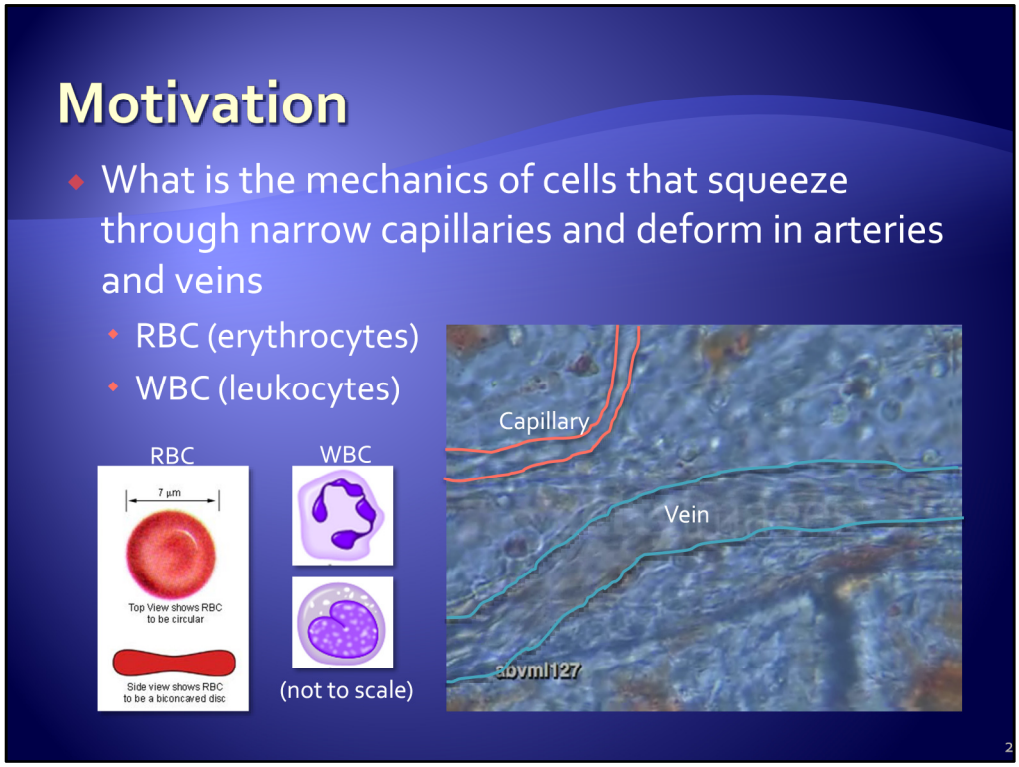
A classic technique…



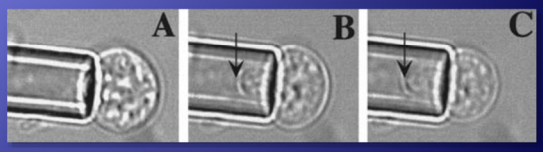
RBC begin with adult stem cells in the bone marrow that differentiate into erythroblasts. While in the bone marrow, these cells expel their nuclei and become erythrocytes (RBCs). Their final form at rest is a biconcaved disc. Under hemodynamic shear or when forced to squeeze through narrow capillaries, these cells exhibit high deformations that some have called ”bullets” or “slippers”. These shape changes occur without volume change.

WBCs are bigger than RBCs and have nuclei, but often the shapes of the nuclei are elongated or kidney-shaped and do not have the typical egg shape seen in most eukaryotes.

The Approach

- Pipette vacuum pressure and observed cell deformation used to obtain material constants of the constitutive relationships

micromanipulator



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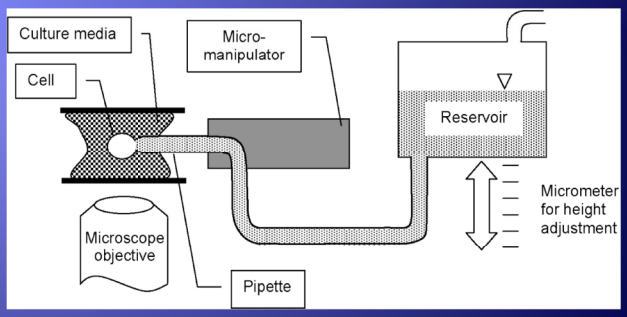
Micropipette can be consider the oldest technique for cell biomechanical measurements. A fine glass micropipette is used to suck on the surface of a cell to observe its deformation and determine its mechanical behavior. Typically, the glass pipette has an inner diameter of 1-10 microns and can be precisely positioned to the cell with a micromanipulator.

The System

- Hydrostatic vacuum pressure from fluid reservoir
  - $\Delta P = \rho g \Delta h$
  - Min: 0.1 Pa
  - Max: 96 kPa



- Concerns:
  - Evaporation leads to slow drift

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Once the pipette tip has been brought into contact with the cells, a suction pressure ΔP is generated by lowering the water tank height to create hydrostatic vacuum pressure. A microscope is positioned to observe the deformation of the cell intro the pipette tip. The tanks are placed on vertical stages that have micrometer resolution. As a result of evaporation, there are practical limits to the suction pressure. The minimum vacuum pressure that can be applied is 0.1-0.2 $pN/\mu m^2$ (Pa) and the maximum vacuum pressure is 96 kPa.

Erythrocyte Membrane

- EM images show spoke-and hub arrangement to RBC cortical CSK
- Spectrin spokes
- Actin hubs

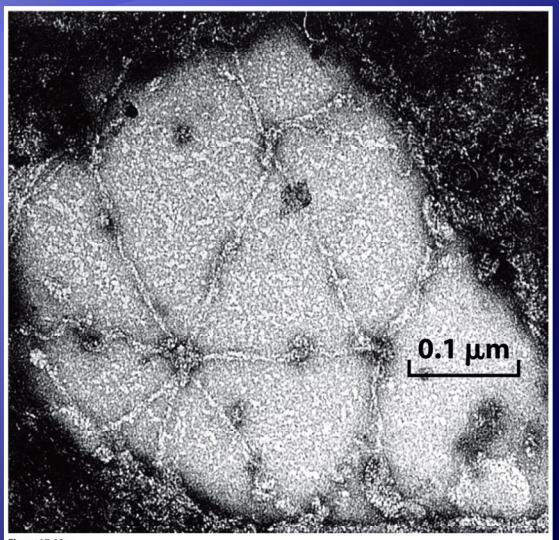

Figure 17-19a
Molecular Cell Biology, Sixth Edition

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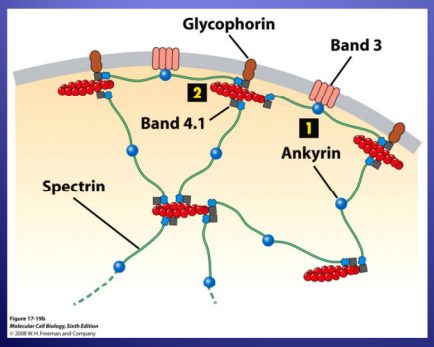
RBC membranes show a spoke-and-hub organization of the cortical cytoskeleton that supports the cell's membrane. The long spokes are composed of spectrin and can be seen to intersect at the hubs. The hubs are where the structures attaches to the cell membrane through gylcophorin.

Membrane connections

- CSK linker – Transmembrane protein
  - Ankyrin-Band 3
  - Band 4.1-Glycophorin



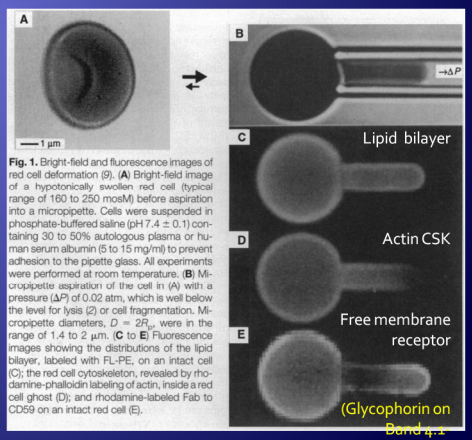


Fig. 1. Bright-field and fluorescence images of red cell deformation (9). (A) Bright-field image of a hypotonically swollen red cell (typical range of 160 to 250 mosM) before aspiration into a micropipette. Cells were suspended in phosphate-buffered saline (pH 7.4 ± 0.1) containing 30 to 50% autologous plasma or human serum albumin (5 to 15 mg/ml) to prevent adhesion to the pipette glass. All experiments were performed at room temperature. (B) Micropipette aspiration of the cell in (A) with a pressure (ΔP) of 0.02 atm, which is well below the level for lysis (2) or cell fragmentation. Micropipette diameters, $D = 2R_p$, were in the range of 1.4 to 2 μm. (C to E) Fluorescence images showing the distributions of the lipid bilayer, labeled with FL-PE, on an intact cell (C); the red cell cytoskeleton, revealed by rhodamine-phalloidin labeling of actin, inside a red cell ghost (D); and rhodamine-labeled Fab to CD59 on an intact red cell (E).

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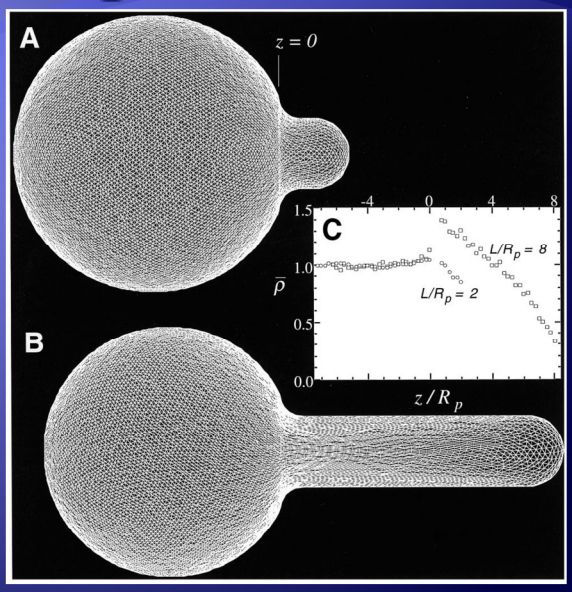
The membrane is connected to the cortical CSK through two links: 1) The transmembrane protein Band 3 connects to Ankyrin, which links together two spectrin proteins as a "spoke". 2) The transmembrane protein Gylcophorin connects to the protein Band 4.1, which links to the actin-spectrin "hubs".

Dennis Discher conducted a study on the mechanical response of RBC cortical CSK using micropipette aspiration. He used different fluorescent stains to mark the location of the (c) lipid bilayer that forms the cell membrane, (d) the actin cytoskeleton that supports the membrane, and (e) a membrane protein called CD59 which is a "self-recognition" receptor on the surface of cells that prevents the immune system recognizing the cell as foreign so it won't be destroyed.

As seen by the fluorescent imaging, lipid membrane matches the distorted shape as seen by phase contrast (panel b)i. However, the actin cytoskeleton is not uniformly distributed. There is a high density at the entrance of the pipette tipe but at the "cap" structure at the end of the cell, there is a much lower density of actin. The theory is that there is stretching the cytoskeleton that leads to a lower density at the stretched end. CD59 does not directly couple to the cytoskeleton, so it should be free to diffuse in the lipid bilayer of the cell membrane, but what we are seeing here is steric exclusion ("crowded out"). The high density of the actin at the entrance, and with it is the large CSK-linked proteins like Band 3 that prevent the membrane bound protein to diffused into this region.

Cortical Stretching

- Polymer network model built by Discher and Boal to describe the micropipette experiment



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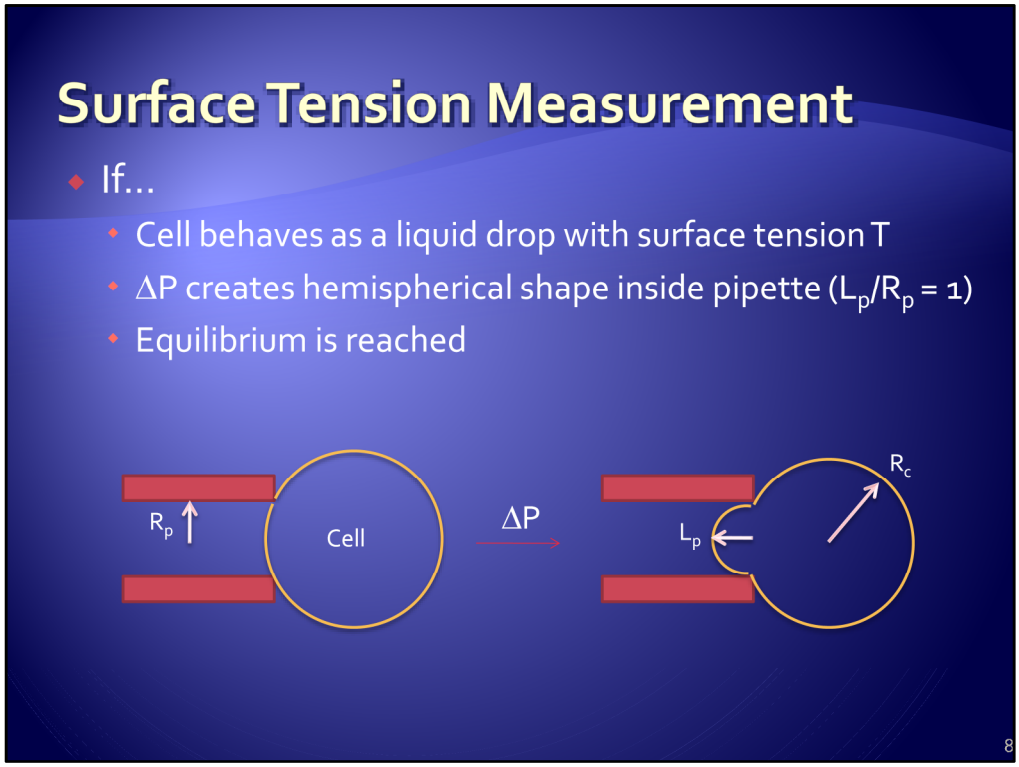
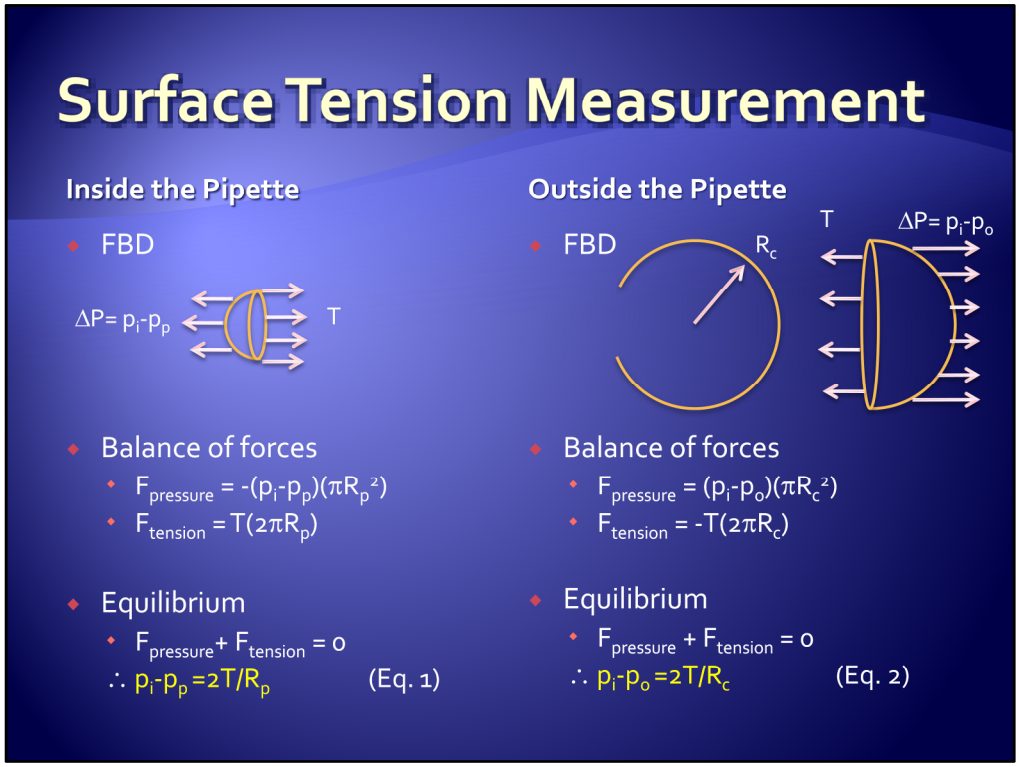
The results of Dennis' experiments was used by him and David Boal to construct a polymer network model for spectrin that shows the higher density (bunching-up) at the entrance and the low density at the stretched cap.

This model is rather complex and so we will look at it again later. Now, let's turn our attention to the simple biomechanical measurements of cells that is possible with micropipette aspiration.



The surface tension of a cell can be measured if sufficient vacuum pressure is applied so that a hemisphere is formed inside the pipette. There are a few assumptions to be applied to this measurement.



Using static equilibrium, we can determine at the surface tension in the cell from its radius inside and outside. The pressure force acts on the project area of the cell. The force of tension acts on the circumference of the hemisphere.

Surface Tension Measurement

- Combine equations 1 and 2

$$p_i - p_p = \frac{2T}{R_p} \qquad p_i - p_o = \frac{2T}{R_c}$$

- Yields… (Eq. 3)

$$p_o - p_p = 2T\left(\frac{1}{R_p} - \frac{1}{R_c}\right)$$

- $\Delta P = p_0 - p_p$ is vacuum pressure

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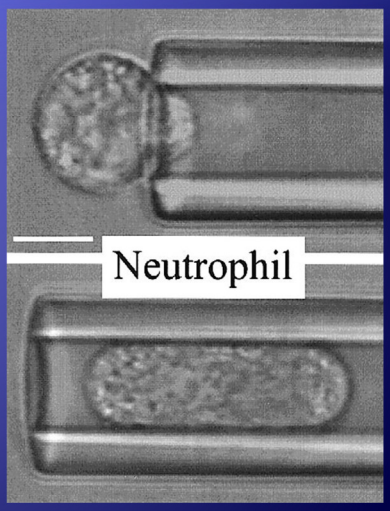
Critical Pressure

- Increasing the pressure when $L_p/R_p = 1$ does not allow for equilibrium.
- This causes the cell to flow into the pipette



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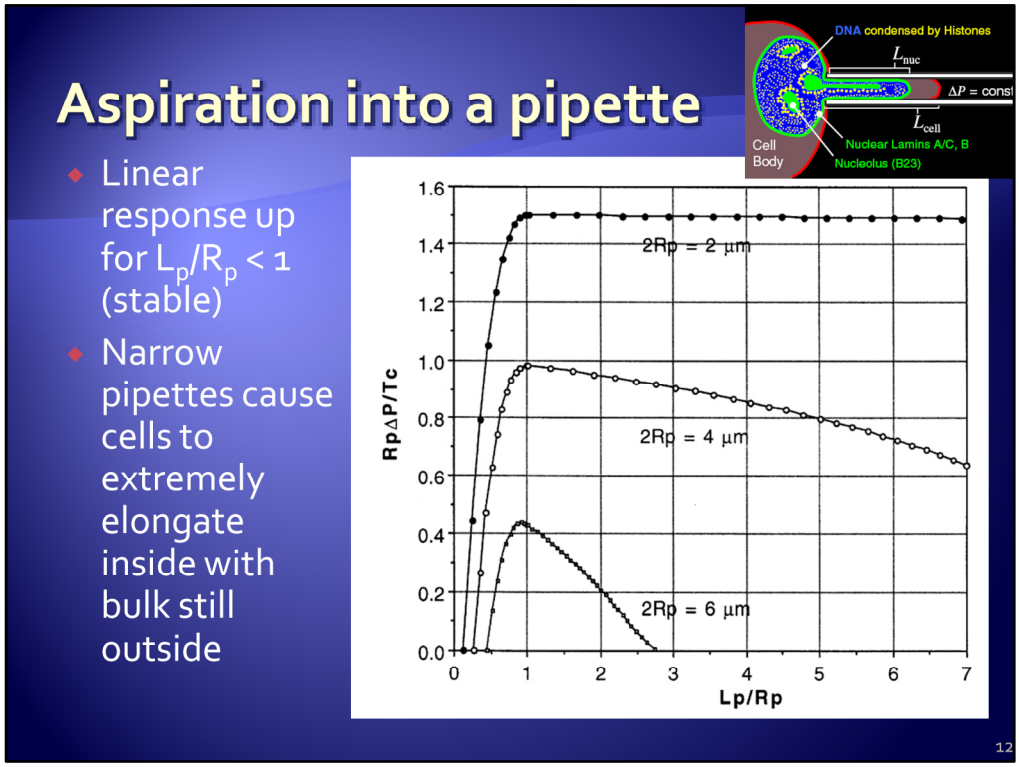
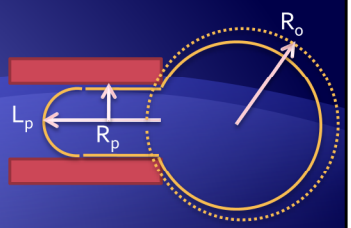


Before the cell is sucked completely into the pipette, the membrane extension into the pipette is linear (elastic). The amount of pressure applied (ΔP) is proportional to the increase in length. However, if the critical pressure is surpassed, the cell will be sucked into the pipette. The amount that the cell is sucked in depends on the radius of the pipette. A small pipette diameter will only bring a small amount of the cell volume inside and so more pressure is required to pull it inside because of the large amount of bulk cell outside the pipette.. A large pipette will easily suck the whole cell inside.

No volume change



- Original volume: $V = (4/3)\pi R_o^3$
- Original area: $A = 4\pi R_o^2$
- Pipetted volume:

  $V = (4/3)\pi(R_o - \Delta R_o)^3 + \pi R_p^2 L_p + (2/3)\pi R_p^3$
- Pipetted area:

  $A = 4\pi R_o^2 + 2\pi R_p L_p + 2\pi R_p^2$
- Change in volume (assume constant volume):

  $0 = 4R_o^2 dR_o + R_p^2 dL_p$
- Change in area

  $dA = 8\pi R_o dR_o + 2\pi R_p dL_p$
- Combining

  $dA = 2\pi R_p^2(1/R_p - 1/R_o)dL_p$ (Eq. 4)

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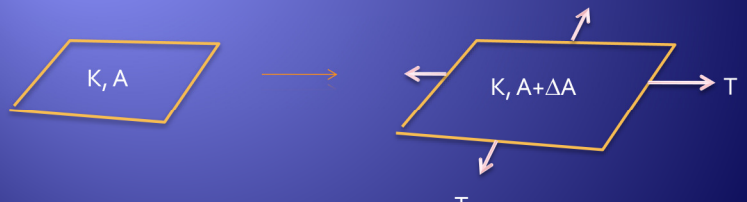
What this last equation shows is that small changes in cell surface area (dA) can be converted to a measurable change in the length of the cell inside the pipet (dL_p). Furthermore, if $R_0 >> R_p$, the terms in front of dL_p reduce to $2\pi R_p$.

Area Expansion Modulus

- Y.C. Fung calls it "Areal Modulus of Elasticity"
- D. Discher calls it "surface dilation modulus"
- Let's call it "K".
- Hooke's law on a surface: $T = K \Delta A/A$



- Using Eq. 4, one can obtain K from dL_p
- For RBC, K = 500 dyne/cm = 500 nN/μm
- For WBC, K = 39 pN/μm

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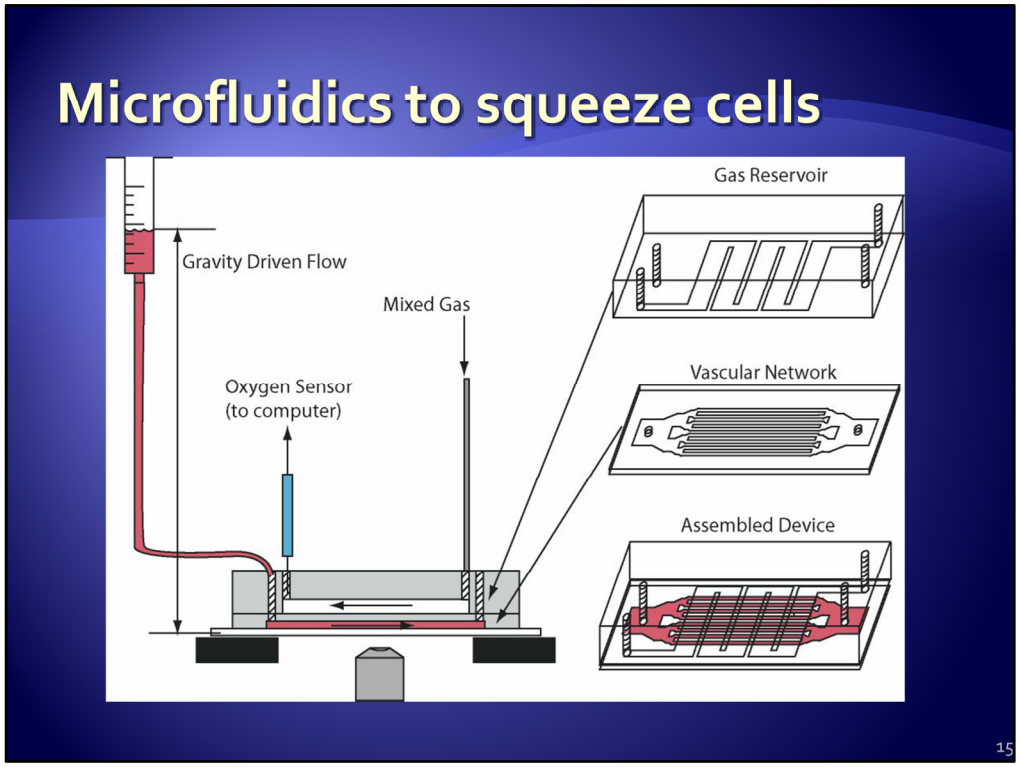
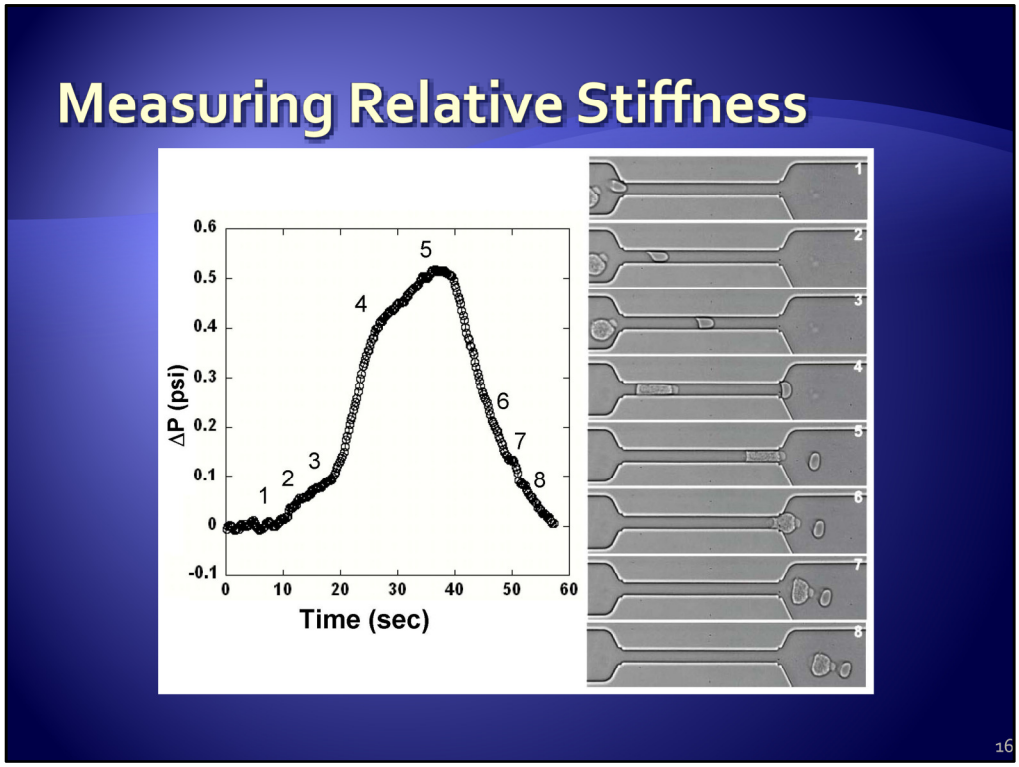
From micropipette aspiration measurements, we can also find the areal modulus of elasticity. RBC have 2-4 times stiffer dilation moduli than shear moduli. This indicates that they are easy to deform with shear but there is not likely to be an area change.

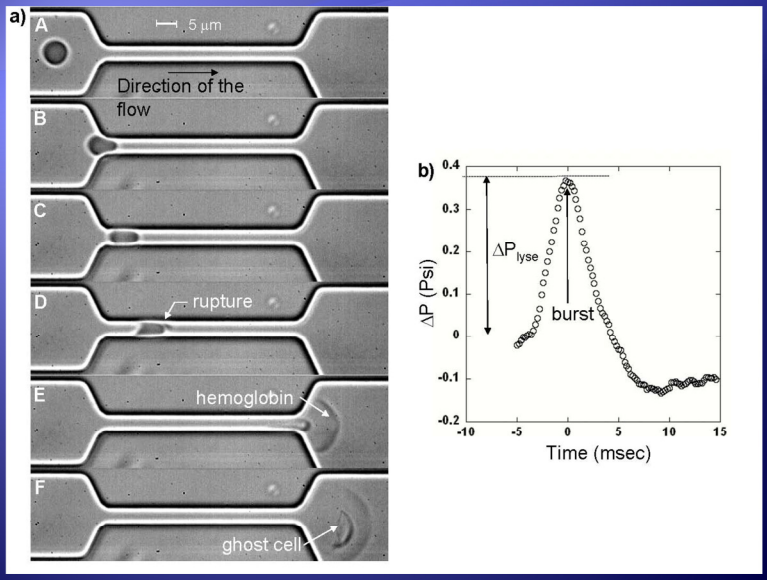


Microchannels can be created in silicone or glass materials that have dimensions that are the size of capillary walls. This allows us to watch as the RBCs sequeeze through the narrow channels and watch them deform. Using a second layer for gas control we can watch their role in as oxygen and CO2 transporters.



Using microfludics, you can measure the deformation and pressure drop when the cells flow in a narrow channel. A red cell enters the channel followed shortly thereafter by a larger (and stiffer) white blood cell (WBC). You can compare the time trace of the pressure drop with the corresponding image sequence. When the first cell enters the channel, there is a rise in the pressure that rises as the cell moves through the channel. When the stiffer, WBC enters the channel, the pressure rise greatly increases and has a slow rise when the RBC exits. Once everything exits, the pressure drop goes away.

Hemolysis



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A pressure drop of 0.4 psi (2.7 kPa) leads to rupture of the cell. We're sorry, little guy!