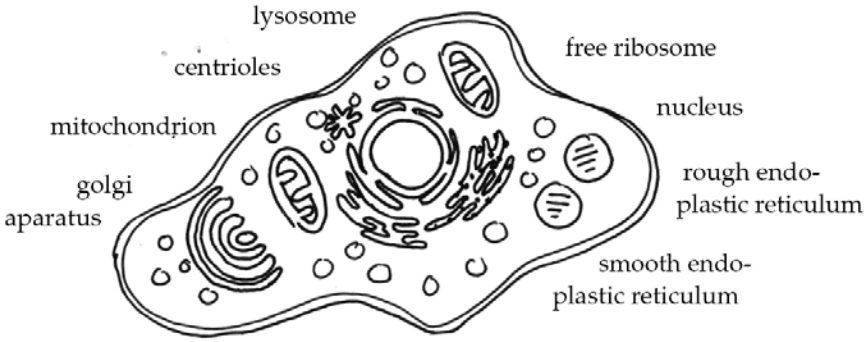
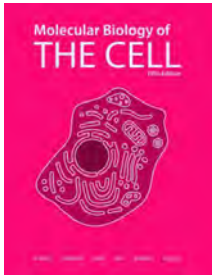
me239 - mechanics of the cell

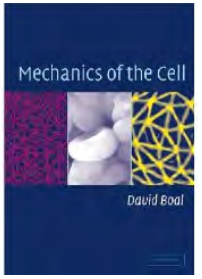


me239 mechanics of the cell

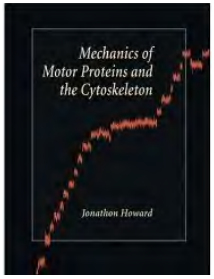
recommended textbooks / additional reading



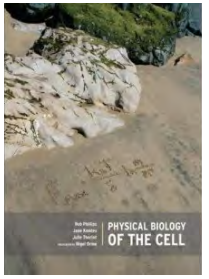
alberts et al. [2008]



boal [2002]



howard [2001]



phillips et al. [2008]

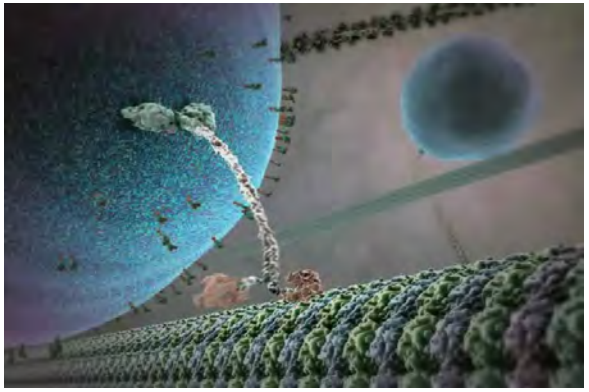
me239 mechanics of the cell - literature

topics covered in class

01	Introduction	Motivation, movies	3.29
02	Introduction	Cell biology	3.86
03	Introduction	Cell mechanics	4.00
04	Biopolymers	Polymerization kinetics	3.86
05	Biopolymers	Energy, tension, bending	3.71
06	Biopolymers	Entropy, persistence length	4.14
07	Cytoskeleton	Filopodia buckling	4.14
08	Cytoskeleton	Red blood cells	4.71
09	Cytoskeleton	Tensegrity model	3.00
10	Biomembranes	Micropipette aspiration	3.14
11	Biomembranes	Lipid bilayers	3.86
12	Biomembranes	Energy, tension, bending	4.29
13	Mechanotransduction	Signaling, probing	4.57
14	Mechanotransduction	Membrane potential	4.29
15	Mechanotransduction	Action potential	4.71

me239 mechanics of the cell - overview

1. introduction to cell biology



the inner life of a cell, viel & lue, harvard [2006]

1 introduction to cell biology

the classical cell theory

- all living things are composed of cells
- cells are the basic unit of structure and function in living things
- cells are produced from other cells

hooke [1665] schwann & schleiden [1839], virchow [1858]

some facts and figures

- humans consists of approximately 100 trillion,i.e., 10^{14} cells
- humans consists of > 200 different cell types
- a typical cell size is 10 μm
- the smallest cells are less than 1μm in diameter while nerve cells can be up to a 1m long
- a typical cell mass is 1 nanogram

eukaryotic cells

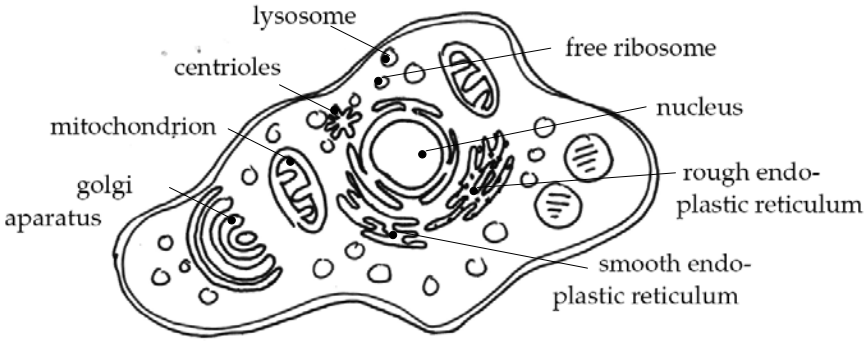


Figure 1.2 Eukaryotic cell. Cell without a distinct nucleus.

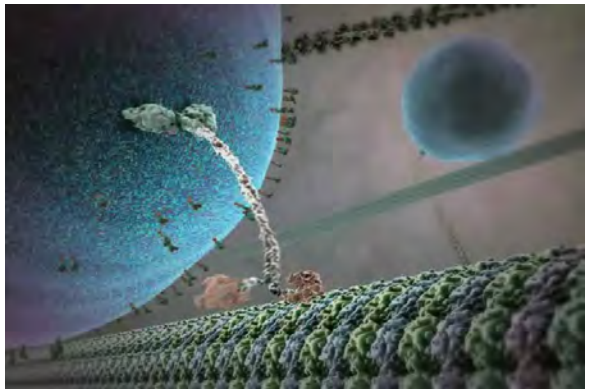
organelles

organelles are **specialized subunits** within a cell that are enclosed by their own **lipid membrane**. the name organelle indicates that these subunits have a similar function to the cell as have organs to the human body. larger organelles such as the nucleus are easily visible with a light microscope. different types of organelles may be found in a cell depending on the cell's function.

typical organelles and their characteristic functions

- nucleus - maintenance of DNA and transcription of RNA
- endoplastic reticulum - translation and folding of new proteins
- golgi apparatus - storage and sorting of proteins
- mitochondrion - energy production / conversion of glucose to ATP

2. introduction to mechanics



the inner life of a cell, viel & lue, harvard [2006]

trusses, beams, walls, plates, membranes, shells

	dimension	geometry	loading	deformation	gov eqn
truss	1d straight	$w, h \ll l$	axial	tension	2^{nd} order
beam	1d straight	$w, h \ll l$	transverse	bending	4^{th} order
wall	2d flat	$h \ll w, l$	in plane	tension/shear	2^{nd} order
plate	2d flat	$h \ll w, l$	transverse	bending	4^{th} order
membrane	3d curved	$h \ll w, l$	in plane	tension/shear	2^{nd} order
shell	3d curved	$h \ll w, l$	transverse	bending	4^{th} order

Table 2.1: Classification of structural elements based on dimension, geometry and loading

3. biopolymers

the inner life of a cell, viel & lue, harvard [2006]

biopolymers

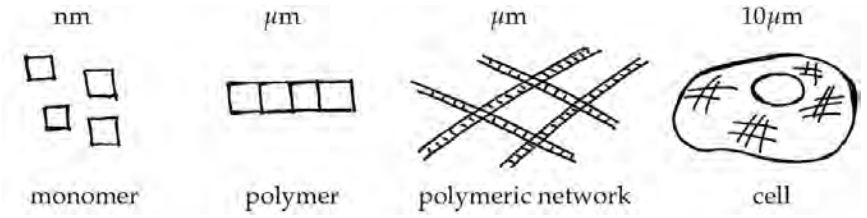


Figure 3.1. Biopolymers. Characteristic length scales on the cellular and sucellular level..

cytoskeletal filaments

actin filaments are 7nm in diameter and consist of two intertwined actin chains. they are tension bearing members of the cell. being located close to the cell membrane, they are responsible for inter- and intracellular transduction. together with myosin, they from the contraction apparatus to generate muscular contraction of skeletal and cardiac muscle.

intermediate filaments are 8-12nm in diameter and thus more stable than actin filaments. they are also tension bearing within a cell. anchoring at organelles, they organize and maintain the three dimensional structure of the cell.

microtubules are hollow cylinders, 25nm in diameter with a 15nm lumen. they are comprised of 13 protofilaments consisting of α and β tubulin. microtubules are organized by the centrosome, but reassemble dynamically. unlike actin and intermediate filaments, microtubules can also bear compression. in addition, they form a highway for intracellular transport.

actin filaments

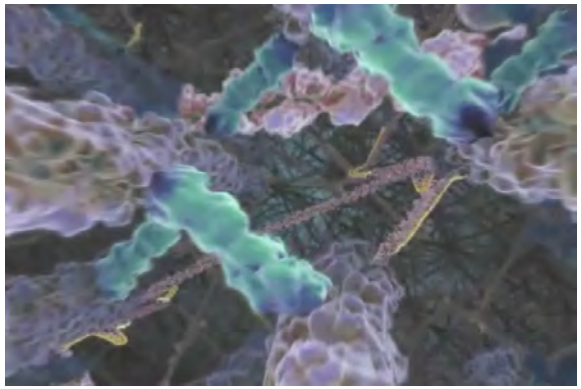


Figure 1.4.1 Actin filaments form tight parallel bundles which are stabilized by cross-linking proteins. Deeper in the cystol the actin network adopts a gel-like structure, stabilized by a variety of actin binding proteins.

the inner life of a cell, viel & lue, harvard [2006]

microtubules

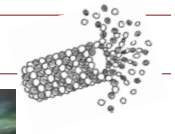


Figure 1.4.3 The cytoskeleton includes a network of microtubules created by the lateral association of protofilaments formed by the polymerization of tubulin dimers.

the inner life of a cell, viel & lue, harvard [2006]

axial deformation - tension

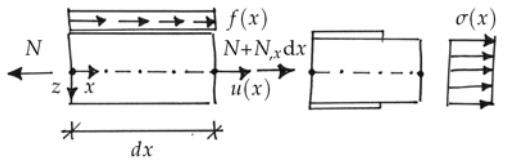


Figure 3.1: Axial loading of one dimensional structure ◦ Stresses σ are constant across the cross section

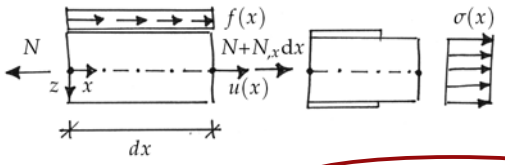
◦ kinematics $\varepsilon = \lim_{x\to 0} \frac{u}{x} = \frac{\mathrm{d}u}{\mathrm{d}x} = u_{,x}$ homogeneous $\varepsilon = \frac{\Delta l}{l}$

◦ constitutive equation $\sigma = \sigma(\varepsilon)$ linear elastic $\sigma = E\,\varepsilon$

◦ stress resultant $N = \iint \sigma \,\mathrm{d}y\mathrm{d}z$ homogeneous $\sigma = \frac{N}{A}$

◦ equilibrium $\sum f \doteq 0$ in axial direction $N_{,x} + f = 0$

axial deformation - tension



$EA\,u_{,xx} + f = 0$ with EA ... axial stiffness

cross section area $A = \pi r^2$

scales with radius²

	r	A	E	EA
microtubule	12.5 nm	491 nm^2	1.9·10^9 N/m^2	93·10^{-8} N
intermediate filament	5.0 nm	79 nm^2	2.0·10^9 N/m^2	15·10^{-8} N
actin filament	3.5 nm	39 nm^2	1.9·10^9 N/m^2	7·10^{-8} N

Table 3.1: Axial stiffness EA of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

transverse deformation - bending

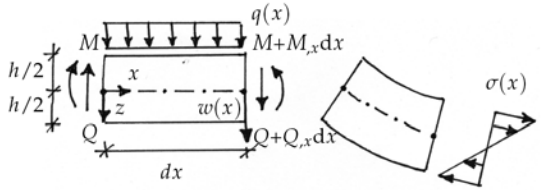
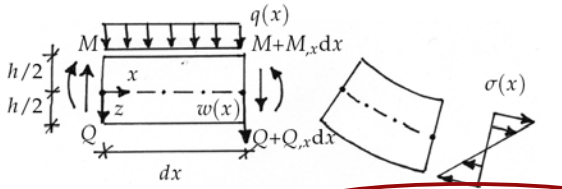


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

- kinematics $\varepsilon = -w_{,xx}\,z = \kappa\,z$
- constitutive equation $\sigma = E\,\varepsilon = -E\,w_{,xx}\,z = E\,\kappa\,z$
- stress resultants $M = \int_{-h/2}^{+h/2} \sigma\,z\,\mathrm{d}z = \int_{-h/2}^{+h/2} E\,\kappa\,z^2\,\mathrm{d}z = EI\,\kappa$
- equilibrium $\sum f_z \doteq 0 \quad Q_{,x} + q = 0$; $\sum m \doteq 0 \quad M_{,x} - Q = 0$

transverse deformation - bending



$q = EI\,w_{,xxxx}$ with EI ... bending stiffness

for circular cross sections $I = \pi r^4 / 4$

scales with radius⁴

	r	I	E	EI
microtubule	12.5 nm	19,175 nm^4	1.9·10^{9}N/m^2	364·10^{-25}Nm^2
intermediate filament	5.0 nm	491 nm^4	2·10^{9}N/m^2	10·10^{-25}Nm^2
actin filament	3.5 nm	118 nm^4	1.9·10^{9}N/m^2	2·10^{-25}Nm^2

Table 3.2: Bending stiffness of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

free energy - energy and entropy

$\psi = W - T\,S$ ... free energy

$W = W(\varepsilon)$ ... strain energy

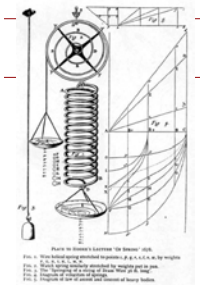
$T = 300K$ ... absolute temperature

$S = k\,\ln(p)$ ... Boltzmann equation

$k = 1.38 \cdot 10^{-23}$ J/K ... Boltzmann constant

$$\psi = W - T\,S \approx -T\,S = -T\,k\,\ln(p)$$

example - entropic spring



"The power of any spring is in the same proportion with the tension thereof: that is, if one power stretch or bend it one space, two will bend it two, and three will bend it three, and so forward. Now as the theory is very short, so the way of trying it is very easy."

Robert Hooke [1678] De Potentia Restitutiva

Entropic spring Do you remember Hooke's law for a linear elastic spring? For that simple model, the spring stiffness k could be calculated as the second derivative of the spring energy $\psi = \frac{1}{2} k^{\text{spr}} u^2$ such that $\partial^2\psi/\partial u^2 = k^{\text{spr}}$. We can do the same thing for the entropic polymer. The second derivative of its energy $\psi^{\text{fjc}} = \psi_0^{\text{fjc}} + kTN\frac{3}{2} r^2/L^2$ with respect to r gives us $\partial^2\psi/\partial r^2 = 3kTN/L^2$. This is the equivalent stiffness of a spring that had the same stretch resistance as the biopolymer modeled with as an uncorrelated Gaussian chain. The biopolymer can thus be understood as an entropic spring with the spring stiffness $3kTN/L^2$.

concept of persistence length

- stiffer filaments are straighter ∝ bending stiffness EI
- cooler filaments are straighter ∝ inverse temperature kT

$$A = \frac{EI}{kT} \quad \ldots \quad \text{persistence length} \quad l \leq A \leq L$$

scales with radius⁴

	r	E	EI	$A = [EI]/[kT]$
microtubule	12.5 nm	$1.9{\cdot}10^9$N/m^2	$364{\cdot}10^{-25}$Nm^2	8.800 mm
intermediate filament	5.0 nm	$2{\cdot}10^9$N/m^2	$10{\cdot}10^{-25}$Nm^2	0.240 mm
actin filament	3.5 nm	$1.9{\cdot}10^9$N/m^2	$2{\cdot}10^{-25}$Nm^2	0.048 mm

Table 3.6: Persistence lengths of major consituents of cytoskeleton at room temperature: microtubules, intermediate filaments and actin filaments

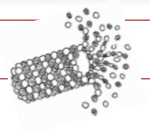
concept of persistence length

$$A = \frac{EI}{kT} \quad \ldots \quad \text{persistence length}$$

- the persistence length is a measure of the length scale over which a **polymer remains roughly straight**
- the persistence length is a measure of the **competition between** the **entropic** parts of the free energy randomizing the orientation of the polymer and the **energetic** cost of bending.
- the persistence length is the scale over which the **tangent-tangent correlation function decays** along the chain

suggested reading: 8.2 macromolecules as random walks / 10.2.2 beam theory and the persistence length physical biology of the cell, phillips, kondev, theriot [2009]

polymerization of idealized polymers



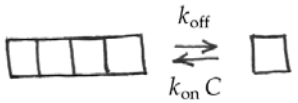


Figure 3.3: Model of idealized polymerization with addition and removal of subunits.

$$\frac{dn}{dt} = +k_{\text{on}}\,C \quad \ldots \text{ monomer capture}$$

$$\frac{dn}{dt} = -k_{\text{off}} \quad \ldots \text{ monomer release}$$

$$\frac{dn}{dt} = +k_{\text{on}}\,C - k_{\text{off}}$$

critical free monomer concentration

$$\frac{dn}{dt} = +k_{\text{on}}\,C_{\text{crit}} - k_{\text{off}} \doteq 0 \quad \text{thus} \quad C_{\text{crit}} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

polymerization of actin and tubulin

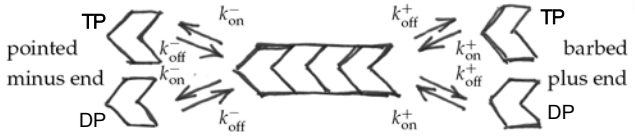


Figure 3.4: Eight rate constants for polymerization capture and release in non-symmetric actin and microtubules filaments.

	k^+_{on} [1/(μMs)]	k^+_{off} [1/s]	k^-_{on} [1/(μMs)]	k^-_{off} [1/s]	C^+_{crit} μM	C^-_{crit} μM
ATP-actin	11.60	1.40	1.30	0.80	0.12	0.62
ADP-actin	3.80	7.20	0.16	0.27	1.90	1.70
GTP-tubulin	8.90	44.00	4.30	23.00	4.90	5.30
GDP-tubulin	0	733	0	915	n/a	n/a

Table 3.1: Measured rate constants of actin filaments and microtubules

polymerization of actin - treadmilling

Figure 3.5: During treadmilling, monomers move along the filament from the plus end to the minus end while the overall filament might remain at constant length.

Treadmilling The special situation when one end of a filament polymerizes while the other end depolymerizes is referred to as treatmilling. Treadmilling can be observed in many cellular cytoskeletal filaments, especially in actin filaments and microtubules. The filament grows at the plus or barbed end while, at the same, it shrinks at the minus or pointed end. From the outside, it seems as if segments of the filament move across the cytosol [1]. In general, treadmilling may occur at different rates at both ends. At a particular concentration at which the speed of growth at the plus end is equal to the rate of growth at the minus end the net length of the treadmilling filament remains unchanged. This is state is called steady-state treadmilling.

polymerization of actin - steady state treadmilling

Figure 3.5: During treadmilling, monomers move along the filament from the plus end to the minus end while the overall filament might remain at constant length.

$$C^-_{crit} = \frac{k^-_{off}}{k^-_{on}} = \frac{0.8}{1.3}\,\mu M = 0.62\,\mu M \qquad C^+_{crit} = \frac{k^+_{off}}{k^+_{on}} = \frac{1.4}{11.6}\,\mu M = 0.12\,\mu M$$

$$\frac{dn^+}{dt} + \frac{dn^-}{dt} = k^+_{on} C_{std} - k^+_{off} + k^-_{on} C_{std} - k^-_{off} \doteq 0 \qquad C_{std} = \frac{k^+_{off} + k^-_{off}}{k^+_{on} + k^-_{on}}$$

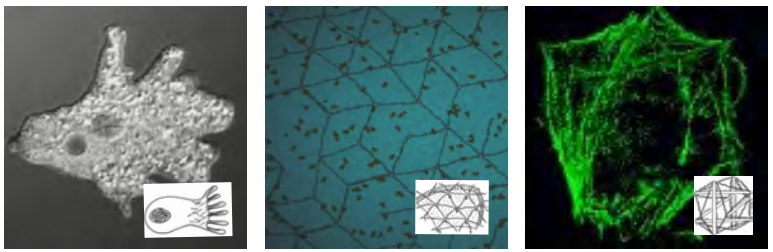
$$C_{std} = \frac{k^+_{off} + k^-_{off}}{k^+_{on} + k^-_{on}} = \frac{1.4 + 0.8}{11.6 + 1.3}\,\mu M = 0.17\,\mu M \quad \text{steady state concentration}$$

$$C^+_{crit} = 0.12\,\mu M \leq C_{std} = 0.17\,\mu M \leq C^-_{crit} = 0.62\,\mu M$$

from molecular level to cellular level

three examples

- **fiber bundle model** for filopodia
- **network model** for red blood cell membranes
- **tensegrity model** for generic cell structures



from molecular level to cellular level

assuming we know the mechanical properties of the individual filaments, what does that actually tell us about the assembly of filaments that we find in the cell?

- could we then predict the **stiffness of the overall assembly**?
- how does the filament microstructure affect **cytoskeletal properties**?
- how can we calculate the **macroscopic network properties** from the individual microscopic filament properties?

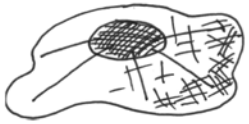


Figure 4.1: The cytoskeleton provides structural stability and is responsible for forces during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

filopodia and other fiber bundels of F-actin

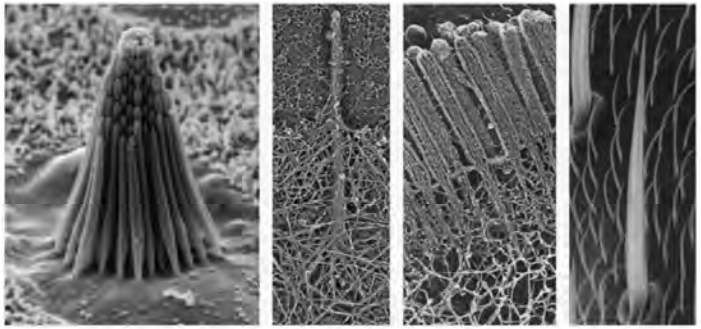


Figure 4.2: Fiber bundles of F-actin. Ciliary bundle from the sensory epithelium of a bullfrog saccule consisting of stereocilia, filopodium protruding from the lamellipodium of a mouse melanoma cell, epithelial microvilli, and drosophila neurosensory micro- and macrochaete bristles,

bathe, heussinger, claessens, bausch, frey [2008]

assembly of crosslinked actin filaments

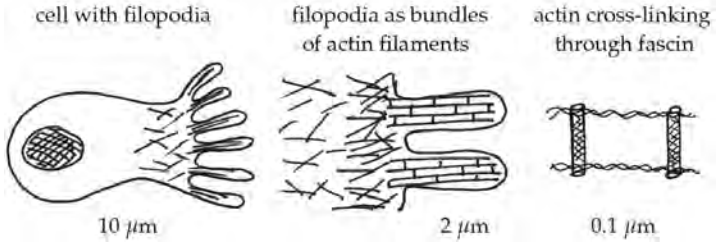


Figure 4.3. Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth.

filopodia are very thin structures approximately 0.2 um in diameter. they can easily extend up to 1.5um. they typically polymerize and depolymerize at rates of approximately 10 um/min. the mechanical properties of filopodia play an essential role in various different physiological processes, including hearing, cell migration, and growth. despite their importance to cell function, the structural architecture responsible for their overall mechanical behavior remains largely unknown.

pushing the envelope

simplified model for cell locomotion

- protrusion … polymerization at the leading edge of the cell
- attachment … formation of focal adhesions to link the cell to the surface
- retraction … contraction of stress fibers to retract the rear of the cell

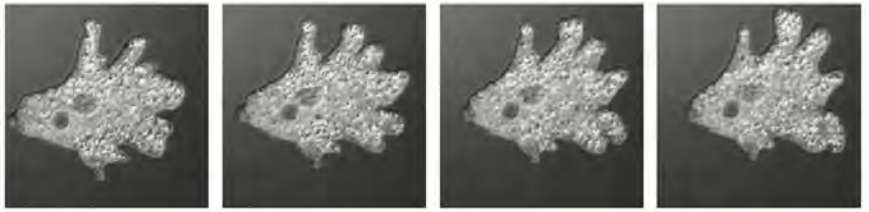
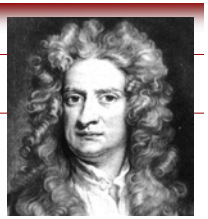


Figure 4.4: Single-celled amoeba crawling around by using actin polymerization to push out pseudopods to explore new territory. Organelles move in complex patterns within the cell,

alberts, johnson, lewis, raff, roberts, walter [2002]

pushing the envelope - critical length



Newton's third law: actio = reactio

$$F_{\text{fil}} \doteq F_{\text{mem}}$$

$$F_{\text{fil}} = \frac{\pi^2 EI}{[2L]^2} = \frac{\pi^2 EI}{4L} \qquad F_{\text{mem}} \approx 5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}$$

$$\frac{\pi^2 EI}{4L_{\text{crit}}^2} = 5\sqrt{n}\, r_{\text{act}} \frac{\text{pN}}{\text{nm}} \quad \text{thus} \quad L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{EI}{5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}}}$$

$E = 1.9 \cdot 10^9\ \text{N/m}^2 = 1.9\ \text{GPa}$ $\quad r_{\text{act}} = 2.5$ $\quad$ moment of inertia I

- loose assembly
- tightly crosslinked

case I - loosely assembled actin filaments

$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{EI}{5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}}}$$

moment of inertia I

$$I = n\, I_{\text{act}} \quad \text{with} \quad I_{\text{act}} = \frac{\pi r_{\text{act}}^4}{4}$$

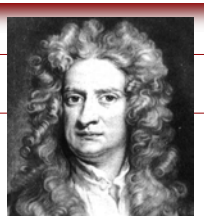
$E = 1.9 \cdot 10^9\ \text{N/m}^2 = 1.9\ \text{GPa}$ $\quad r_{\text{act}} = 2.5$

$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{1.9 \cdot 10^9\,\text{N/m}^2 \cdot n\,\pi/4\,[3.5 \cdot 10^{-9}]^4\text{m}^4}{5\sqrt{n}\; 3.5 \cdot 10^{-12}\,\text{N}}} \approx 0.17769\,\mu\text{m}\; n^{1/4}$$

$n = 30$ filaments $\quad L_{\text{crit}} = 0.416\,\mu\text{m}$

much too low - disagrees with observations of 2um

case II - tightly crosslinked actin filaments



$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{EI}{5\sqrt{n}\, r_{\text{act}}\, \text{pN/nm}}}$$

moment of inertia I

$$I = \frac{\pi r_{\text{fil}}^4}{4} = n^2 \frac{\pi r_{\text{act}}^4}{4} \quad \text{with } r_{\text{fil}} = \sqrt{n}\, r_{\text{act}}$$

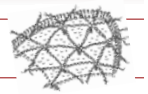
$E = 1.9 \cdot 10^9\ \text{N/m}^2 = 1.9\ \text{GPa}$ $\quad r_{\text{act}} = 2.5$

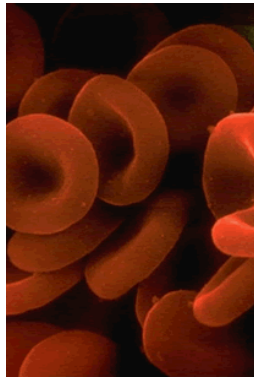
$$L_{\text{crit}} = \frac{\pi}{2}\sqrt{\frac{1.9 \cdot 10^9\,\text{N/m}^2 \cdot n^2\,\pi/4\,[3.5 \cdot 10^{-9}]^4\text{m}^4}{5\sqrt{n}\; 3.5 \cdot 10^{-12}\,\text{N}}} \approx 0.17769\,\mu\text{m}\; n^{3/4}$$

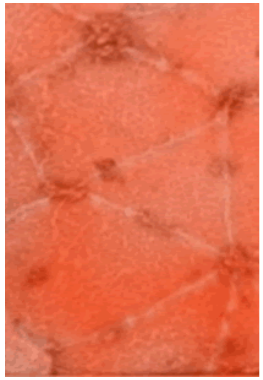
$n = 30$ filaments $\quad L_{\text{crit}} = 2.278\,\mu\text{m}$

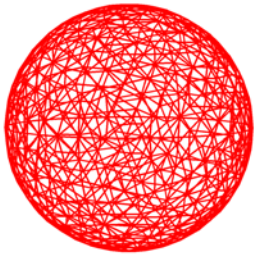
better model - agrees with observations of 2um

network model for red blood cells









the human red blood cell membrane skeleton is a network of roughly 33,000 protein hexagons that looks like a microscopic geodesic dome

network model for red blood cells

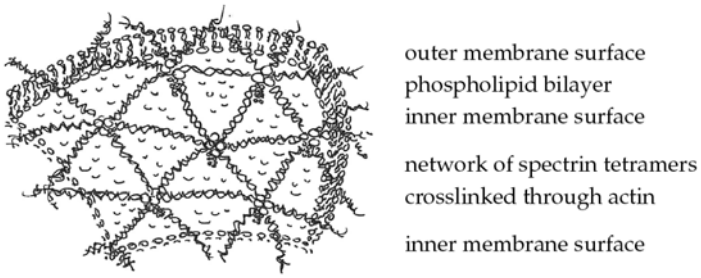


Figure 4.6: Microstructural architecture of the cell membrane of a red blood cell. A six-fold connected network of spectrin tetramers which are crosslinked through short actin filaments, anchored to the phospholipid bilayer, provides structural support to the inner cell membrane.

homogenization - hill-mandel condition

aim. to determine the overall material properties κ and μ of the network of spectrin chains in terms of the spectrin chain stiffness k

ENERGY APPROACH

$$W^{\text{mac}} \doteq W^{\text{mic}}$$

It has been shown how the central problem is reducible to the calculation of average stress or strain in one or other phase. A more versatile approach stems directly from classical theorems in elasticity and focusses attention on strain energies.

hill, r. elastic properties of reinforced solids: some theoretical principles, journal of the mechanics and physics of solids, 1963, 11:357–372.

different network kinematics

six-fold connected network **four-fold connected network**

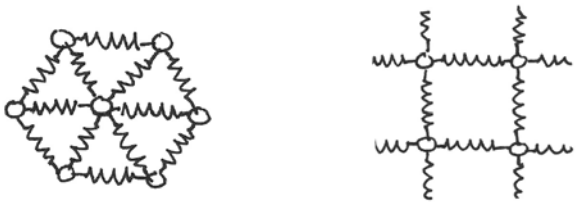


Figure 4.8: Microstructural architecture of a six-fold and four-fold connected network. The theory of homogenization helps to explain why nature prefers a six-fold connected network geometry.

single spring energy

free energy W^{spr} of a single spring

$$W^{\text{spr}} = \tfrac{1}{2}\, k\, \delta^2 = \tfrac{1}{2}\, k\, [\, l - l_0\,]^2 \qquad \text{where} \qquad \delta = l - l_0$$

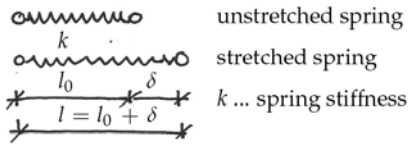
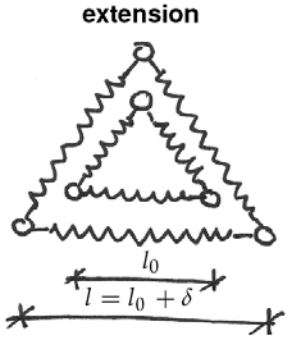


Figure 4.7: Spectrin can be modeled as Gaussian chain which we can conceptually replace by an equivalent linear entropic spring with a spring stiffness of $k = 3\,\mathrm{k}\,T\,N\,/\,L$. The strain energy of this spring can then be expressed as $W^{\text{spr}} = \frac{1}{2}\, k\, \delta^2$.

discrete microscopic network energy



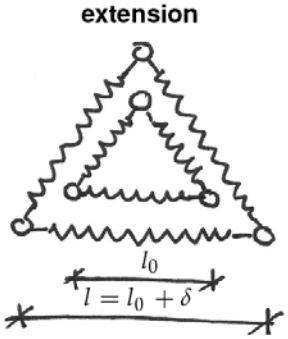
$$W^{\text{mic}} = \frac{\sum_{i=1}^{3} W_i^{\text{spr}}}{\sum_{i=1}^{3} A_i^{\text{spr}}}$$

$$\sum_{i=1}^{3} W_i^{\text{spr}} = 3\,W^{\text{spr}} = 3\left[\tfrac{1}{2}\,k\,\delta^2\right]$$

$$\sum_{i=1}^{3} A_i^{\text{spr}} = 3\,A^{\text{spr}} = \tfrac{1}{2}\sqrt{3}\,l_0^2$$

$$W^{\text{mic}} = \frac{3\,\frac{1}{2}\,k\,\delta^2}{\frac{1}{2}\sqrt{3}\,l_0^2} = \sqrt{3}\,k\left[\frac{\delta}{l_0}\right]^2$$

equivalent macroscopic energy



$$W^{\text{mac}} = \tfrac{1}{2}\,\kappa\left[\varepsilon_{xx} + \varepsilon_{yy}\right]^2 + \tfrac{1}{2}\,\mu\left[\varepsilon_{xx} - \varepsilon_{yy}\right]^2 + 2\,\mu\,\varepsilon_{xy}^2$$

micro-to-macro kinematics

$$\varepsilon_{xx} = \varepsilon_{yy} = \delta / l_0 \qquad \varepsilon_{xy} = 0$$

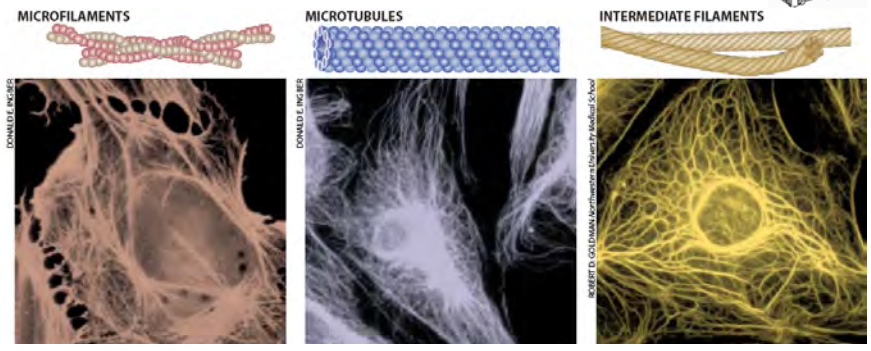
$$W^{\text{mac}} \doteq W^{\text{mic}}$$

$$\frac{1}{2}\,\kappa\left[\frac{\delta}{l_0} + \frac{\delta}{l_0}\right]^2 = \sqrt{3}k\left[\frac{\delta}{l_0}\right]^2$$

$$\kappa = \tfrac{1}{2}\sqrt{3}\,k$$

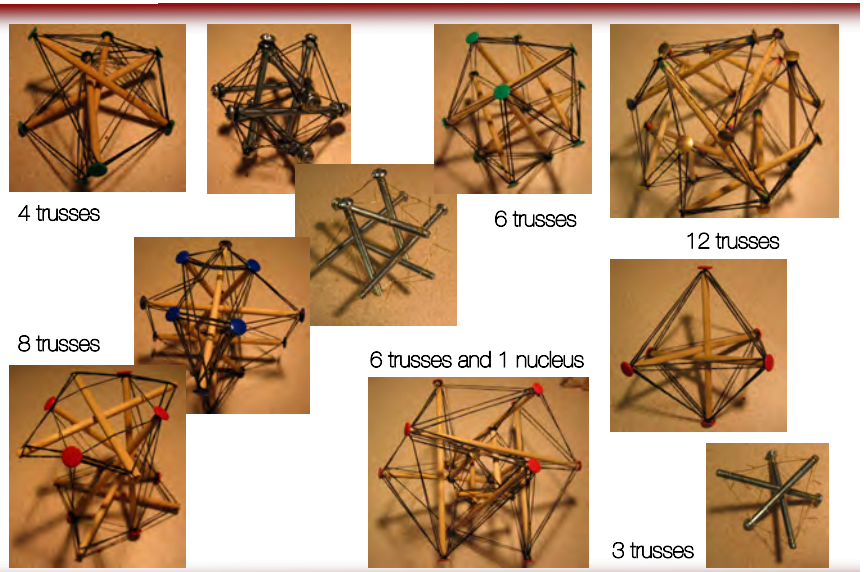
scales with $\sqrt{3}$ spring stiffness

tensegrity = tension + integrity



balanced interplay between tension and compression

ingber [1998]



tensegrity models for cells

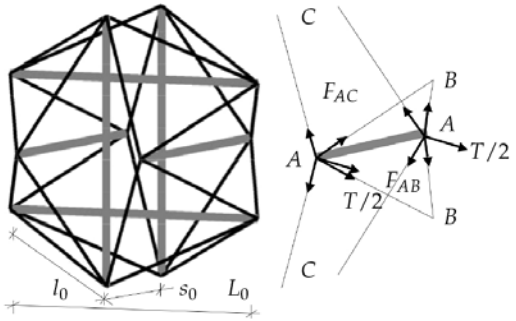
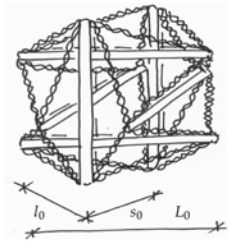


Figure 4.12: Kinematics of simple tensegrity cell model consisting of six compressive trusses (grey) and 24 tensile ropes (black). In the original state, all trusses are of the same length L_0, the rope lengths are $l_0 = \sqrt{3/8}\,L_0$, and the distances between two parallel trusses are $s_0 = 1/2\,L_0$.

tensegrity models for cells



E_0 ... incremental modulus
F_0 ... resting force in actin filaments
L_0 ... length of microtubules
l_0 ... resting length of actin filaments
ε_0 ... strain in actin filaments

Figure 1. Kinematics of simple tensegrity cell model consisting of six compressive trusses and 24 tensile ropes. In the original state, all trusses are of the same length L_0, the rope lengths are $l_0 = \sqrt{3/8}\,L_0$, and the distances between two parallel trusses are $s_0 = 1/2\,L_0$.

$$W^{\text{mac}} \doteq W^{\text{mic}} \qquad W^{\text{mac}} = \frac{1}{2}\,\varepsilon\,E\,\varepsilon \qquad W^{\text{mic}} = \frac{1}{V_0}\int_{s_0}^{s_x} T\,\mathrm{d}x$$

$$E = \frac{2\sqrt{3}}{5\sqrt{2}l_0}\,\frac{T}{s_x - s_0} \qquad \text{small strain} \qquad E_0 = 5.85\,\frac{F_0}{l_0^2}\,\frac{1+4\,\varepsilon_0}{1+12\varepsilon_0}$$

prestress - analytically predicted

$$P \approx \tfrac{1}{3}\,\nu^{\text{actin}}\,\sigma^{\text{actin}}$$

$$\nu^{\text{actin}} = \frac{V^{\text{actin}}}{V_0} = \frac{24A^{\text{actin}}l_0}{[5\sqrt{2}]/[3\sqrt{3}]l_0^3} = \frac{24A^{\text{actin}}}{1.3608l_0^2}$$

$$\sigma^{\text{actin}} = \frac{F_0}{A^{\text{actin}}}$$

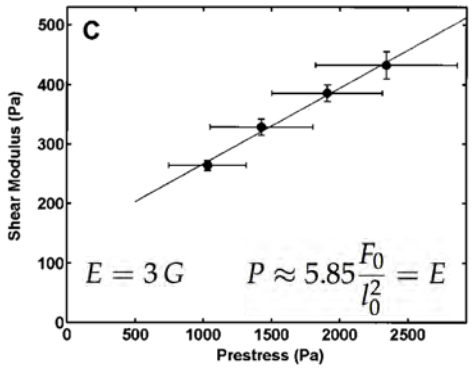
$$P \approx \frac{1}{3}\nu^{\text{actin}}\,\sigma^{\text{actin}} = \frac{1}{3}\,\frac{24\,A^{\text{actin}}}{1.3608l_0^2}\,\frac{F_0}{A^{\text{actin}}}$$

$$P \approx 5.85\frac{F_0}{l_0^2} = E$$

young's modulus scales with pre-stress

prestress is of the same order as young's modulus

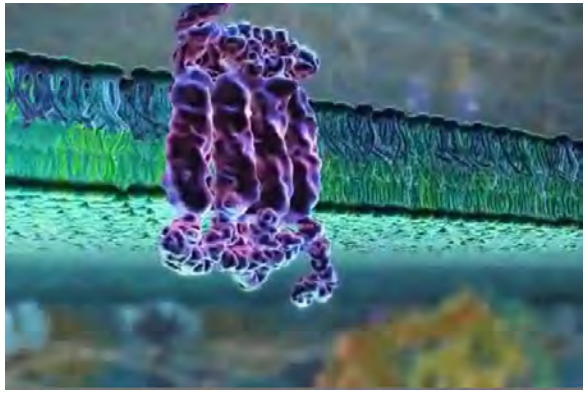
prestress - experimentally measured



prestress is of the same order as young's modulus

wang, naruse, stamenovic, fredberg, mijailovich, tolic-norrelykke, polte, mannix, ingber [2001]

5. biomembranes



the inner life of a cell, viel & lue, harvard [2006]

the lipid bilayer

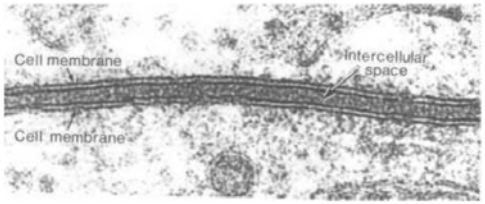


Figure 5.1: Electron microscopy of the cell membrane stained with osmium tetroxide illustrating the polar head groups with a light 2nm space of hydrophobic tails sandwiched between them, adopted from [4]

the cell membrane

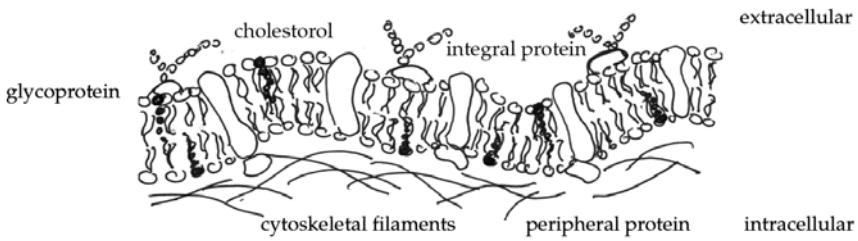


Figure 1.3. Cell membrane. Phospholipic bilayer with hydrophobic water-avoiding tails and hydrophilic water-loving heads.

the cell membrane

all cellular components are contained within a cell membrane which is **extremely thin**, approximately 4-5nm, and **very flexible**. inside the cell membrane, most cells behave like a liquid as they consist of more than 50% of water. the cell membrane is **semi-permeable** allowing for a controlled exchange between intracellular and extracellular components and information.

mechanisms of transport through the membrane

- passive transport driven by gradients in concentration
- active transport that does require extra energy; it is regulated by ion channels, pumps, transporters, exchangers and receptors

the cell membrane

the barrier between the inner and outer cell is the cell membrane, a **bilayer** consisting of **phospholipids** of a characteristic structural arrangement. in aqueous solutions, these phospholipids essentially display two kinds of non-covalent interactions.

non-covalent interactions of phospholipids

- hydrophobic, water avoiding non-polar residues
- hydrophilic, water loving polar head groups

this behavior is similar to fatty acids or **oil in water**, where the hydrophilic polar heads tend to be oriented towards the water phase while the hydrophobic tails are oriented towards the oil phase.

micropipette aspiration

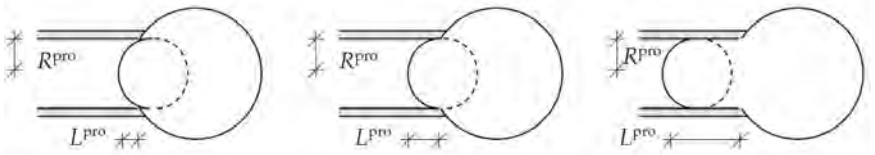


Figure 5.4: The three stages during mircopipette aspiration. The initial state with $L^{pro}/R^{pro} < 1$, left, the critical state with $L^{pro}/R^{pro} = 1$, middle, and the final state with $L^{pro}/R^{pro} > 1$, right.

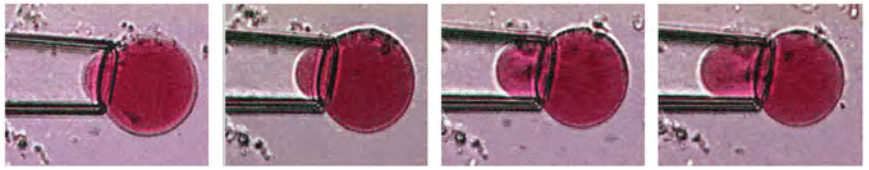


Figure 5.5: Experimental observation of different stages during micropipette aspiration adopted from http://newton.ex.ac.uk/research/biomedical/membranes.

law of laplace from free body diagram

$$p^{int} - p^{out} = 2\frac{n}{R} \quad \text{... Law of Laplace}$$

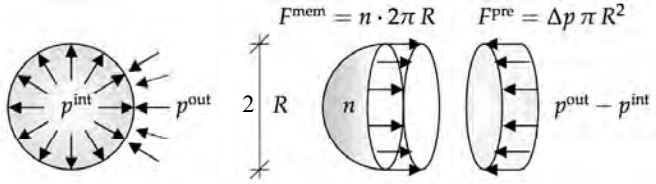


Figure 5.7: Law of Laplace. The membrane force $F^{mem} = n \cdot 2\pi R$ is the result of the surface tension n acting on the cell membrane along the circumference $C = 2\pi R$. It is in equilibrium with the forces F^{pre} resulting from the pressure difference Δp acting on the cell area $A = \pi R^2$.

law of laplace

$$p^{pip} + p^{int} - p^{out} = 2n\frac{1}{R^{pip}} \quad \text{... law of Laplace for the protrusion}$$

$$p^{int} - p^{out} = 2n\frac{1}{R^{cell}} \quad \text{... law of Laplace for the cell}$$

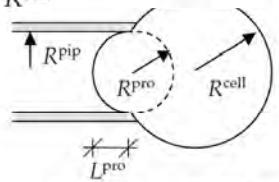
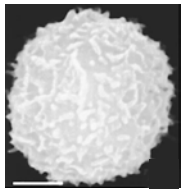


Figure 5.8: Kinematics of micropipette aspiration. For the limit state, at $L^{pro}/R^{pro} = 1$, the Law of Laplace can be used to determine the surface tension n.

$$p^{pip} = 2n\left[\frac{1}{R^{pip}} - \frac{1}{R^{cell}}\right]$$

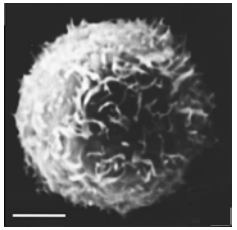
surface tension relates relates pressure and cell radius

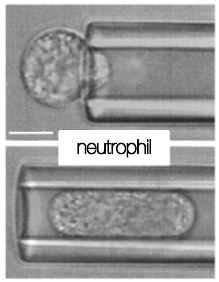
micropipette aspiration

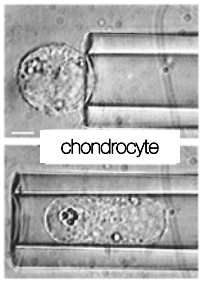


human neutrophil
with diameter of 8um

human chondrocyte
with diameter of 12-16um



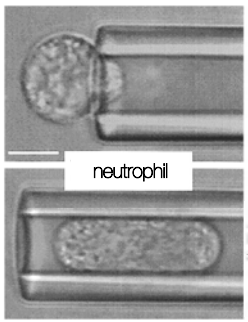


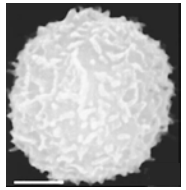


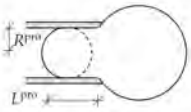
the figure on the left shows a human neutrophil with a diameter of ~8um and a human chondrocyte with a diameter of ~12-16um. scale bars indicate 2um.the figure on the right shows a neutrophil and a chondrocyte each being aspired into a micropipette. scale bars indicate 5um.

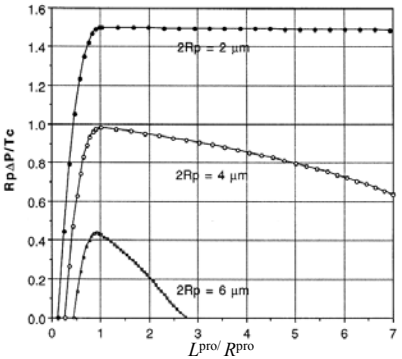
hochmuth [2000]

micropipette aspiration - neutrophil



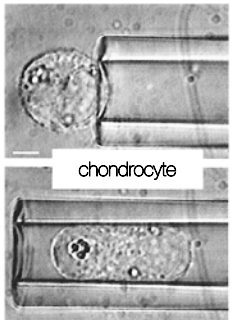


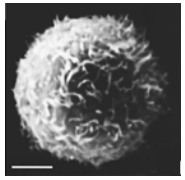


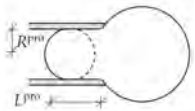


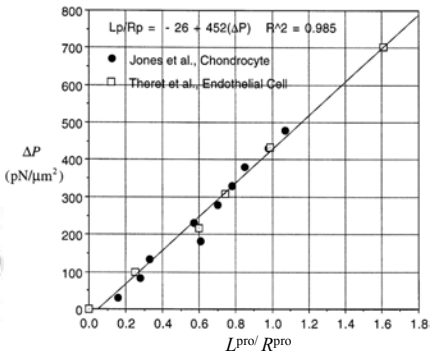
micropipette aspiration of a liquid drop with a constant cortical tension T_c. L^{pro} is the length of the protrusion of the drop into the pipette and R^{pro} is the radius of the protrusion. when L^{pro}/R^{pro} >1, the results are no longer stable to an increase in pressure. the cell flows freely into the pipette when the pressure is increased beyond L^{pro}/R^{pro} =1. cells as neutrophils that flow into the pipette freely at this point behave as liquid drops.

micropipette aspiration - chondrocyte





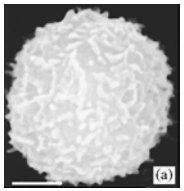




micropipette aspiration of a chondrocyte and an endothelial cell. chondrocytes and endothelial cells continue to behave as an elastic solid for values L^{pro}/R^{pro} >1 that are significantly larger than one. cells that do not flow into the pipette freely behave as elastic solids.

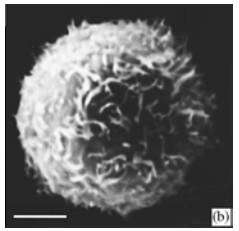
hochmuth [2000]

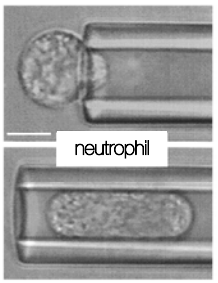
micropipette aspiration

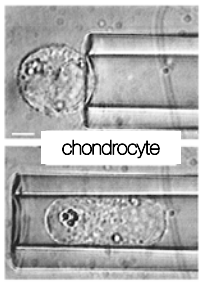


human neutrophil
with diameter of 8um

human chondrocyte
with diameter of 12-16um



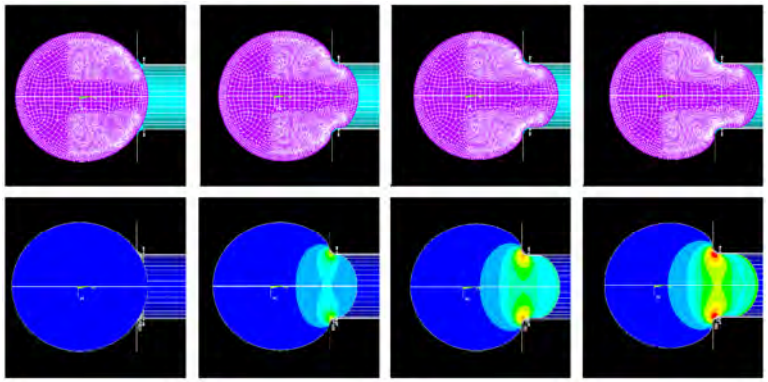




summary neutrophils behave as a liquid drop with a cortical surface tension of about 30pN/um and a viscosity of the order of 100Pa. chondrocytes and endothelial cells behave as a solid with an elastic modulus of the order of 500pN/um=0.5kPa.

hochmuth [2000]

finite element simulation of micropipette aspiration



zubin huang [2007]

Figure 5.10: Finite element simulation of micropipette aspiration of a chondrocyte modeled as an elastic solid. In contrast to analytical results, finite element simulations can account for large deformations, heterogeneous stress distributions, and a more realistic representation of the boundary conditions [21].

concept of surface tension

$$n = \sigma\, h \qquad \text{with} \qquad [\,n\,] = [\,\text{force / length}\,]$$

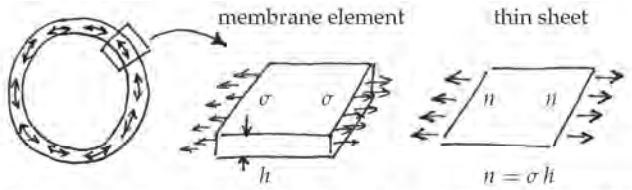
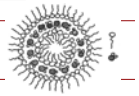


Figure 5.6: Liquid drop model. The internal fluid pressure is balanced by a thin elastic shell. The membrane element of thickness h is subjected to membrane stresses σ. Equivalently, the membrane can be represented as a thin sheet subjected to the surface tension n which results from the integration of the membrane stress over the thickness as $n = \sigma h$.

concept of surface tension



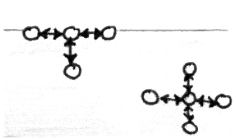
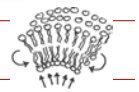


Figure 5.15: Air water interface - molecular interpretation of surface tension

Surface tension Surface tension is typically measured in force per length related to the units dynes per cm. Since 1 dyne = 10 mN, 1 dyne/cm = 1 mN/m. Alternatively, especially in thermodynamics, the notion surface energy is used instead. Surface energy is measured in ergs per length squared, where one eng, the force of one dyne exerted for a distance of one cm is equal to gram centimeter squared per second squared g cm^2/s^2 or, equivalently, 10^{-7} joules. The surface tension of water at room temperature is γ^{water}=72 dynes/cm, ethanol has a lower surface tension of $\gamma^{ethanol}$=22 dynes/cm and mercury has a surface tension as large as $\gamma^{mercury}$=465 dynes/cm.

tension vs bending - membranes vs shells



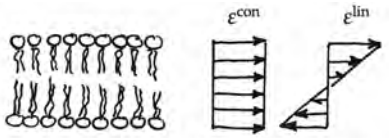
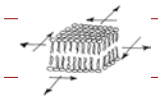


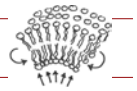
Figure 5.18: Von Kármán strains in cross section – constant terms ε^{con} related to in plain strains and linear terms ε^{lin} related to out of plane bending

overall strain = in plane (constant) + transverse (linear)

$$\begin{aligned}
\varepsilon_{xx} &= u_{,x} + \tfrac{1}{2} w_{,x}^2 - z\, w_{,xx} \\
\varepsilon_{yy} &= v_{,y} + \tfrac{1}{2} w_{,y}^2 - z\, w_{,yy} \\
\varepsilon_{xy} &= \tfrac{1}{2} [u_{,y} + v_{,x} + w_{,x} w_{,y} - 2z\, w_{,xy}]
\end{aligned}$$



membrane stiffness characteristics



$$n_{xx} = K_N\,[\varepsilon_{xx} + \nu\,\varepsilon_{yy}]$$
$$n_{yy} = K_N\,[\varepsilon_{yy} + \nu\,\varepsilon_{xx}]$$
with $K_N = \dfrac{E\,h}{[1-\nu^2]}$ ... extensional stiffness

$$n = K_A\,\frac{\Delta A}{A} \quad \text{with} \quad K_A = \frac{E\,h}{2\,[1-\nu]}$$ ... area expansion modulus

red blood cells $K_A = 0.45\,\mathrm{N/m}$

$$n_{xy} = K_S\,\varepsilon_{xy} \quad \text{with} \quad K_S = 2\,G\,h = \frac{E\,h}{1+\nu}$$ ... membrane shear stiffness

red blood cells $K_S = 6 - 9 \cdot 10^{-6}\ \mathrm{N/m}$

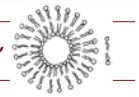
this is super low!

$$p_z = K_B\,\Delta^2 w \quad \text{with} \quad K_B = \frac{E\,h^3}{12\,[1-\nu^2]}$$ ... membrane bending stiffness

red blood cells $K_B = 10^{-19}\ \mathrm{Nm}$

this is super low!

the fluid mosaic model



$K_S = 6 - 9 \cdot 10^{-6}\ \mathrm{N/m}$

fluids have now shear stiffness

The fluid mosiac model What does a low shear stiffness mean for a cell? We have seen that different biological membranes have different functions depending on the proteins associated with their membrane. The low shear resistance indicates that membrane proteins and lipids can easily diffuse laterally or sideways throughout the membrane, giving it its characteristic appearance of a fluid rather than a solid. This property was first recognized by Singer and Nicolson in 1972 who coined the notion of the fluid mosaic model [42]. The fluid mosaic model of lipid bilayer membranes is a two-dimensional fluid, or liquid crystal, in which the hydrophobic integral components such as lipids and membrane proteins are constrained within the plane of the membrane, but are free to diffuse laterally. From a mechanics point of view, biomembranes can thus be understood as fluids as they bear very little resistance to shear.

singer & nicolson [1972]

mechanotransduction

the process of **converting physical forces into biochemical signals** and **integrating these signals into the cellular response** is referred to as mechnotransduction. to fully understand the molecular basis for mechanotransducion, we need to know how externally applied forces are transmitted into and throughout the cell. different techniques have been developed to **probe mechanotransduction** by mechanically stimulate cells to address the following questions.

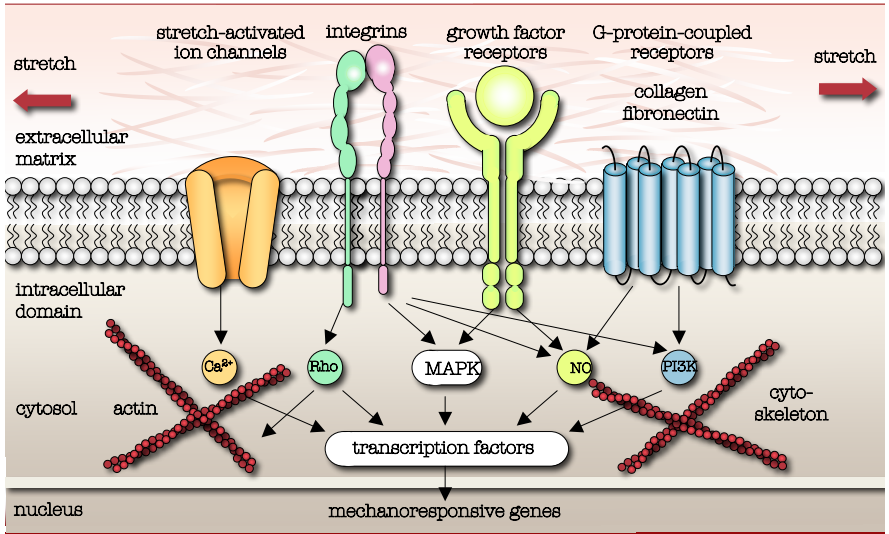
What do we study in mechanotransduction? How do cells respond to mechanical forces? ◦ How do mechanical forces lead to biochemical and molecular responses? ◦ How can we strengthen bone? ◦ How can we grow cartilage? ◦ How can we strengthen muscle? ◦ How can we improve cardiac contractility? ◦ How can we engineer tissues for artificial organs? ◦ How can we mimic the mechanical loading environment of cells in vitro? ◦ What can we learn from mechanical stimulation of cells with precisely controlled forces?

mechanotransduction

the process of mechanotransduction can be divided into three steps

- **mechanoreception**
  detection of the stimulus and transmission of the signal from outside the cell to its inside
- **intracellular signal transduction**
  transduction of the stimulus to location in the cell where a molecular response can be generated
- **target activation**
  activation of proteins that cause alterations in cell behavior through a variety of different mechanisms

mechanotransduction pathways during skin expansion



mechanotransduction pathways during skin expansion

mechanotransduction in *growing skin* consists of three steps

- **mechanoreception**
  detection of the stimulus, *stretch beyond the physiological limit*, and transmission of the signal from outside the cell to its inside
- **intracellular signal transduction**
  transduction of the stimulus *to the nucleus*, to the location in the cell where a molecular response can be generated
- **target activation**
  activation of proteins that cause alterations in cell behavior through *increased mitotic activity and increased collagen synthesis*

mechanoreception

integrins
mediate attachment between a cell and the extracellular matrix, play a central role in force transmission across the cell membrane, triggering targets such as nitric oxide NO signaling, mitogen-associated protein kinases MAPK, Rho GTPases, and phosphoinositol-3-kinase PI3K

stretch-activated ion channels
open in response to elevated membrane strains, allowing positively charged calcium ions Ca2+ and other cations to enter the cell, changes in the intracellular calcium concentration regulate intracellular signaling and cytoskeletal remodeling

growth factor receptors
bind to growth factors outside the cell, thereby turning on several receptor mediated pathways inside the cell, such as nitric oxide NO signaling and mitogen-associated protein kinases MAPK

G protein-coupled receptors
seven-transmembrane proteins, can be activated by mechanical stretch outside the cell to initiate mechanotransduction pathways inside through second messengers such as nitric oxide NO signaling and phosphoinositol-3-kinase PI3K

intracellular signal transduction

- **physical transduction.** the **cytoskeleton** serves as scaffold for the transduction of mechanical into biochemical signals. strain can induce conformational changes in the cytoskeleton, which may affect binding affinities to specific molecules and activate signaling pathways
- **biochemical transduction.** signaling molecules, small intracellular mediator molecules, second messengers, and network of intracellular signaling molecules

Ca2+ **Ca2+** changes in the intracellular calcium concentration are known to regulate intracellular signaling and cytoskeletal remodeling

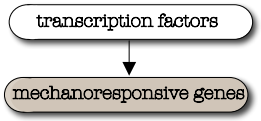
Rho **Rho** GTPases regulates many aspects of intracellular actin dynamics, Rho proteins have been described as molecular switches and play a role in cell proliferation, apoptosis, gene expression, and multiple other common cellular functions

MAPK **MAPK** mitogen-associated protein kinase pathways convey information to effectors, coordinate incoming information from other signaling cascades, amplify signals, and initiate a variety of response patterns

NO **NO** nitric oxide acts as a second messenger, it is a free radical that can diffuse through the plasma membrane and affect nearby cells

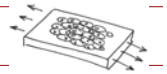
PI3K **PI3K** phosphoinositol-3-kinase is an intracellular signaling pathway regulating apoptosis

target activation



mechanical activation initiates multiple signaling pathways, which can have a substantial overlap and crosstalk. however, since mechanically-induced signaling pathways may be shared with classical receptor-mediated pathways, they are typically difficult to study in isolation. it is clear, however, that **all these signaling pathways converge to activate transcription factors**, which **stimulate gene expression and other nuclear events**. overall, the underlying principle is that stretch invokes a cascade of events that trigger **increased mitotic activity** and **increased collagen synthesis**, which ultimately result in **increased skin surface area** to restore the homeostatic equilibrium state.

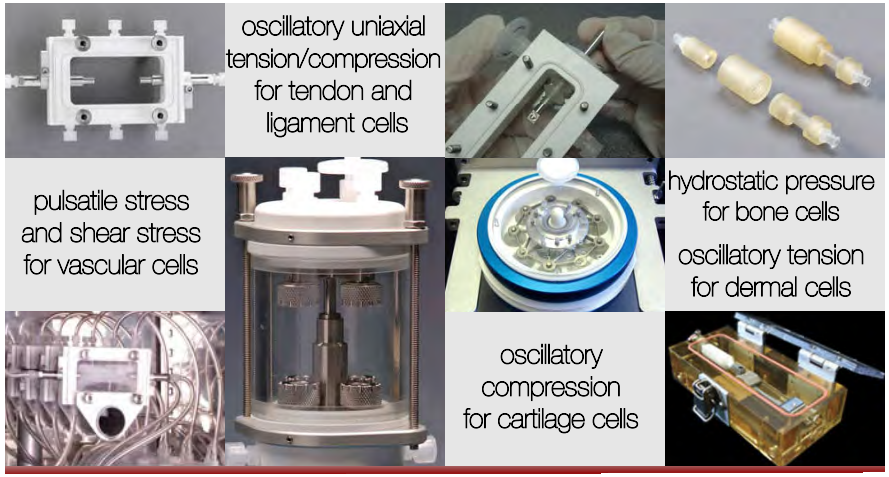
probing mechanotransduction



in their physiological environment, cells are subjected to **various combinations of mechanical stimuli** and it is difficult to predict which stimulus is responsible for which change within the cell. in an attempt to better understand the response of the cell to individual mechanical stimuli, experiments are performed under **controlled laboratory conditions** in which different loading scenarios can be applied in a selective way. some of the classical devices that are used to **probe mechanotransduction in living cells** include the following tests.

- uniaxial and biaxial tension
- uniaxial and hydrostatic compression
- uniaxial and circumferential shear

probing mechanotransduction



me239 - midterm - may 22, 2012

ME239 Mechanics of the Cell — Midterm Exam

ME239 - Mechanics of the Cell

Instructions

A. This is an closed note / closed book exam allowing for one sheet of paper.
B. Multiple choice questions might have more than one correct answer.
C. Questions are not all worth the same number of points.
D. You have the full class period (75 minutes) to complete the exam.

Name _____ Student ID _____

1. Multiple choice questions may have more than one correct answer
32 points, 1 point per correct answer

1f. What are the assumptions of the Euler Bernoulli beam theory?

- ☒ Upon deformation, normals remain straight.
- ☒ Upon deformation, normals remain unstretched.
- ☐ Upon deformation, normals remain unrotated.
- ☒ Upon deformation, normals remain normal.

2. Complete the following texts. 32 points, 1 point per correct answer

2e. van Kármán shell theory

In the van Kármán shell theory, strains can be decomposed into a constant and a linear contribution. Constant strains are related to *in plane* deformations and lead to a *second order* partial differential equation. Linear strains are related to *transverse* deformations and lead to a *fourth order* partial differential equation.

2. Complete the following texts. 32 points, 1 point per correct answer

2f. Mechanotransduction

Mechanotransduction is the process of converting *mechanical forces* into *chemical signals*. Mechanotransduction consists of three phases (state the technical terms and/or describe them with your own words):

(i) *mechanoreception*
(ii) *intracellular signal transduction*
(iii) *target activation*

To test a particular cell type, three loading scenarios can be studied to probe mechanotransduction. *tension* can be applied by seeding cells on biocompatible sheets and stretching them at both ends. *shear* is typically applied to probe osteocytes or endothelial cells in flow chambers. *compression* can be applied to test cells such as chondrocytes in a hydrostatic or uniaxial setting.

3.-7. Problems like the homework problems 6-12 points each

In class, we have assumed microtubules to be solid cylinders with a Young's modulus of $E = 1.9 \cdot 10^9 \text{N/m}^2$ and a radius of approximately $r^{\text{solid}} = 12.5\text{nm}$. We have calculated their cross section area $A^{\text{solid}} = \pi r^{\text{solid}\,2}$ to $A^{\text{solid}} = \pi\,(12.5\text{nm})^2 = 491\ \text{nm}^2$ and their moment of inertia $I^{\text{solid}} = 1/4\,\pi\,r^4$ to $I^{\text{solid}} = 1/4\,\pi\,(12.5\ \text{nm})^4 = 19{,}175\ \text{nm}^4$. Actually this was an oversimplification! In reality, microtubules are hollow cylinders. The outer and inner radii have been determined to $r^{\text{outer}} = 13.5$ nm and $r^{\text{inner}} = 11.5$ nm.

1.1 Calculate the cross section area $A^{\text{hollow}} = \pi[\,r^{\text{outer}\,2} - r^{\text{inner}\,2}\,]$ of microtubules when considered as a hollow cylinders.

1.2 Calculate the moment of inertia $I^{\text{hollow}} = 1/4\pi[\,r^{\text{outer}\,4} - r^{\text{inner}\,4}\,]$ of microtubules when considered as a hollow cylinders.

1.3 Calculate the radius r^{solid} of an imaginary solid cylinder which would have the same cross section area as microtubules.

1.4 Calculate the moment of inertia of I^{solid} of this imaginary solid cylinder of equal cross section area.