Analytical model for extracting mechanical properties of a single cell in a tapered micropipette

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A simple solid mechanical model has been developed to extract the mechanical properties of a single cell in a tapered micropipette. This analytical model is derived using the definition of elastic modulus and force equilibrium. Using the authors' model, an elastic modulus of 21.80±4.91 Pa, a Poisson ratio of 0.46±0.03, and a friction coefficient of 0.0274±0.0077 are extracted for a neutrophil cell. The model is verified by finite element software and shows good agreement with experiments. The biophysical basis of the model and application in microfluidic channels for cancer cell research are discussed, while a comparison is made with other models. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2430936]

There has recently been extensive interest in studying the biomechanical properties of living cells as an aid for disease study. Diseases such as gastrointestinal cancer and malaria can induce structural and molecular alterations in cells, which in turn change cells' elastic properties.[1,2] For example, the shear modulus of red blood cells has been found to increase up to tenfold during parasite development.[1] Elastic moduli of bladder cancerous cells are about one order of magnitude lower than those of normal cells.[3]

Micropipette aspiration,[4] microplate manipulation,[5] and optical tweezer stretching[6] have been developed to study cell mechanical properties. Micropipette aspiration uses a negative pressure to suck a single cell until it forms a hemispherical projection in the micropipette. Microplate manipulation squeezes a cell between two microplates, and the optical tweezer method stretches a single cell by trapping two microbeads that are attached to the cell.

Tapered micropipetting is a technique for the manipulation of a single cell and was used by Needham and Hochmuth[7] (1992) to study the membranes of a neutrophil cell by regarding it as a liquid drop in order to extract elastic properties of the cell membrane. There is, however, currently no solid model for the tapered micropipette experiment that can be used to extract the properties of the whole cell, including global mechanical and biophysical information during the deformation.

Figure 1 shows a single cell being aspirated into a tapered cylindrical micropipette. When a small positive pressure is applied to the micropipette, the cell moves forward, deforms, and rests at a place inside the pipette where force equilibrium is established. Any further increase in the applied pressure drives the cell to a new equilibrium position. To extract a particular cell's elastic properties, several such equilibrium measurements are made.

The schematic diagram for analysis is shown in Figure 2(a). Figure 2(b) shows the force diagram for a deformed cell. There are three forces acting on the cell: a force due to the applied positive pressure ΔP, a reaction force N (P is the pressure due to the force N) from the micropipette wall, and a frictional force f between the cell and the micropipette. Figure 2(c) shows a cross section of the deformed cell.

The elastic modulus for a neutrophil cell can be defined as

$$E_x = \frac{\sigma_x - \upsilon\sigma_y - \upsilon\sigma_z}{\varepsilon_x},$$

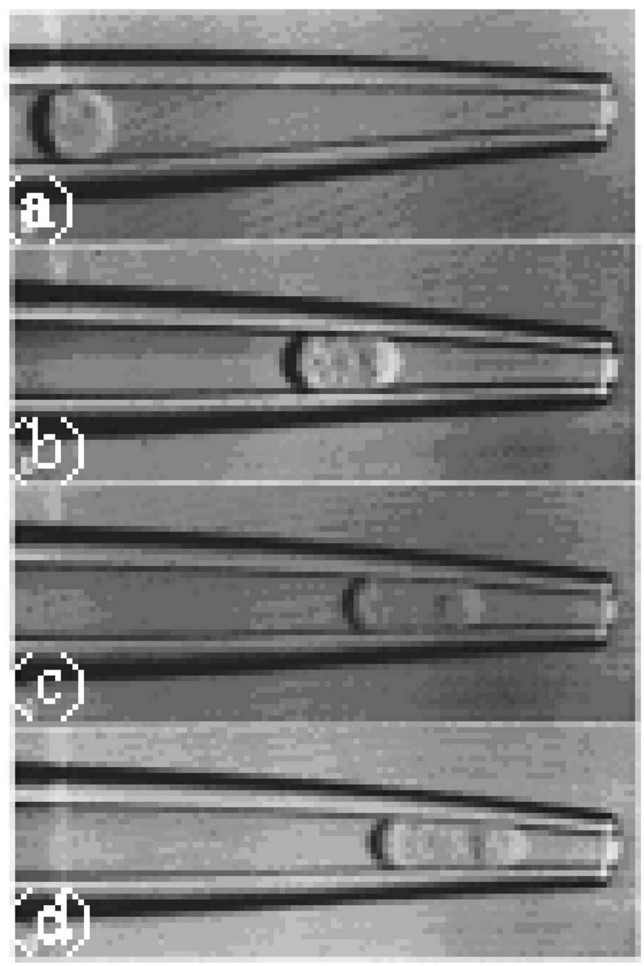

FIG. 1. Images from experimental measurement in Needham and Hochmuth's paper (Ref. 7). (a) ΔP=0 Pa, (b) ΔP=2.5 Pa, (c) ΔP=5 Pa, and (d) ΔP=7.5 Pa (reprinted with Biophysical Journal's permissions).

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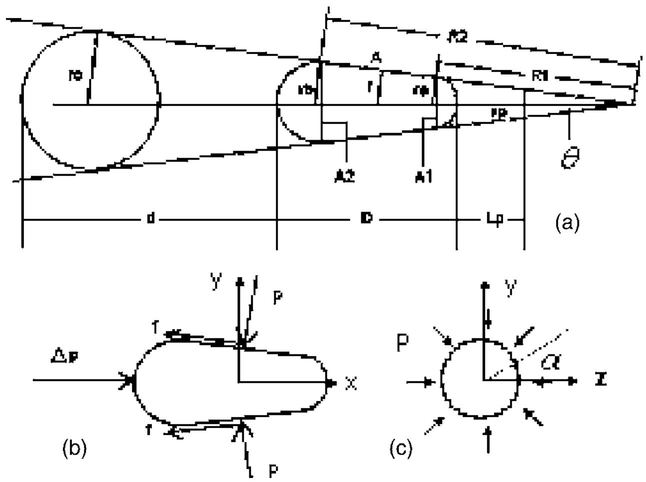

FIG. 2. (a) Linear drawing of a cell in a tapered pipette with a half angle θ. r_a and r_b are the radii of the spherical end caps of the cell. r is the average value of r_a and r_b. r_p is the radius of pipette opening. r_0 is the radius of a single passive cell. L_p is the distance from the small end cap to pipette opening. D is the length of the deformed cell. A_1 and A_2 are cross section areas of the two spherical end caps of the cell. A is the contact area between the cell and the micropipette wall. (b) Force diagram. P is the pressure from the pipette wall perpendicular to the cell surface. f is the frictional force between the cell and pipe wall. ΔP is the applied pressure to one side of the cell. (c) Cross section of cell center. α is an angle from the z axis.

$$E_y = \frac{\sigma_y - \upsilon\sigma_x - \upsilon\sigma_z}{\varepsilon_y}, \tag{1}$$

where υ is Poisson's ratio. In order to extract the elastic modulus of the cell, the stresses and strains in the x, y, and z directions need to be established. The stress in the x direction is induced by the pressure and is expressed as

$$\sigma_x = -\Delta P. \tag{2}$$

Since the channel is cylindrical, we can integrate the pressure over half of the channel wall to get the total force exerted on the cell for both the reaction force N and frictional force f based on Fig. 2(c).

$$\sigma_{y-N} = \frac{-\int_0^\pi P(1/2)(r_a d\alpha + r_b d\alpha) l \cos\theta}{(r_a + r_b) l \cos\theta} = -\frac{\pi}{2}P,$$

$$\sigma_{y-f} = \frac{\mu\int_0^\pi P(1/2)(r_a d\alpha + r_b d\alpha) l \sin\theta}{(r_a + r_b) l \cos\theta} = \frac{\pi}{2}\mu P \tan\theta,$$

$$\sigma_y = \sigma_{y-N} + \sigma_{y-f} = \frac{\pi}{2}\mu P \tan\theta - \frac{\pi}{2}P,$$

$$\sigma_z = \sigma_y = \frac{\pi}{2}\mu P \tan\theta - \frac{\pi}{2}P, \tag{3}$$

where μ is the coefficient of friction. Strains in the x and y directions are shown in Eqs. (3) and (4).

$$\varepsilon_x = (D - 2r_0)/2r_0, \tag{4}$$

$$\varepsilon_y = (r\cos\theta - r_0)/r_0. \tag{5}$$

Substituting Eqs. (2)–(5) into (1), we obtain an expression for the elastic modulus with two unknown parameters, υ and μ. Other parameters relate to the geometry and the pressure applied. Geometrical analysis leads to

TABLE I. Parameters measured from experimental images (Fig. 1).

Figure used	θ (deg)	r_0 (μm)	ΔP (Pa)	D (μm)	Displacement (μm)	L_p (μm)
(b)	2	4.5	2.5	13	35.12	25.7
(c)	2	4.5	5	15.5	43.7	15.8
(d)	2	4.5	7.5	17.7	48.7	11

$$r_a = \frac{L_p \tan\theta + r_p}{\cos\theta - \tan\theta(1 - \sin\theta)},$$

$$r_b = \frac{D\sin\theta + r_a(1 - \sin\theta)}{1 + \sin\theta},$$

$$r = (r_a + r_b)/2;$$

$$A_1 = \pi(r_a\cos\theta)^2,$$

$$A_2 = \pi(r_b\cos\theta)^2,$$

$$A = \pi(r_a\cos\theta + r_b\cos\theta)(r_b/\tan\theta - r_a/\tan\theta).$$

When a cell rests in a micropipette, the three forces reach an equilibrium in both the x and y directions. Assuming that the static frictional force reaches its maximum limiting value, it equals μPA. The force equilibrium in the x direction leads to $\mu PA\cos\theta + PA\sin\theta = \Delta PA_2$. Rearranging the equation, we obtain $P = \Delta PA_2/\mu A\cos\theta + A\sin\theta$ for Eq. (3).

The cell is assumed homogeneous in our model; therefore, we can equate the expressions for the elastic modulus in the x and y directions for various applied pressures. Two equations are sufficient to extract the two unknown parameters: Poisson's ratio υ and friction coefficient μ. After that, the elastic modulus of the cell can be calculated by substituting them into Eq. (1).

The analytical model is applied to extract the elastic properties of a neutrophil cell, based on the images of the tapered micropipette experiment by Needham and Hochmuth.[8] The radius of the micropipette opening r_p is 2 μm, and all the other parameters needed for our model are summarized in Table I. After substituting all parameters into the expression for E_x and E_y, we obtain six expressions for E_{xb}, E_{yb}, E_{xc}, E_{yc}, E_{xd}, and E_{yd} with only two unknown parameters: υ and μ. Each time, two expressions for E from the same figure in the x and y directions and a third expression from another figure in the y direction are set equal to solve for υ and μ. The results are shown in Table II. The average elastic modulus for a single neutrophil cell is 21.80±4.91 Pa, Poisson's ratio is 0.46±0.03, and the coefficient of friction is 0.0274±0.0077.

To verify, these values obtained above were fed into the finite element analysis software ANSYS (ANSYS, Inc., Canonsburg, PA) for simulation using a contact model. A three-dimensional finite element model was built up using the same physical condition as Needham and Hochmuth's experiment. ANSYS element type SOLID187 was used for meshing, and element types CONTA174 and TARGE170 were used to mesh the contact surfaces of the cell, and the micropipette channel wall, respectively. In the simulation,

TABLE II. Results from the analytical model for a neutrophil cell based on Needham and Hochmuth's experiment.

Figures used	E expressions to be equated	Poisson ratio	Friction coefficient	Elastic modulus (Pa)
(b) and (c)	$E_{xb}=E_{yb}=E_{yc}$	0.4367	0.0186	27.160 3
(c) and (d)	$E_{xc}=E_{yc}=E_{yd}$	0.4949	0.0310	17.533 9
(b) and (d)	$E_{xb}=E_{yb}=E_{yd}$	0.4405	0.0327	20.711 0
	Average value	0.4574	0.0274	21.801 7
	Standard deviation	0.0326	0.0077	4.905 04

2.5 Pa forward pressure was applied to the hemispherical surface of a cell. Figure 3(d) shows the simulation results. The displacement of the cell is 33.35 μm, in agreement with the experimental value[7] of 35.12 μm in Fig. 3(b), implying the usefulness and accuracy of the analytical model.

Cells are composed of a membrane, cytoplasm, and a nucleus, and they are inhomogeneous in nature. Our model, however, treats the cell as a homogeneous solid. Simulation results suggest that a homogeneous model is indeed satisfactory; this is due to the special nuclear morphology of neutrophil cells, and the nuclei of these cells are lobulated (separated into small pieces) and highly variable in shape. The nuclei spread out in the cytoplasm and make these cells appear more homogeneous than other types of cell. Furthermore, if a cell's nucleus is very small and located at the center of the cell, then the effect of inhomogeneity will be insignificant, and this model can be applied. The neutrophil cell is assumed to have a Poisson ratio of 0.5 in liquid models[8,9]—similar to that of a real liquid drop—but the cell actually shrinks in compression. Our solid model does not assume a fixed cell volume and can extract some important properties of a cell that cannot be obtained by a liquid model, such as Poisson's ratio and stiffness.

The extracted elastic modulus in our case ranges from 17 to 27 Pa, exhibiting considerable variation among the results obtained from one single cell. Similar results were obtained in the work by Schmid-Schonbein *et al.*,[10] in which the parameter k_1 varied from 10 to 15.2 Pa on a single neutrophil (k_1 is an elastic constant in their homogeneous viscoelastic model). The shear modulus G of a neutrophil cell is $G=k_1/2$ and was determined to be 5–7.6 Pa for an infinitely slow motion, similarly to our situation. Assuming that $E=2G(1+v)$ and that v equals 0.5, the elastic modulus E extracted by Schmid-Schonbein *et al.*[10] is in the range of 15–22.8 Pa, in reasonable agreement with our results in Table II. The considerable variation in both sets of results may arise due to unconsidered factors, such as the nuclear position in the cell.

To conclude, a simple analytical model was developed to extract solid mechanical properties of a single living cell using a tapered micropipette. It is verified by ANSYS finite element simulation. The displacement of a cell under pressure in the simulation is in fair agreement with the experimental results. The extracted elastic modulus of the neutrophil cell is also in good agreement with values extracted by other models.[10] It is an advantage to study a cell's global mechanical properties in a tapered micropipette without substantially altering the cell's living conditions. Moreover, there is scope to integrate tapered channels into microfluidic devices to study cancer metastasis and diagnosis, with the concomitant advantages to biomedical research of small sample sizes, disposability, and high throughput. This analytical method is simple and effective and could be developed for use in tapered channels in microfluidic devices, which are currently being fabricated at Institute of Microelectronics.

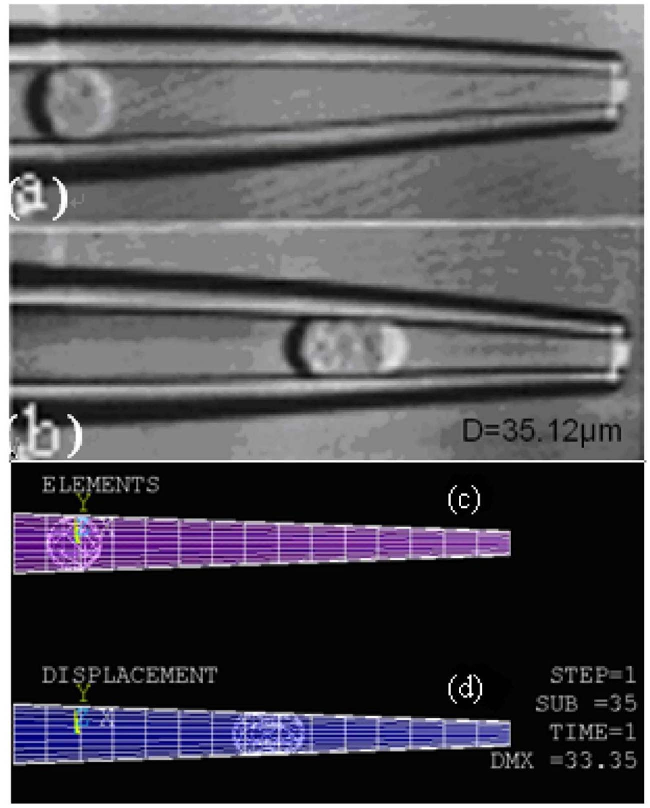

FIG. 3. (Color online) Displacement diagram (a) before and (b) after 2.5 Pa pressure applied in Needham and Hochmuth's experiment and simulated displacement diagram (c) before and (d) after 2.5 Pa pressure applied in ANSYS.

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