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Review

Micropipette aspiration of living cells

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Abstract

The mechanical behavior of living cells is studied with micropipette suction in which the surface of a cell is aspirated into a small glass tube while tracking the leading edge of its surface. Such edges can be tracked in a light microscope to an accuracy of $\pm$ 25 nm and suction pressures as small as 0.1–0.2 pN/μm^2 can be imposed on the cell. Both soft cells, such as neutrophils and red cells, and more rigid cells, such as chondrocytes and endothelial cells, are studied with this technique. Interpretation of the measurements with basic continuum models leads to values for a cell's elastic and viscous properties. In particular, neutrophils are found to behave as a liquid drop with a cortical (surface) tension of about 30 pN/μm and a viscosity on the order of 100 Pa s. On the other hand, chondrocytes and endothelial cells behave as solids with an elastic modulus of the order of 500 pN/μm^2 (0.5 kPa).

1. Introduction

We study the mechanical properties of living cells in order to understand their response to stress in the circulation and the tissues. For example, by characterizing the response of white cells to an applied pressure we learn how these cells flow through the smallest vessels of the body and migrate within tissue to sites of infection. In addition, by measuring the response of cells to applied forces and stresses, we learn about the underlying structure of a cell. Does it behave like a liquid or a solid? What molecular structure is responsible for its behavior? How do mechanical and chemical stimuli alter its behavior?

How do we study and measure the mechanical properties of the cell (Hochmuth, 1987,1993; Waugh and Hochmuth, 1995)? The cell must be deformed in some way by a known force or stress and its deformation must be measured. Usually, the surface of the cell is depressed (indented) or extended. Several instruments including the atomic force microscope (AFM), the optical trap (laser tweezers) and micropipette suction, (the subject of this paper) can do this. Each has its own strengths and weaknesses. The AFM (Weisenhorn et al., 1993; Radmacher et al., 1996), which is available commercially, and a predecessor called the cell poker (Daily and Elson, 1984; Zahalak et al., 1990), depress (indent) a surface with a probe that moves at constant velocity, hence producing an increasing force. The force produced by the AFM is proportional to the deflection of a beam that is very stiff compared to the apparent surface stiffness of the cell that is being deformed. Thus, small forces that are of the order of 10–15 pN will be masked by thermal fluctuations[1]. Also, the variable shape of a typical AFM probe will determine the nature of the force-deformation curve. The laser tweezers usually are limited to the measurement of small forces on the order of 50 pN, although more powerful lasers and better alignment of the system can produce forces that are an order of magnitude larger than this. Here a small bead is captured in an optical trap, the bead is touched to the surface of a cell and then the cell is moved away from the adherent bead, thus extending the surface and, usually, pulling membrane tethers from the surface (Dai and Sheetz, 1995; Dai et al., 1997; Hochmuth et al., 1996). The force of extension is determined by the deflection of the bead in the trap perpendicular to the optical axis. Indenting the surface with a bead in an

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[1] By equating the Brownian fluctuations kT, where k is Boltzmanns constant, to the energy stored in a cantilever beam with spring constant k_b, we see that the equivalent force caused by the fluctuations is $F = \sqrt{k_b(kT)}$. At 300 K and for a spring constant of 40,000 pN/μm, $F = 13$ pN. Smaller spring constants, if and when available, will reduce this force in proportion to the square root of this constant.

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optical trap is unstable in that the bead will tend to slide along the optical axis, although a restoring force is generated when the bead is displaced along the optical axis and this may act to stabilize the bead. Finally, micropipette suction, discussed here, produces a deformation that is the opposite of that produced by the AFM in that the cell surface is extended into the pipette rather than depressed into the interior of the cell. A novel application of micropipette suction is the biointerface probe (Evans et al., 1995). Here a nearly inflated red cell or lipid bilayer vesicle is aspirated into a small micropipette until the surface of the membrane outside of the pipette is spherical. A bead is attached to the membrane at a point that is diametrically opposite to the mouth of the pipette. The deflection of the bead is proportional to the force exerted on it and the spring constant for this deflection (force per unit deflection) can be adjusted by adjusting the suction pressure. The device can measure piconewton-level forces. It is ideally suited for measuring molecular bond forces (Merkel et al., 1999) although it also could be used to depress a surface in a manner akin to the AFM, but with a spring constant that can be adjusted to be several orders of magnitude less than that of the AFM.

How do we model the mechanical behavior of cells? We are at a primitive stage of our modeling in that we use simple continuum models. The two most popular models for analyzing cellular deformation treat the cell either as a homogeneous elastic solid or as a liquid surrounded by an elastic cortical shell. Surprisingly, these models make good predictions of the deformation response of the cell to known suction forces produced by the pipette. This is so in spite of the fact that the cells in general consist of a complex cytoplasm with granules, various types of filaments, a nucleus, and dissolved proteins. A model at the other extreme from a continuum one is the tensegrity model (Ingber, 1998). Here the loads are borne by struts in compression and cables in tension. Clearly, complex cells have tensegrity-like elements (Wang et al., 1993) although it is not likely that a significant compressive load is borne by struts in compression. Rather, because the water content of most cells in of the order of 70% or more, compressive loads are borne either in their entirety by water's hydrostatic, isotropic pressure or partially by a hydrostatic pressure plus a normal stress produced by an isotropic compression of a cell's internal elastic structure. The modeling of cellular deformation in the future may combine elements of both continuum models and tensegrity models. This may be especially important for cells attached to surfaces at points of focal adhesion where molecular cross bridging occurs.

2. Units at the cellular level

A set of derived units of the SI system is naturally suited to studies of the mechanical properties of the neutrophil. These "natural" units are shown in Table 1. Distance is measured in μm, because cells are of the order of 10 μm in diameter and, therefore, cellular deformation will be of the order of 1 μm or more. Forces needed to deform soft cells, such as red cells or white cells, are of the order of 10–100 pN while those exerted on stiff cells such as endothelial cells or chondrocytes are of the order of 1 nN. Because the diameter of pipettes are of the order of 1–10 μm and the forces range from 10 pN to 1 nN, we measure stresses and pressures in units of either $pN/\mu m^2$ or $nN/\mu m^2$. These units are very convenient and, it so happens, familiar because $1\ pN/\mu m^2 = 1$ Pa and, thus, $1\ nN/\mu m^2 = 1$ kPa. Some cells behave as liquid drops and, thus, have a characteristic surface tension or "cortical" tension. The natural unit for this constant is pN/μm. The shear modulus of red cell membrane also is measured in pN/μm. On the other hand, the expansion of a lipid bilayer membrane, which behaves as a two-dimensional liquid, requires much larger tensions and, therefore, the natural unit in this case is nN/μm. Again, this is a very familiar unit because $1\ nN/\mu m = 1\ mN/m =$ 1 dyne/cm. The traditional way of reporting values for surface tension is in units of dyne/cm.

Table 1
"Natural" SI units at the level of the cell

	"Micro SI"	Application
Distance (m)	1 μm (10^{-6} m)	All
Force (N)	1 pN(10^{-12} N)	Molecular bonds "soft" cells
	1 nN(10^{-9} N)	Stiff cells
Pressure, stress (Pa)	1 pN/μm^2(1 Pa)	Soft cells (blood cells)
	1 nN/μm^2(1 kPa)	Stiff cells
Tension (mN/m)	1 pN/μm (10^{-3} mN/m)	Cortical elasticity of soft cells
	1 nN/μm(1 mN/m)	Elasticity of lipid bilayer

3. Micropipette aspiration

A schematic diagram of the micropipette aspiration system, excluding the manometer and the micromanipulator, is shown in Fig. 1. Fig. 1(a) shows a common application where a cell suspended in a saline solution is partially aspirated into the mouth of the pipette. The round shape occurs either naturally in the case of white cells or spontaneously when cells are detached from a surface. As we will discuss, cells can behave as either a solid or a liquid. The response in either case to an aspiration pressure is similar until a hemispherical projection is formed in the pipette. Beyond that point, a further increase in the suction pressure causes a liquid-like cell with a constant cortical tension to flow completely into the pipette (Evans and Yeung, 1989) while the surface of a solid cell will extend (project) into the pipette to

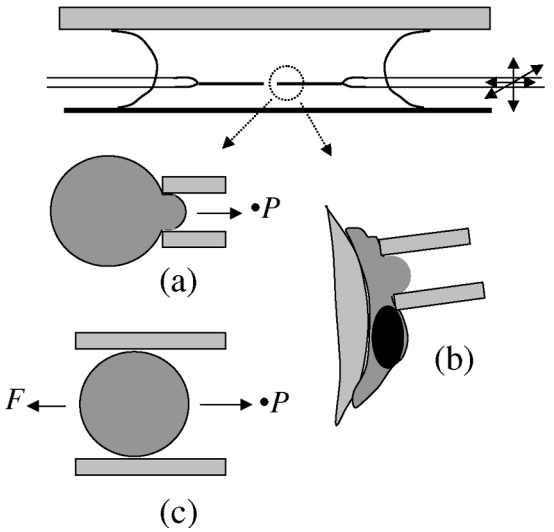

Fig. 1. Two micropipettes in a chamber. A pneumatic micromanipulator controls the movement of a micropipette along three orthogonal axes. (a) A spherical cell being aspirated into a micropipette with a suction pressure ΔP. (b) An attached cell being aspirated into a pipette. (c) A closely fitting cell or bead moving freely in a pipette like a piston in a cylinder. When static, the suction pressure times the cross-sectional area of the pipette equals the attachment force F.

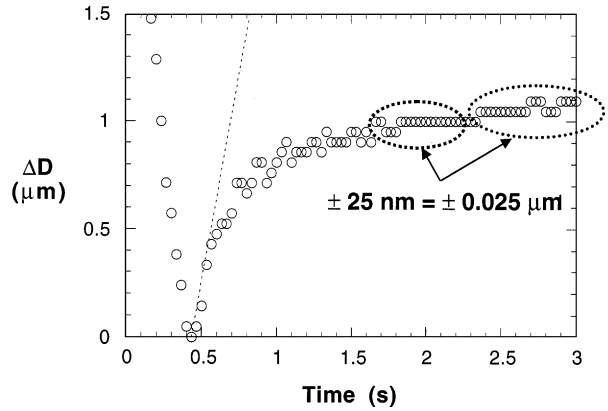

Fig. 2. The tracking of a neutrophil as it moves freely in a pipette towards a fixed bead held by a second pipette. When $\Delta D = 0$, the cell has just touched the bead. At this point, the pressure is reversed and the cell either moves freely in the pipette and away from the bead (dashed line) or adheres to the bead and, eventually ceases its motion (open circles). The cell motion of an adhering cell does not cease immediately because of the stretching of an elastic microvillus. This figure shows that the edge of a cell can be tracked manually in monochromatic light with a cursor imposed electronically on the video screen to an accuracy of approximately $\pm$ 25 nm. (Figure adapted from Shao et al., 1998.)

a new equilibrium position (Theret et al., 1988). Fig. 1(b) depicts the aspiration of a cell into a micropipette when the cell adheres to a bead. For example, the cell could be an endothelial cell cultured on a microcarrier bead with the microcarrier bead being held on its opposite side by another pipette. The resolution of the image of the aspirated portion of an attached cell is greatly improved when the cell is attached to a curved surface. Fig. 1(c) shows a relatively new application in which a freely moving cell or bead subjected to a pressure drop in the pipette is allowed to adhere to another cell or bead held by a second pipette (Shao and Hochmuth, 1996). In all cases, the suction pressure and the location and movement of the edge of the cell in the pipette must be measured.

The minimum value for the suction pressure ΔP is given by the minimum displacement downward of the water reservoir, which controls the hydrostatic head Δh. Thus,

$$\Delta P_{\min} = \rho g \Delta h_{\min} \approx 0.025 \frac{\mathrm{pN}}{\mu\mathrm{m}^2}, \quad (1)$$

where the minimum height adjustment of the reservoir is $\Delta h_{\min} \approx 2.5\,\mu$m. In practice this resolution is not achieved because of fluctuations in the system and because of a slow drift in the null (zero) setting, probably because of evaporation. A practical lower bound is of the order of 0.1–0.2 pN/μm^2. It is likely that the drift in the null setting, which is the point where there is no movement of the fluid in the pipette, is caused by evaporation which changes the curvature of the interface shown in Fig. 1. For a nominal water surface tension of 50 nN/μm at the air–water interface and a radius of curvature of 1000 μm, the pressure drop across this interface would be $(50 \times 10^3/1000)$ pN/μm^2 = 50 pN/μm^2. Thus, a 1% change, say, in this curvature caused by evaporation would cause a change in the zero pressure setting of 0.5 pN/μm. In the winter when the dew points are low, evaporation can be significant and the null point must be checked every few minutes when working at the lower range of the suction pressures.

The vapor pressure of water at room temperature limits the maximum suction pressure. Thus, the maximum suction pressure is of the order of 96 nN/μm^2.

The results of the manual tracking of an edge with a cursor on the video screen is shown in Fig. 2, which is adapted from the work of Shao et al. (1998). Here a neutrophil that moves freely in a pipette, as illustrated by Fig. 1(c), has adhered at a single point to a bead coated with an antibody to the membrane receptor. The bead is held in place by a second pipette. Initially, we create a small, fixed suction pressure by lowering the reservoir so that the cell moves slowly away from the bead. In Fig. 2 the suction pressure is 0.5 pN/μm^2. We impose a small, positive air pressure on the water surface in the reservoir to move the cell towards the bead. Once the cell contacts the bead, we suddenly release the air pressure, which causes the cell to move away from the bead. If no adhesion occurs, the cell moves along the dashed line. Because adhesion occurs in this example, the motion of

the cell is retarded and an equilibrium state is approached asymptotically as shown by the experimental data points. In this case a small microvillus is being stretched to a length of about 1 μm. As we see in Fig. 2, the fluctuations in the measurements near and at equilibrium are of the order of ± 0.025 μm. Thus, the movement of an edge as observed in the optical microscope in monochromatic light can be tracked manually to an accuracy that is at least one-tenth of the wavelength of light.

The force F on a static cell in a micropipette is simply the suction pressure ΔP times the cross sectional area of the pipette πR_p^2. In the case of Fig. 2, with a pipette diameter of about 10 μm, the force acting on the *static* cell is about $(0.5\ \mathrm{pN/\mu m^2})\ (\pi/4)\ (10\ \mu\mathrm{m})^2 = 40$ pN. But as long as the cell is moving because of the stretching of the microvillus in this case, the force on the cell will be less than that exerted on the static cell. Its value is (Shao and Hochmuth, 1996)

$$F = \Delta P \pi R_p^2 \left(1 - \frac{U_t}{U_f}\right), \tag{2}$$

where U_f is the free motion velocity of the cell (the dashed curve in Fig. 2) and U_t is the velocity of the attached (tethered) cell. Note the U_t varies from an initial value equal to U_f when the force is zero according to Eq. (2) to a value of zero which gives the maximum force on the cell equal to the pressure drop times the cross-sectional area.

We see that the fundamental independent (imposed) experimental variable in micropipette suction is the suction pressure ΔP. The dependent variable is the response of the cell surface to the suction pressure. This response is either the extension of the surface of the cell into the pipette L_p or the movement of a cell or bead away from a point of attachment as shown by Fig. 2. Often L_p is scaled with respect to the radius of the pipette: L_p/R_p. Hence, when this ratio is unity, we have a hemispherical or a hemispherical-like projection into the pipette.

4. Continuum models at equilibrium: liquid vs. solid

Fig. 3 shows a scanning electron micrograph of two cells, a human neutrophil (a) and a chondrocyte (b), that look nearly identical to each other except for their difference in size. (The diameter of a neutrophil is about 8 μm (Ting-Beall et al., 1993) while that of a chondrocyte varies between 12 and 16 μm (Jones et al., 1999). Fig. 4 shows the same two kinds of cells after they have been aspirated completely into a micropipette. It is impossible to say if a cell behaves as a solid or a liquid just from an examination of these two figures. In fact, the neutrophil behaves as a liquid (Evans and Yeung, 1989) while the chondrocyte behaves as a solid (Jones et al., 1999).

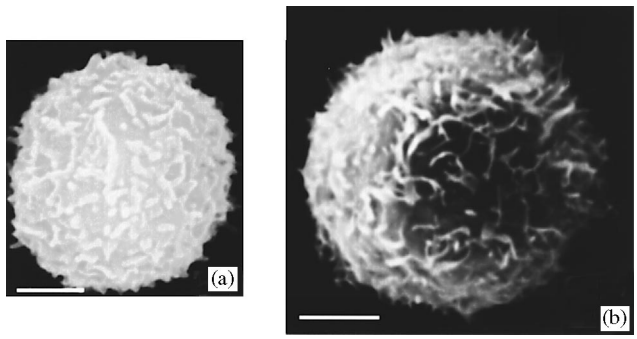

Fig. 3. Comparison of a human neutrophil (a) to a chondrocyte (b). The neutrophil has a diameter of about 8 μm while the majority of chondrocytes have diameters between about 12 and 16 μm. The scale bars indicate 2 μm, but note that the significant shrinkage of the cell has occurred during the preparation of the cells for scanning electron microscopy.

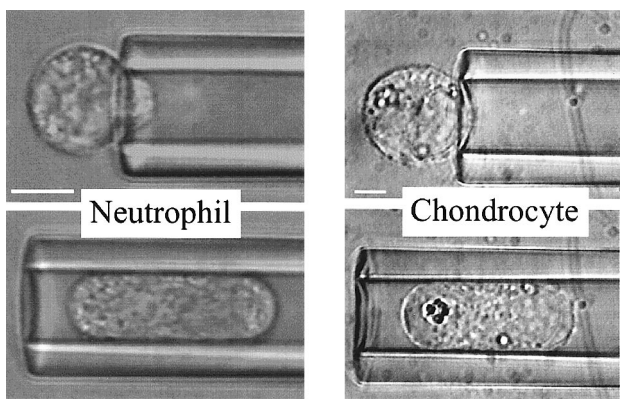

Fig. 4. A neutrophil and a chondrocyte each being aspirated into a micropipette. The photomicrographs of the chondrocyte are adapted from Jones et al. (1999). The scale bars indicate 5 μm.

Micropipette suction is unique in which it can clearly and dramatically show if a cell behaves as a liquid drop with constant cortical tension. This comes directly from the law of Laplace when applied to the suction of a cell into a micropipette until $L_p/R_p = 1$, the point where the cell forms a hemispherical projection into the pipette. For this case,

$$\Delta P = 2T_c\left(\frac{1}{R_p} - \frac{1}{R_c}\right), \quad \left(\Delta P = \Delta P_c \text{ when } \frac{L_p}{R_p} = 1\right), \tag{3}$$

where T_c is the cortical tension, R_c is the radius of the cell outside the pipette and ΔP_c is the critical pressure when $L_p/R_p = 1$. Because of the small suction pressures relative to the osmotic pressure of isotonic saline, the cell will deform at constant volume. A further increase in the suction pressure beyond the critical value will cause the radius of the cell outside the pipette to *decrease* and its

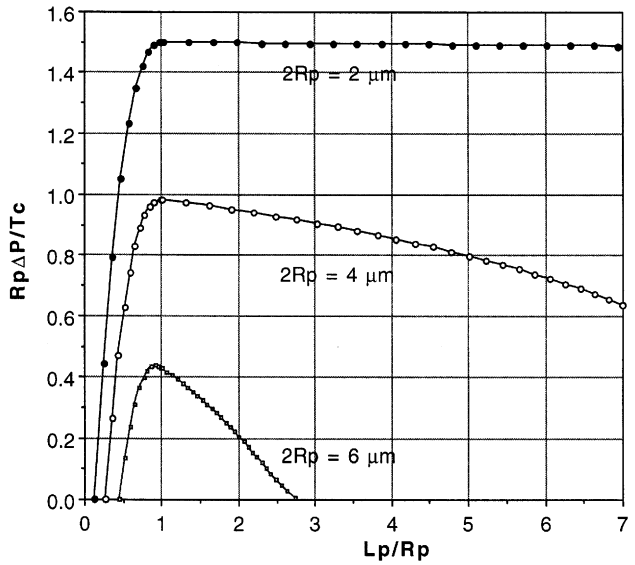

Fig. 5. The aspiration into a pipette of a liquid drop with a constant cortical tension T_c according to (3). L_p is the length of extension of the drop into the pipette and R_p is the radius of the pipette. When $L_p/R_p > 1$, the results are no longer stable to an increase in pressure. Thus, the cell flows freely into the pipette when the pressure is increased beyond the point where $L_p/R_p = 1$. Cells such as neutrophils that flow freely into pipettes at this point behave as liquid drops.

reciprocal to *increase*. It will be impossible to satisfy Eq. (3) for a cell at equilibrium and, therefore, the cell will flow into the pipette, as shown for the human neutrophil by Evans and Yeung (1989). A plot of Eq. (3) for the constant volume deformation of an 8 μm diameter liquid drop into pipettes of different diameters is shown in Fig. 5. We can see in this figure that the initial response of the cell for $L_p/R_p < 1$ is almost linear, which led others to treat the neutrophil as an elastic solid (Schmid-Schönbein et al., 1981). Nevertheless, it is impossible to satisfy Eq. (3) when the pressure is increased beyond the point where $L_p/R_p = 1$. Note, however, that for small diameter pipettes, the pressure is almost constant beyond this point. This is so because the cell radius barely decreases as the cell is drawn further into the pipette.

There is another piece of evidence for the liquid behavior of the neutrophil besides its ability to flow smoothly into a pipette once the suction pressure exceeds the critical pressure. That is, the cell behaves according to Eq. (3) when a neutrophil is aspirated to its critical point (when $L_p/R_p = 1$) into pipettes of different diameters for a constant value for T_c of 35 pN/μm (Evans and Yeung, 1989). Nevertheless, when Needham and Hochmuth (1992) aspirated cells into tapered pipettes so as to produce a series of stable states and a significant amount of area expansion, they saw evidence that the cortical tension of the neutrophil could increase. They correlated this with the relative increase in apparent surface area of the neutrophil as it was aspirated further down the tapered tube and proposed a relative area expansion modulus of $K_A = 39$ pN/μm and an extrapolated value for the cortical tension before any expansion of area of $T_c = 24$ pN/μm.

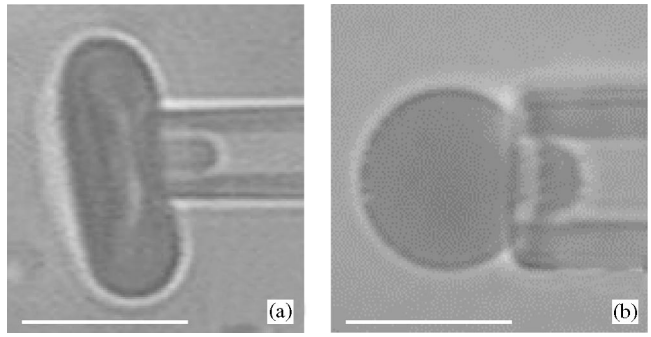

Fig. 6. Aspiration of a flaccid (a) and swollen (b) red cell into a pipette. The diameter of the flaccid red cell is approximately 8 μm and that of the swollen cell is about 6 μm. The scale bars indicate 5 μm.

Other analyses of neutrophils deformed by micropipette aspiration based on the solid model of Schmid-Schönbein et al. (1981) include work by Chien et al. (1987). However, in their work only small deformations of the cell were studied whereby the cell appears to behave as an elastic solid as shown in Fig. 5 for the case where $L_p/R_p < 1$.

In an attempt to duplicate the flow behavior of neutrophils when they are aspirated into a pipette (Evans and Yeung, 1989; Needham and Hochmuth, 1990), Dong et al. (1991) used a Maxwell liquid to model the flow of a neutrophil. However, even this more complicated model, compared to a Newtonian one of Evans and Yeung (1989), would not fit the experimental data unless the values for the viscosity and elasticity of the cytoplasm were allowed to change continuously as the cell flowed into the pipette. A completely satisfactory constitutive equation for the flow behavior of the cytoplasm of neutrophils and other granulocytes has yet to be found.

The natural shape of the human red cell, unlike most cells in suspension, is not spherical. Rather, it is shaped like a biconcave disk as shown in Fig. 6(a). Because its interior is a Newtonian liquid, the shape of the red cell comes from the natural shape of its membrane. The membrane has a shear rigidity (Evans, 1973) in which it does not flow into a pipette when subjected to a suction pressure as shown in Fig. 6(a). This resistance to shear comes from extension of the membrane in the radial direction and compression along the circumference as the cell is aspirated further into the pipette. This deformation has been analyzed based upon a constant area assumption (Evans, 1973; Chien et al., 1978; Waugh and Evans, 1979). Chien et al. linearized this result to give

$$\frac{\Delta P \cdot R_p}{\mu} = 2.45\frac{\Delta L_p}{R_p}, \quad \left(\frac{L_p}{R_p}\right) > 1, \tag{4}$$

where μ is the shear elastic modulus of the membrane. From Eq. (4) we see that the experimental determination of the slope of the ΔP vs. ΔL_p line gives a value for the elastic shear modulus μ. Note that this value is proportional to the pipette radius *squared*. Because pipettes with radii that are only a factor of two to four times the wavelength of green light are used in these experiments, as shown in Fig. 6(a), errors in measurement of at least 20% are common. In all cases, typical values for μ are of the order of 6–9 pN/μm (Hochmuth, 1987; Waugh and Hochmuth, 1995).

We make an interesting observation at this point and note that the shear modulus for the red cell membrane is about one-third to one-fourth that of the cortical tension of the neutrophil. Discher et al. (1994,1998) have shown that the underlying cytoskeleton of the red cell membrane possesses both a shear elasticity and a network dilatation elasticity and the ratio of the shear to the area elasticity is of the order of 1 : 2 to 1 : 4. This suggests that a similar cytoskeletal network may exist at the inner surface of the neutrophil.

When swollen as shown in Fig. 6(b), the red cell membrane has an extraordinary resistance to further expansion (Waugh and Evans, 1989). The membrane behaves as a two dimensional liquid with a tension at lysis of the order of 10 nN/μm and with an expansion modulus on the order of 500 nN/μm (Hochmuth, 1987; Waugh and Hochmuth, 1995). Lipid bilayers made from a variety of phospholipids with and without cholesterol show an extraordinary range in values for the area expansion modulus from about 57 to 1734 nN/μm (Needham and Nunn, 1990).

When cells that behave as solids are aspirated into a micropipette, obviously they do not flow into the pipette when the aspiration length L_p exceeds the pipette radius R_p. The aspiration length increases linearly with the suction pressure regardless of the value for L_p as shown by the experimental data in Fig. 7 for chondrocytes (Jones et al., 1999) and endothelial cells (Theret et al., 1988). Jones et al. (1999) further show that chondrocytes continue to behave as an elastic solid for values for L_p/R_p that are significantly greater than one. In both cases the cells are spherical in shape. This is the natural shape for the chondrocyte and the shape assumed by an endothelial cell when it is detached from the surface on which it is cultured. Note that the plot in Fig. 7 has the independent experimental variable plotted along the y-axis (ordinate) so that the slope of the line is proportional to the elasticity of the material and that Fig. 7 can be compared to the theoretical plots shown in Fig. 5. Probably it is a coincidence that the results in Fig. 7 for both chondrocyte and endothelial cell are identical. Similar but not identical responses are expected because both cells do have similar values for their elastic moduli.

The analysis of Theret et al. (1988) for an infinite, homogeneous half-space drawn into a micropipette gives

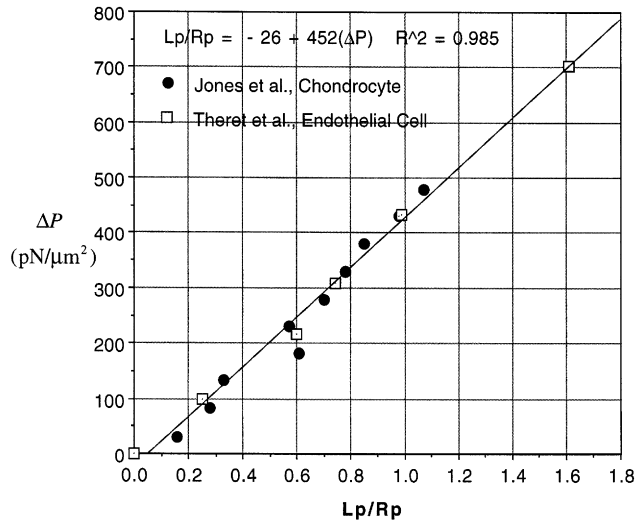

Fig. 7. Aspiration of a chondrocyte and an endothelial cell into a micropipette. Data for the chondrocyte are taken from Jones et al. (1999) and that for the endothelial cell are taken from Theret et al. (1988). Chondrocytes continue to behave as an elastic solid for values for L_p/R_p that are significantly greater than one (Jones et al., 1999).

the following result:

$$\Delta P = \frac{2\pi}{3} E \frac{L_p}{R_p} \Phi, \tag{5}$$

where E is the Young's modulus for the homogeneous solid, ΔP is the suction pressure and Φ is a term that depends weakly on the ratio of the thickness of the pipette wall to the radius of the pipette. A typical value for Φ is $\Phi \approx 2.1$. Thus, the coefficient in Eq. (5) is $(2\pi\Phi/3) = 4.4$. Because the slope of the line in Fig. 7 is 452 pN/μm^2, the value for E in this case is only $E = 103$ pN/μm^2. Endothelial cells subjected to a shear stress of 1 pN/μm^2 for 3 and 14 h had a value for the Young's modulus, as calculated by Eq. (5), of E ≈ 400 pN/μm^2 (Theret et al., 1988). (In a later study by Sato et al. (1990) of the viscoelastic response of endothelial cells to be discussed below, the sum of the two elastic constants from this study gives a value of about 280 pN/μm^2.) Young's modulus for chondrocytes is slightly larger: $E = 650$ pN/μm^2 with a very large standard deviation approximately equal to the value for E (Jones et al., 1999).

It is possible to calculate an equivalent cortical tension for solid cells that are aspirated into pipettes by equating Eqs. (3)–(5) at the point where $L_p/R_p = 1$:

$$(T_c)_{\text{equivalent}} \approx 2.2 \frac{E R_p}{1 - \left(\frac{R_p}{R_c}\right)} \approx 2.2 E R_p, \tag{6}$$

where we have assumed in Eq. (6) that the radius of the pipette is significantly smaller than the radius of the cell. Thus, for a typical value of $E = 500\ \mathrm{pN/\mu m^2}$ and a typical pipette radius of 2 μm, $(T_c)_{\mathrm{equivalent}} = 2200\ \mathrm{pN/\mu m} = 2.2\ \mathrm{nN/\mu m}$. This value is 100 times larger than the cortical tension of the neutrophil, indicating that endothelial cells and chondrocytes are significantly stiffer than the extraordinarily soft neutrophil (and red cell).

The process of detachment of endothelial cells from their substrate could change their mechanical properties. Experiments should be performed with the cells attached to the surface on which they are cultured, as illustrated schematically by Fig. 1(b). However, in this case the infinite half-space model is not appropriate. Other models, probably numerical ones using finite element analysis, need to be developed. The other difficulty in determining the mechanical properties of endothelial cells is that the endothelial cell remodels when subjected to mechanical stress (Sato et al., 1987). The process of deforming an attached cell with a micropipette may cause it to stiffen as it remodels its mechanical structure in response to the deformation. However, if the remodeling process is sufficiently slow, then the baseline elasticity could be measured and the change in the elasticity with time as the cell remodels could be studied.

Portions of cells, such as the microvilli on the surface of neutrophils (Fig. 3(a)) also respond elastically to a force as shown by Fig. 2 (Shao et al., 1998). This experiment, described above, determines the elastic spring constant for neutrophil microvilli. Its value is about 40 pN/μm, which is similar to the value for the cortical tension of the neutrophil and about four times bigger than the shear modulus of red cell membrane. It is similar in value to the area expansion modulus of the neutrophil measured by Needham and Hochmuth (1992). Again we are led to speculate that the elastic cytoskeleton of the red cell membrane is similar to that of the neutrophil membrane.

5. Viscous continuum models: liquid and solid

As we have discussed, the neutrophil behaves as a liquid drop and flows readily into a micropipette of smaller diameter. The rate at which a cell flows into a pipette $\mathrm{d}L_p/\mathrm{d}t$ is constant over much of the entry phase, indicating a Newtonian-like behavior[2]. Thus, it is easily measured and then used to calculate the viscosity of the cytoplasm of the neutrophil (Evans and Yeung, 1989; Needham and Hochmuth, 1990). A simple expression for the calculation of a value for the viscosity η is (Needham and Hochmuth, 1990)

$$\eta = \frac{R_p \Delta p}{\left(\frac{\mathrm{d}L_p}{\mathrm{d}t}\right) m(1 - R_p/R)}, \tag{7}$$

where L_p is the length of the extended portion of the cell in the pipette and R is the radius of the spherical portion outside the pipette at the point where $\mathrm{d}L_p/\mathrm{d}t$ is measured. The constant m is obtained from the theory of Evans and Yeung (1989) and has a value of $m \approx 6$. Somewhat larger values for m are suggested by the recent and extensive numerical solutions to this problem by Drury and Dembo (1999). In any case, Needham and Hochmuth (1990) used Eq. (7) to calculate a value of 135 $\mathrm{pN\,s/\mu m^2}$ (135 Pa s) for the viscosity of cytoplasm at room temperature for 151 neutrophils from five different donors. Recall that the viscosity of water is about 10^{-3} Pa s at room temperature, so the viscosity of cytoplasm is about 10^5 times larger than that of water. With fewer cells and donors and for studies at lower suction pressures, Evans and Yeung (1989) obtained a value of 200 Pa s for the viscosity of cytoplasm.

Micropipette suction also can be used to study the viscous response of solid cells such as endothelial cells and chondrocytes. This is done by subjecting the surface of the cell to a step change in pressure and measuring the rate at which the extended length into the pipette L_p approaches a steady-state value such as those shown in Fig. 7. Sato et al. (1990) analyzed this problem based upon the semi-infinite half-space model and a standard viscoelastic solid model in which a Maxwell element (elastic and viscous elements in series) is in parallel with another elastic element. Their experiments were done with cultured aortic endothelial cells from pigs. The cells were detached from the surface either by chemical or mechanical means. In either case the typical value for the viscosity based on calculations with the standard viscoelastic solid model is about 7500 Pa s. This result indicates that the viscosity of the cytoplasm of endothelial cells is significantly larger than that of neutrophils. Although no value for viscosity was given by Jones et al. (1999) in their study of chondrocytes, the very slow viscoelastic creep into a suction pipette when the chondrocyte was subjected to a step change in pressure indicates similarly large values for the viscosity of the chondrocyte in comparison to that of the neutrophil.

6. Discussion

It should be clear that micropipette suction offers a versatile method for measuring the material properties of living cells. Its range of suction pressures, from $0.1\ \mathrm{pN/\mu m^2}$ to almost atmospheric, and forces, from about 10 pN to 10^4 nN, is unsurpassed by other

[2] When the cell first contacts the pipette, the leading edge "jumps" into the pipette, indicating a decidedly non-Newtonian behavior at the beginning of the entry process.

techniques. Edges can be tracked to an accuracy of $\pm$ 25 nm. It can measure the elastic and viscous properties of very soft materials like red cells and white cells and those of stiffer, more viscous cells such as endothelial cells and chondrocytes. However, it is not a perfect instrument because in its basic form there can be significant evaporation in the chamber and a drift in the null (zero) setting for the pressure. Also, it demands training and skill on the part of the experimenter. Finally, as with all experiments that deform cells and cell surfaces, the results must be interpreted with the use of mechanical models. At present, only continuum and, usually, homogeneous models have been solved, either exactly or by finite element analyses. In the future, more complex models with internal structure, multiple phases and domains and complex boundary conditions are needed for the correct interpretation of experiments with the micropipette.

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