MOLECULAR, CELLULAR, & TISSUE BIOMECHANICS

Spring 2015

Problem Set #8 – Membrane mechanics, cell motility and combined problems

Distributed: Wednesday, April 29, 2015
Not to be handed in

Primary Cilium

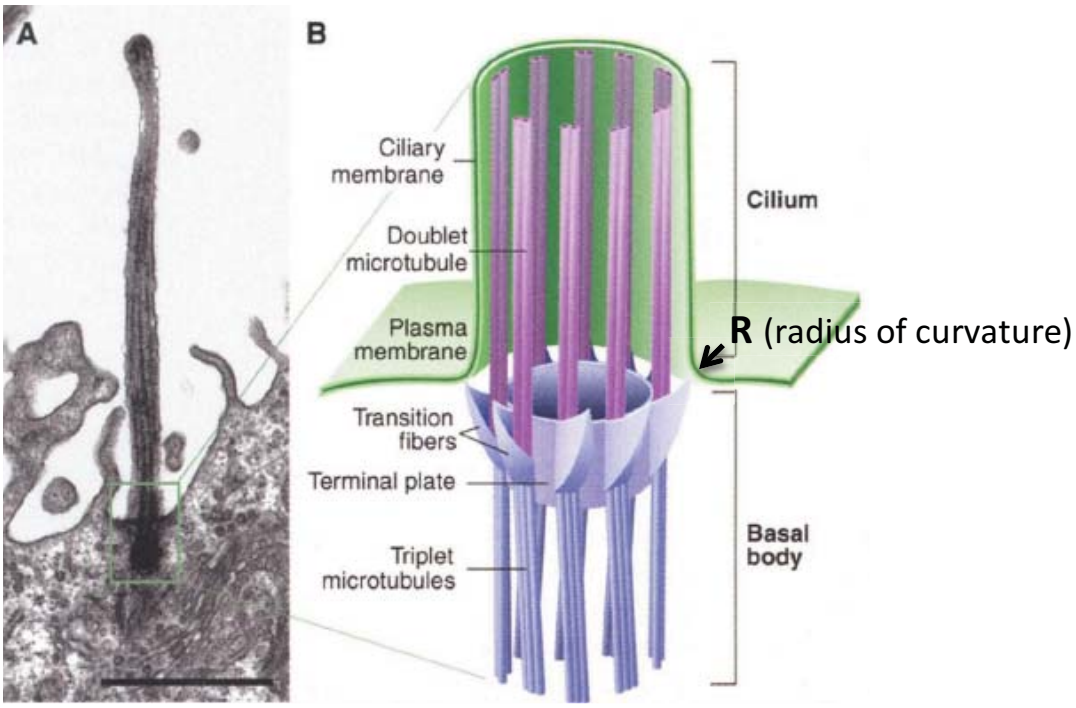
The primary cilium (PC) is a single cilium that protrudes from most mammalian cells and plays a critical role in chemical and mechanical signaling, and although discovered more than 100 years ago, only recently has the PC been linked to essential phenomena in cases ranging from embryonic development to cancer[1][2]. PC in kidney epithelial cells sense the flow of urine and use this as a means to regulate cell proliferation. Being microtubule-based, the PC is relatively rigid and can transmit forces and bending moments to various intracellular structures. In this problem, we will examine several different issues relating to PC mechanics.

PC Radius = a = 100 nm
Microtubule radius = a_m = 12 nm
EI = bending stiffness of the PC treated as a beam = 1.4×10^{-23} Nm^2
Young's modulus of a microtubule, treated as a solid rod of radius a_m, = 1 GPa
PC Length = L = 6 μm
Membrane bending stiffness = $K_b = 2 \times 10^{-18}$ Nm
Membrane tension (assumed uniform) = $N = 10^{-5}$ N/m

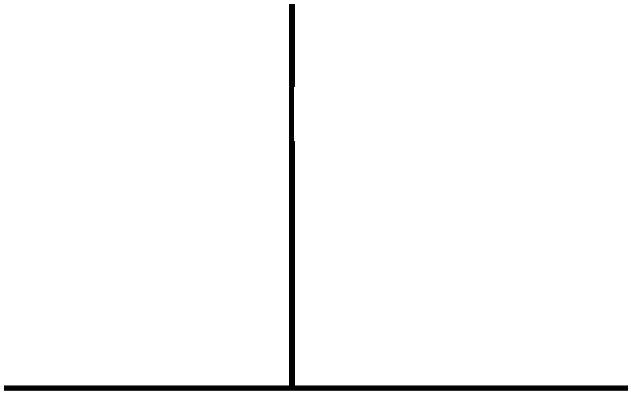
a) Approximate the PC (neglecting the basal body) as a beam connected to a stiff support at its base that can support a bending moment. Calculate the bending moment at the membrane and sketch the deformed shape of the beam, assuming that the flow-induced force can be modeled as a point force F acting at the tip of the PC.

b) Using any method you choose, estimate the force F (if applied at the tip and in a direction perpendicular to the axis of the PC) required to deflect the tip of the PC a distance of 1 μm. You may treat the PC as a solid, homogeneous beam with the characteristics given above.

[1] Michaud EJ and Yoder BK, Cancer Research, 66: 6463, 2006.
[2] Singla V and Reiter JF, Science, 313: 629, 2006



c) Neglecting the effects of the cell membrane and assuming that all of the stress is supported by the 9 doublet microtubules that dominate the internal structure of the PC (see figure), describe the distribution of forces among these microtubules at the point where the PC meets the cell body. That is, show (sketch) qualitatively the relative magnitude and direction of the force in each doublet as a function of distance from the center of the PC (x) in a plot as shown below, where x is the coordinate parallel to the direction of force application. Pay particular attention to whether the forces are tensile (positive) compressive (negative) or both.



d) Estimate by an approximate analysis the maximum level of tensile force acting in just one of the doublet microtubules at the base of the PC, where it enters into the cell body, using the values given above and corresponding to the deflection described in (b).

e) Now, treat the internal structure of the cell as a linear elastic material (shear modulus G and Poisson ratio ν) that extends far in all directions, and that the forces just described are acting at the surface of

this elastic material. Sketch the deflections at the surface caused by the force distribution from (c) and magnitude from (d) and estimate by an approximate analysis the maximum vertical deflection of the surface. (You don't need to calculate the actual value, just provide the scaling relationship in terms of the defined variables.)

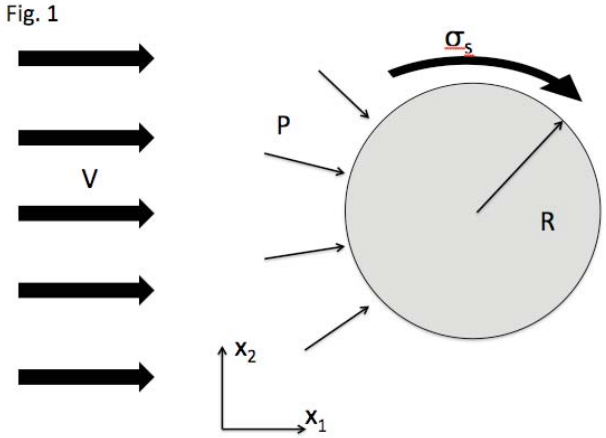
f) Now focusing on the lipid bilayer, estimate the radius of curvature at the base of the PC where it meets the cell body. Consider the case when the PC is not subjected to a force (F=0), and looks as shown in the left hand sketch in the figure. The radius of curvature of interest is labeled R in the figure.

Cancer cell with interstitial flow

Recent experiments (Polacheck et al., PNAS, 2011) have produced the intriguing result that the directional persistence of cancer cells migrating through extra cellular matrix (ECM) is influenced by flow of interstitial fluid as induced by pressure gradients in the vicinity of a tumor. This problem probes a number of questions that relate to the underlying mechanisms for this observation.
For purposes of this problem, consider the cancer cell as a sphere of radius R, within an infinite, homogeneous ECM and exposed to a uniform fluid flow velocity V (Fig. 1). The ECM has a hydraulic permeability of k_{ECM} and a shear modulus, G_{ECM}. The viscosity of the interstitial fluid is η.

a) Due to the flow, the cell experiences a net force in the direction of flow, F, due to a combination of fluid pressure (normal stress, p) and fluid shear (shear stress = σ_s). The latter can be shown to scale as $V / \sqrt{(k_{ECM}/\eta)}$. Use Darcy's equation (the equation that relates to the pressure gradient and flow velocity through a porous medium):

Fig. 1



$$\nabla p = \frac{V}{k}$$

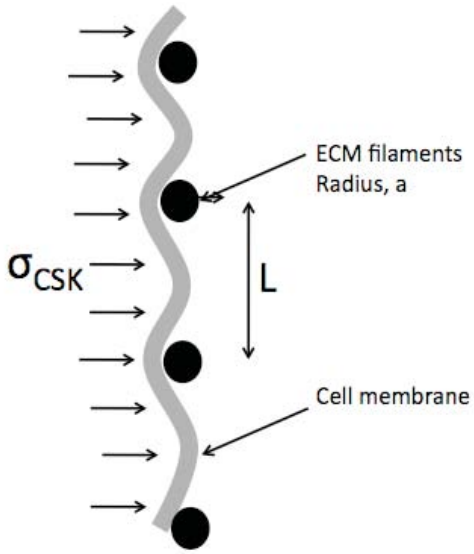
and scaling analysis to **estimate the net force acting on the cell due to pressure, F_p,** and **obtain a ratio of F_p to the total force due to shear stress, F_s.** Using the typical values given at the end of this problem, **obtain a numerical value for this ratio**.

b) Given the total force due to the combined effects of fluid pressure and fluid shear F as computed in (a), and the shear modulus of the ECM given below, **find an approximate (scaling) expression for the distance δ that the cell is displaced due to the combined effects of fluid pressure and shear.** Assume that the cell retains its spherical shape, but that the surrounding ECM is locally distorted due to the force exerted on it by the cell.

c) Now assume that the entire fluid force acting on the cell is supported by the membrane-ECM interactions on the downstream side of the cell, where the membrane pushes against the filaments that comprise the ECM (see Fig. 2). Assume that all of the force computed in (b) is transmitted by the cytoskeleton to the cell membrane, which is supported externally by forces exerted by the matrix filaments in contact with the membrane. If, in electron micrographs the cell membrane appears as shown in the figure, so that the characteristic length is the spacing between filaments, L, **which is more important in terms of supporting the force, membrane bending or membrane**

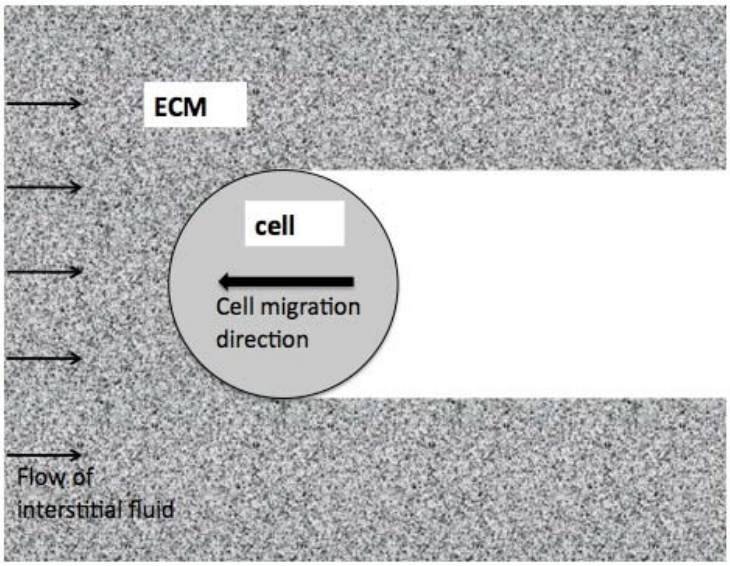
tension? You may assume that the membrane has a tension of 10^{-5} N/m (The values for bending stiffness, K_b, and L are given below).

Fig. 2



d) In reality, as the cell migrates in the upstream direction (to the left in Fig. 3), it leaves behind it a region void of matrix material due to the enzymatic degradation of the matrix metalloproteases released by the cell. The cell must therefore support itself (balance the force F, which acts to the right in the figure) by adhering to the matrix via the integrin adhesion receptors located in the upstream facing side of the cell membrane. These integrins form adhesive molecular bonds tethering the cell membrane to the ECM filaments. Given that (i) the solid fraction of the ECM, $\Phi_{ECM} = (a/L)^2 = 0.01$, (ii) the integrin molecule density on the cell membrane is 300 molecules/μm^2, and (iii) there is excess ligand on the filaments to which the integrins can bind, **estimate the average force per integrin bond supporting the cell. Is this level of force sufficient to rupture the integrin-ECM bond? Is it sufficient to activate the cell** (that is, to elicit a biological response from the cell)? **Be sure to explain your answers.** (Hint: Start by estimating the area of contact between the cell membrane and the filaments of the matrix.)

Fig. 3



Parameter Values:

$k_{ECM} = 10^{-11}$ $m^2 \cdot Pa^{-1} \cdot s^{-1}$ $G_{ECM} = 10^3$ Pa

$\eta = 10^{-3}$ Pa·s $R = 10$ µm

$a = 10$ nm $L = 100$ nm

$V = 2 \times 10^{-6}$ m/s $K_b = 10^{-19}$ N·m

Bead forces on a cell

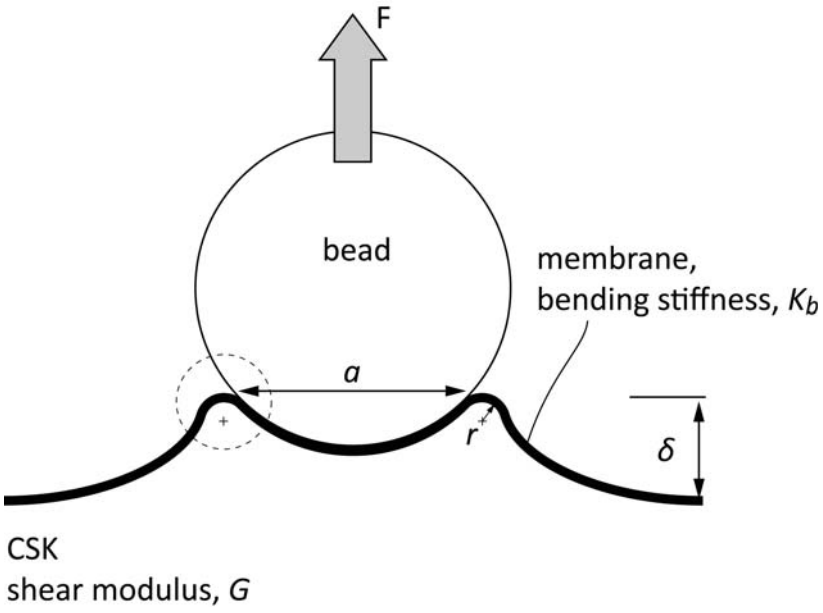
In many of the experiments used to probe cell mechanics, a bead is attached to the cell membrane via surface-bound receptors and force is applied either by a magnetic or optical trap. Here we consider such an experiment in which the force is applied in a direction normal to the plane of the cell membrane. We consider in the following questions the relative importance of the membrane and cytoskeleton (CSK) in resisting or balancing this force.

In parts (a) – (c), consider only the cell membrane, ignoring the effects of the CSK.

a) Draw the shape of the membrane in the vicinity of the bead for the case in which there is no membrane bending stiffness (K =0) and only membrane tension acts to balance the force. See the figure for an example in which both tension and bending are present.

b) In reality, bending will be important at least in a small region close to where the bead is attached (see the region inside the dashed circle in the figure). Given that bending and tension must be comparable in magnitude within this region, use an order of magnitude analysis to estimate the surface tension, N, in terms of the bending stiffness of the membrane, K , and the radius of curvature of the membrane close to the bead (r).

c) In a figure similar to that of Fig. 1 draw a control volume that will allow you to calculate an approximate relationship between the vertical force, F, applied to the bead and the surface tension in the membrane, N. Taking a value of 1 nN for F, calculate the surface tension, N, and a value for r defined in the figure. Note that the bead is spherical.



Now, for part (d) neglect membrane stiffness and consider the case in which the force on the bead is balanced by the stiffness of the CSK.

d) Using a scaling analysis, estimate the distance δ that the cell would be deflected, given the shear modulus of the CSK, *G*, and the radius of the region of contact between the cell and the bead, *a*.

Now consider the combined effects of the membrane and the CSK.

e) We now wish to determine whether the membrane or the CSK is the dominant structure in terms of resisting deformation. Using your answers from (b) and (d) above, obtain a dimensionless ratio that represents the force supported by the membrane divided by the force supported by the CSK. Given the following values:

- G = 1000 Pa
- δ = 500 nm
- r = 100 nm
- $K = 10^{-18}$ N·m
- F = 0.1 nN
- a = 1 μm

Which structure dominates?

Horse racing

While watching the Kentucky Derby (and thinking about the biomechanics exam), I recalled some studies done years ago by a prominent pulmonary physiologist, John West, that addressed a common problem in racehorses (but has also been observed in humans, camels and greyhounds!). Apparently, exercise induced pulmonary hemorrhage (EIPH) occurs in 50-75% of racing quarter horses and results from ruptured pulmonary capillaries. The cause, interestingly, has not been convincingly demonstrated, although it is thought to be associated with a combination of elevated pulmonary blood pressure, and the increased negative gas pressures created in the lung during inspiration under intense exertion. Here we explore the plausibility of this theory, based on the mechanics of the endothelial monolayer.

Capillaries in the lung run through the narrow walls that separate one gas-filled alveolus from another (see Figure 1). Consider the blood pressure inside the capillaries to be 20 kPa higher than the air pressure in the alveolus (acting external to the capillary), and (in a worst case scenario) assume that all the pressure difference is supported by the endothelial monolayer. The capillary radius can be taken to be 5 μm. Assume also that the load on the capillary wall due to the pressure difference is equally distributed between the two endothelial lipid bilayers (inside and outside), and that the capillary can be envisioned as a thin-walled tube of circular cross-section (Figure 2).

a) Calculate the surface tension acting in the circumferential (θ) direction in a single bilayer.

b) Is this surface tension sufficient to cause rupture of the bilayer? You may assume that the bilayer is 5 nm thick, has an effective Young's modulus of 1 GPa , a Poisson ratio of 0.4, and that rupture occurs at strains of >3%.

c) An alternative explanation for the hemorrhaging is that the cell junctions rupture. If the cells are assumed to be arranged in such a way that, for each cell, 500 cadherin junctions support the load (assume that one cell layer extends 20 microns in the axial (z) direction, perpendicular to the plane of the paper), what is the force per cadherin junction?

d) Is the force calculated in (c) high enough to cause the bond to rupture? (You are not expected to know the rupture force of a cadherin junction, but should be able to estimate it, and provide a brief explanation of your answer.)

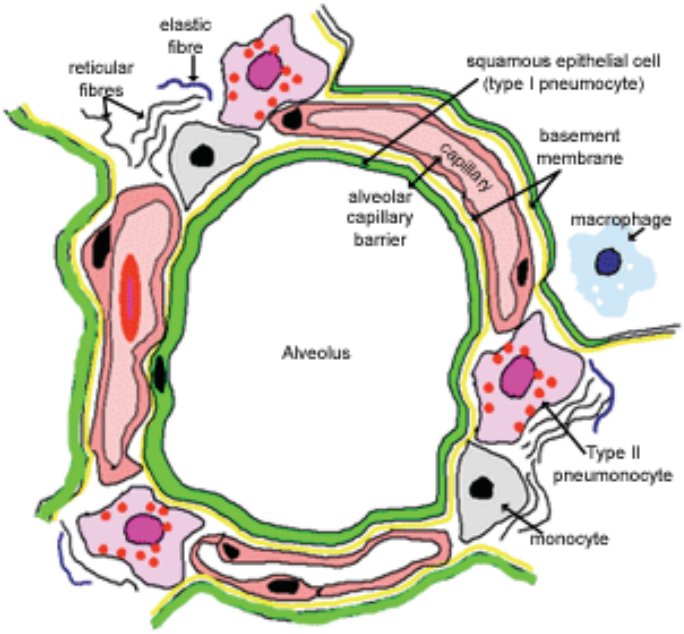
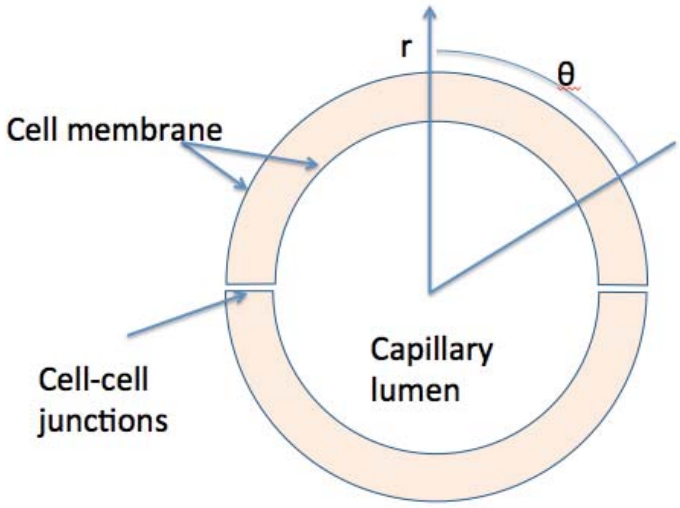


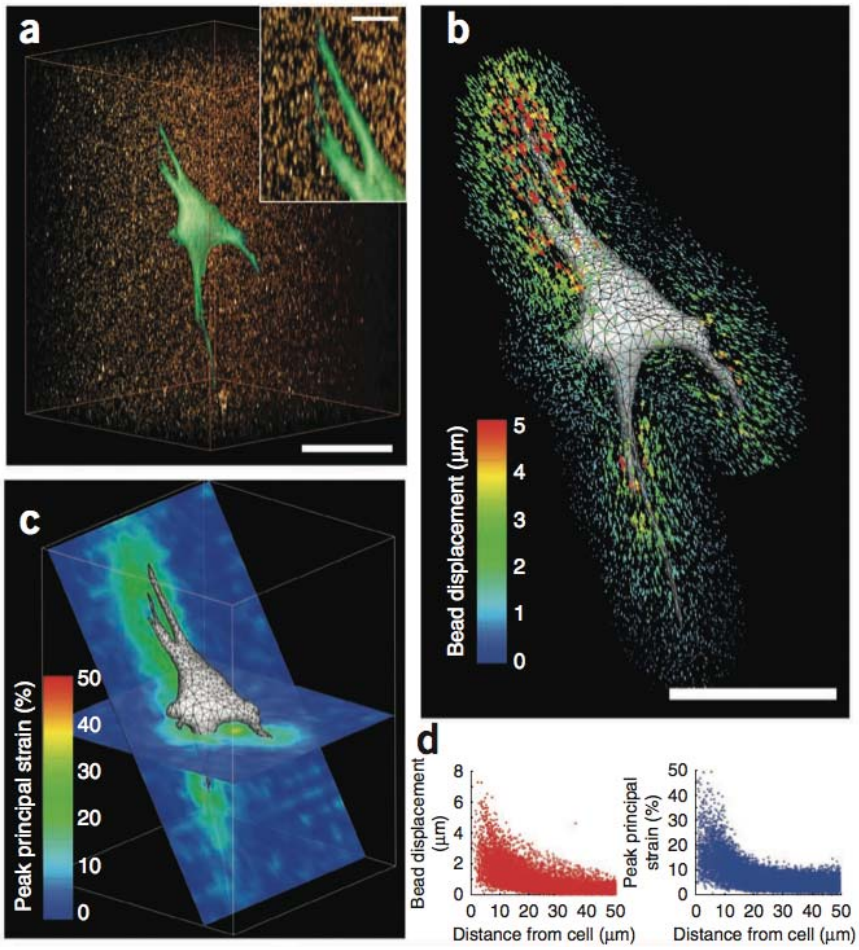
Figure 1. A single alveolus showing the epithelial layer lining the inner wall with vascular capillaries in the wall between neighboring epithelial layers. The capillary is shown flattened, but in our example, would be circular in cross-section.

Figure 2. Cross-sectional view of capillary



Migrating cell in three dimensions

There is currently considerable interest among the cancer research community in determining the forces generated by a cell as it migrates through a three-dimensional matrix or gel such as the extracellular matrix. Migrating cells often have the appearance shown in the figure below, with actively extending, finger-like protrusions (filopodia) in the front that push through the matrix material, and long tube-like tethers in the rear where the cell adheres to the matrix, that eventually must be released if the cell is to make forward progress. This problem considers both the forward protrusions and the tethers at the rear.



a) In a set of *in vitro* experiments designed to determine the force with which the cell pushes forward into the matrix (see Legant, et al., *Nature Materials*, 2010, see figure), the cell is induced to migrate through a gel with a known elastic or Young's modulus, E. In order to estimate the force, small marker beads are seeded into the gel and their displacement is observed during cell migration. (These beads can be assumed to be rigidly fixed to the gel, but have no effect on the gel's elastic properties.) As the cell sends a protrusion forward, the beads just in front of the leading edge are observed to move a distance δ. Given that the protrusion can be pictured as a "finger" of radius a, and the deformations are observed to extend into the gel a distance that scales with a, estimate the force with which the protruding "finger" pushes against the gel.

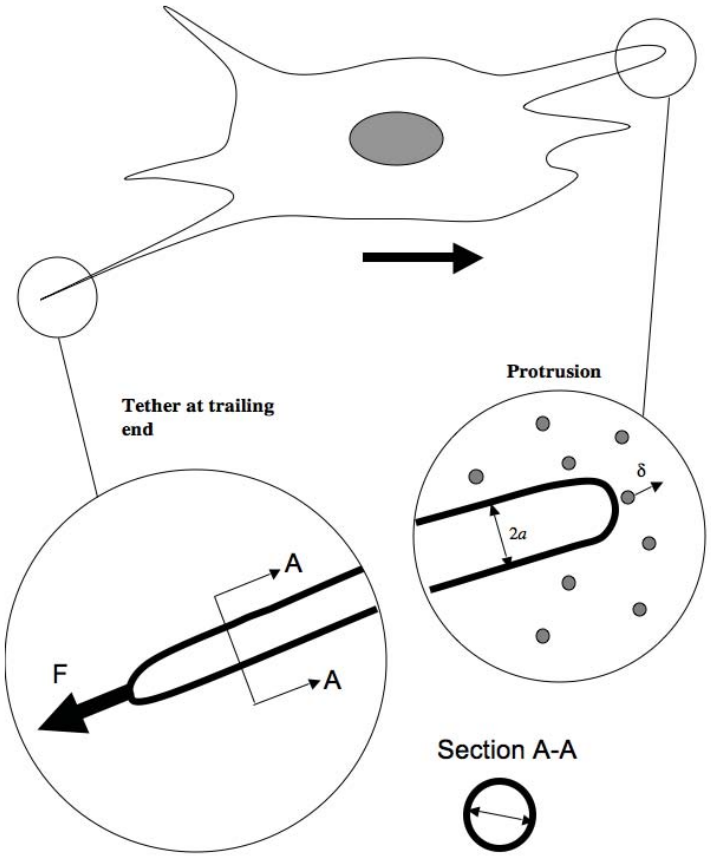
b) At the trailing tether, the gel is assumed to pull backward with a point force F as shown in the magnified view and in the cross-sectional view, **A-A**. If the tether is viewed as a cylinder with radius R, use a force balance to determine the tension N in the membrane that comprises the tether.

c) In the vicinity of the externally acting force at the end of the tether, the membrane shape is determined by a combination of membrane tension and bending. What relationship must be satisfied if bending and tension effects are approximately in balance? Use this expression in combination with your answer in (b) to obtain an estimate for the radius of the tether, R.

d) Fragments of membrane that have been torn away from the cell are often observed attached to the matrix behind the cell. Given the parameter values below and the fact that membranes (lipid bilayers) rupture when they stretch about 3% ($\varepsilon_{11} \sim 0.03$), estimate the maximum force F that can be sustained by the tether.

Parameter Values:

$K = 10^{-19}$ N·m $\qquad$ $E = 10^{8}$ N/m^2 $\qquad$ $h = 6\text{x}10^{-9}$ m



Micropipette aspiration

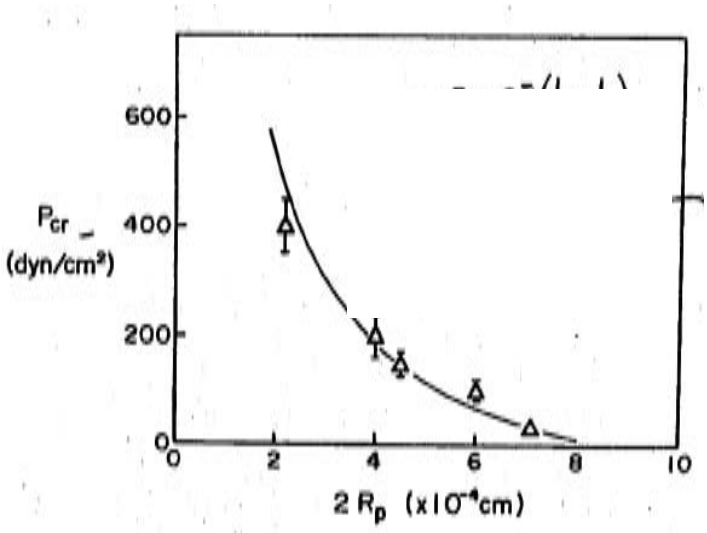
Experiments using micropipettes have provided evidence suggesting that neutrophils possess a membrane (or membrane plus "cortex") that behaves mechanically as though it were in a state of uniform and constant surface tension. To determine the magnitude of this surface tension (and demonstrate support for the concept of a constant surface tension), micropipettes with different diameters are used to aspirate the cell. In each experiment with a given diameter pipette, the pressure within the pipette is incrementally reduced (made more negative). For pressure magnitudes below a critical threshold, the cell attains an equilibrium position and stops. For pressure magnitudes above this threshold value, the cell continues to flow and eventually is drawn entirely into the pipette. The unique value of the *critical pressure* is noted for each diameter pipette.

a) *Obtain an expression for the critical pressure required to draw a neutrophil into the pipette* on the assumption that:

- The initial cell radius (before aspiration), R_c, is known from the measurement to be 4 μm.
- The cell volume remains constant during the course of an experiment.
- The cell membrane can increase its total surface area while maintaining a constant effective surface tension, N.
- The cell interior behaves as a viscous fluid with G'=0.
- The bending stiffness of the cell membrane can be neglected.

Express your answer in terms of a (known) pipette radius, R_p.

b) *Using the experimental data shown in the figure below, calculate the effective surface tension of the cell, N.*



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