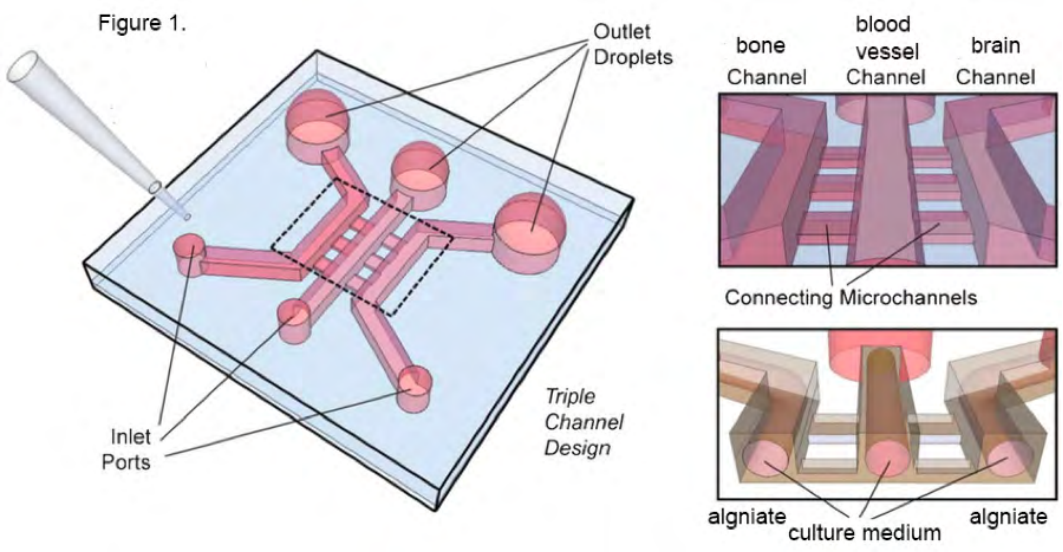
**Name and UID:**

**BE 167L: Bioengineering Laboratory** **December 3, 2018**
**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)**

Eliot is a Ph.D. student who is studying metastasis of breast cancer tumors to bone and brain tissues. To do this he is constructing an "organ-on-a-chip" device to serve as an *in vitro* test environment for his studies. Eliot's device consists of two adjacent microfluidic chambers, one designed to mimic the small-diameter vasculature and the other designed to mimic lung tissue (Figure 1).




During cancer metastasis, tumor cells circulating in the bloodstream have to cross the endothelium, a layer of endothelial cells connected by tight junctions to prevent other cells from freely passing in and out of the blood vessel. To create an endothelial layer in one of the microfluidic chambers, Eliot plans to seed Human Umbilical Vein Endothelial Cells (HUVECs), which are a well-characterized, immortalized cell line.

a) Briefly, what are some cautions you would express to Eliot when interpreting data from HUVEC as opposed to primary endothelial cells (i.e., how might HUVECs differ from endothelial cells found in a working blood vessel of the body (6 pts)?

b) After fabricating his device, Eliot plans to culture HUVECs inside the "blood vessel" chamber. Eliot knows that a special type of extracellular matrix called basement membrane surrounds endothelial cells in blood vessels in the body. What are the major constituents of this matrix (e.g., nucleic acids)? How does the overall structure of basement membrane differ from many other matrix structures? (4 pts)

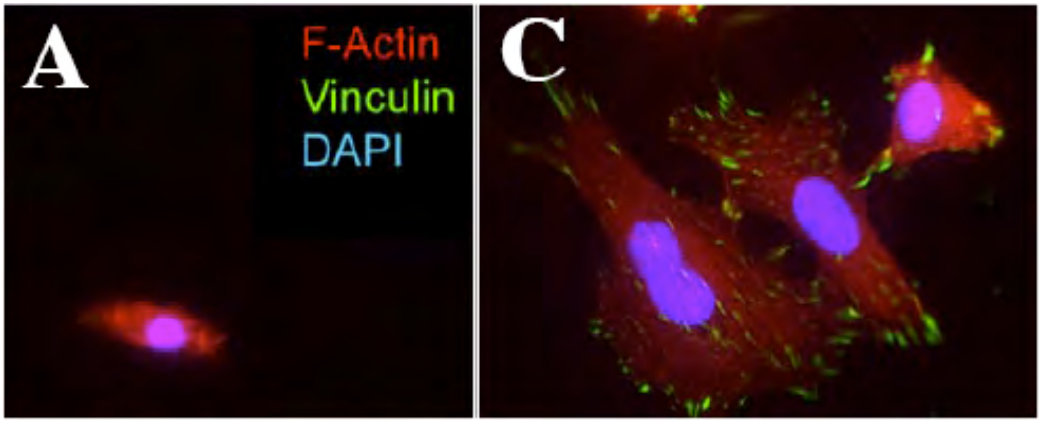
c) Eliot tries his device a first time, and although the medium in his flask remains pinkish red in color, the medium in his device is yellow. He seeded both the flask and the device at the same time passaged from the same flask of HUVECs in the same medium under identical conditions. Assuming that both cultures remained sterile, why do you think the device medium turned yellow while the flask medium remained pinkish red? (3 pts)

d) One advantage of the alginate hydrogel network is that Eliot can control the mechanical properties so that they match the mechanical properties of native brain and lung tissues. Is native brain harder or softer than tissue culture plastic? Does this correspond to a greater or lesser elastic modulus? (3 pts)

e) After characterizing the mechanical properties, Eliot plans to encapsulate lung fibroblasts in the alginate hydrogels to establish a 3D culture. To provide lung tissue-specific sites for cell adhesion, he mixes several matrix proteins isolated from lung tissue into the alginate/cell solution before gelling. What tripeptide is a common adhesive site in many extracellular matrix proteins? (3 pts)

f) Name one assay that Eliot could use to evaluate cell viability after encapsulating in alginate AND describe how the assay works. (8 pts)

g) Eliot immunostains lung fibroblasts in 3D alginate cultures and in 2D cultures on tissue culture-treated polystyrene for F-Actin and Vinculin (DAPI shows the cell nucleus). Given the images below, which (A or C) do you think more likely came from the 2D culture? Why? (6 pts)




Eliot has finally constructed his entire device, including adding culture cells, and is ready to perform experiments modeling migration of metastatic breast cancer cells. He suspects that the cancer cells that cross the endothelial migrate at different speeds in the “brain” and “lung” chambers. Thus, he decides to set up an experiment to model migration based on the persistent random walk model of cell migration:

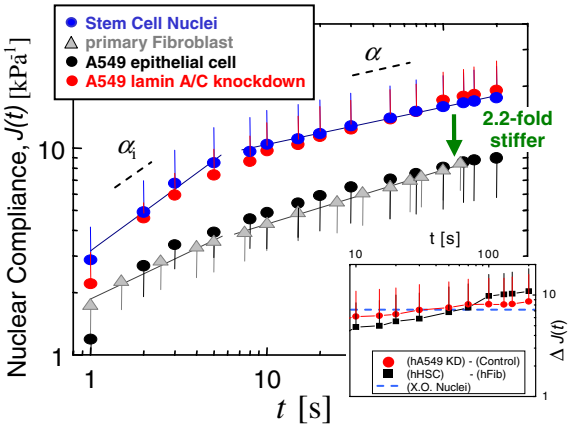
h) First, Eliot determines that the persistence time of migrating breast cancer cells is 25 min (assume this is the same in brain-like and lung-like hydrogels). If he chooses an experimental time step of 5 min, would you expect the cell direction at the second time point to be highly correlated with its direction at the first measurement? What if he chooses a time step of 5 hrs? Why? (6 pts)

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

i) Eliot takes his images and calculates the distance a cell has traveled across his images when taken every 5 min and 5 hrs. He determines that just imaging the cells more often (5 min) causes the cells to migrate faster. What is wrong with Eliot’s interpretation? (7 pts)

j) Describe the role of retrograde actin flow (or “treadmilling”) in cell migration and how it relates to force generation within the cell. Where does actin polymerization happen? (7 pts)

k) Eliot is interested in the role the nucleus might play in limiting cell migration. Why might Eliot have this concern? He finds the following plot from Pajerowski *et al*, PNAS, 2007. Does this demonstrate that he should think of the nucleus as an *elastic* or *viscoelastic* material? Justify your answer. (7 pts)




**Question 2 (40 pts)**

You are designing a medical device to provide measurements of blood oxygenation from skin spectroscopy performed on the wrist. You know that the device provides a voltage that is proportional to blood oxygenation, but have to calibrate it for each patient to values measured separately.

a) What method could you use to quickly determine this conversion factor from your calibration points? (5 pts)

b) A team member suggests that the voltage-oxygenation relationship is log-linear instead of linear, and so suggests using log(V) instead. When would this be alright? What is an alternative approach? Are there any concerns with calculating the answer in either case? (10 pts)

c) How could you test whether a set of points follow the key assumption above? Very briefly describe. (5 pts)

d) You now want to use this device in a study testing the effect of a daily exercise regimen on blood oxygenation. You expect that there is considerable person-to-person variation in blood oxygenation that is unrelated to exercise. 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)

e) You decide to enroll 20 patients in your study with one of your designs from above. You 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)