

BioE 116 Midterm #1 SOLUTIONS

Spring 2006

Please write your name and SID on each page of the exam. Write LEGIBLY and clearly. Only exams written in PEN will be considered for regrades.

Part 1. Multiple Choice: Clearly mark the letter of your choice in the space provided.. (2-pts each.)

- D** ___ 1. Which of the following is NOT a form of cell signaling:
- A. Endocrine
 - B. Autocrine
 - C. Paracrine
 - D. Exocrine
 - E. Neuronal signaling
- C** ___ 2. An ideal scaffold has the following properties EXCEPT:
- A. No foreign body response
 - B. Can recruit host cells to help populate the scaffold
 - C. Can incite encapsulation
 - D. Cells are able to integrate the material into the host tissue
 - E. Scaffold will degrade without toxic residue
- A** ___ 3. What is the correct sequence of cell migration:
- A. Cell extension, attaching to ECM at the front, cell contraction, releasing of ECM at the end, recycling molecules.
 - B. Cell extension, attaching to ECM at the front, recycling molecules, cell contraction, releasing of ECM at the end,
 - C. Cell extension, releasing of ECM at the end, cell contraction, recycling molecules, attaching to ECM at the front.
 - D. Cell extension, attaching to ECM at the front, cell contraction, recycling molecules, releasing of ECM at the end.
 - E. Cell extension, recycling molecules, attaching to ECM at the front, cell contraction, releasing of ECM at the end.
- H** ___ 4. Cell migration can be affected by the following environmental cues:
- A. Growth factor gradients
 - B. Fluid shear stress
 - C. Collagen fiber direction
 - D. Stiffness of substrate
 - E. Cell density
 - F. D, E
 - G. A, C, D, E,
 - H. A, B, C, D, E

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H_____ 5. The following are soluble molecules used in cell-cell communication EXCEPT:

- A. Steroids
- B. Cadherins
- C. Amino acids
- D. Cytokines
- E. Nucleotides
- F. Nitric oxide
- G. Integrins
- H. B, G
- I. B, F, G
- J. B, D, E, F

Part 2. True or False: Write “True” if the statement is true or “False” if the statement is false. If “False”, provide a *brief* sentence on why it is false. (1 pt each)

_____ 1. Induction, effector (e.g., caspase), and protein/DNA degradation are all phases associated with necrosis. **False, this is characteristic of apoptosis, not necrosis.**

_____ 2. Actin filaments are the major intracellular cytoskeletal attachments found in desmosomes. **False, intermediate filaments instead of actin filaments; or tight junctions instead of desmosomes.**

_____ 3. Cartilages can resist compressive force because they have a lot proteoglycans that are covalently linked with highly positively charged polysaccharide chains. **False; negatively charged.**

_____ 4. Immobilization can increase the stability of growth factors. **True**

_____ 5. The loading/unloading curve of collagen shows hysteresis due to the elastin content. **False; due to collagen content.**

_____ 6. Different growth factors may have the same effect, but one growth factor has only one effect. **False; growth factors can have more than one effect.**

_____ 7. Boyden chamber can be used to measure chemotaxis, but the chemical gradient in the pores of the membrane is not stable. **True**

_____ 8. During cell migration, some components of the cell will be left behind. **True**

_____ 9. In quantifying DNA content with flow cytometry, the cells with less DNA content than the normal cells are apoptotic cells. **True**

_____ 10. DNA microarray, polymerase chain reaction, and immunoblotting are methods to quantify gene expression. **False; not immunoblotting.**

Part 3. Short Essay.

1. Research shows that pleiotrophin (PTN) is a growth factor that stimulates angiogenesis. (15 points)

- A. Describe one experiment to test the ability of soluble PTN protein to stimulate endothelial cell migration by chemotaxis *in vitro*. What parameters would you use to quantify the level of cell migration? What controls would you use? (8 sentences max)

Boyden Chamber: Seed endothelial cells seeded on the upper surface of a porous membrane in media, while PTN protein is added to the media in the lower reservoir. To reduce the migrational speed, a thin layer of collagen gel can be coated on top of the porous membrane. For the negative control, the setup is the same except that PTN is not added to the lower reservoir. In the presence of a chemotactic gradient, the cells will sense the gradient and migrate downwards through the porous membrane. At different timepoints, one can quantify the amount of chemotaxis by staining the cells with a nuclear dye and then counting the cells on the lower surface of the membrane. The presence of a chemotactic gradient will correlate with a greater number of cells on the porous membrane as a function of time.

- B. To deliver PTN in a controlled-release manner, you conjugate PTN onto the surface of a biodegradable porous scaffold. What assay would you use to quantify the release kinetics of PTN over time *in vitro*? How does the assay work? (8 sentences max)

The PTN-conjugated scaffolds can be incubated with a buffered-saline for different timepoints. At specified timepoints, samples of the saline can analyzed for the presence of PTN by Enzyme-Linked Immunosorbent Assay (ELISA). Alternatively, the scaffold could be chemically dissolved before taking a sample for ELISA analysis. ELISA is carried out by immobilizing PTN onto a substrate. A saline sample is allowed to incubate with the substrate. During the incubation time, any PTN present in the solution will bind to the antibody. Afterwards, another anti-PTN antibody is incubated with the substrate to bind to PTN. The anti-PTN antibody is conjugated to an enzyme such as horse radish peroxidase (HRP), which precipitates when incubated with the substrate chromogen. The precipitation can then be measured by spectrophotometry and correlated to a concentration.

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- C. To test the angiogenic potential of the PTN-conjugated scaffold *in vivo*, you then implant it subcutaneously (below the skin) in mice for 3 weeks. After 3 weeks when you remove the scaffold from the mice, describe 2 methods to determine whether the scaffold was effective in promoting angiogenesis? (8 sentences max)

Histology: Cryosection thin sections of the samples for histology (hematoxylin & eosin staining) to visualize the new vessels in the scaffold.

Immunofluorescence: Cryosection thin sections of the sample for immunofluorescence staining of vascular-specific proteins.

Polymerase chain Reaction: Lyse the cells in the scaffold for RNA isolation and then PCR analysis of vascular-specific genes

Immunoblotting: Lyse the samples for immunoblotting of angiogenic proteins.

2. To engineer skin, you initially seed 2×10^9 fibroblasts onto a porous PLGA scaffold. After 7 days, you notice less cells attached to the scaffold than you initially seeded, so you suspect that the cells had undergone apoptosis. (25 points)

- A. List 4 specific cellular changes associated with apoptosis.

- Mitochondrial membrane loses function
- Nuclear envelope disassembles
- Cytoskeleton collapses
- DNA breaks into fragments (50-500 bp)
- Cell shrinks

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B. Describe 2 methods to quantify the number of cells remaining in the scaffold after 7 days (8 sentences max)

- Label the cells with a fluorescent dye and count the cells on a fluorescence microscope
- Detach the cells from the scaffold (by trypsin) and count the cells using a hemocytometer
- Detach the cells from the scaffold and count the cells using an electronic particle counter
- Lyse the cells for DNA and quantify the amount of DNA. By calibrating the sample DNA with the DNA in known quantities of cells, you can determine the number of cells in the sample.
- Lyse the cells for protein and quantify the amount of protein in the sample. By calibrating the protein sample with protein concentrations in known quantities of cells, you can determine the number of cells in the sample.

C. To calculate the rate of apoptosis, you count the number of remaining cells in your scaffold, which you determined from part B to be 5×10^4 cells. Using the model below, determine the rate of apoptosis (μ). $X(t)$ is the number of cells at time t .

$$\frac{dX}{dt} = -\mu X$$

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Problem 2C

$$\frac{dX}{dt} = -\mu X$$

$$X_f = 5 \times 10^4 \text{ cells (at } t = 7\text{d)}$$

$$X_0 = 2 \times 10^9 \text{ cells (at } t = 0)$$

$$\int \frac{dX}{X} = \int -\mu dt \Rightarrow \ln X = -\mu t + C_1 \Rightarrow X = C_2 e^{-\mu t}$$

Boundary Conditions -

$$t = 0$$

$$X = X_0$$

$$X_0 = C_2 e^0$$

$$C_2 = X_0$$

$$X = X_0 e^{-\mu t}$$

$$\text{For } t = 7\text{d} \quad X_f = X_0 e^{-\mu(7)} \Rightarrow \frac{X_f}{X_0} = e^{-\mu(7)}$$

$$\ln\left(\frac{X_f}{X_0}\right) = -7\mu = \ln\left(\frac{5 \times 10^4}{2 \times 10^9}\right)$$

$$\mu = 1.514 \frac{1}{\text{day}}$$

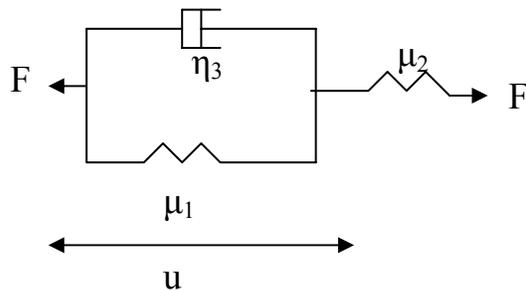
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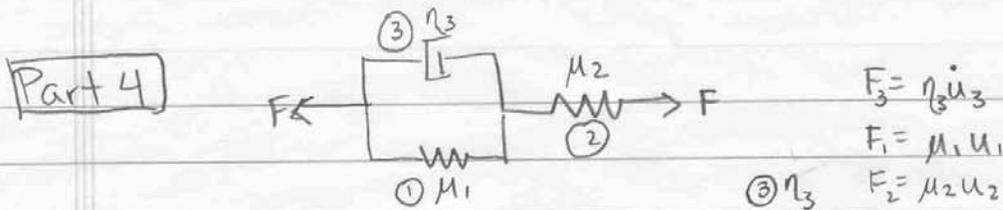
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Part 4. Quantitative Analysis. State your assumptions and define symbols that you introduce. (30 points)

1. To study the viscoelastic properties of tendon, you model it with the following arrangement of springs and dashpots. As shown in the diagram, μ_1 and μ_2 are the elastic moduli of springs 1 and spring 2, respectively, and η is the coefficient of viscosity for the dashpot element.

- A. Derive a constitutive equation for the model (the relationship between F and u).
- B. Graph the creep and stress relaxation responses for loading and unloading processes. You can assume there is a limited loading time for the creep model. Label the graph with spring and dashpot responses at points of interest.





A) Consider Kelvin-Voigt First

$F = F_1 + F_3 \Rightarrow F_3 = F - F_1$
 $u = u_1 = u_3 \Rightarrow \dot{u} = \dot{u}_1 = \dot{u}_3$

$$\dot{u}_3 = \frac{F_3}{\eta_3} = \frac{F - F_1}{\eta_3} = \frac{F - \mu_1 u}{\eta_3} = \dot{u} \quad (1)$$

Add Spring in series

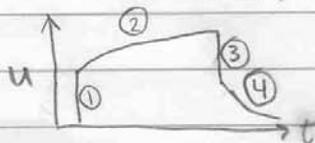
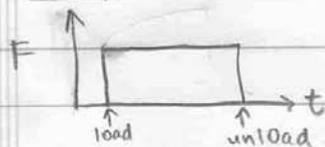
$F = F_1 = F_2 \quad F_2 = \mu_2 u_2$
 $u = u_1 + u_2 \Rightarrow \dot{u} = \dot{u}_1 + \dot{u}_2 \Rightarrow \dot{u}_1 = \dot{u} - \dot{u}_2$

Rearranging (1), $F = \dot{u}_1 \eta_3 + u \mu_1 = \left(\dot{u} - \frac{\dot{F}}{\mu_2} \right) \eta_3 + \mu_1 \left(u - \frac{F}{\mu_2} \right)$

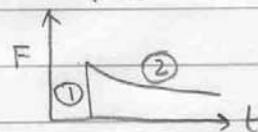
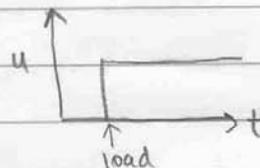
$$F = \dot{u} \eta_3 - \frac{\dot{F}}{\mu_2} \eta_3 + u \mu_1 - \frac{F \mu_1}{\mu_2}$$

$$\frac{\dot{F}}{\mu_2} \eta_3 + F \left(1 + \frac{\mu_1}{\mu_2} \right) = \dot{u} \eta_3 + u \mu_1$$

B) Creep (F constant)



Stress Relaxation



- ① Instant response by spring #2
- ② Dashpot and spring #1 elongate slowly
- ③ Instant response by spring #2
- ④ Delayed response of spring #1 and dashpot

- ① Maximum force by dashpot
- ② Force decreases as dashpot deforms with time