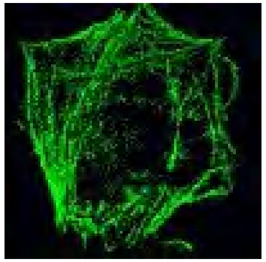
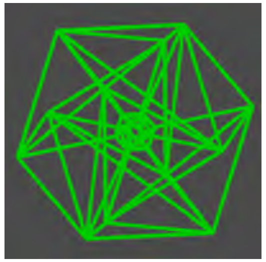


me239
introduction



me239 mechanics of the cell 1

ME 239 - Cell Mechanics

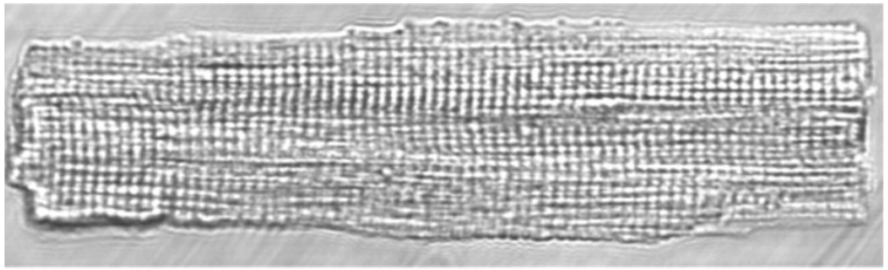
Tue/Thu 12:50-2:05pm, edu 128

Ellen Kuhl, ekuhl@stanford.edu, Durand 217
Manuel Rausch mkrausch@stanford.edu, Durand 226

Cells are the fundamental building blocks of life. The understanding of their characteristic biological features, their motility, their biochemistry and their interaction with the environment is crucial when cells are to be applied, modified or engineered in health care and modern medical therapies. This class focuses on the mechanical aspects of the cell, which can be two fold: On the one hand, cell biology and biochemistry influence the mechanical properties of the cell. On the other hand the mechanical environment, load, pressure, stress, or strain can influence the cell's shape and integrity, and eventually its biology and biochemistry. In the first part of this class, we will discuss how cellular properties can be measured experimentally and how they can be characterized in the form of equations. We will elaborate concepts of energy and entropy for different structural units of the cell: biopolymers, i.e., microtubules, actin, and intermediate filaments and biomembranes, i.e., the lipid bi-layer that forms the cell membrane. To explore the cell's behavior in silico, we will introduce computational simulation tools. In the second part, we address aspects of mechanotransduction. We discuss different aspects of how cells sense loads and how signals are transmitted within the cell and through the extracellular matrix.

me239 mechanics of the cell - overview 2

mechanogenomics - can we engineer better cells?

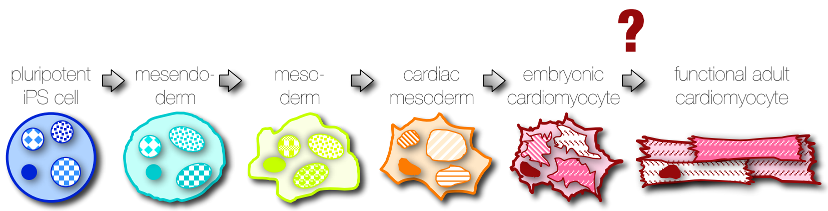


can we make functional cardiomyocytes? 

isolated functional adult cardiomyocyte from inya-agha, klauke, davies, smith, cooper [2007]

me239 mechanics of the cell - motivation 3

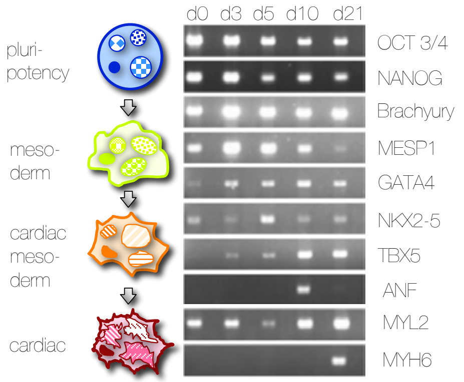
stem cell differentiation - from pluripotent to functional



functionality is a major road block in stem cell research

me239 mechanics of the cell - motivation 4

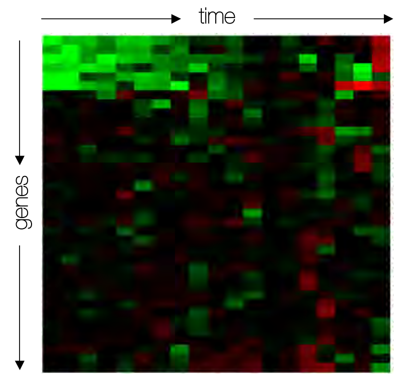
fundamental building blocks - quantification



qPCR - up- & downregulation of specific markers

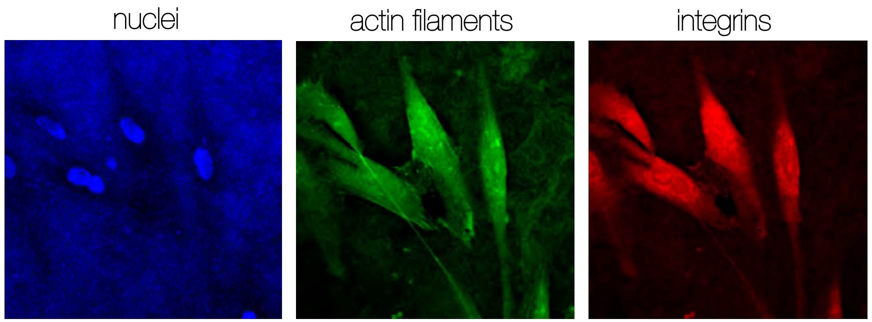
mauritz, schwanke, reppel, neef, katsimtaki, maier, nguemo, menke, haustein, hescheler, hasenfuss, martin [2008]

fundamental building blocks - quantification



microarrays - up- & downregulation of specific markers

fundamental building blocks - structural arrangement



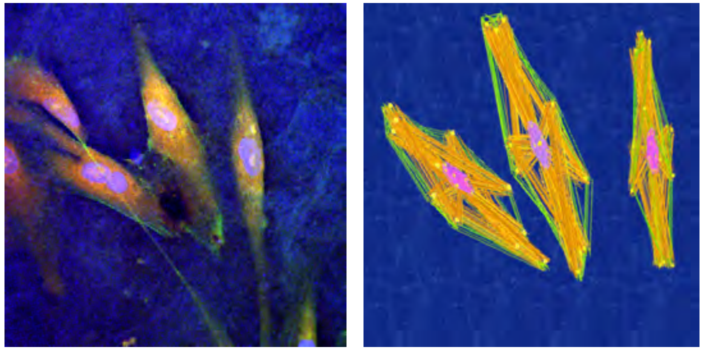
imaging - identification of cellular microstructure

hESC-derived cardiomyocytes - courtesy of jayakumar rajadas

fundamental building blocks - assembly

and that's why we need engineers!

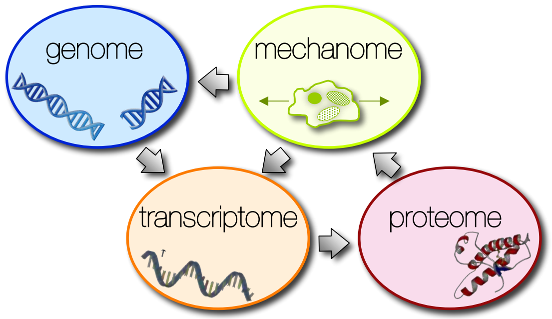
fundamental building blocks - assembly



in vitro measurement vs *in silico* prediction

hESC-derived cardiomyocytes - courtesy of jayakumar rajadas

and that's why we need engineers!



functionality is controlled through the mechanome

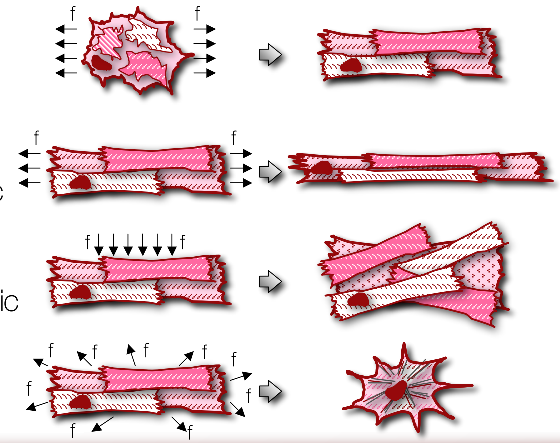
functionality is controlled through the mechanome

#1 differentiation
from embryonic
to functional adult

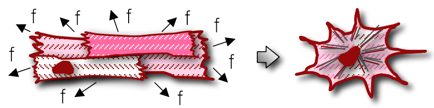
#2 heart failure
from healty to
eccentric hypertrophic

#3 wall thickening
from healthy to
concentric hypertrophic

#4 dedifferentiation
from functional adult
back to embryonic



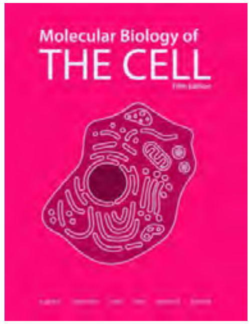
#4 dedifferentiation - from functional adult to embryonic



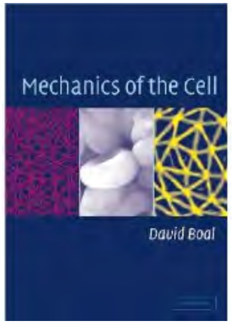
in long term culture cells revert to embryonic phenotype

bugaisky & zak [1989]

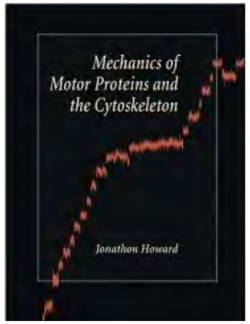
… additional reading - some textbooks on cell mechanics…



alberts et al. [2008]



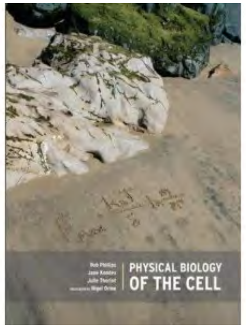
boal [2002]



howard [2001]

me239 mechanics of the cell - literature 13

… additional reading - *the* textbook on cell mechanics…



phillips, kondev, theriot [2008]

me239 mechanics of the cell - literature 14

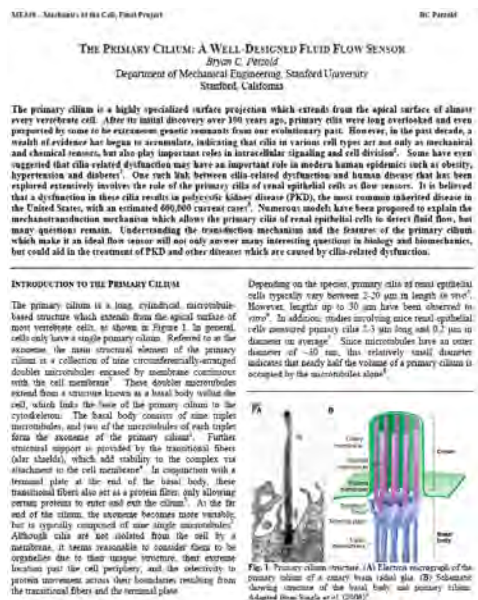
	Grading	
Homework	30 %	three homework assignments, 10% each
Midterm	30 %	one single letter format page of notes allowed
Final Project	20 %	oral presentations graded by the class,
Final Project	20 %	written essay graded by myself ;-)

Tue 05/22 **Midterm**

Tue 06/05 **Final projects**
Oral presentations evaluated by the class

Fri 06/08 **Final projects due**
Written essays due

me239 mechanics of the cell - grading 15



ME239 - Mechanics of the Cell, Final Project BC Petzold

THE PRIMARY CILIUM: A WELL-DESIGNED FLUID FLOW SENSOR

Bryan C. Petzold

Department of Mechanical Engineering, Stanford University

Stanford, California

The primary cilium is a highly specialized surface projection which extends from the apical surface of almost every vertebrate cell. After its initial discovery over 100 years ago, primary cilia were long overlooked and even purported by some to be extraneous genetic remnants from our evolutionary past. However, in the past decade, a wealth of evidence has begun to accumulate, indicating that cilia in various cell types act not only as mechanical and chemical sensors, but also play important roles in intracellular signaling and cell division. Some have even suggested that cilia-related dysfunction may have an important role in modern human epidemics such as obesity, hypertension and diabetes. One such link between cilia-related dysfunction and human disease that has been explored extensively involves the role of the primary cilia of renal epithelial cells as flow sensors. It is believed that a dysfunction in these cilia results in polycystic kidney disease (PKD), the most common inherited disease in the United States, with an estimated 600,000 current cases. Numerous models have been proposed to explain the mechanotransduction mechanism which allows the primary cilia of renal epithelial cells to detect fluid flow, but many questions remain. Understanding the transduction mechanism and the features of the primary cilium which make it an ideal flow sensor will not only answer many interesting questions in biology and biomechanics, but could aid in the treatment of PKD and other diseases which are caused by cilia-related dysfunction.

INTRODUCTION TO THE PRIMARY CILIUM

The primary cilium is a long, cylindrical, microtubule-based structure which extends from the apical surface of most vertebrate cells, as shown in Figure 1. In general, cells only have a single primary cilium. Referred to as the axoneme, the main structural element of the primary cilium is a collection of nine circumferentially-arranged doublet microtubules encased by membrane continuous with the cell membrane. These doublet microtubules extend from a structure known as a basal body within the cell, which links the base of the primary cilium to the cytoskeleton. The basal body consists of nine triplet microtubules, and two of the microtubules of each triplet form the axoneme of the primary cilium. Further structural support is provided by the transitional fibers (alar sheets), which add stability to the complex via attachment to the cell membrane. In conjunction with a terminal plate at the end of the basal body, these transitional fibers also act as a protein filter, only allowing certain proteins to enter and exit the cilium. At the far end of the cilium, the axoneme becomes more variable, but is typically composed of nine single microtubules. Although cilia are not isolated from the cell by a membrane, it seems reasonable to consider them to be organelles due to their unique structure, their extreme location past the cell periphery, and the selectivity to protein movement across their boundaries resulting from the transitional fibers and the terminal plate.

Depending on the species, primary cilia of renal epithelial cells typically vary between 2-20 μm in length in vivo. However, lengths up to 30 μm have been observed in vitro. In addition, studies involving mice renal epithelial cells measured primary cilia 2-3 μm long and 0.2 μm in diameter on average. Since microtubules have an outer diameter of ~30 nm, this relatively small diameter indicates that nearly half the volume of a primary cilium is occupied by the microtubules alone.

Fig. 1. Primary cilium structure. (A) Electron micrograph of the primary cilium of a canary brain radial glia. (B) Schematic drawing structure of the basal body and primary cilium. Adapted from Singla et al. (2006).

me239 mechanics of the cell - final project 16

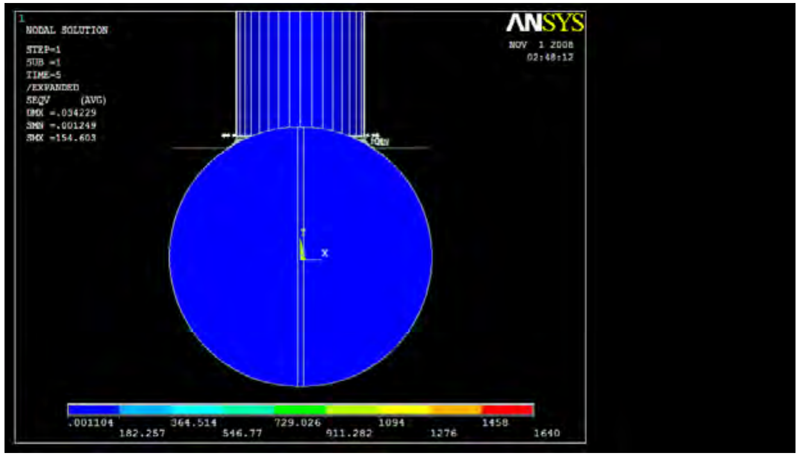

Mechanisms of Neuron Repair and Stem Cell Neural Differentiation:

Neural Stem Cell Grafts and Current Advances in Spinal Cord Injury Research

José Solomon

me239 mechanics of the cell - final project 17

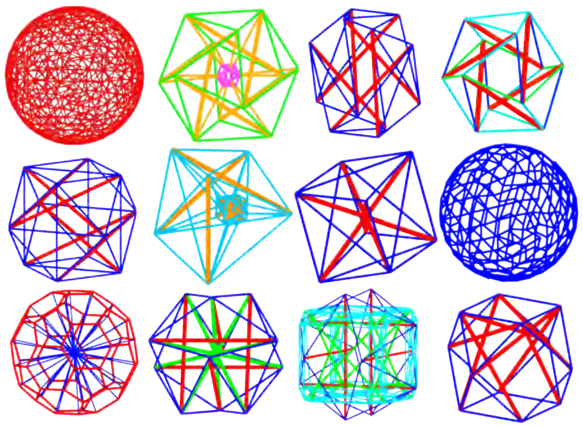
example: finite element simulation of pipette aspiration

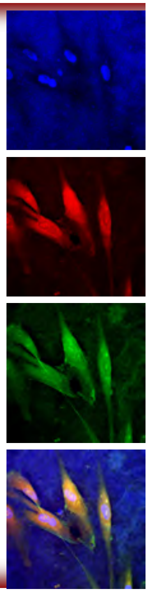


zubin huang [2007]

me239 mechanics of the cell - final project 18

cell design contest





joey doll, emily gu,lizzie hager-barnard, zubin huang, amir ali kia, monica ortiz, bryan petzold, yufen shi, sang do suk, takane usui, chun hua zheng

me239 mechanics of the cell - homework 19


Proceedings of the ASME 2008 Summer Bioengineering Conference (SBC2008)
June 25-29, Marriott Resort, Marco Island, Florida, USA

SBC2008-192407

EXPLORING CELLULAR TENSEGRITY:
PHYSICAL MODELING AND COMPUTATIONAL SIMULATION

Chun hua Zheng*, Joseph Doll*, Emily Gu†, Elizabeth Hager-Barnard‡, Zubin Huang*, AmirAli Kia*, Monica Ortiz†, Bryan Petzold, Yufen Shi, Sang Do Suk*, Takane Usui*, Ronald Kwon*, Christopher Jacobs*†, Ellen Kuhl*†

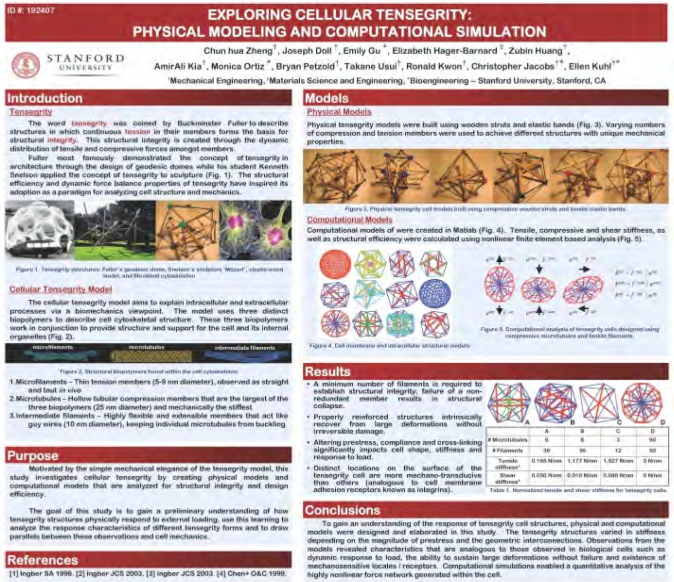
Department of Mechanical Engineering*
Department of Materials Science and Engineering‡
Department of Bioengineering†
Stanford University, Stanford, CA-94305, USA

TENSEGRITY: FROM ARCHITECTURE TO CELL MECHANICS

Figure 1. Tensegrity structures: Snelson's sculpture Mozart, elastic-wood model, and cytoskeleton of the cell.

Figure 2. Tensegrity in the cell: Cell membrane models and intracellular structural models.

me239 mechanics of the cell - homework 20



me239 mechanics of the cell - homework 21

add'l information http://biomechanics.stanford.edu and coursework

day	date		topic	notes	material
tue	apr	03	introduction I - cell biology		s01 q01
thu	apr	05	introduction II - cytoskeletal biology, stem cells	n02	s02 l02
tue	apr	10	introduction III - structural mechanics	n03	s03
thu	apr	12	biopolymers I - energy, tension, bending	n04	s04
thu	apr	12	homework I - biopolymers, directed stem cell differentiation	h01	m04
tue	apr	17	biopolymers II - entropy, FJC and WLC model	n05	s05
thu	apr	19	biopolymers III - polymerization kinetics in amoeba	n06	s06 m06
tue	jan	24	cytoskeletal mechanics I - fiber bundle model for filopodia	n07	s07 m07
thu	jan	26	cytoskeletal mechanics II - network model for red blood cells	n08	s08
thu	jan	26	homework II - cytoskeleton, cell mechanics challenges	h02	m10
tue	may	01	cytoskeletal mechanics III - tensegrity model for generic eukaryotic cells	n09	s09 m09
thu	may	03	biomembranes I - micropipette aspiration in white blood cells and cartilage cells	n10	s10
tue	may	08	biomembranes II - lipid bilayer, soap bubble, cell membrane	n11	s11
thu	may	10	biomembranes III - energy, tension, shear, bending	n12	s12
tue	may	15	mechanotransduction I - inter- and intracellular signaling, bone cells	n13	s13
tue	may	15	homework III - micropipette aspiration, final project	h03	m12
thu	may	17	summary and midterm preparation	n14	s14
tue	may	22	midterm		
thu	may	24	mechanotransduction II - electrophysiology in nerve cells	n16	s16
tue	may	29	mechanotransduction III - excitation contraction in skeletal muscle and heart cells	n17	s17
thu	may	31	mechanics of the cell - the inner life	n18	l01 l02
tue	jun	05	final projects - oral presentations	p02	
thu	jun	07	no class		
fri	jun	08	final projects - written projects due	p01	

me239 mechanics of the cell - syllabus 22

Introduction I - Cell biology
Overview of the cell
Biochemistry
Biopolymers
Biomembranes

Introduction II - Cytoskeletal biology
Cytoskeletal composition and structure
Regulating cell structure and function
Stem cells

Introduction III - Structural mechanics
Equilibrium - stress
Kinematics - strain
Material behavior – stress strain relation
Energy and entropy

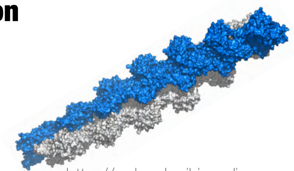

alberts et al. [2008]

me239 mechanics of the cell - introduction 23

Biopolymers I – Energy
Structural mechanics of biopolymers
Tension, bending, and buckling

Biopolymers II – Entropy
Introduction to statistical mechanics
Freely jointed chain model
Worm like chain model

Biopolymers III – Polymerization
Polymerization kinetics
Actin, tubulin, and microtubules
Treadmilling
Amoeba

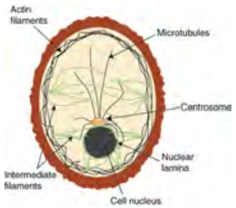

http://upload.wikimedia.org

me239 mechanics of the cell - biopolymers 24

Cytoskeletal mechanics I
Fiber bundle model
Filopodia

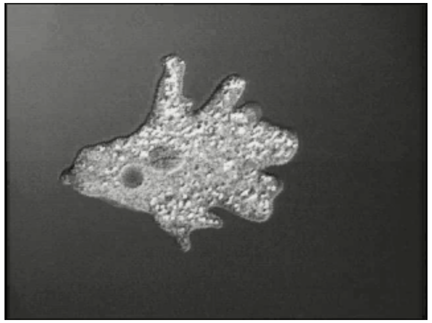
Cytoskeletal mechanics II
Chain network models
Red blood cells

Cytoskeletal mechanics III
Tensegrity models
Generic eukaryotic cells



http://img.sparknotes.com

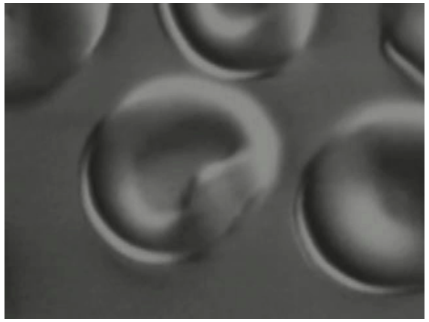
example: amoeba



this single-celled amoeba crawls around by using actin polymerization to push out pseudopods, or false feet, to explore new territory. at the same time, organelles move in complex patterns within the cell.

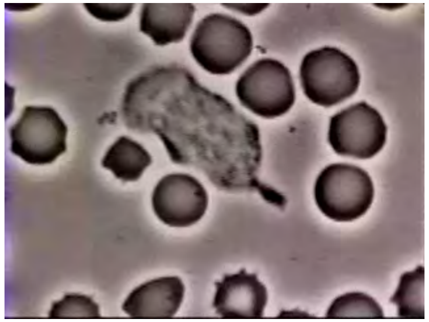
alberts et al. [2008]

example: red blood cells



red blood cells must deform when they squeeze through small blood vessels. In this experiment, a red blood cell is pushed and deformed with laser tweezers. it quickly springs back to its original shape because it has an extremely tough cytoskeleton to which the plasma membrane is anchored. when the cell is placed in high salt solution, however, the shape changes dramatically. driven by the difference in osmotic pressure, water rushes out of the cell causing spikelike protrusions to form as the cell collapses. alberts et al. [2008]

example: white blood cells

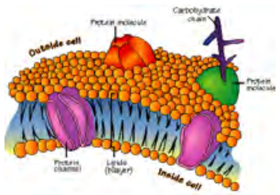


neutrophils are white blood cells that hunt and kill bacteria. in this spread a neutrophil is seen in the midst of red blood cells. a staphylococcus aureus bacterium has been added. the bacterium releases a chemoattractant that is sensed by the neutrophil. the neutrophil becomes polarized, and starts chasing the bacterium which, powered by its flagella, swims in a random path, seemingly avoiding its predator. eventually, the neutrophil catches up with the bacterium and engulfs it by phagocytosis. alberts et al. [2008]

Biomembranes I
Pipette aspiration
Laplace's law
Liquid drop model
White blood cells and cartilage cells

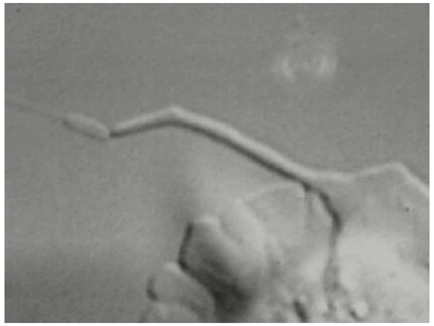
Biomembranes II
Lipid bilayers
Soap bubbles
Cell membranes

Biomembranes III
Mechanics of biomembranes
Tension, shear, and bending



http://library.thinkquest.org

example: neuronal cells

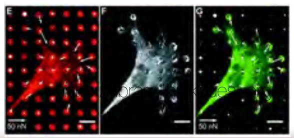


to demonstrate the fluidity of the lipid bilayer, a piece of the plasma membrane of this neuronal cell is pulled out with laser tweezers. remarkably, moving this membrane tubule rapidly back and forth does not rupture the plasma membrane, which flows quickly to adapt to the mechanical distortion. alberts et al. [2008]

Mechanotransduction I
Intercellular and intracellular signaling
Ion channels
Bone cells

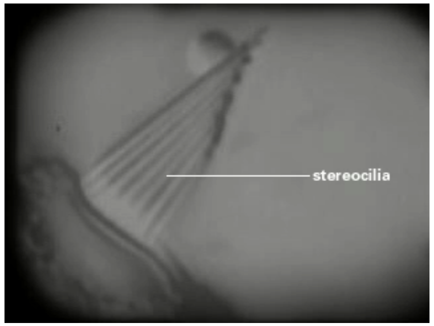
Mechanotransduction II
Electrical signaling and electrophysiology
Huxley Hodgkin model
Nerve cells

Mechanotransduction III
Electromechanical signaling and excitation contraction
FitzHugh Nagumo model
Skeletal muscle cells and heart cells



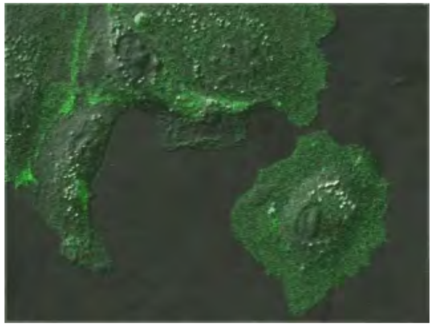
tan et al. [2003]

example: hair cells



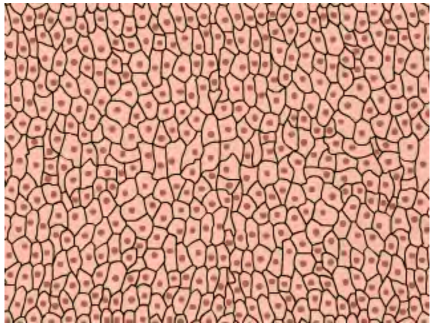
the stereocilia that project from hair cells vibrate in response to sound waves. here the bundle of stereocilia projecting from a single hair cell is pushed with laser tweezers to simulate this movement. movement opens stress-activated ion channels in the plasma membrane, leading to membrane depolarization. this is translated into the perception of sound. moving an individual stereocilium demonstrates the flexible attachment of these structures to the cell body. alberts et al. [2008]

example: epithelial cells



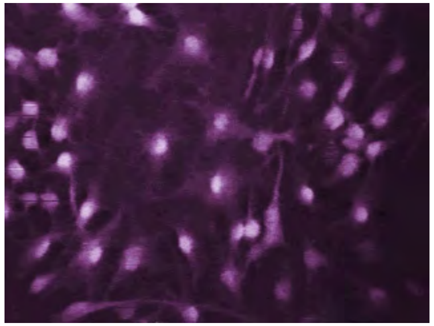
these epithelial cells express green fluorescent cadherin. they are grown at low density, so that isolated cells can be observed. Initially, labeled cadherin is diffusely distributed over the whole cell surface. as cells crawl around and touch each other, cadherin becomes concentrated as it forms the adhesion junctions that link adjacent cells. eventually, as the cell density increases further, the cells become completely surrounded by neighbors and form a tightly packed sheet of epithelial cells. alberts et al. [2008]

example: fibroblasts



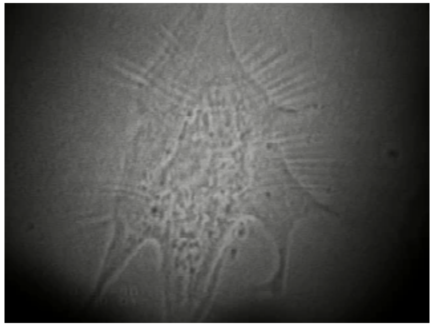
fibroblasts grown in vitro in a culture dish form a confluent monolayer of cells. cells in a monolayer are relatively static; contacting each other inhibits their migration. such cell layers can be wounded experimentally by scratching them with a needle. in such an experiment, we can observe that the fibroblasts at the edge of the wound become migratory and quickly move to repair the gap. such cell migration is important for wound repair in an intact organism. alberts et al. [2008]

example: glial cells



in this experiment, glial cells from the rat brain are grown in cell culture. calcium concentrations are visualized with a fluorescent dye that becomes brighter when calcium ions are present. in the presence of small amounts of a neurotransmitter, individual cells light up randomly as ion channels open up and allow calcium ions to enter the cell. occasionally, calcium waves are transmitted to adjacent cells through gap junctions at regions where the cells contact each other. alberts et al. [2008]

example: heart cells



single heart muscle cells spontaneously contract when grown in cell culture. This cell is grown on a flexible rubber substratum. each time the cell contracts, it pulls on the substratum which becomes wrinkled. although individual heart cells can beat with their own rhythms, they are coordinated in an intact heart so that all cells beat synchronously. alberts et al. [2008]

different cell types covered in class

01	all cells	filament growth
02	stem cells	differentiation is partially based on micro-environmental stimuli and growth factors, such as ECM
03	eukaryotic cells	tensegrity structure
04	amoeba	movement via polymerization
05	red blood cells	representative volume elements, 4-fold vs 6-fold network models, characteristic shape to fit through small cross sections, lack of shear resistance when going through capillaries
06	neutrophils (white blood cells)	liquid drop model, micropipette aspiration
07	chondrocytes (cartilage cells)	elastic solid model, micropipette aspiration
08	endothelial cells	elastic solid model, micropipette aspiration mechanotransduction, probing in flow chambers, shear
09	neurons (nerve cells)	really long length, communication via action potentials
10	skeletal muscle and cardiomyocytes (cardiac muscle cells)	mechanotransduction, ion channels, action potentials, contraction probe contractile forces on force posts
11	bone cells	density adaptation due to stress/loading conditions
12	skin cells	mechanotransduction in wound healing
13	hair cells	mechanotransduction through stereocilia

favorite topics in class - from last year's survey

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

… the 42 things to remember …

01 Even simple mechanics can give a lot of insight…
02 ... but different cell types can have totally different mechanical characteristics!
03 Most cells consist of a cytoskeleton and organelles embedded in a membrane.
04 And as always, energy minimization rulez!
05 ... but the free energy can consist of an energetic and an entropic contribution!
06 For jiggly filaments, the entropic term dominates the energetic term.
07 Biofilament entropy can be modeled by the statistics of long chain molecules.
08 Based on the chain shape uncorrelated or correlated chain models can be used.
09 Correlated chains can be characterized through the persistence length.
10 Polymerization governs the dynamic assembly and disassembly of filaments.
11 Cell movement is driven by filament assembly at the leading edge.
12 Treadmilling is the simultaneous growth and shrinkage at opposite filament ends.
13 Filament growth is limited by buckling when pushing against the outer envelope.
14 The Euler buckling modes explain filopodia buckling and filament crosslinking.
15 The interaction with the environment lowers the critical buckling length.
16 Homogenization can relate subcellular and cellular mechanical properties.
17 The flexible membrane of red blood cells can be modeled as a spring network.
18 Six fold networks explain the rigidity of red blood cells, four fold networks don't.
19 The cytoskeleton is made of microtubules, intermediate filaments and actin.
20 Cytoskeletal filaments possess a highly organized hierarchical mircostructure.
21 Tensegrity models view the cell as trusses tied together by pre-stressed ropes.

… the 42 things to remember …

22 Lightweight engineering structures use tensegrity concepts similar to some cells.
23 Membrane phospholipids consist of hydrophilic heads and hydrophobic tails.
24 The lipid bilayer is the energetically favorable configuration of phospholipids.
25 The Law of Laplace can describe both soap bubbles and cell membranes.
26 Surface tension is important in thin membranes and in micropipette aspiration.
27 Depending on their stiffness, cells can act as elastic solid or liquid drop.
28 Structural elements display in plane tension and shear and out-of-plane bending.
29 The tension and shear equation is of 2nd order, the bending equation of 4th order.
30 Mechanotransduction is the conversion of forces into biochemical signals.
31 Its complex cascades of biochemical events are illustrated in funny figures.
32 To improve understanding, it is usually probed in tension, compression, or shear.
33 The cell membrane is selectively permeable.
34 Membrane transport is passive along and active against concentration gradients.
35 Cells consist mainly of water with charged sodium, potassium, and chloride ions.
36 At the resting state, cells are negatively charged.
37 At rest, concentration gradient and membrane potential are balanced.
38 Action potentials are responsible for an all-or-none response of excitable cells.
39 Pacemaker cells continuously re-excite themselves, muscle cells usually don't.
40 Stem cells differentiate according to their mechanical environment.
41 Cell mechanics uses weird super large and super small units.
42 Cell mechanics still faces lots of exciting open problems that will be fun to solve!