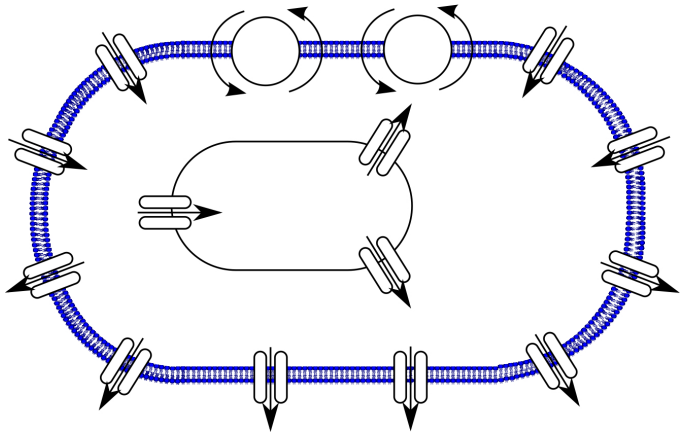
6 mechanotransduction



wong, goktepe, kuhl [2010]

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

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ME239 FINAL PROJECT

Jane Q. Public[1], John I. Doe[2], and G. I. Joe[3]
Department of Mechanical Engineering, Stanford University
Stanford, California

Abstract. The abstract should summarize what you did and what you learned quantitatively. Summarize the important results for easy reference. Don't just write something along the lines of "This paper describes the design of X, outlines the fabrication and testing methods, and analyzes expected performance." Instead be specific about the main features of the design, results of your analysis, and summarize key features of how you would make and test it. The summary should be substantive but generally should not include figures or references. Your paper should summarize expected device performance quantitatively; describe methods, materials, challenges of your design. Formatting and content descriptions are provided here.

Background. In this section, discuss what you set out to do, your design requirements, and compare and contrast to prior work.

The Annual Poster Sessions for Stanford University's E240, Introduction to Micro and Nano Electromechanical Systems (M/NEMS), will be held on December 3 and 5, 2008, from 2:15 to 3:45 pm on the steps of the Durand building on Stanford University Campus. Papers for each project should be submitted electronically as PDF files by 5pm Tuesday, December 2. These papers will be printed and bound into "ENGR240 Class Proceedings" and distributed at the poster sessions.

- Affiliation: 11 points, regular;
- City, State: 12 points, regular;
- Text body: 10 points, regular; paragraphs without indent
- Figure captions: 10 points, *italic*;
- Table captions: 10 points, *italic*;
- References: 10 points, regular, numbered in [ ].

Analysis of Performance. In this section, you should quantify the expected performance of your design and how you will test it. Justify your assumptions and compare expected performance to existing devices. Graphs, tables, figures summarizing these

downloadable grading criteria from coursework

ME 239 – Mechanics of the Cell

Final Project Presentations
Thu, May 27 & Tue, June 01, 2010

Instructions for Judges
according to ASME / SBC conference review guidelines

The presentation format includes the **structure of the presentaiton and its composition**. In general, a presentation should be structured to include an introduction, method, analysis, results, a conclusion, and references. The introduction should define the problem, scope of the study, and a brief background of previous work. The method section also should be brief to leave the majority of the report body for results and discussion. The final paragraph should be a brief paragraph on inference or conclusions reached.

Technical merit should be judged on the completeness of what is reported. For scientific studies, the result should support the conclusions presented. The key is validation of the express conclusion with results and data. Unsubstantiated conclusions or results should receive minimum points. However, not all papers represent basic research. Some papers present the design of a hardware system or a new software development. Both require the development of tests and measurement procedures to validate the product.

After the scoring is complete, please indicate a final grade. Please provide a comment in the designated area that describes why you think this presentation suitable/not suitable. These comments will be collected and provide to the students for feedback.

Is not necessary for the judge to be an expert in the field represented by the paper to evaluate its technical merit using these criteria. Subjective rating of the paper's scientific contribution is not encouraged unless there is evidence that the conclusions are incorrect. A judge should feel free to consult colleagues who are experts in the field, if you are unsure about the correctness of the conclusions. Since presentations can vary from hardware designs to software technique, or simulations and modeling to basic research, each reviewer will have to use his/her own best judgment about the technical merit of the work that is presented.

Mechanotransduction I

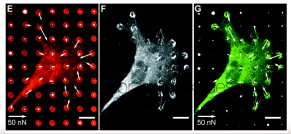
Mechanoreception, intracellular signaling, target activation
Probing mechanotransduction

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]

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

disease	dysfunctional cell type
deafness	hair cells in the inner ear
glaucoma, loss of vision	optical neurons
muscular dystrophy	myocytes, endothelial cells, fibroblasts
cardiomyopathy	cardiomyocytes
osteoporosis	bone cells
arteriosclerosis	endothelial cells, smooth muscle cells
immune system disorders	leukocytes
central nervous system disorders	neurons

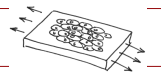
Table 6.1: Typical diseases associated with defects in mechanotransduction.

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

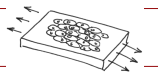
probing mechanotransduction



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 - tension



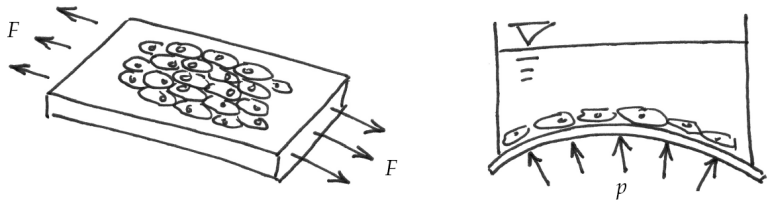


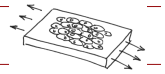
Figure 6.1: Uniaxial and biaxial tension devices stretching cells cultured on a thin sheet.

uniaxial tension

culture cells on a flexible thin sheet and stretch the sheet uniaxially

- advantage: relatively simple
- advantage: long sheets relatively homogeneous in loading dircetion
- disadvantage: lateral compression due to poisson's effect

probing mechanotransduction - tension



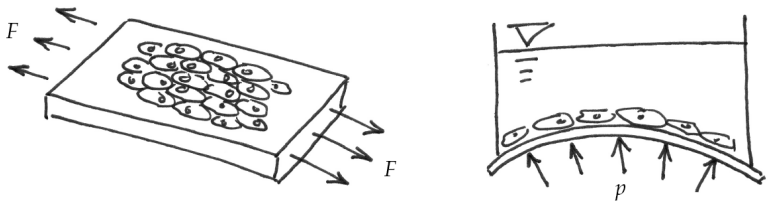


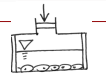
Figure 6.1: Uniaxial and biaxial tension devices stretching cells cultured on a thin sheet.

biaxial tension

culture cells on circular membrane and pressurize it from underneath

- advantage: ideally, all cells experience the same strain in all directions
- disadvantage: pure membrane state is difficult to achieve
- disadvantage: cell membrane needs to slide along frictionless support

probing mechanotransduction - compression



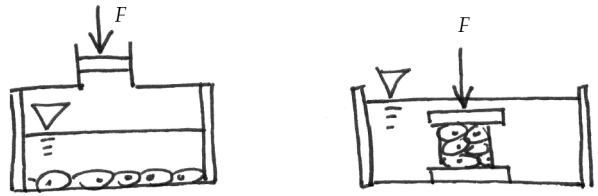


Figure 6.2: Hydrostatic and uniaxial compression devices compressing plain cells and cells in a matrix.

hydrostatic compression

culture cells in media and increase gas pressure in culture system

- advantage: ideally, all cells experience similar hydrostatic compression
- disadvantage:changes in gas composition affect chemical environment
- disadvantage: might affects cytoplasm rather than mechanoreceptors

probing mechanotransduction - compression

Figure 6.2: Hydrostatic and uniaxial compression devices compressing plain cells and cells in a matrix.

uniaxial compression

culture cells in 3d matrix and subject cell matrix to compressive loading

- advantage: mimics response of cells in their in vivo environement
- disadvantage: difficult to back out stress state of individual cells
- disadvantage: influence of poisson effect, matrix viscosity, fluid flow

probing mechanotransduction - shear

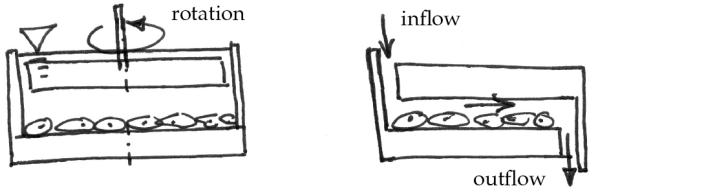


Figure 6.3: Circumferential and uniaxial flow devices applying shear stress to the cell culture.

circumferential flow

culture cells on flat plate and expose them to fluid flow by rotating disk

- advantage: single cells can be tested in fluidic environment
- disadvantage: rotational device generates inhomogeneous flow profile
- advantage: different shear profiles can be tested in one experiment

probing mechanotransduction - shear

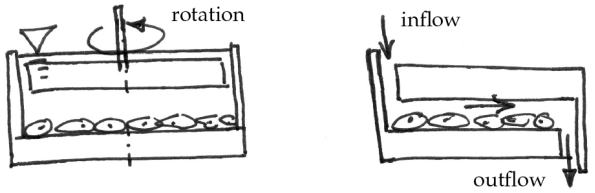


Figure 6.3: Circumferential and uniaxial flow devices applying shear stress to the cell culture.

uniaxial flow

culture cells on substrate and expose them to laminar flow field

- advantage: single cells can be tested in fluidic environment
- advantage: flow chambers can be studied under a microscope
- disadvantage: fully developed laminar flow might be non-physiological

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

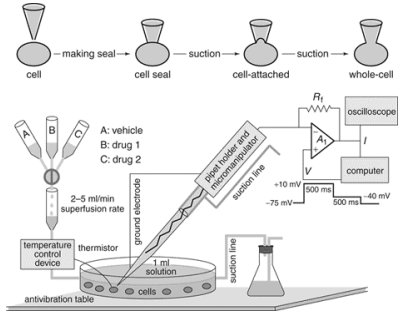
the cell membrane

the cell membrane contains water-filled pores with diameters of about 0.8nm and **protein-fined pores called channels** which allow for the **controlled passage** of specific molecules, in particular Na^+, K^+, and Cl^-. the phospholipid bilayer acts as a barrier to the free flow of these ions maintaining a well-regulated **concentration difference** across the cell membrane which is referred to as **membrane potential**. this implies that the membrane can selectively separate charge.

$$\phi = \phi^{\text{int}} - \phi^{\text{ext}} \qquad \text{... membrane potential}$$

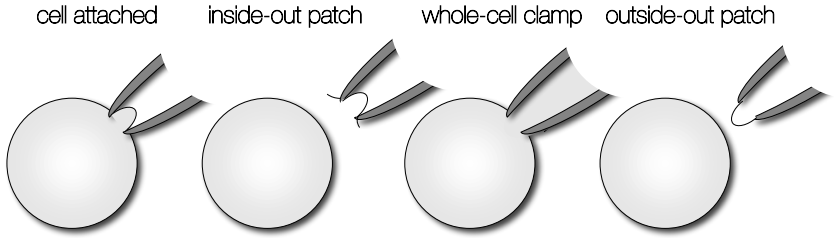
virtually all cells are **negatively charged**, i.e., their membrane potential is negative. but how can we measure membrane charge?

patch clamp



the experiment that allows the study of single or multiple ion channels is called **patch clamp**. it uses a glass **micropipette** to measure the membrane potential. the pipette can have a tip diameter of only 1um enclosing a membrane surface area or patch that contains one or just a few ion channels.

patch clamp



depending on the goal of the study, several variations of **patch clamp** technique can be applied. in **inside-out** and **outside-out** techniques the patch is removed from the main cell body. inside-out, outside-out, and **cell attached** techniques can be used to study the behavior of individual channels whereas **whole-cell clamp** is used to study the behavior of the entire cell.

membrane potential

	Na^+_{int} mM	Na^+_{ext} mM	K^+_{int} mM	K^+_{ext} mM	Cl^-_{int} mM	Cl^-_{ext} mM	resting pot. mV
nerve cell	50	437	397	20	40	556	ϕ =-65
skeletal muscle cell	13	110	138	2.5	3	90	ϕ =-99
cardiac muscle cell	10	145	135	4	25	140	ϕ =-90
red blood cell	19	155	136	5	78	112	ϕ =- 8

Table 6.2: Typical values for intracellular and extracellular concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions.

- why is there a potential difference across the cell membrane?
- what are the mechanisms that are responsible for generating, maintaining, and regulating membrane potentials?

membrane potential

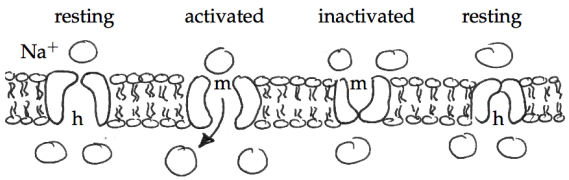


Figure 6.4: The fast sodium channel has two gates, an activation gate (m-gate) shown at the top and an inactivation gate (h-gate) shown at the bottom. In the resting state (left), activtion gates (m-gates) are closed and inactivation gates (h-gates) are open. Rapid depolarization opens voltage-gated m-gates enabling sodium to enter the cell (second from left). Upon repolarization, inactivation gates (h-gates) close to inactivate the channel (third from left).

- **passive** discontinuous transport through **ion channels**
- **active** continuous transport through **ion pumps**

membrane potential

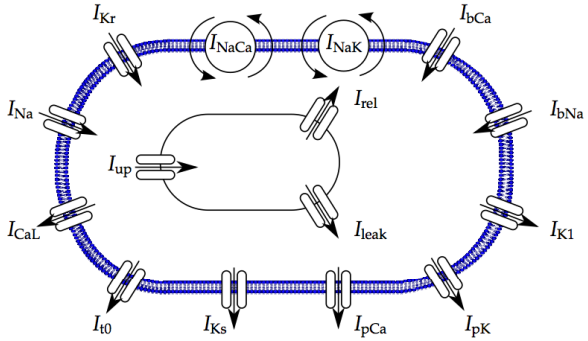


Figure 6.5: Human ventricular cardiomyocyte. In this model, the chemical state of the cardiomyocyte is characterized in terms of four ion concentrations: the free intracellular sodium, potassium, and calcium concentrations and the free calcium concentration in the sarcoplastic reticulum. Ion concentrations are controlled through 15 ionic currents,

wong, goktepe, kuhl [2010]

passive transport through ion channels

passive transport is driven by **directed diffusion** to equilibrate concentrations. it is directed **along concentration gradients**, from high to low.

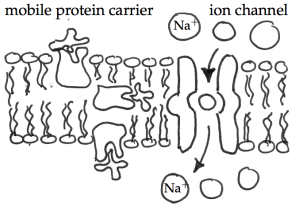


Figure 6.5: Passive transport through protein lined ion channel. Ion channels a specified for a particular class of ions and their pores are usually so small that only one ion can pass through it at a time.

- osmosis, transport of water through the membrane
- simple diffusion through pores and through lipid bilayer
- carrier-mediated diffusion by means of carrier molecules

passive transport through ion channels

ion channels are integrated membrane proteins through which ions can diffuse through the membrane. they can be either fully open or fully closed. ionic current is dependent on both **concentration gradient** and **membrane potential**.

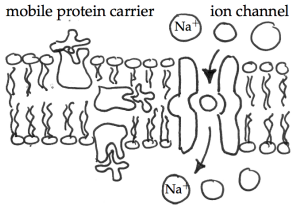
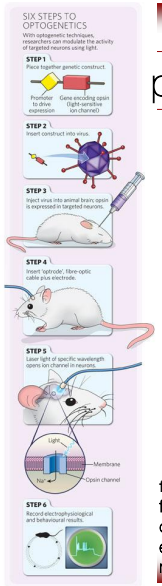


Figure 6.5: Passive transport through protein lined ion channel. Ion channels a specified for a particular class of ions and their pores are usually so small that only one ion can pass through it at a time.

- voltage-gated channels
- ligand gated channels
- mechanically gated channels
- light gated channels



passive transport through ion channels

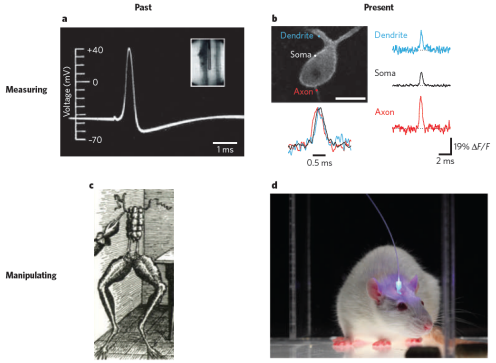


figure 1 recording and stimulation: past and present. **a** first action potential recorded intracellularly from a neuron inset, the electrode inserted into a giant squid axon [hodgkin, huxley 1939] **b** multisite optical recording of action potentials in a cerebellar purkinje neuron by using voltagesensitive dyes. **c** electrical stimulation of frog nerve [galvani 1791]. **d** optical deep-brain stimulation of neurons expressing microbial opsin genes [deisseroth lab, stanford, 2010]

active transport - ion pumps

active transport requires extra energy in the form of ATP. it is directed **against concentration gradients**, from low to high.

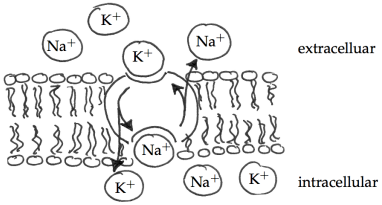
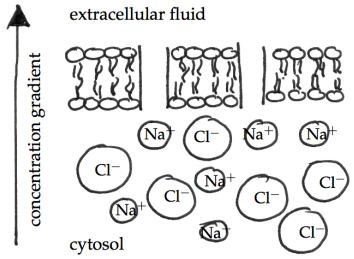


Figure 6.6: Active transport through cell membrane containing sodium/potassium pump. The Na^+/K^+ pump is the most important ion pump that consumes up to one third of the total energy requirement of a typical animal cell to actively pump cells against concentration gradients.

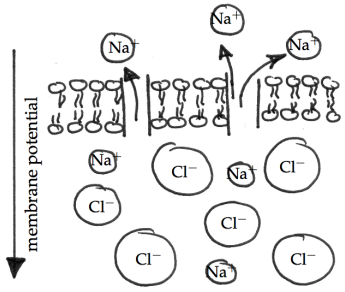
- example sodium potassium pump
- requires about 1/3 of all the energy of a typical animal cell

membrane potential



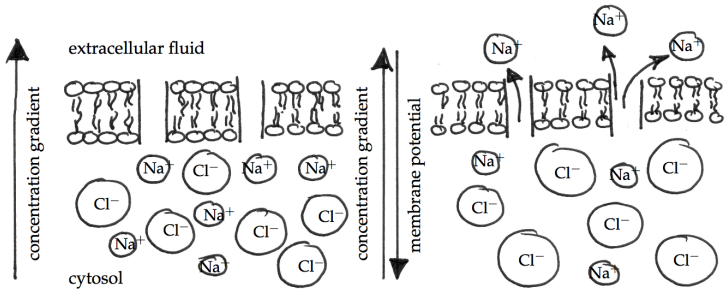
phase I electrically neutral state initially, both reservoirs contain the same ions, but at different concentrations. both sides are electrically neutral. each + ion is balanced with a - ion on each side of the membrane.

membrane potential



phase II selective permeability now the membrane is made permeable to sodium but not to chloride. concentration difference initiates passive transport of Na^+ along concentration gradients while Cl^- distribution remains unchanged.

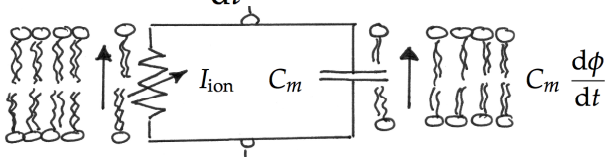
membrane potential



phase III resting state an equilibrium state is reached when concentration-gradient driven diffusion is balanced by membrane-potential driven forces that keep ions from diffusing

electric circuit model

$$C_m \frac{\mathrm{d}\phi}{\mathrm{d}t} + I_{\text{ion}} = 0$$



Here, $C_m = c_m A$ where c_m is the capacitance per area measured in farad per meter squared, i.e., $[c_m] = [\mathrm{F}/\mathrm{m}^2]$. The capacitance of the cell membrane is typically of the order of 0.01 - 0.1 F/m^2. Remember that one farad F is defined as the amount of capacitance for which a potential difference of one volt V results in a static charge of one coulomb C, i.e., $[F] = [C]/[V]$ and one coulomb C corresponds to $6.24 \cdot 10^{18}$ ions of elementary charge. Moreover, A is the membrane surface area, ϕ is the membrane potential, and I_{ion} is the ionic current. The most challenging task is to determine a good model for the ionic currents I_{ion} and we will address this issue later in this chapter.