1 Biomembranes

1.1 Micropipette aspiration

1.1.1 Experimental setup

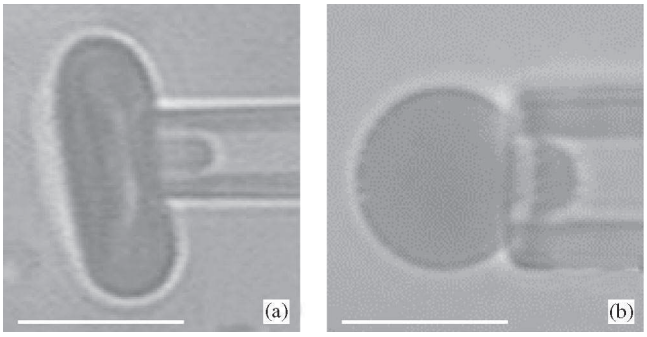

Figure 1.1: Flaccid (a) and swollen (b) red blood cells being drawn into a micropipette. The scale bars represent 5 μm. Figure adapted from [2].

Some of the first mechanical measurements of cell membranes and lipid bilayers were made using micropipette aspiration experiments. However, their versatality and ease of interpretation continue to make them an important experimental tool for studying the mechanics of cells. As we will see, these experiments not only allow one to make measurements of cell membrane mechanical properties, but they also allow insight into the fundamental mechanical behavior of cells.

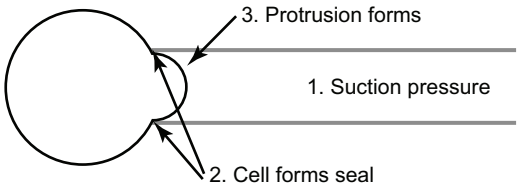

Figure 1.2: In a micropipette aspiration experiment, the micropipette tip is placed in the proximity of the cell, and a suction pressure is applied. A seal forms between the cell and the micropipette, forming a protrusion into the micropipette.

A micropipette is a glass tube that tapers to a diameter of several microns at the tip. It is hollow all the way through its length. In a typical micropipette asipiration experiment, a suction pressure is applied within the micropipette. If the

end is brought in proximity of a cell and a negative (suction) pressure is applied, a seal will form and the cell will be drawn into the micropipette, forming a protrusion (Figure 1.1). The negative pressure can be applied in a variety of ways. One way is to apply the pressure by mouth (this method actually provides a lot of control, and researchers commonly do this when forming the seal)! Another common (and safer!) way is to connect the micropipette to tubing that runs to a water-filled reservoir with controllable height. In this case, decreasing the height of the water surface in the reservoir relative to the height of the fluid surface in the dish in which the cells are cultured will create a suction pressure within the micropipette. In theory, the minimum suction pressure that can be applied is determined by the minimum change in height of the fluid reservoir that can be acheived ($\sim$0.01 Pa). However, in practice, the resolution is much higher ($\sim$1 Pa) due to drift caused by water evaporating from the reservoir [2]. Typically, the maximum pressure that can be applied is on the order of atmospheric pressure, resulting in a wide range of forces, from ($\sim$10 pN) to ($\sim$100 nN) [2].
Once the cell is drawn into the micropipette, the morphology of the cell relative to the pipette can be divided into three regimes, as in Figure 1.3. The first regime is when the length of the protrusion of the cell into the pipette L_{pro} is less than the radius of the pipette R_{pip}, or $L_{pro}/R_{pip} < 1$. The second regime is when the protrusion length is equal to the pipette radius, or $L_{pro}/R_{pip} = 1$. In this case, the protrusion is hemispherical. The third regime is when $L_{pro}/R_{pip} > 1$, and the protrusion is cylindrical with a hemispherical cap. The radius of the hemispherical cap is R_{pip}, since the radius of the protrusion can not change once the hemispherical cap is formed.

1.1.2 Liquid drop model

When researchers performed early micropipette aspiration experiments on cells such as neutrophils (a type of white blood cell), they noticed that after the micropipette pressure exceeded a certain threshold, the cells would continuously deform into the micropipette (in other words, the cells would rapidly "rush into" the pipette). This observation led to the development of the liquid drop model.
In this model, the cell interior is assumed to be a homogeneous Newtonian viscous fluid and the cell cortex is assumed to be a thin layer under a constant surface tension, and without any bending resistance. The surface tension has units of force per unit length, and can be thought of as the tensile stress within the membrane integrated through the depth of the membrane. For example, if a membrane of thickness d is subject to a tensile stress of σ that is constant through its depth, then we can model it as infinitely thin, with a surface tension of $N = \sigma d$.

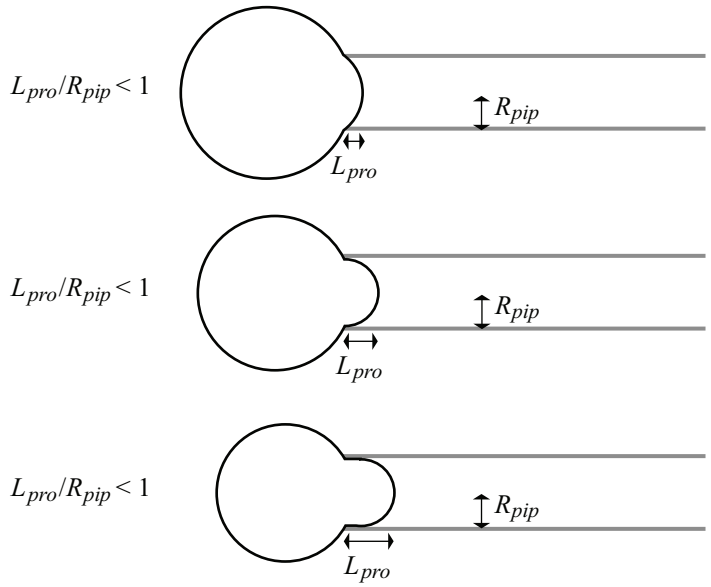

Figure 1.3: Three regimes as a cell is aspirated into a micropipette. (Top) The length of the protrusion of the cell into the pipette is less than the radius of the pipette, or $L_{pro}/R_{pip} < 1$. (Middle) $L_{pro}/R_{pip} = 1$. In this case, the protrusion is hemispherical. (Bottom) $L_{pro}/R_{pip} > 1$. The protrusion is cylindrical with a hemispherical cap of radius R_{pip}

So why then is this model called the liquid drop model? A drop of liquid (such as water) suspended in any other fluid (such as air) will have a thin layer of molecules surrounding the bulk that, because of the imbalance of intermolecular forces at the surface, will pack together and cause surface tension. This surface tension is what allows some types of insects to walk on the surface of water.

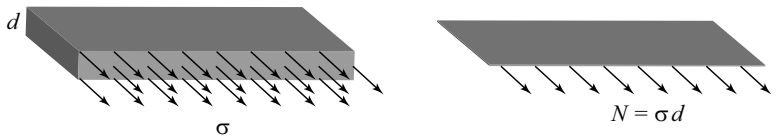

Figure 1.4: For a membrane of thickness d subject to a tensile stress of σ that is constant through its depth, we can model it as infinitely thin with a surface tension of $N = \sigma d$.

1.1.3 Law of Laplace

By modeling the cell as a liquid drop, we can analyze micropipette aspiration experiments using the Law of Laplace, which relates the difference in pressure between the inside and outside of a thin walled pressure vessel with the surface tension within the vessel wall. The Law of Laplace can be derived from the

Laplace Equation by assuming zero bending stiffness and a spherical geometry. Alternatively, it can be derived from a simple free body diagram, as seen below. Consider a spherical thin walled pressure vessel with radius R, a pressure of P_i inside the vessel, and a pressure of P_o outside the vessel. If we cut the sphere in half, then there are two equal and opposite resultant forces acting on the cut plane. The first is due to pressure, and it is calculated as $F_p = (P_i - P_o)\pi R^2$. The second resultant force is due to surface tension on the wall. If the surface tension is given by N, then the resultant force due to surface tension F_t is calculated as $F_t = N2\pi R$. Setting $F_p = F_t$, we arrive at the Law of Laplace:

$$P_i - P_o = \frac{2N}{R} \tag{1.1.1}$$

Analyzing micropipette aspiration experiments using the Law of Laplace

We can now use the Law of Laplace to analyze micropipette aspiration experiments by relating suction pressure with the morphology of the cell as it enters the pipette. Consider the scenario in Figure 1.5 where P_{atm} is the pressure of the environment, P_{cell} is the pressure within the cell, P_{pip} is the pressure within the pipette, R_{cell} is the radius of the cell outside the pipette, R_{pip} is the radius of the pipette, R_{pro} is the radius of the protrusion, and $L_{pro}/R_{pip} = 1$.

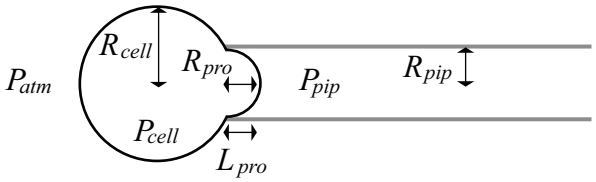

Figure 1.5: Schematic depticing quantities used in analyzing micropipette aspiration experiment.

In this case, for the portion of the cell which is not in the micropipette, from 1.1.1 we know that

$$P_{cell} - P_{atm} = \frac{2N}{R_{cell}} \tag{1.1.2}$$

where N is the surface tension of the cell. For the protrusion, or the portion of the cell in the pipette,

$$P_{cell} - P_{pip} = \frac{2N}{R_{pro}} \tag{1.1.3}$$

Combining 1.1.2 and 1.1.2, we get

$$P_{atm} - P_{pip} = \Delta P = 2N\left(\frac{1}{R_{pro}} - \frac{1}{R_{cell}}\right) \tag{1.1.4}$$

which relates the difference in the pressure in the surroundings and the pipette with the radius of the cell in and outside of the pipette for a cell with a given surface tension (this is only valid if the cell is stationary).

Measuring surface tension and areal expansion modulus

One can now easily measure surface tension from 1.1.4. For example, since the cell is drawn into the micropipette such that the protrusion is hemispherical ($L_{pro}/R_{pip} = 1$), then the radius of the protrusion also equals the radius of the pipette, $R_{pro} = R_{pip}$. In this case,

$$\Delta P = 2N\left(\frac{1}{R_{pip}} - \frac{1}{R_{cell}}\right). \tag{1.1.5}$$

The pressure ΔP is controlled by the user, and R_{pip} is also known. The cell radius R_c can be measured optically under microscope, allowing one to calculate the surface tension N. Evans and Yeung [1] performed this experiment using different micropipette diameters and found a surface tension of ($\sim$35 pN/μm) in neutrophils. This tension was found to be independent of the pipette diameter.
Although the surface tension within a liquid drop will remain constant as it is aspirated into a micropipette, in reality, the cells do not behave perfectly like a liquid drop. This is because the membrane area increases as it is aspirated and results in a slight increase in surface tension. The increase in tension per unit areal strain is given by the areal expansion modulus. Needham and Hochmuth [3] quantified the areal expansion modulus in neutrophils by aspirating them through a tapered pipette (Figure 1.6). By applying larger and larger pressures, the cell would advance further and further into the taper, and increase its surface area while maintaining constant volume. In this case, the radii at either end of the cell, R_a and R_b, the total volume V, and the total apparent surface area A (we will soon discuss what the apparent surface area is) were measured from the geometry. The surface tension was calculated using the Law of Laplace as

$$\Delta P = N\left(\frac{1}{R_a} - \frac{1}{R_b}\right) \tag{1.1.6}$$

The "original" radius R_0 of the cell was calculated from the volume (assuming the volume remains constant) as $V = 4/3\pi {R_0}^3$, allowing the "original" or undeformed apparent surface area to be calculated as $A_0 = 4\pi {R_0}^2$. Thus, the areal strain $(A - A_0)/A_0$ and surface tension could be found for the same cell as the pressure was increased and the cell advanced through the taper. The surface tension was plotted as function of areal strain, and the data was fit to a line. The areal expansion modulus was found from the slope of the line and was calculated to be 39 pN/μm. Extrapolating the fit line to zero areal strain resulted in a surface tension of 24 pN/μm. This is a slightly lower tension than obtained by Evans and Yeung, meaning that the cells increase their surface tension very little as they are aspirated. The lower surface tension can be interpreted as the surface tension in the undeformed state. This tension likely arises due to metabolic processes (i.e., actin contraction) within the cortex to keep it under isometric tension.

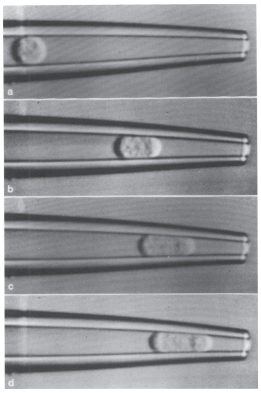

Figure 1.6: Cell being aspirated within a tapered pipette. The radius of the pipette opening is 4 μm. (A) A cell was aspirated into the tapered pipet and allowed to recover to its resting spherical shape. A positive pipette pressure was then applied, and the cell driven down the pipette. Final resting configuration after (B) $\Delta P = 2.5$ Pa, (B) $\Delta P = 5.0$ Pa, and (B) $\Delta P = 7.5$ Pa. Adapted from [3].

Why is this tension important in neutrophils? Remember that neutrophils circulate in the blood and therefore need to squeeze through small capillaries with diameters smaller then the cells themselves, similar to red blood cells. As it squeezes through a capillary, the shape of the cell transforms from a sphere into a "sausage" (i.e., a cylinder with hemispherical caps at both ends). Since the cells contain mostly fluid, it is incompressible and so must maintain constant volume during this shape change. It can be shown that the surface area of a "sausage" is greater than a sphere of the same volume, and the increase in surface area grows larger as the radius of the "sausage" decreases. However, we will see later that biomembranes are quite inextensible, so how do neutrophils undergo this

increase in surface area when squeezing through small blood vessels?

Figure 1.7: Electron micrograph of a neutrophil. Ruffles in the membrane can be clearly seen. Figure adapted from [2].

As can be seen in Figure 1.7, neutrophils contain many microscopic folds in their membrane. What this means is that their "apparent" surface area is much less than the actual surface area of the membrane if one were to take into account all the fold and ruffles. The folds allow neutrophils to substantially increase their apparent surface area without actually increasing the surface area of the membrane, as long as the folds are not completely smoothed out. The tension within the cortex of these cells thus has a crucial role: it allows the cells to have folds and ruffles in the membrane while maintaining their spherical shape!

1.1.4 Why do cells "rush in"?

Remember that the liquid drop model was developed in large part to the observation that some cells would "rush in" after applying any pressure greater than the critical pressure at which $L_{pro}/R_{pip} = 1$. Why do liquid drops do this? Remember that Equation 1.1.4 is a relation that must be satisfied for equilibrium. Suppose we apply the critical pressure such that $L_{pro}/R_{pip} = 1$, and then we increase ΔP. Lets examine what happens to the terms on the righthand side of Equation 1.1.4. We already learned that N is constant for liquid drops, and approximately constant for neutrophils as they are aspirated. The radius of the protrusion, R_{pro}, will also remain constant, since the radius of the hemispherical cap will be equal to R_{pip} for any $L_{pro} > R_{pip}$. R_{cell} can not get bigger, since the experiments are performed in isotonic solution, meaning the volume of the cell remains essentially constant over the time of the experiment. Taken together, what this means is that we have increased the lefthand side of Equation 1.1.4 with no way to increase the

righthand side. The result is that equilibrium can not be satisfied. This produces an instability, and the result is the cell will rush into the pipette!

1.1.5 Final notes: elastic solid or liquid drop?

Micropipette aspiration is an extremely versatile measurement technique for measuring the properties of membranes. It can apply a wide range of forces, and the experiments are conducive to mechanical analysis. However, besides making mechanical measurements of membranes, micropipette aspiration experiments play perhaps an even more important role in understanding cell mechanics, in that they easily allow insight into the fundamental mechanical behavior of different cell types. For example, when researchers performed experiments on endothelial or chondrocytes, they found that these cells would not rush into the pipette, even after $L_{pro}/R_{pip} > 1$). Why? Put simply, these cells do not behave like a liquid drop. Subsequent experiments and analyses showed that their mechanical behavior is much more like that of an elastic solid, so will not have this instability. Therefore, by observing whether a particular cell does or does not rush into the pipette after $L_{pro}/R_{pip} = 1$, one can easily distinguish whether its mechanical behavior is like a liquid drop, or an elastic solid. Note that this simple method for classification is only possible if the critical pressure is exceeded. If the experiment is terminated before $L_{pro}/R_{pip} = 1$, then one can not distinguish between elastic solid and liquid drop behavior, as can be seen in Figure 1.8. This seems obvious, but it wasn't to early researchers! They would unknowingly terminate their experiments before $L_{pro}/R_{pip} = 1$, and after seeing the linear increase in pressure with protrusion length, incorrectly conclude that neutrophils behave like elastic solids.

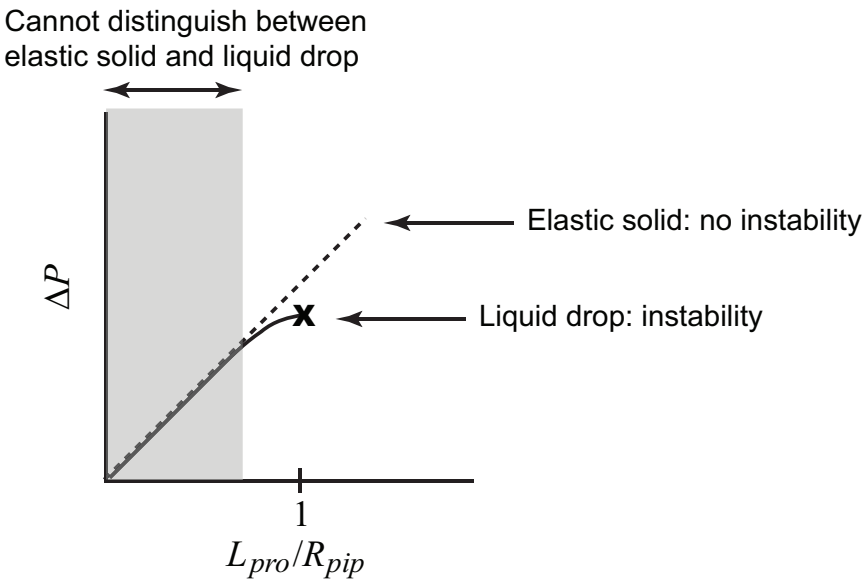

Figure 1.8: ΔP as a function of L_{pro}/R_{pip} for a cell which behaves like an elastic solid (dotted line) and a cell which behaves like a liquid drop (solid line). When $L_{pro}/R_{pip} = 1$, an instability will occur for the cell that behaves like a liquid drop, and the cell will rush into the micropipette. In constrast, a cell that behaves like an elastic solid will not have this instability.

Bibliography

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