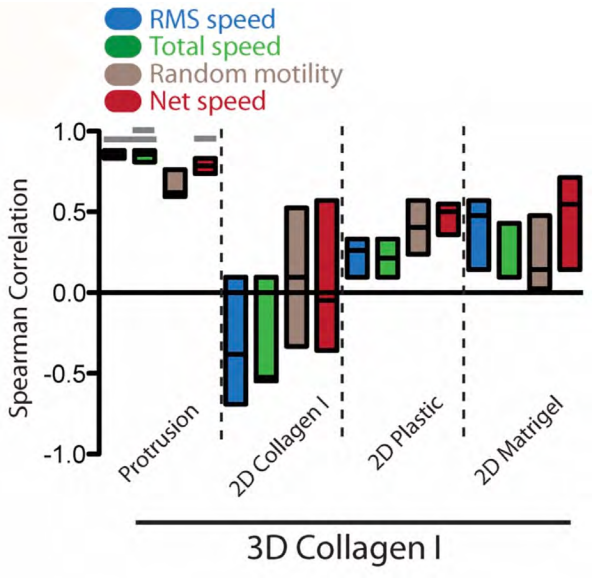
Name and UID:

BE 167L: Bioengineering Laboratory
June 4, 2019
Exam 2

Be sure to fill out your course evaluation online. It will be worth 2 extra points on the exam!

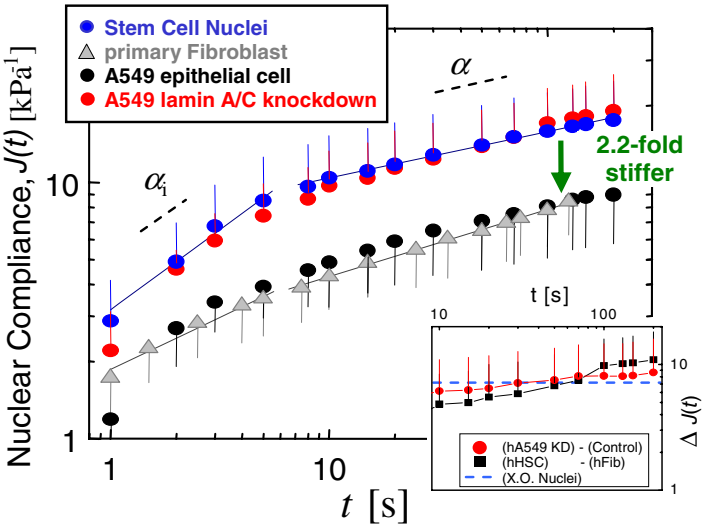


Question 1 (60 pts)
Sarah is interested in following up on the work in Fraley *et al*, *Nat Cell Bio*, 2010. Specifically, she wants to explore whether the findings in the paper hold for ECM environments other than collagen. She asks for your assistance designing these experiments based on what you know from 167L.

a) Sarah is planning to use a PEG hydrogel with adhesion sites conjugated to the gel polymer. Cells are then embedded in this matrix. This allows her to vary the amount of cell adhesion to the gel over a very wide range. What do you expect to be the relationship between the amount of adhesion and cell migration? Plot your expectation. What is happening at each extreme?
b) Sarah next wants to check that her cells are viable in both her 2D and 3D environments after seeding. What is one assay she can use to assess cell viability? Describe how the assay works and the expected outcome (e.g., what it looks like if the cells are alive).
c) Like in Fraley *et al*, Sarah wants to evaluate both the persistence and speed of cells in her ECM environments. She finds that the cells she is using have a persistence time of 25 minutes from a previous, similar paper. At what frequency would you recommend she image her cells? Justify your answer.
d) Using the images she collects, Sarah wants to evaluate the migration of her cells using a persistent random walk model. For different time intervals (t), she quantifies the mean squared displacement ($\langle d^2(t) \rangle$). What fitting method can she use to solve for the speed and persistence of her cells? What are the assumptions of using this method?

$$\langle d^2(t) \rangle = 2S^2P\left[t - P\left(1 - e^{-t/P}\right)\right]$$

e) Sarah decides she doesn't care about cell persistence after all, so she images once a day so there are fewer images to analyze. Will this give her an accurate measurement of cell speed? If not, how will it be off? Explain.
f) Following up on Fraley *et al*, Meyer *et al*, *JCB*, 2012 identified that cell protrusion in 2D can predict cell migration response in 3D (see top figure). What are the three other essential steps of cell migration?
g) Sarah plans to use her microscopy images to identify cells that are constrained from migrating by their nuclei. By tracking these cells when they cross through a pore in the matrix, she wants to see whether fast or slow cells are more constrained by nuclear constriction. She finds the plot above from Pajerowski *et al*, *PNAS*, 2007. Should she think of the nucleus as an elastic or visco-elastic material? Does this indicate cell's nuclei will move through pores more easily when transiting quickly or slowly? (Compliance is inverse of deformation modulus. Note axis labels.)



h) In Engler *et al*, *Cell*, 2006, what do they change about the environment to influence cell response? What are the three cell outcomes/responses, and how are they related to this variable?

Question 2 (40 pts)
You are designing a device for patient monitoring during clinical trials, to quantify blood pressure and pulse features upon drug treatment. A number of concerns related to analysis of the data arise while preparing the device.

a) For blood pressure, you are measuring a voltage from a wrist monitor. Blood pressure is proportional to signal, but the monitor has to be calibrated for each patient individually. You collect a few points with different blood pressures measured via another device. How can you calculate your conversion factor? (5 pts)
b) Can you test whether a set of points follow any of the key assumptions of your analysis above? Very briefly describe. (5 pts)
c) You now want to use this device in a study testing the effect of a new blood pressure medication. You expect that there is considerable pre-existing person-to-person variation. What are two experimental design approaches you could use to eliminate this issue? You don't need their names, just how they're designed. (10 pts)
d) You decide to enroll 20 patients in your study with one of your designs from above. Your statistician colleague warns you that your experiment will be underpowered. What does this mean, and what is the risk? What could you do to remedy the situation? (10 pts)

Question 3 (30 pts)
Sun *et al*, *Nature*, 2012 observed that a critical difference between many synthetic and natural hydrogels is that the former are less stretchable. (30 pts)
a) In the figure, label the elastic and plastic deformation phases. (3 pts)
b) Ligaments are known to soften with exercise. What does this indicate about the type of deformation occurring? What is happening on a molecular scale? Why might this be less concerning for living materials (as opposed to, say, an airplane)? (12 pts)
c) A benefit of large deformations proposed by Sun *et al* is increased strength, sometimes measured by energy absorbed before rupture. With the properties below, roughly estimate the strength (energy to rupture) of the two materials above. (λ is the same as ε, starting length 1 mm.) (15 pts)

energy = force × displacement
$\epsilon = \delta / L$