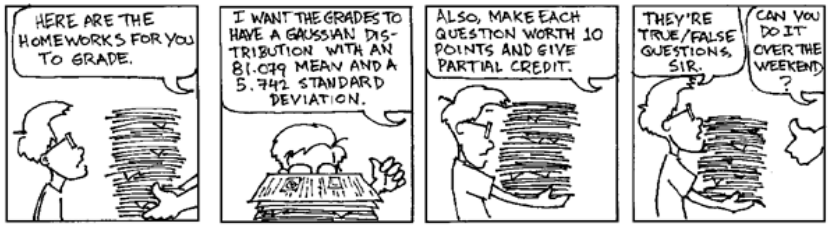
3. biopolymers

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

Homework I - Mechanical stiffness

due 04/19/12, 12:50pm, edu 128

Late homework can be dropped in a box in front of Durand 217. Please mark clearly with date and time @drop off. We will take off 1/10 of points for each 24 hours late.



biopolymers

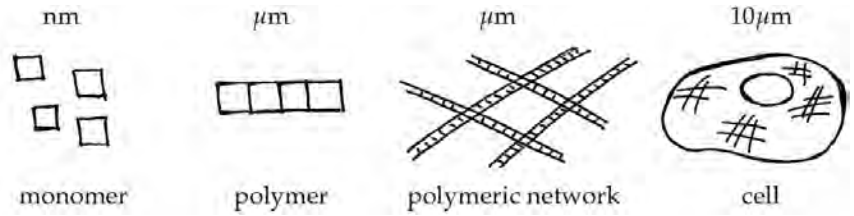


Figure 3.1. Biopolymers. Characteristic length scales on the cellular and sucellular level..

biopolymers

biopolymers are made up of **monomers** and **polymers**. monomers are smaller micromolecules such as nucleic acids, amino acids, fatty acid, and sugar. assembled together as repeating subunits, monomers form long macromolecules which are referred to as polymers.

typical examples of biopolymers

- genes: RNA and DNA
- gene products: peptides and proteins
- biopolymers not coded by genes: lipids, polysaccharides, and carbohydrates

biopolymers are **extremely flexible**. upon **thermal fluctuations**, they may bend from side to side and jiggle around. this is the nature of **soft matter** related to the notion of **entropy**.

the cytoskeleton

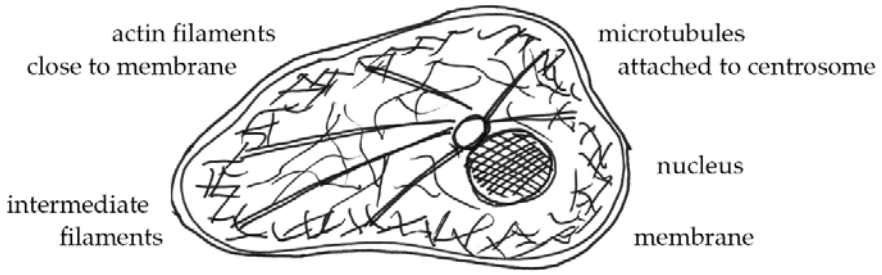


Figure 1.3. Eukaryotic cytoskeleton. The cytoskeleton provides structural stability and is responsible for force transmission during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

actin filaments

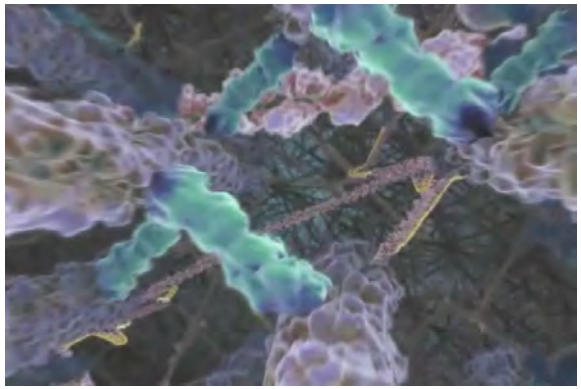


Figure 1.4.1 Actin filaments form tight parallel bundles which are stabilized by cross-linking proteins. Deeper in the cystol the actin network adopts a gel-like structure, stabilized by a variety of actin binding proteins.

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]

the cytoskeleton

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.

axial deformation - tension

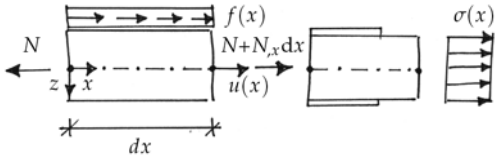
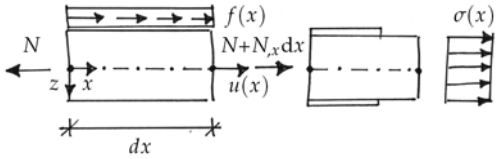


Figure 3.1: Axial loading of one dimensional structure ∘ Stresses σ are constant across the cross section

- ∘ 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



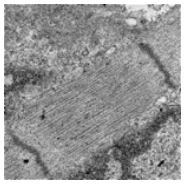
$EA\,u_{,xx} + f = 0$ with EA ... axial stiffness

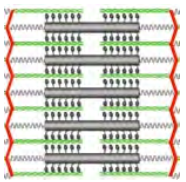
cross section area $A = \pi\, r^2$

	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

axial deformation - tension





Example Determine the elongation of an active muscle with Young's modulus $E = 40\text{MPa} = 4 \cdot 10^7\text{N/m}^2$, a cross section of $A = 1000\text{mm}^2 = 10^{-3}\text{m}^2$ and a total length $l = 10\text{mm} = 0.01\text{m}$! Assume that the muscle is loaded by a weight of $m = 10\text{kg}$. What is its elongation Δl and its its strain ε? ∘ The force acting on the muscle is $N = m\,g$ with the acceleration due to gravity $g = 10\text{m/s}^2 = 10\text{N/kg}$, thus $N = 10\text{kg}\cdot 10\text{N/kg} = 100\text{N}$. The elongation Δl then follows as $\Delta l = \varepsilon l = \sigma l / E = N l / [EA] = 100\text{N} \cdot 0.01\text{m} / [4 \cdot 10^7\text{N/m}^2 \cdot 10^{-3}\text{m}^2] = 2.5 \cdot 10^{-2}\text{mm}$. The strain simply follows as $\varepsilon = \Delta l / l = 2.5 \cdot 10\text{mm} / 10\text{ mm} = 0.0025 = 0.25\%$.

tension vs bending - trusses vs beams

	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

tension vs bending - trusses vs beams

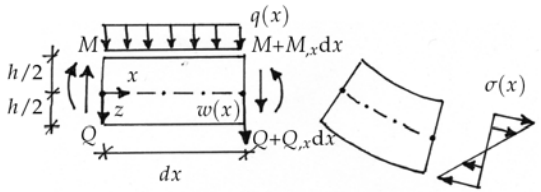


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

overall deformation = axial + transverse deformation

$$u^{\text{tot}}(x,z) = u(x) - z\,w(x)_{,x}$$
$$\varepsilon = u^{\text{tot}}_{,x} = u_{,x} - z\,w_{,xx}$$

- axial deformation $u(x)$
- transverse deformation, scaled rotation of beam axis $z\,w(x)_{,x}$

transverse deformation - bending

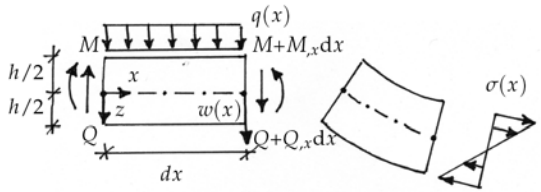


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

euler bernoulli beam theory

- normals remain straight (they do not bend)
- normals remain unstretched (they keep the same length)
- normals remain normal (they remain orthogonal to the beam axis)

transverse deformation - bending

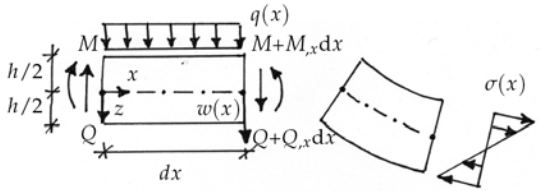
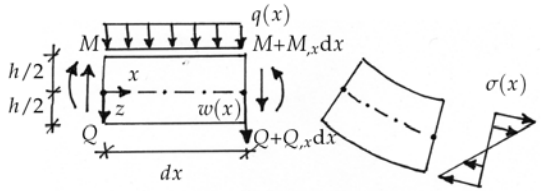


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

transverse deformation - bending



$$q = EI\,w_{,xxxx} \qquad \text{with} \qquad EI \;\ldots \text{bending stiffness}$$

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

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

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

bending stiffness of microtubules
hawkins, mirigian, yasar, ross [2010]

mechanics of microtubules

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Mechanics of microtubules

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ABSTRACT

Microtubules are rigid cytoskeletal filaments, and their mechanics affect cell morphology and cellular processes. For instance, microtubules for the support structures for extended morphologies, such as axons and cilia. Further, microtubules act as tension rods to pull apart chromosomes during cellular division. Unlike other cytoskeletal filaments (e.g., actin) that work as large networks, microtubules work individually or in small groups, so their individual mechanical properties are quite important to their cellular function. In this review, we explore the past work on the mechanics of individual microtubules, which have been studied for over a quarter of a century. We also present some prospective on future endeavors to determine the molecular mechanisms that control microtubule rigidity.

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mechanics of microtubules

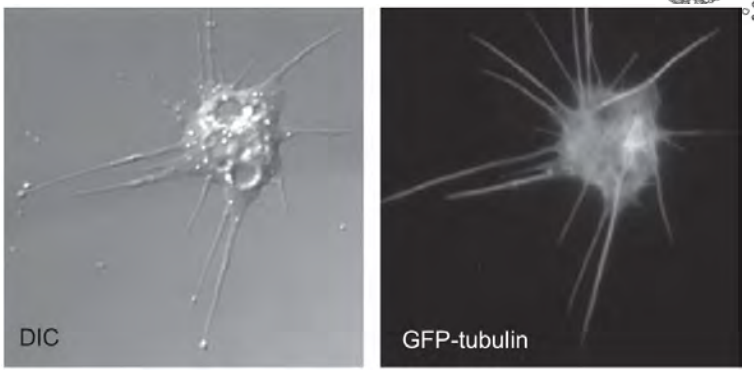


Figure 1. Microtubules as cellular supports. Microtubules are support structures for the cell. When S2 cells are depleted of actin filaments, long, mircotubule-filled processes remain. The outline of the cell is clearly seen in differential interference contrast microscopy (DIC) on the left. Fluorescence imaging of GFP-tubulin reveals that long extensions are supported by microtubules. Before actin was depleted, the cells were almost perfectly round.

hawkins, mirigian, yasar, ross [2010]

mechanics of microtubules

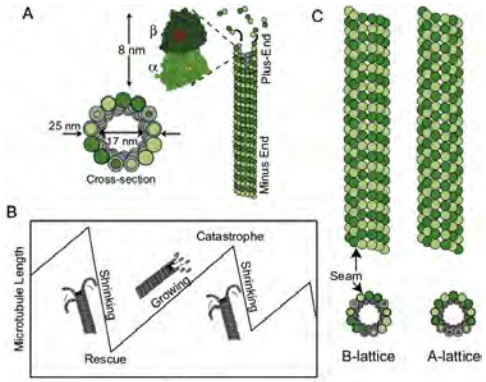
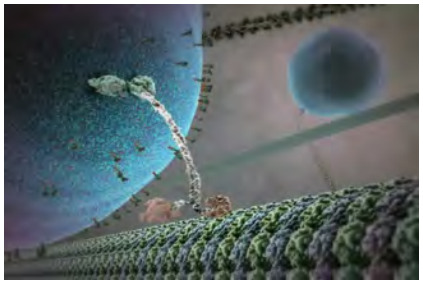


Figure 2. Microtubule structure and dynamics. Microtubules are polymer filaments made from tubulin dimers. The tubulin heterodimer is made of a beta (dark) and alpha (light) subunit. A few hundred dimers bind together to nucleate the polymer, and individual dimers add on to the ends to growt the microtubule. The plus end is the more dynamic and rapidly growthing and shrinking end, the minus end is less dynamic.

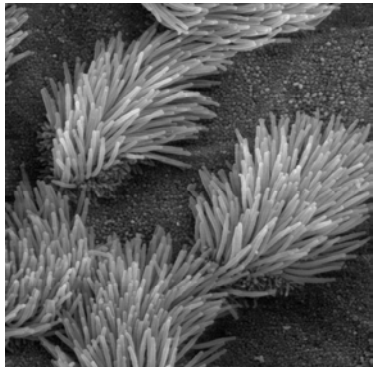
hawkins, mirigian, yasar, ross [2010]

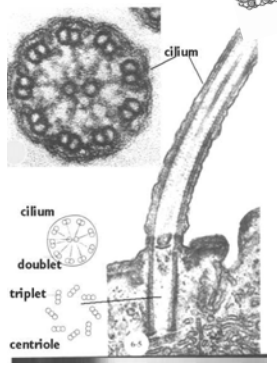
mechanics of microtubules



- essential structural elements, outline cell shape
- serve as support for extended morphologies
  axons, dendrites, and cilia
- enable efficient, long-range transport
- work individually instead of as a network

mechanics of microtubules - cilia

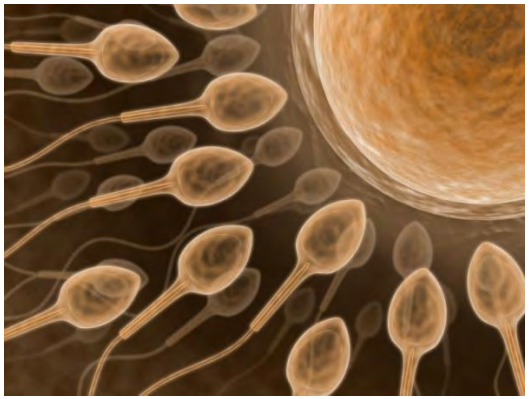




Scanning electron microscope image of lung trachea epithelium. Inside cilia and flagella is a microtubule-based cytoskeleton called the axoneme. The axoneme of primary cilia typically has a ring of nine outer microtubule doublets, and the axoneme of a motile cilium has two central microtubule doublets in addition to the nine outer doublets. The axonemal cytoskeleton acts as a scaffolding for various protein complexes and provides binding sites for molecular motor proteins such as kinesin II, that help carry proteins up and down the microtubules.

daghlian [2006]

mechanics of microtubules - flagella



Though eukaryotic flagella and motile cilia are ultrastructurally identical, the beating pattern of the two organelles can be different. In the case of flagella, e.g., the tail of a sperm, the motion is propeller-like. In contrast, beating of motile cilia consists of coordinated back-and-forth cycling of many cilia on the cell surface.

Problem 1

In class, we have assumed microtubules to be solid cylinders with a Young's modulus of $E = 1.9 \cdot 10^9 \mathrm{N/m^2}$ and a radius of approximately r^{solid} =12.5nm. We have calculated their cross section area $A^{\mathrm{solid}} = \pi\, r^{\mathrm{solid}\,2}$ to $A^{\mathrm{solid}} = \pi\,(12.5\mathrm{nm})^2$ = 491 nm^2 and their moment of inertia $I^{\mathrm{solid}} = 1/4\,\pi\, r^4$ to $I^{\mathrm{solid}} = 1/4\,\pi\,(12.5\ \mathrm{nm})^4$ = 19,175 nm^4. Actually this was an oversimplification. In reality, microtubules are hollow cylinders. The outer and inner radii have been determined to r^{outer} =14 nm and r^{inner} =11 nm.

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

1.2 Calculate the moment of inertia $I^{\mathrm{hollow}} = 1/4\pi[\, r^{\mathrm{outer}\,4} - r^{\mathrm{inner}\,4}\,]$ 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.

Problem 2

To gain a better understanding of the bending stiffness of microtubules, consider microtubules as cantiliver beams of length $L = 5\mu$m, clamped at the centrosome end and loaded by a point load F at the other end. We are interested in the transverse force F that creates a beam deflection of $w = 1\mu$m on the free end.

2.1 Compare the forces needed to deform microtubulues when considered as hollow cylinders (use the moment of inertia I^{hollow} calculated in the previous problem) with the forces needed to deform an imaginary solid cylinder of equal volume (use the value I^{solid} calculated at the end of the previous problem).

2.2 Discuss the results! Why, you think, does nature prefer hollow structures over solid structures?

Hints: To solve this problem, you might need the equation for the Euler Bernoulli beam $EI\, w_{,xx} - M = 0$ as derived in class. In addition, you need to know that the bending moment for a cantiliver beam is $M = [L - x]F$. Combine this equation with the beam equation. You then need to integrate the beam equation twice. To determine the integration constants, you need to use the boundary conditions of a cantiliver $w(0) = 0$ and $w'(0) = 0$. Solve the final equation for the force F for the different moments of inertia I^{hollow} and I^{solid}!

Problem 3

The 2006 manuscript *"Matrix elasticity directs stem cell lineage specification"* by Engler, Sen, Sweeney, and Discher discusses the importance of mechanical environments during stem cell differentiation. Read the manuscript carefully.

3.1 Summarize the manuscript in no more than 150 words.

3.2 What are the three cell types discussed in this manuscript? Make a table to compare (i) their elastic stiffnesses, (ii) their microstructural appearances, and (iii) their cellular functions. Feel free to consult other sources of information to complement the table, i.e., cell images from the web, etc.

3.3 Figure 1c) given below shows microarray profiling for cells cultured on matrices with different stiffnesses. Compare the first column of each of the three marker sets. Explain the findings in less than 100 words.

3.4 For each of the three columns, pick one of the specific markers, look it up, and explain in two or three sentences what its upregulation means for the cell. Example: *MYOD1 is a protein with a key role in regulating muscle differentiation. MYOD1 is one of the earliest markers of myogenic commitment. It is upregulated in MSCs cultured on 11 kPa stiff gels indicating the lineage specification towards muscle cells.*

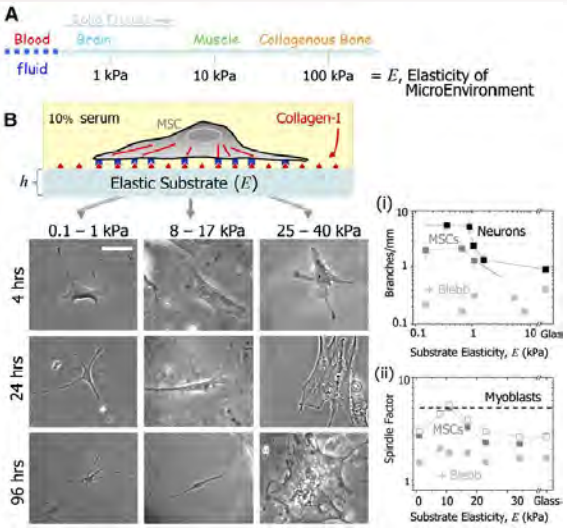
3.5 Discuss the impact of the major findings in this manuscript on stem cell therapies.

Matrix Elasticity Directs Stem Cell Lineage Specification

Adam J. Engler,[1,2] Shamik Sen,[1,2] H. Lee Sweeney,[1] and Dennis E. Discher[1,2,3,4,*]

abstract. microenvironments appear important in stem cell lineage specification but can be difficult to adequately characterize or control with soft tissues. native mesenchymal stem cells are shown here to specify lineage and commit to phenotypes with extreme sensitivity to tissue level elasticity. soft matrices that mimic brain are neurogenc, stiffer matrices that mimic muscle are myogenic, and comparatively rigid matrices that mimic collagenous bone prove osteogenic. during the initial week in culture, reprogramming of these lineages is possible with addition of soluble induction factors, but after several weeks in culture, cells commit to the lineage specified by matrix elasticity, consistent with the elasticity-insensitive commitment of differentiated cell types. inhibition of nonmuscular myosin II blocks all elasticity directed lineage specification - without strongly perturbing many other aspects of cell function and shape. the results have significant implications for understanding physical effects of the in vivo microenvironment and also for therapeutic use of stem cells.

engler, sen, sweeney, discher [2006]



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