Homework #3

Problem #1 – The Power Stroke Model (20 pts)

The Power Stroke Model is a mechanical model of actomyosin interaction developed by Sir Andrew Huxley in order to quantitatively understand actin-myosin force generation. Earlier you explored the polymerization of actin. The polymerization of actin is helical and the pitch of this helix has been measured as Δ = 36 nm. This means the polymer completes one turn of the helix every 36 nm along its length. Each of these actin subunits has only one binding site for myosin heads. The distance a myosin molecule stretches when it is cocked in preparation for a power stroke, δ, is only ~ 5 nm. Thus, the myosin molecule can only occur once in each full twist. However, the idea here is that many of these molecules work together to produce a sliding. Thus, myosin molecules link adjacent actin polymers together (cross-bridge model). We also know this process is regulated by ATP/ADP.

We can estimate the duty ratio or the time myosin is engaged, as

$$r_{duty} = \delta/\Delta = 5 \text{ nm} / 36 \text{ nm} = 0.14$$

However, it is know that myosin actually doesn't even bind this frequently and this is based on the force generation capacity of the entire actin-myosin bundle. So then, let's consider the force generation by stating the following:

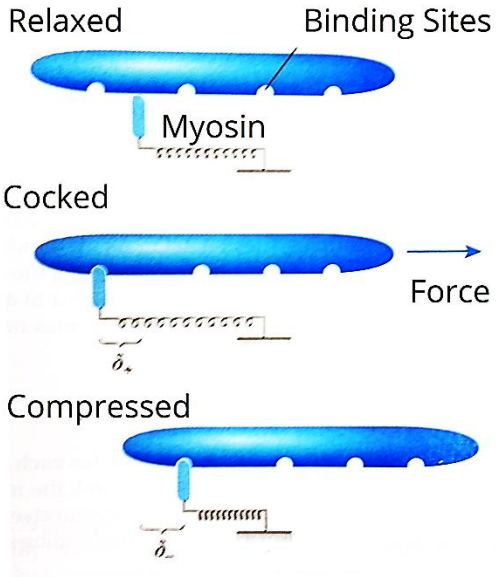
$$< F > = \frac{t_{on} < F_{on} > + t_{off} < F_{off} >}{t_{on} + t_{off}}$$

Here, we are saying the time average of the force is dependent on the on and off rates. However, the force generated when myosin is not engaged to actin must be zero $<F_{off}> = 0$. Thus, this simplifies:

$$< F > = \frac{t_{on}}{t_{on} + t_{off}} < F_{on} > = r_{duty} < F_{on} >$$

$$r_{duty} = \frac{\delta}{\Delta} = \frac{t_{on}}{t_{on} + t_{off}} = \frac{< F >}{< F_{on} >}$$

Now we have an idea of engagement of myosin with actin. Let's consider the power stroke model itself. We will treat the myosin heads like linear springs with some spring constant k_m. The model considers three possible positions (as seen below).



The spring moves forward by a distance δ_+ when it is cocked and imparts a force of $k_m\delta_+$ on the actin filament. In the "compressed position," the spring compresses by δ_- past the zero position before releasing acting. **Based on this illustration, can you figure out the average force $<F_{on}>$?** (2 pts)

$$< F_{on} > = \frac{k_m(\delta_+ - \delta_-)}{2}$$

With the expression you wrote down for force, the limiting condition below, and the previous expressions derived, **rewrite the expression for <F> in terms of k_m, δ_+, δ_-, and Δ.** (2 pts)

$$\delta = \delta_+ + \delta_-$$
$$< F > = r_{duty} < F_{on} >$$
$$< F > = \frac{\delta}{\Delta} \frac{k_m(\delta_+ - \delta_-)}{2}$$
$$< F > = \frac{(\delta_+ + \delta_-)}{\Delta} \frac{k_m(\delta_+ - \delta_-)}{2}$$
$$< F > = \frac{k_m}{2\Delta}\delta_+^2 - \frac{k_m}{2\Delta}\delta_-^2$$
$$< F > = F_+ - F_-$$

You should have derived a positive and negative term, or F_+ and F_-.

Let's see if we can use what you derived to relate it to sliding velocity. What does the positive term actually mean? It depends on distance that the myosin head move forward when cocked. At this point, most myosin heads are bound so there is no reason to expect that this would depend on velocity. The second term depends on distance the heads overshoot the zero force position before releasing. Thus, it seems reasonable to assume this compression distance depends on sliding velocity. Let's introduce a new parameter for release time, t_r. **Rewrite the <F> expression for sliding velocity. You can keep the first term as F_+.** (2 pts)

$$< F > = F_+ - \frac{k_m}{2\Delta}(vt_r)^2$$

Now, let's examine the force-velocity behavior. Which features are consistent with the Hill model? Which are not?

It is consistent with some of the features of the force-velocity behavior like the maximum force is at zero velocity and maximum contraction velocity is when force is zero. However, the drag predicted the model increased with the square of the sliding velocity and this is not consistent with Hill's model.

Let's resolve the issue. Consider Δ, the distance between myosin binding sites on actin. Let's call the minimum distance d_s. From the duty ratio argument, we expect Δ to exceed d_s by some amount but the myosin head and actin-binding site must align eventually. Therefore,

$$\Delta = nd_s \; where \; n \; is \; an \; integer.$$

To estimate what n might be, consider some effective sweet spot of width w_{bs}. The time available for binding within this width is inversely related to velocity.

$$t = \frac{w_{bs}}{v}$$

Further, we assume that while the myosin is in this sweet spot, it will bind actin at a constant on rate of k_+. **Write the rate equation for [M] based on previous lectures.** (2 pts)

$$\frac{d[M]}{dt} = -k_+[M]$$

Solve this differential equation, which was done in class to understand the Bell model for adhesive bonds. (2 pts)

$$[M](t) = [M(0)]e^{-k_+ t}$$

What you just solved is from the perspective of an individual myosin molecule and you can use it to define the probability of transitioning from an unbound to bound state in the time available for binding. **Do this here.** (2 pts)

$$p(t_{bs}) = 1 - e^{-k_+ t} = 1 - e^{-k_+ \frac{w_{bs}}{v}}$$

From this expression, what happens to the probability when the velocity is too high? (2 pts)

The probability gets very small and the myosin heads would pass by many potential binding spots without binding.

Consider another limiting case, Δ>>d and n is large. [Relatively few of the binding sites are occupied]. Because each binding site is separated from the next by a distance δ and the distance between an occupied site is Δ, **then the probability of a given head being bound is also given by…** (2 pts)

$$p(t_{bs}) = \frac{1}{n} = \frac{d_s}{\Delta}$$

Set your two expressions for probability equal to each other and solve for Δ. (2 pts)

$$\Delta = \frac{d_s}{1 - e^{-k_+ \frac{w_{bs}}{v}}}$$

Replace Δ in your expression for <F>. **Does this resemble Hill's model?** Try out the following parameters.

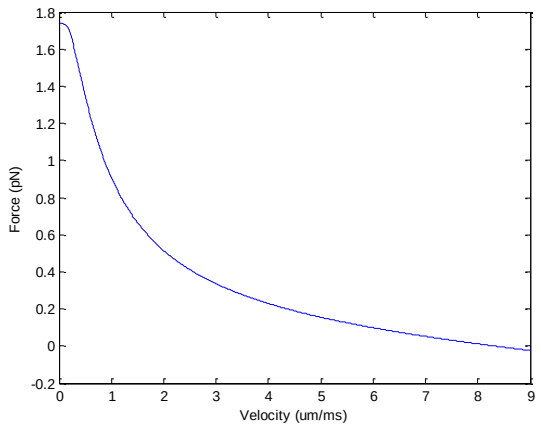
For these parameters, plot the curve. You'll find it is not precisely hyperbolic but within physiological range. (2 pts)

$$< F > = \frac{k_m}{2\Delta}\delta_+^2 - \frac{k_m}{2\Delta}\delta_-^2$$

$$< F > = \frac{k_m \delta_+^2}{2\Delta}(1 - \frac{\delta_-^2}{\delta_+^2})$$

$$< F > = \frac{k_m \delta_+^2}{2d_s}(1 - \frac{v^2 t_-^2}{\delta_+^2})(1 - e^{-k_+ \frac{w_{bs}}{v}})$$

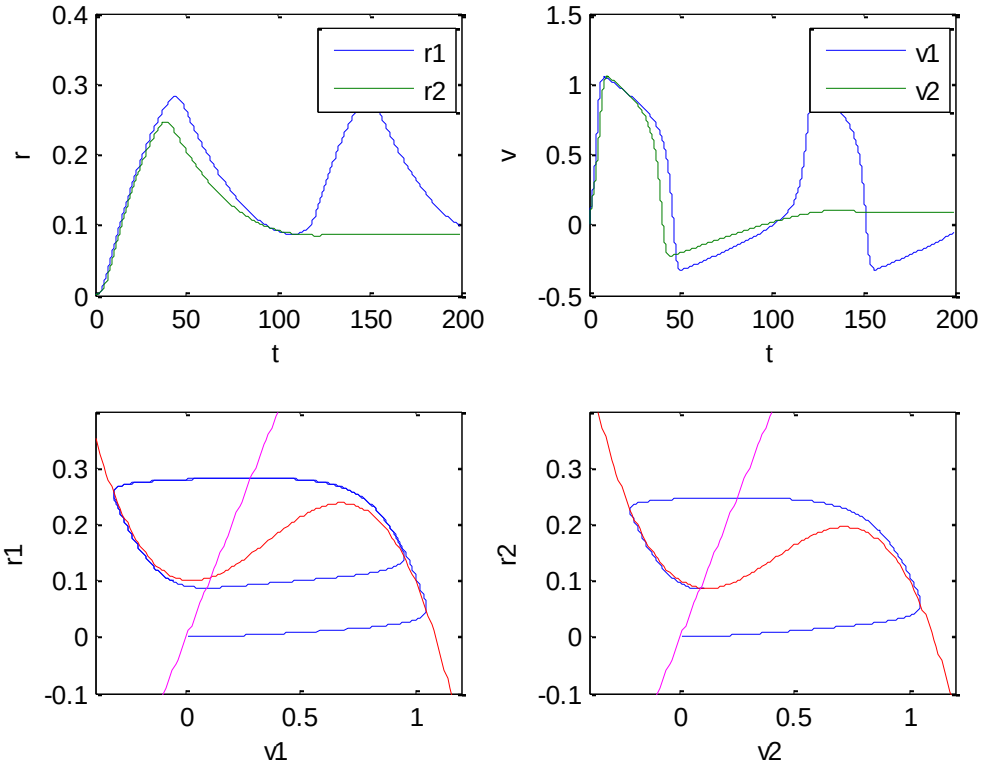
(2 pts for plot – units should be correct plot below with either per ms or per s and the x axis is multiplied by 1000)



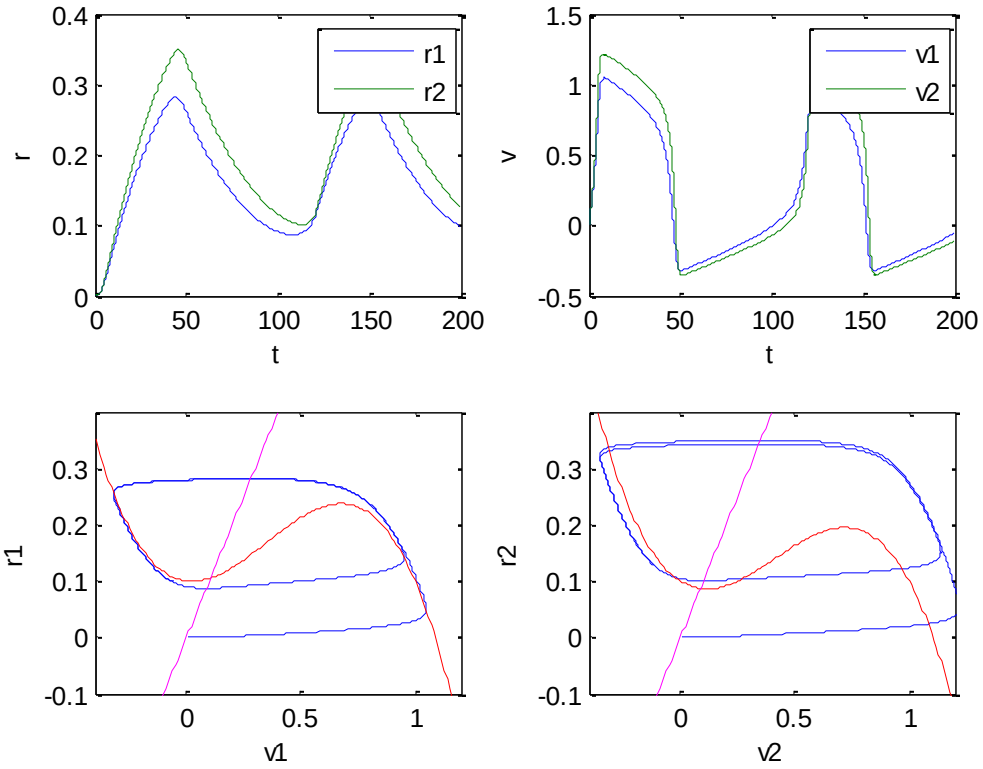
Not exactly Hill's model but resembles the hyperbolic behavior of F vs. velocity.

Problem #2 – Coupled Neurons Simulation (10 pts for correct plots, 10 pts for conclusions --- for plots, make sure people used the right initial conditions. An older version of code set one of the a's differently and changed the plots)

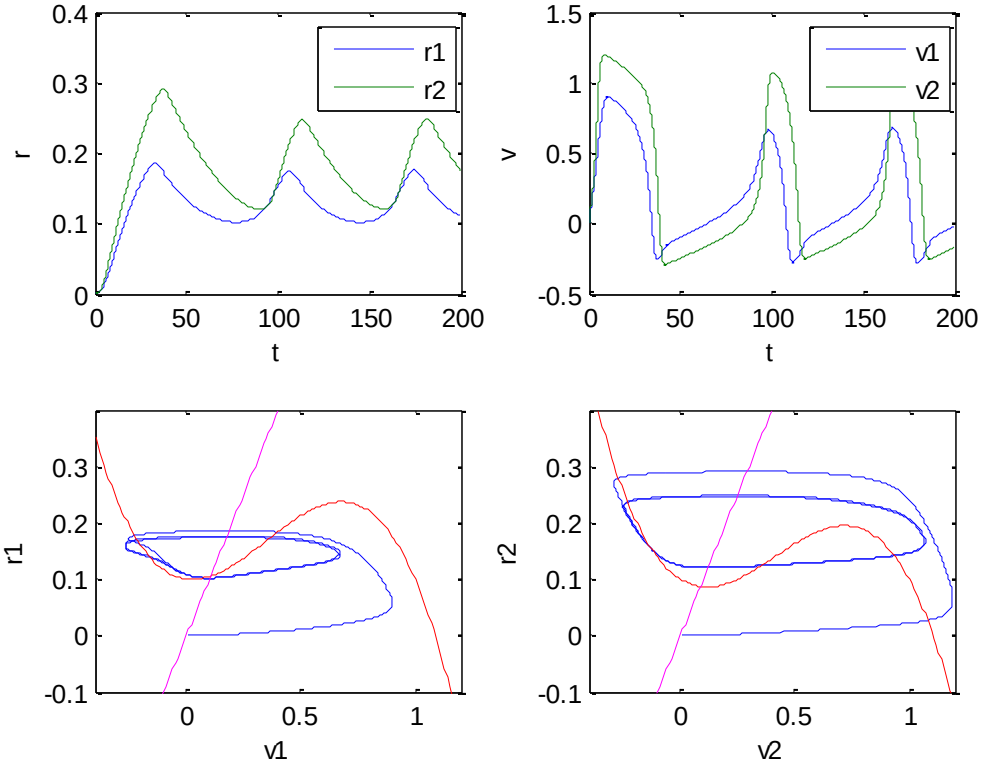
For (d_{12}, d_{21})= (0,0)



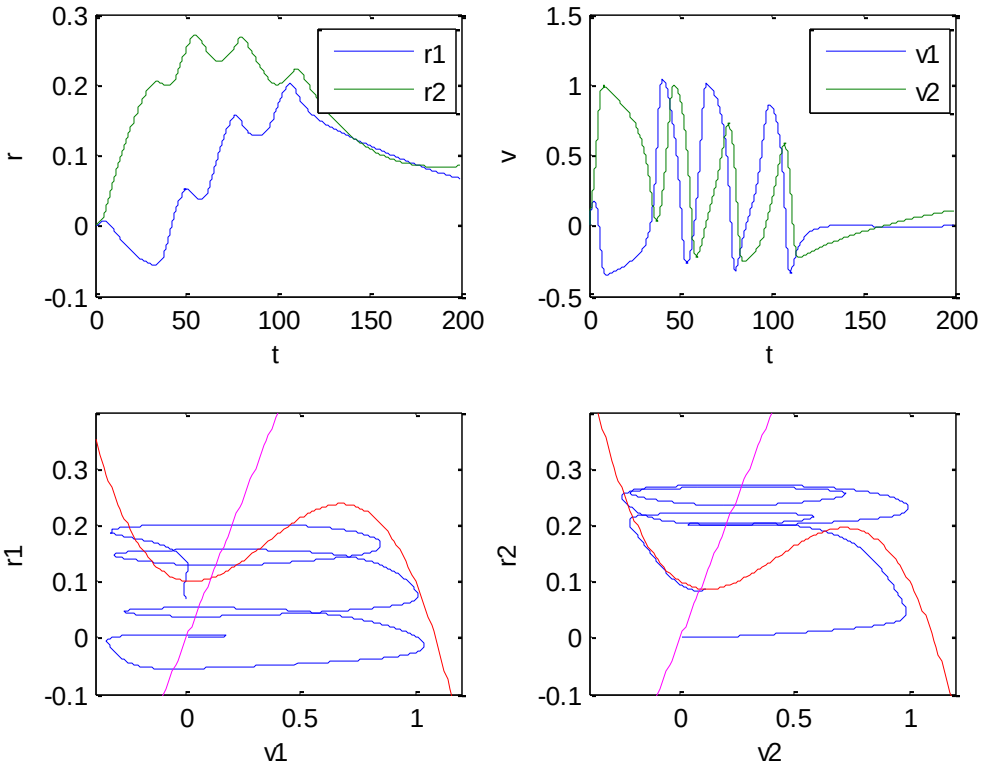
For (d_{12}, d_{21})= (0,0.2)



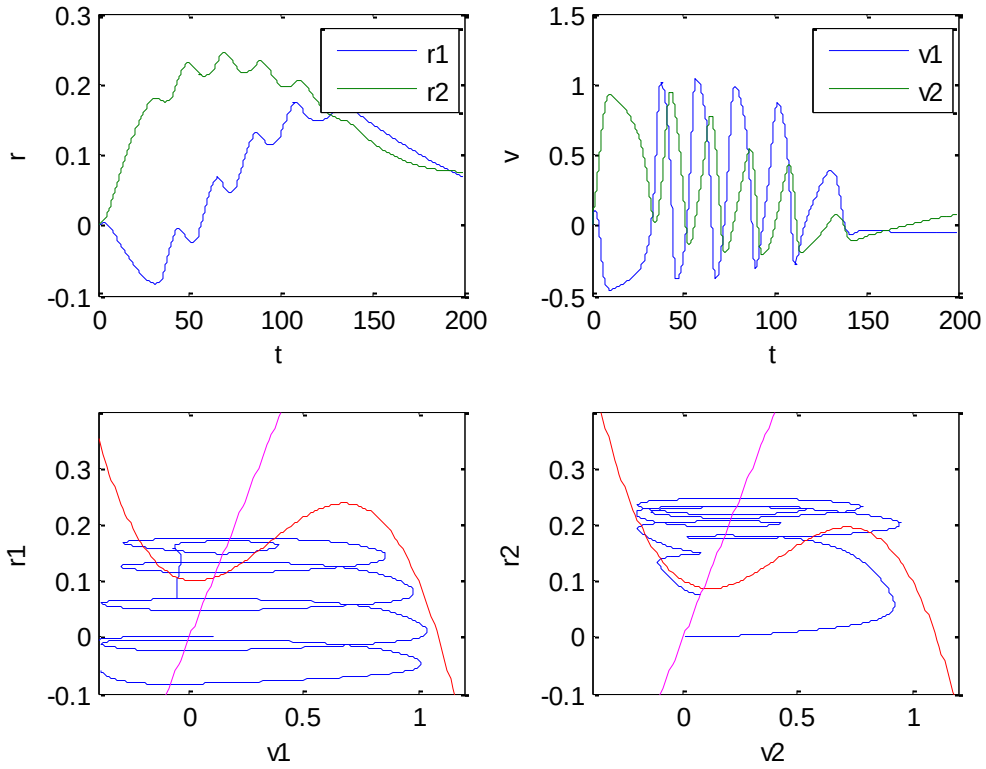
For (d_{12}, d_{21})= (-0.1,0.2)



For (d_{12}, d_{21}) = (-0.3,0.2)



For (d_{12}, d_{21}) = (-0.5,0.2)



Conclusions:

1) The first plot represents zero interactions between the neurons. You can clearly see all four phases that describe the phase plot. It is important to note that the second neuron is a single spike and not periodic. It is clearly shown in the phase plot, where the signal does not complete the next cycle and does not enter the regenerative phase again, while the first neuron does re-enter the cycle.
2) When the second neuron begins to linearly couple with the first, it is able to enter a regenerative phase. It has a stronger potential than the first neuron now. Both signals are essentially periodic.
3) When the interaction of the first neuron is a negative linear coupling, while the second neuron has a positive linear coupling, they are more signals but much weaker. There is a slight decay from the initial potential until it reaches a steady state. Both signals still appear to be periodic.
4) From the last two plots, you can see how the magnitude of this negative linear coupling knocks the neurons out of its typical oscillatory behavior. It is unsteady behavior where you clearly see the signal both signals overcome by an exponential decay rather than this periodic behavior in examples prior to these last two plots.

Bonus:

1) Can you change the different initial parameters to explain intuitively what the constant mean? What happens if both interactions are positive?
    - Must illustrate through different plots and explain clearly what happens. (5 pts)
2) What happens if you have three neurons? Can you write the equations for this sort of system successfully? (2 pts)

$$\frac{dv_1}{dt} = -{v_1}^3 + (1 + a_1){v_1}^2 - a_1 v_1 - r_1 + I + d_{12}v_2 + d_{13}v_3$$
$$\frac{dr_1}{dt} = bv_1 - cr_1$$
$$\frac{dv_2}{dt} = -{v_2}^3 + (1 + a_2){v_2}^2 - a_2 v_2 - r_2 + I + d_{21}v_1 + d_{23}v_3$$
$$\frac{dr_2}{dt} = bv_2 - cr_2$$
$$\frac{dv_3}{dt} = -{v_3}^3 + (1 + a_3){v_3}^2 - a_3 v_3 - r_3 + I + d_{31}v_1 + d_{32}v_2$$
$$\frac{dr_3}{dt} = bv_3 - cr_3$$

Can you alter my code to reflect 3 linearly coupled neurons?

- Must attach code (4 pts)

Can you show what happens when you have different interaction parameters?

- Must attach plots (4 pts)

Problem # 3 - Choose either the Engler paper or Spatz paper and answer the questions regarding to each paper. (30 pts – 10 pts per problem)

For the 2006 manuscript "Matrix elasticity directs stem cell lineage specification"…

1. Summarize the manuscript in no more than 150 words.

This study altered the mechanical properties of the extracellular matrix to illustrate its effect on stem cell differentiation. They Start with a mesenchymal stem cell and place it on different matrices. They find the stem cells reprogram to choose a specific lineage. When they inhibited myosin – the force generation molecular protein, it blocked this mechanism for the stem cell while the cell itself, in function and shape, remained the same. This further illustrates that the stem cells sense these mechanical properties of their microenviorment before choosing a path.

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.

Cell Type	Elastic Stiffness	Microstructural	Function should align with what you look up for:
Neurogenic	0.1-1kPa	branched	Nerve cells
Myogenic	8-17 kPa	spindle	Muscle cells
Osteogenic	25-40 kPa	polygonal	Bone cells

3. Discuss the impact of the major findings in this manuscript on stem cell therapies. As a typical example, you might think of the direct injection of undifferentiated human embryonic stem cells into the infarcted region of the heart. In response to the infarct, compliant contracting heart muscle cells are replaced by stiff scar tissue. What are the dangers of stem cell injection therapies in view of the experimental results of the manuscript?

With the discover that matrix elasticity can help regeneration of tissue, there are many implication for diseases in which people need help recovering damaged tissue in the heart, muscle, nerves, or bone. However, most of this experiment was done in vitro and in a 2D environment so purely injecting stem cells would not necessarily work – since it was not studied in this particular paper. In addition, stem cells are in a 'baby' state while our body is in an adult state. The initial matrix elasticity to trigger this change may not work directly in the body and could trigger more problems.

For the 2014 manuscript "Nanoparticle Tension Probes Patterned at the Nanoscale: Impact Integrin Clustering on Force Transmission" …

1. Summarize the manuscript in no more than 150 words.

The goal is to develop a method to control focal adhesion clusters and measure the molecular tension between the receptor and ligand bonds. In order to do this, this group developed something called Molecular Tension Fluorescence Microscopy. Essentially, the molecular probe is immobilized on the surface and when the cell binds to it and applies tension, it has a fluorescence signal. The clustering was controlled based on the spacing of gold nanoparticles. With this tool, this group was able to illustrate that cells sense ligand spacing through molecular forces.

2. What is a focal adhesion and its role in different cellular processes? What conclusion is reached about the formation of focal adhesions and force generation?

Focal adhesions are dynamic protein complexes at the plasma membrane that connect the extracellular matrix outside of the cell to the actin cytoskeleton, inside of the cell. Because of this, they are involved in processes like cell migration, differentiation, and proliferation. Because of this direct linkage, the focal adhesion feels force via the integrins and these forces play a role in the activation and function of these integrins. The study finds that actin polymerization increases the integrin tension and there is a critical spacing required in order for the integrin to handle this tension and allow for the focal adhesion to stabilize and mature.

If you could use this technology, what would you study and why?

Open ended – I personally want to study single platelets with this technology.

Problem #4 – Mechanotransduction Gone Awry (30 pts – 10 pts per problem)

The recent review "Mechanotransduction gone awry" by Jaalouk and Lammerding discusses defects in mechanotransduction and their effects on various different disease types.

1. Read the manuscript carefully and summarize it in approximately 150 words.

   The manuscript is a review on mechanotransduction and in particular how different mutations or modifications can interfere with the signaling process and the cell's ability to sense mechanical forces. They had a table, which I also had in class.

Disease	Primary cells/tissues affected	Selected references
Deafness	Hair cells in the inner ear	6
Arteriosclerosis	Endothelial and smooth muscle cells	10–13, 19
Muscular dystrophies and cardiomyopathies	Myocytes, endothelial cells and fibroblasts	35, 36, 41, 85
Osteoporosis	Osteoblasts	20
Axial myopia and glaucoma	Optic neurons and fibroblasts	43–45
Polycystic kidney disease	Epithelial cells	51, 52
Asthma and lung dysfunction	Endothelial cells and alveolar tissue	15, 21–23
Premature ageing (HGPS)	Multiple cell types and tissues	55, 57
Developmental disorders	Multiple cell types and tissues	46–50, 86
Cancer	Multiple cell types and tissues	2, 58–60, 68, 71, 73, 87
Potential immune system disorders	Leukocytes	24–26
Potential central nervous system disorders	Neurons	27, 28

2. Select your favorite mechanotransduction pathway and describe how it is altered under diseased conditions.

   Open ended. The paper references several papers in the table above. They specifically talk about cardiac hypertrophy, muscular dystrophies, glaucoma, Kartagener's syndrome, Hutchinson–Gilford progeria syndrome, and cancer cells.

3. What are the implications of this article? Write a paragraph of about 150 words.

Studying mechanotransduction can help us

- Understand diseases and uncover new mechanosensors
- develop new treatments to these particular diseases or in the case of gene mutations, help correct downstream signaling.