the length unit is μm . This is the expected shape of the deformed membrane. The order of magnitude of the bending modulus is

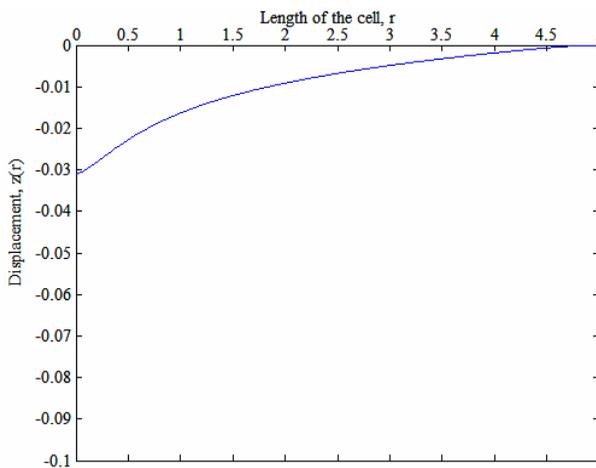


Figure 2. Plot of the deformed shape of the membrane.

reasonable because the order of magnitude of the displacement obtained here is the same as that of the actual displacement [McElfresh et al. 2002].

We remark that experiments to obtain the whole deformed shape are a great experimental challenge. Two different experimental set-ups to obtain the whole deformed shape of the cell membrane are in trial: one at the Lawrence Livermore National Laboratory and another at Prof. Turner's laboratory at the University of Nebraska. As experimental results become available, Equation (29) can be very easily used to obtain material constants for the membrane. Also, the exact solution (29) serves as a benchmark solution for comparison with future numerical simulations.

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Received 12 Apr 2007. Accepted 20 Apr 2007.

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