MECHANICAL CHARACTERISTICS OF VERY SMALL CELLS

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Abstract

The smallest terrestrial cells have few structural elements: a fluid membrane to isolate the cell's contents, a very large molecule to carry its genetic information, and, often, a cell wall to offset the osmotic pressure of the cell's interior. There are few theoretical obstacles to constructing cells with radii as small as 50 nm using the same molecular materials as are found in 300 nm mycoplasmas. The energy required to bend a flat fluid membrane into the shape of a cell is comparatively small, such that closed spherical shapes are energetically favored for radii greater than about 20 nm, depending upon composition. Further, the membrane of a small cell could withstand the osmotic pressures typical of many bacteria without the aid of a cell wall. However, it would be difficult to pack a genetic blueprint with a hundred genes into a small cell using double-stranded DNA, whose rigidity permits only gentle curvature on 50 nm length scales; rather, a small cell would employ most flexible molecules such as RNA or single-stranded DNA.

Introduction

The human body contains about 10^{13} cells—perhaps a hundred times the number of stars in the Milky Way—although only about 200 different cell types are represented in this collection. A minimal set of mechanical components is present in each cell to perform such tasks as isolating its contents, maintaining its shape or, in some cases, facilitating its movement. The *chemical* composition of these *structural* components does not vary strongly from one cell type to another, permitting us to understand, in a somewhat systematic fashion, the architecture that nature has chosen for the cell. Small cells, such as bacteria, have a particularly simple construction:

- a fluid membrane (and possibly a cell wall) forming the cell boundary,
- an interior fluid region likely at higher pressure than the cell's immediate environment,
- at least one large molecule carrying the cell's genetic information.

Some questions that we might ask about the mechanical characteristics of these components are illustrated in Figure 1.

The properties of many of the cell's structural elements are known as a function of their size. For example, the filaments of the cytoskeleton (the molecular scaffolding that helps a cell organize its internal compartments and maintain its shape) display a resistance against bending that grows rapidly with their radius, just as rope is stiffer than string. Thus, we can predict, if crudely, the size of the cytoskeletal components needed for a cell to function under various conditions. In addition, limits or bounds exist on the minimum mechanical strength required of these components: for example, the fluid membrane enclosing the cell must possess a certain minimal resistance against rupture on a time scale appropriate to the cell's lifetime.

Because a given structural element may play several different roles in a cell, a limit based solely on

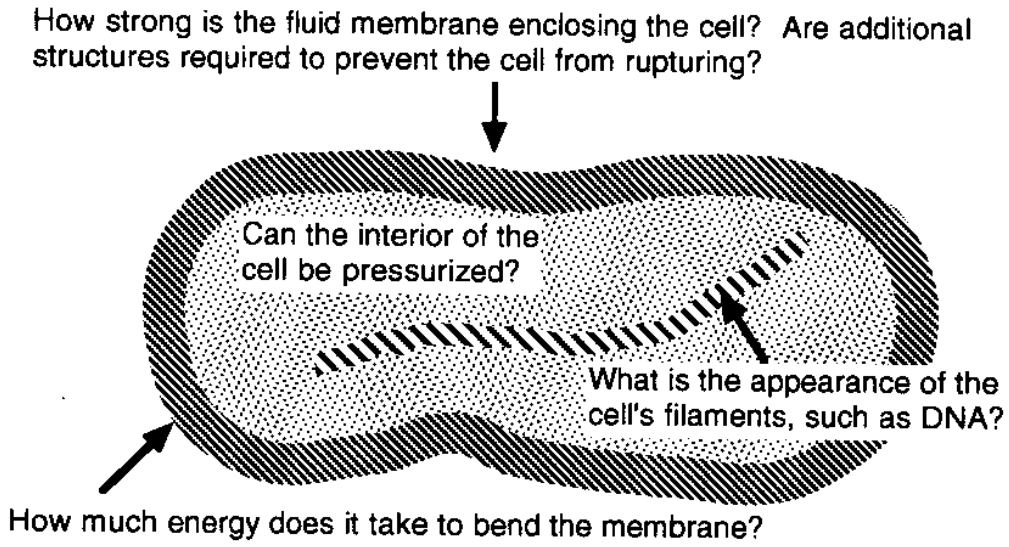

Figure 1. Some civil engineering issues facing the designer of very small cells.

a single mechanical characteristic may not truly reflect the complete architectural specifications of that element. As an illustration, a membrane with a molecular composition providing good rupture resistance may be so viscous that proteins are unable to diffuse readily within it. Thus, the actual molecular composition of the membrane reflects not only the strict limit on its mechanical strength, but also a softer constraint arising from the functionality of its constituents. Further, the limits are not inviolable and should be regarded more as challenges to Nature. The constraints that we obtain here assume only the most rudimentary architecture and the simplest chemical compositions. We make these assumptions in the belief that the smallest cells arise early in the history of a planet and have not had sufficient time to develop a complex architecture. However, there is nothing to prevent Nature from finding ingenious strategies to circumvent mechanical constraints that strictly apply only to the most structurally simple cells.

Viability of Very Small Cells

In this paper, we focus on just a few of the cell's mechanical properties: the resistance of the boundary membrane to bending and rupture and the elasticity of a cell's filaments. We then discuss the implications of these characteristics to the mechanical functionality of cells much smaller in size than typical terrestrial cells. Our benchmark is a structurally simple cell of radius 50 nm. We demonstrate that:

- In simple models of fluid membranes, the bending energy of a spherical shell is independent of its radius, so that it takes the same amount of energy to bend a flat membrane into a small spherical shell as

a large shell. Whether such a cell is stable depends upon its energy compared to other configurations such as a flat disk with a free boundary. The creation of a hole or a free edge in a membrane requires an input of energy that is proportional to the length of the edge boundary. Except for very small membrane segments, it is energetically more favorable for a membrane with a free boundary to close up into a spherical shape, eliminating the boundary. The estimated minimum sphere radius arising from this argument is about 20 nm.

- There is a minimum stress that a membrane can tolerate before it ruptures on conventional time scales. Because the (surface) stress on a spherical shell is proportional to its radius, a small cell can tolerate higher internal pressures than can a large cell for a given membrane composition. Thus, a very small cell would not require a cell wall in order to function at the osmotic pressures typical of many bacteria.
- The bending resistance of a filament rises rapidly with its radius, so that thick filaments are relatively inflexible. Although a very small cell does not have sufficient volume to accommodate a conventional cytoskeleton (whose elements may be 10-25 nm in diameter), even a filament of double-stranded DNA would appear somewhat stiff on the scale of 50 nm. In order to code sufficient genetic information in a linear sequence, small cells would need very flexible molecules with perhaps half the mass per unit length of DNA, a requirement that is consistent with the idea that RNA or some other single-stranded molecule is the evolutionary precursor of DNA as the genetic template.

Detailed Analysis

Membrane Curvature

All cells are bounded by a plasma membrane consisting of a bilayer of dual-chain lipid molecules within which are embedded proteins and other molecules such as cholesterol. Bilayers are self-assembled structures whose equilibrium configuration is spatially flat if the molecular composition is the same within both layers. Such symmetric bilayers resist bending with an energy cost per unit area ε whose simplest parameterization is

$$\varepsilon = (\kappa / 2) (1 / R_1 + 1 / R_2) ^2 + \kappa_G / (R_1 R_2), \quad (1)$$

where the constants κ (bending rigidity) and κ_G (Gaussian curvature modulus) have units of energy [for a review of more complete descriptions of bilayer bending, building on the original approach of Helfrich (1973), see Lipowsky (1991)]. The quantities R_1 and R_2 are the two principal radii of curvature displayed in Figure 2. As an illustration, a sphere of radius R has $R_1 = R_2 = R$, while a cylinder has an infinite radius of curvature along the axis of cylindrical symmetry. To find the bending energy of a particular surface, one simply integrates ε over the entire surface: for example, a spherical shell has a bending energy of $8\pi\kappa + 4\pi\kappa_G$, independent of the shell's radius.

What is the magnitude of the bending energy for typical cells? Lipid bilayers in terrestrial cells are found to have $\kappa = 10\text{-}25\ k_B T$, where k_B is Boltzmann's constant and T is the temperature [see Evans and Rawicz (1990) and references therein]. The value of κ_G is much less well known, but is expected to have a similar magnitude as κ. With $\kappa = \kappa_G$, the energy of a spherical shell is $12\pi\kappa$. Considering only the contribution from κ, the bending energy of a spherical cell would be 250-600 $k_B T$. Although this is not really a large amount of energy (recall that $k_B T$ is roughly the kinetic energy of an atom in a gas), why would nature expend this energy to form a closed surface from an open bilayer sheet? To answer this question, we examine how a bilayer might rupture.

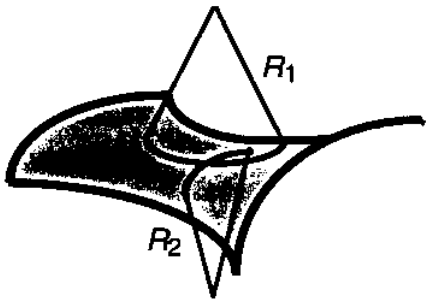

Figure 2. Principal radii of curvature for a saddle-like surface.

Membrane Rupture

The fluid membrane not only resists bending, but also resists in-plane stretching. Under tensile stress, the membrane first stretches then ruptures once the area has expanded a few percent beyond its unstressed value. The creation of a hole in a membrane likely involves reconfiguring the lipid molecules around the boundary of the hole in order to reduce contact between the aqueous medium surrounding the bilayer and the water-avoiding hydrocarbon chains of the lipid molecules, which are normally buried within the bilayer. In general, the orientation of the lipids at the hole boundary is energetically unfavorable compared to that of an intact bilayer, so that there is an energy penalty if the membrane has a hole or a free edge.

The boundary of the hole can be characterized by an edge tension λ (energy per unit length along the boundary), which has been measured to be in the 10^{-11} J/m range (for example, Fromherz, 1983); the measured values are larger than the minimum edge tension for membrane stability estimated from computer simulations of membrane rupture (Boal and Rao, 1992). For example, the edge energy of a flat disk of radius R_{disk} and perimeter $2\pi R_{disk}$ is $E_{disk} = 2\pi R_{disk}\lambda$. A membrane having this shape will be energetically favored over the closed sphere considered above ($E_{sphere} = 12\pi\kappa$ for $\kappa = \kappa_G$) if $R_{disk} < 6\kappa/\lambda$. If the disk and the sphere have the same surface area then $R_{sphere} = R_{disk}/2$ (see Figure 3). Thus we expect $R_{sphere} > 3\kappa/\lambda$ (after Fromherz, 1983). Using typical values of $\kappa \sim 15\ k_BT$ and $\lambda = 10^{-11}$ J/m leads to $R_{sphere} > 20$ nm, a bound whose exact value depends upon the membrane composition. Experimentally, one finds that pure bilayer vesicles (simple artificial cells in some sense) can be produced in the lab with radii as small as 30 nm (Fromherz, 1983; Frisken, 1998, private communication). Once the membrane has adopted a closed shape, the configuration could be further stabilized by the addition of lipids to the outer layer, thus reducing the strain in the bilayer.

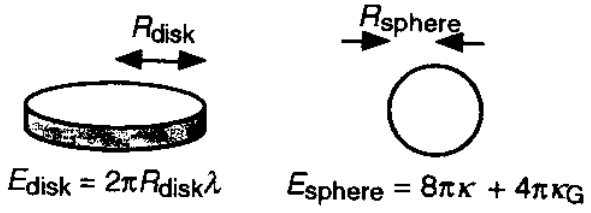

Figure 3. Energetics of disks and spheres. The two shapes have the same areas if $R_{sphere} = R_{disk}/2$.

Experiments on membrane failure find that typical bilayers rupture at tensile stresses of 1×10^{-2} J/m^2 on laboratory time scales (Needham and Hochmuth, 1989). In cells, a (two-dimensional) surface stress Π can result from the osmotic pressure difference P between the cell's interior and its external environment. For a spherical shell of radius R, the stress and pressure are related by (Fung, 1994)

$$\Pi = PR / 2. \tag{2}$$

Thus, a spherical shell of radius 1-micron can support a pressure difference of up to 2×10^4 J/m^3, if the two-dimensional bursting stress is 1×10^{-2} J/m^2 on laboratory timescales. However, many bacteria operate at much higher internal pressures, ranging up to many atmospheres, where 1 atmosphere = 10^5 J/m^3. Most varieties of bacteria accommodate this pressure by the use of a cell wall.

Because the surface stress is proportional to R in Equation (2), a smaller cell would experience a lower stress for a given osmotic pressure P. In fact, a bilayer alone could handle an osmotic pressure of 4 atmospheres for a cell with a radius of just 50 nm, so that very small cells would not need a cell wall to function at moderate osmotic pressures. The absence of a cell wall would reduce the functional tasks of the cell and hence eliminate that part of DNA required to produce the proteins associated with cell wall construction. Alternatively, a small cell could choose to have a cell wall and increase the osmotic pressure at which it operates. Because the osmotic pressure is directly proportional to the concentration of proteins, ions, etc., then small cells could have a higher concentration of chemical reactants. Given that the rate of chemical reactions is proportional to the product of the reactant concentrations, an increase in the concentrations would result in an increase of the chemical reaction rates.

Flexible Filaments

The most evolutionarily advanced cells—eucaryotic cells—contain a filamentous cytoskeleton, which helps maintain the cell's shape, along with its other duties. Components of the cytoskeleton frequently include actin, intermediate filaments, and microtubules, with diameters in the range of 10 to 25 nm. Compared to a typical eucaryotic cell diameter of 10 microns or more, the transverse dimension of a cytoskeletal filament is trivial. Smaller cells such as bacteria, whose evolutionary origin predates eucaryotes, do not contain a cytoskeleton, but may instead possess a strong cell wall surrounding the pressurized bag bounded by a fluid membrane. Even bacteria, with a typical diameter of 1 micron, could accommodate the size of cytoskeletal filaments found in eucaryotes. However, cells with a radius as small as 50 nm would probably not have sufficient interior volume to permit a conventional cytoskeleton.

The absence of a cytoskeleton within a small cell does not imply that there are no filaments present. Cells must have some means of carrying hereditary information; the earliest cells may have used RNA but today's cells use DNA, both of which are linear molecules. Now, the visual appearance of a flexible rope, string, or linear molecule depends on the length scale of observation. For example, a human hair may be curly as seen by the eye on a length scale of centimeters, but a segment of the hair would seem straight if viewed through a microscope on a length scale of less than a millimeter. A quantity called the persistence length can be used to describe the straightness of a linear molecule. Figure 4 illustrates two linear objects; part [a] is convoluted with a short persistence length while [b] is much straighter with a long persistence length. Mathematically, the persistence length is a measure of the length scale over which a curve undergoes a significant change in direction. The arrows in Figure 4b are about a persistence length apart, as measured along the curve.

Now, double-stranded DNA has a persistence length of about 50 nm (Bustamante et al., 1994),

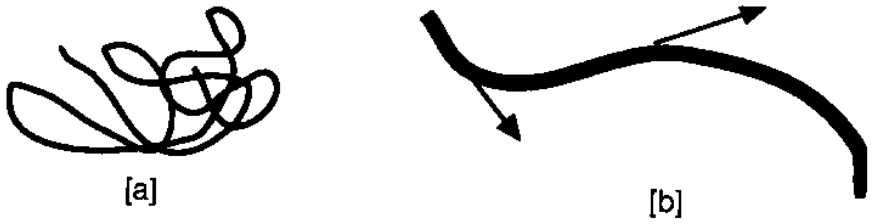

Figure 4. Schematic representation of strings with short [a] and long [b] persistence lengths. The arrows in [b] are about a persistence length apart, as measured along the contour of the string.

meaning that a 100 nm filament of DNA might look like the configuration in Figure 4b: it would appear to be neither a straight rod, nor a tangled ball of thread. At 0.34 nm per base pair, a 100 nm filament of DNA traversing the cell once would contain just 300 base pairs, not a lot of genetic information. This means that cells probably would have to be larger than 50 nm in radius to accommodate a moderate amount of DNA if it were present as a random chain. It is more likely that small cells would use RNA or another flexible molecule to carry genetic information, consistent with the idea that RNA predated DNA in evolution. Many biopolymers display a persistence length that varies as the square of the mass per unit length along the polymer, a scaling behavior consistent with the theoretical expectation that the persistence length varies as the fourth power of the radius for uniform cylindrical rods (Doi and Edwards, 1986; Landau and Lifshitz, 1986). Thus, a molecule with the same mass density as double-stranded DNA, but only half the mass per unit length, would have a persistence length of one-quarter that of DNA, just 13 nm. With a persistence length closer to 10 nm, a long molecule could be balled up in a cell of 100-nm diameter. Self-interactions along the molecule's length, as might be expected for RNA, would reduce the size of the genetic ball even further.

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