Problem 1 - Filament buckling

In class, we have discussed filament buckling of actin polymers. We have seen that the critical buckling force of filopodia

\[ F_{\text{fil}} = \frac{\pi^2 EI}{2 L_{\text{fil}}^2} = \frac{\pi^2 EI}{4 L_{\text{fil}}^2} \]

can be increased by fascin which can create tightly crosslinked filament bundles.

- In class, we have assumed that there are about \( n = 30 \) actin filaments in one filopodium. Assume we do not know the exact number \( n \). What is the minimum number of actin filaments to obtain a critical filopodia length of \( L_{\text{fil}} = 5 \mu m \) assuming that the actin filaments are (i) loosely assembled (4.2.9) and (ii) tightly crosslinked (4.2.12).

- How would the minimum number of required actin filaments \( n \) change if you assume a critical filopodia length of \( L_{\text{fil}} = 2.5 \mu m \)?

Problem 2 - Forces on the cell membrane

To gain a better feeling for stresses that the cytoskeleton might induce on the cell membrane, this problem deals with the membrane pressure resulting from microtubules. Consider a generic spherical cell of radius 10 \( \mu m \) with a tubulin heterodimer concentration of \( C = n / V = 5 \mu M \).

**Figure.** Microtubules are made up of rings of thirteen tubulin heterodimers which are 8 nm in diameter. The rings of tubulin heterodimers form a cylinder of thirteen protofilaments.

- Calculate the total length of microtubules that could be made from this amount of protein if each heterodimer is approximately 8 nm in diameter. Take into account that microtubules are made up of rings of thirteen tubulin heterodimers. Remember that 1 \( \mu M = 1 \mu mol/liter \) and that 1 mol contains \( 6.02 \cdot 10^{23} \) heterodimers.
Problem 3 - Cell mechanics research

The recent manuscript “Biomechanics: Cell Research and Applications for the Next Decade” by Discher, Dong, Fredberg, Gulik, Ingber, Janmey, Kamm, Schmid-Schönbein and Weinbaum discusses the challenges for cell mechanics now and in the future.

- Read the manuscript carefully and summarize it with approximately 150 words.
- The authors have identified ten major accomplishments in cell mechanics. List all the ten accomplishments by title.
- Select your favorite past accomplishment and describe it in less than 100 words.
- The authors have identified seven major challenges for the future. List all the seven future challenges by title.
- Select your favorite future challenge and describe it in less than 100 words.

Problem 4 - Final project

Inspired by the recent manuscript on major accomplishments and challenges in cell mechanics,

- Identify a title for your final project.
- Identify five key words for your final project.
- Write a tentative abstract of approximately 150-200 words.

Papers in the past have generally been about 4-6 pages long, two columns, with about 3-5 figures and 8-12 references. Here are some examples of individual projects.

- Predicting microtubules structure using molecular dynamics
- The primary cilium: A well-designed fluid flow sensor
- The tensegrity paradigm
- Mechanotransduction in hair cells Translating sound waves into neural signals
- Modeling cell membrane dynamics
- Theoretical and experimental study of the penetration of the cell membrane
- Integrin and its role in mechanotransduction
- Finite element analysis of micropipette aspiration

ME339 FINAL PROJECT
THE ROLE OF CELL-CELL JUNCTIONS IN CARDIOMYOCYTE CONTRACTILITY AND MODIFICATIONS NEEDED IN MECHANICAL MODELS
Chelsey S. Simmons
Department of Mechanical Engineering, Stanford University
Stanford, California

ABSTRACT
As adult cardiomyocytes (CMs) cannot recover from damage due to pathologies like myocardial infarction, researchers are looking for ways to culture functional cardiomyocytes to repopulate diseased cardiac tissue. However, as damaged tissue may interfere with cell-cell communication, the role of cell-cell junctions in cardiomyocyte contractility must be considered. This paper examines current literature on CM contractility, evaluating first the role of the cytoskeleton in CM contractility and then the hypothesized role of cell-cell junctions. Additionally, the likelihood of adapting computational models to reflect abnormal conditions of cell-cell junctions is considered. Further examination of contractile force generation with modification of certain cytoskeletal and junctional proteins is recommended to facilitate incorporation into tissue-scale models and to understand the contribution of these various proteins to heart disease.
ABSTRACT Leukocyte extravasation is an innate immune system process by which white-blood cells leave the circulatory system and enter the inflamed tissue or site of infection. Although leukocyte migration across the walls of microvessels was observed over 200 years ago, the molecular mechanisms of the migration were not clarified until the early part of the twentieth century. Today, leukocyte extravasation is thought to occur in a multistep cascade involving the partial capture of the leukocyte by the endothelium, rolling of the leukocyte, slow rolling, arrest, adhesion and spreading of the white blood-cell, intraluminal crawling, and transmigration of the leukocyte across the endothelium. Over the past two decades, a number of molecular and mechanical models have surfaced that view the leukocyte as a shell membrane, a hard sphere defined by Goldman hydrodynamics and liquid droplets. By exploring each leukocyte model in the context of the extravasation process, a greater insight into the importance of integrin and selectin-based mechanotransduction as well as the shear-induced physics of extravasation can be gained. Such understanding can be particularly valuable in the context of drug and cancer development – two radically different processes that mimic leukocyte extravasation.

INTRODUCTION TO THE PRIMARY CLUM

The primary clump is a long, cylindrical, microbead-based structure which emerges from the surface of macrophage-like cells as shown in Figure 1. In general, cells only have a single primary clump. Failed to insert the main structural elements of the primary clump is a collection of non-covalently associated, distinct, highly flexible molecules used to maintain a tractional force. These beads bear a constant stress of tension that facilitates the uptake of the primary clump. In experiments with a tangential plane at the end of the cellular body, these tensional forces act to promote filopodia, only allowing certain properties to exist for the clump. As the free end, the clump becomes more spherical. The primary clump is a collection of primary filopodia. Although cells are not ordered from the cells by a mechanism, it is more reasonable to consider them to be regulated due to their unique structure, their extracellular matrix par for cell properties, and the specificity of the phospholipid-membrane scaffold resulting from the concentration and the tangential plane.

example: finite element simulation of pipette aspiration

Depending on the species, primary clump of macropipette cells typically vary between 2.5-30 mm in length in vitro, whereas lengths up to 80 mm have been observed in vivo. In general, the primary clump is a long, cylindrical structure with a diameter on average, above micrometer but below 30 μm. The uniformly small diameter indicates that nearly all the volume of a primary clump is occupied by the extracellular matrix.
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.

Figure 4.2.1. A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. The cell cortex underlies the plasma membrane. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments. 

three examples

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

elements of the cytoskeleton

- microtubules
- intermediate filaments
- actin filaments

four molecular level to cellular level

from molecular level to cellular level

microstructural arrangement of actin

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

Figure 4.2.1. A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. The cell cortex underlies the plasma membrane. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments.
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.

4.2 fiber bundle model for filopodia

Pushing the envelope - critical length

Newton’s third law: actio = reactio

\[ F_{\text{fil}} = \frac{\pi^2 EI}{2L^2} = \frac{\pi^2 EI}{4L} \]

\[ F_{\text{mem}} \approx 5 \sqrt{n} r_{\text{act}} \text{pN/nm} \]

\[ \frac{\pi^2 EI}{4L_{\text{crit}}} = 5 \sqrt{n} r_{\text{act}} \text{pN/nm} \]

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

\[ E = 1.9 \cdot 10^9 \text{N/m}^2 = 1.9 \text{GPa} \]

\[ r_{\text{act}} = 2.5 \]

- loose assembly
- tightly crosslinked

Case I - loosely assembled actin filaments

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

\[ n = 30 \text{filaments} \]

\[ L_{\text{crit}} = 0.416 \mu\text{m} \]

Much too low - disagrees with observations of 2um

4.2 fiber bundle model for filopodia

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
4.2 fiber bundle model for filopodia

\[
L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{EI}{5n r_{\text{act}}^4 \text{pN/nm}}}
\]

moment of inertia \( I \)
\[
I = \frac{\pi r_{\text{fil}}^4}{4} = n^2 \frac{\pi r_{\text{act}}^4}{4} \quad \text{with} \quad r_{\text{fil}} = \sqrt{n} r_{\text{act}}
\]

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

\[
L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{1.9 \cdot 10^9 \text{ N/m}^2 \cdot n^2 \pi / 4 [3.5 \cdot 10^{-9}]^4 \text{m}^4}{5 \sqrt{n} 3.5 \cdot 10^{-12} \text{ N}}} \approx 0.17769 \mu \text{m} \quad n = 30 \text{ filaments}
\]

better model - agrees with observations of 2\( \mu \text{m} \)

4.3 network model for red blood cells

human mature red blood cells are **flexible biconcave disks** that lack a cell nucleus and most organelles. typical human erythrocytes have a disk diameter of 6–8\( \mu \text{m} \), a thickness of 2\( \mu \text{m} \), a volume of 90fL, and a surface of 136\( \mu \text{m}^2 \). they can swell to spherical shape of 150fL, without membrane distension. the membrane of the red blood cell plays a key role in regulating **surface deformability**, **flexibility**, and adhesion to other cells. these functions are highly dependent on its composition. the red blood cell membrane is composed of 3 layers: the glycocalyx on the exterior, which is rich in carbohydrates; the lipid bilayer consisting of lipidic main constituents and transmembrane proteins; and the membrane skeleton, a **structural network of proteins** located on the inner surface of the lipid bilayer.

red blood cells

erythrocytes, red blood cells are essential to deliver oxygen to the body via the blood flow through the circulatory system. they take up oxygen in the lungs and release it while squeezing through the body’s capillaries. adult humans have about 2–3 \( 10^{13} \), 20–30 trillion, red blood cells comprising about a quarter of the total amount of cells in the human body.

**red blood cells**

Metabolic remodeling of the human red blood cell membrane

**PNAS**

Remarkable deformability of red blood cells

The concept of adaptive remodeling under stress

Mechanisms of this cooperation affect human health
The human red blood cell membrane skeleton is a network of roughly 33,000 protein hexagons that looks like a microscopic geodesic dome.

**4.3 network model for red blood cells**

**network model for red blood cells**

**4.3 network model for red blood cells**

**homogenization - hill-mandel condition**

**aim.** to determine the overall material properties $\kappa$ and $\mu$ of the network of spectrin chains in terms of the spectrin chain stiffness $k$

**Energy Approach**

$W_{\text{mac}} = W_{\text{mic}}$

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 focuses attention on strain energies.

4.3 network model for red blood cells

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

$$W^{spr} = \frac{1}{2} k \delta^2 = \frac{1}{2} k (l - l_0)^2$$

where $\delta = l - l_0$

unstretched spring

stretched spring

$k$ ... spring stiffness

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

discrete microscopic network energy

$$W^{mic} = \frac{1}{2} \sum_{i=1}^{3} W^{spr}_i$$

$$\sum_{i=1}^{3} W^{spr}_i = 3 W^{spr} = 3 \left( \frac{1}{2} k \delta^2 \right)$$

$h_0 = \frac{1}{2} \sqrt{3} l_0$

$$\sum_{i=1}^{3} A^{spr}_i = 3 A^{spr} = \frac{1}{2} \sqrt{3} l_0^2$$

$$W^{mic} = \frac{3}{2} \frac{k \delta^2}{\sqrt{3} l_0^2} = \sqrt{3} k \left( \frac{\delta}{l_0} \right)^2$$

extension

$$l = l_0 + \delta$$

4.3 network model for red blood cells

equivalent macroscopic energy

$$W^{mac} = \frac{1}{2} \kappa \left[ \frac{\epsilon_{xx} + \epsilon_{yy}}{l_0} \right]^2 + \frac{1}{2} \mu \left[ \epsilon_{xx} - \epsilon_{yy} \right]^2 + 2 \mu \epsilon_{xy}^2$$

micro-to-macro kinematics

$$\epsilon_{xx} = \epsilon_{yy} = \delta / l_0 \quad \epsilon_{xy} = 0$$

$$W^{mac} \overset{\text{def}}{=} W^{mic}$$

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

$$\kappa = \frac{1}{2} \sqrt{3} k$$

extension

$$l = l_0 + \delta$$

4.3 network model for red blood cells
4.3 network model for red blood cells

**Equivalent macroscopic energy**

\[ W^{\text{mac}} = \frac{1}{2} k \left[ \varepsilon_{xx} + \varepsilon_{yy} \right]^2 + \frac{1}{2} \mu \left[ \varepsilon_{xx} - \varepsilon_{yy} \right]^2 + 2 \mu \varepsilon_{xy}^2 \]

Micro-to-macro kinematics

\[ \varepsilon_{xx} = 0 \quad \varepsilon_{yy} = 0 \quad \varepsilon_{xy} = \frac{1}{2} \left( \frac{\delta}{\sqrt{3} l_0} + 0 \right) = \frac{1}{\sqrt{3} l_0} \delta \]

\[ W^{\text{mac}} \approx W^{\text{mic}} \]

\[ 2 \mu \left( \frac{1}{\sqrt{3} l_0} \delta \right)^2 = \frac{\sqrt{3}}{6} k \left( \frac{\delta}{l_0} \right)^2 \]

\[ \mu = \frac{1}{4 \sqrt{3} k} \]

**Discrete microscopic network energy**

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

\[ \sum_{i=1}^{3} W_i^{\text{spr}} = \frac{1}{2} k \left[ \frac{\delta}{\sqrt{3} l_0} + \frac{1}{2} \delta \right]^2 \]

\[ \sum_{i=1}^{3} A_i^{\text{spr}} = 3 A^{\text{spr}} = \frac{1}{2} \sqrt{3} l_0^2 \]

\[ W^{\text{mic}} = \frac{1}{2} \frac{k \delta^2}{\sqrt{3} l_0} = \frac{\sqrt{3}}{6} k \left( \frac{\delta}{l_0} \right)^2 \]

---

**Heterogeneous fluctuating rod models**

for unfolded proteins and application to fibrin networks

Biofilaments, such as, actin and DNA, have for long been modeled as thermally fluctuating elastic rods with homogeneous material properties. Such models are adequate if the length scale of the filaments being studied is much larger than the scale of the heterogeneity. However, advanced single molecule experimental techniques have now made it possible to probe the properties of biomolecules at the scale of a few nanometers. The data emerging from these experiments ought to be greeted with appropriately detailed models. In this presentation we study the mechanics of a thermally fluctuating elastic rod whose moduli are a function of position. Such a rod can be used as a model for DNA whose sequence specific properties are known or for a protein oligomer in an AFM where some of the monomers might be unfolded. The mechanics of these rods is understood by first evaluating a partition function through path integral techniques. Similar methods can also be applied to heterogeneous networks of filaments. In this presentation we will show how protein unfolding at the level of a single filament can determine the macroscopic mechanical behavior of a network.

**Mechanics & computation, me395, thu 02/03/11, 4:15-5:25, 320-105**