BioE 116 Midterm
Spring 2007

Name:_____
SID:_____

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

1. Which of the following is NOT a change experienced by typical cells undergoing apoptosis?

d

a. Loss of mitochondrial membrane functions
b. Cytoskeleton collapses
c. DNA breaks into fragments
d. Cell swells and ultimately bursts
e. Nuclear envelope disassembles

2. The following are soluble molecules used in cell-cell communication:

E

A. Steroid
B. Cadherins
C. Nitric oxide
D. Integrins
E. Both A and C
F. A, B and C

3. When a piece of soft tissue is subjected to mechanical stretch, the deformation of the matrix is due to

E

A. Streching of the matrix fibrils
B. Breaking down the integrin and ECM bonds
C. Cell deformation
D. Sliding between the matrix fibrils
E. A and D
F. A, B and D

4. Which of the following is NOT a form of cell-cell signaling:

D

A. Endocrine
B. Autocrine
C. Paracrine
D. Exocrine
E. Neuronal signaling

5. The process of angiogenesis involves:

F

A. Endothelial cell proliferation
B. Endothelial cell migration
C. Matrix degradation
D. Cell signaling
E. A, B and C
F. A, B, C and D

Part 2. (20 points) 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. (2 pt each)

1. Flow cytometry may be used to measure cell proliferation but not apoptosis.
False, DNA staining will show a characteristic peak of fragmented DNA in flow cytometry indicating the apoptotic cell population.
2. Cell-matrix binding may involve integrins but not cadherins.
True

3. Cell signaling molecules involve small, membrane permeable molecules such as steroids, ions and dissolved gases and non-permeable molecules such as proteins, peptides and nucleotides.
False, ions are not membrane permeable.

4. Aggrecan is a proteoglycan commonly found in cartilage. Aggrecan can attract water and ions via its positively charged polysaccharide chains.
False, aggrecans have negatively charge polysaccharide chains.

5. During cell migration a cell exerts rearward traction forces at the front of the cell and forward traction forces at the rear of the cell.
True

6. Collagen fibers in the extracellular matrix have higher Young's modulus than elastin.
True

7. Immobilization can increase the stability of growth factors.
True

8. Hayflick limit is the growth limit for all types of cells.
False, stem cells and tumor cells have higher growth limits, if any.

9. Allogeneic cells can be made available off-the-shelf.
True

10. During electroporation process, cell plasma membrane is permeable transiently.
True

Part 3. Essay and Quantitative Analysis.

1. (30 points) You are given a task to design experiments to evaluate the suitability of the biocompatible and biodegradable poly(L-lactic acid) (PLLA) nanofiber as engineered ECM material for neural tissue engineering.

A. What would be the advantage of aligned nanofiber over random non-aligned nanofiber matrix? (3 pts)

Aligned nanofiber can induce cell alignment and growth along a predefined direction, thus help to form an aligned nerve fibers.

B. You decide to first evaluate the in vitro performance by culturing cells onto the aligned PLLA nanofiber matrix and investigate cell viability. You will use a neuron-like rat pheochromocytoma cell line, PC12, for your experiments. What assay would you use to quantify the number of PC12 remaining in the scaffold after 10 days?

There are several possible ways to quantify the number of cells: (answers should include how the cells are prepared (direct staining, trypsinized or lysed (1pt), reagents and method to be used(3 pts), and how to calculate the numbers of the cells(1pt))

1. Stain the cells on the matrix with fluorescent dye and count the cells under a fluorescent microscope. Number of cells on the matrix can be estimated from average cell density multiplied by total volume of the matrix.

2. Detach the cells from the scaffold with trypsin, and count the cells with hemocytometer;

3. Detach the cells from the scaffold with trypsin, then label the cells with fluorescent dye and count with FACS machine;

4. 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;

5. 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. You notice that the number of cells attached to the matrix is decreased from $5x10^{10}$ to $2x10^{4}$ after 10 days. To calculate the rate of apoptosis, you will use the model below. Determine the rate constant of apoptosis (μ). X(t) is the number of cells at time t.

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

u = ln(5x10^10/2x10^4)/10 = 1.47/day

D. The result shows that the neural stem cells have poor attachment to the PLLA nanofibers. What would you suggest to improve cell adhesion on the matrix? Please give two examples.
(8 pts)

There are multiple answers. (For each suggestion: theory (2pt), method (2 pts))

Answers should address how to change the surface property of the scaffold to improve cell adhesion. RGD peptides, fibronectin or ECM molecules such as collagen, laminin, or gelatin can immobilized on the nanofiber through adsorption or chemical covalent bonding.

We can also increase the rigidity of the scaffold by increasing the molecular weight of the PLLA polymer, or add crosslinkers.

E. Suppose that your suggestion works and you can move on to test the in vivo performance of the nanofiber as peripheral nerve regeneration material. Nerve conduits made of aligned nanofibers are implanted in rats with damaged peripheral nerve. After 4 weeks the scaffold are removed from the rats. Describe two methods to determine whether the scaffold is effective in promoting nerve regeneration. (8 pts)

Answers could include the following methods: (answers should specify name of the method (1) pt), material used (cryosection, live tissue, trypsinized cells or lysed cells, antibodies etc)(2 pt), target (nerve tissue specific protein or gene)(1 pt))

Histology: *Cryosection thin sections of the samples for histological staining to visualize the new nerve fibers in the scaffold.*

Immunofluorescence: *Cryosection thin sections of the sample for immunofluorescence staining of nerve tissue specific proteins.*

Polymerase Chain Reaction (PCR): *Lyse the cells in the scaffold for RNA isolation and then use PCR to analyze transcription of nerve tissue-specific genes*

Immunoblotting: *Lyse the cells in the scaffold, extract proteins for immunoblotting of neurogenic proteins.*

2. (15 points) A mystery protein B116 has been shown to promote wound healing when apply to the wounding area. You hypothesize that B116 works by increasing the rate of cell migration through chemotaxis.

A. (9 pts) Design an in vitro assay to test your hypothesis. Your answer should include descriptions of the assay (3 pts), cell type used (2 pt), parameters measured for quantifying cell migration (2 pt) and appropriate controls (2 pt).

Sample answer 1: Boyden Chamber Assay
Seed human fibroblast on the upper surface of a porous membrane in the media. B116 protein is added to the lower reservoir in the media. If the hypothesis is correct, the cells will sense the

B116 gradient and migrate downwards through the porous membrane. Membrane samples will be collected at different time points. Cells on the lower surface of the membrane will be stained with nuclear dye or fluorescent dye, and will be counted under a microscope. Cell migration will be quantified as the increasing number of cells on the lower surface of the membrane over time.

The negative control will have bovine serum albumin (BSA) (a protein with no chemotaxis effect on fibroblast) in the lower chamber.

The positive control will have a known chemoattractant, such as TGFβ, in the lower chamber.

Sample answer 2: Microfluidic Chamber Assay
Human endothelial cells will be introduced into a microfluidic chamber with a stable gradient of B116 across the chamber. Timelapse microscope images will be recorded to analyze the cell migration. If the hypothesis is correct, the cells will migrate toward the area with a higher concentration of B116 in th e chamber. Migration velocity, including speed and directionality, will be measured from the timelapse images of the cells.

In the negative control, the cells will be seeded in the same microfluidic chamber with a uniformed B116 concentration everywhere, or with a BSA concentration gradient.

In the positive control, the microfluidic chamber will have a stable gradient of a known chemoattractant, such as TGFβ.

B. Supposed that your initial hypothesis failed. List three other possible mechanisms by which B116 promotes tissue regeneration and wound healing.

2 pts each

B116 may
1. *promote angiogenesis;*
2. *promote cell proliferation;*
3. *promote ECM synthesis;*
4. *induce growth factor synthesis;*
5. *inhibit apoptosis;*
6. *act as a ECM scaffold material to promote cell adhesion and migration;*
7. *other reasonable mechanism.*

3. (25 points) To study the viscoelastic properties of ligament, you model it with the following arrangement of springs and dashpots. As shown in the diagram, μ is the elastic modulus of the spring, and η_1 and η_2 are the coefficients of viscosity for the 2 dashpot elements respectively.

A. Derive a constitutive equation for the model (the relationship between total force F and total deformation u).

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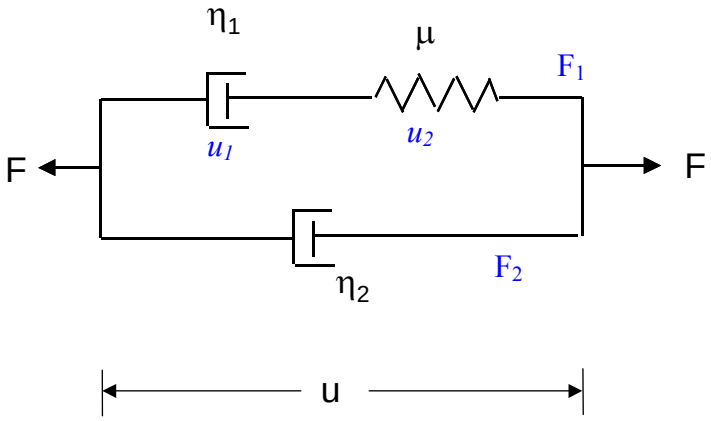

F: Total Force;
u: Total Deformation;
F1: The force on the top spring and dashpot;
F2: The force on the bottom dashpot;
u1: deformation on the top dashpot;
u2: deformation on the top spring.

$$F = F_1 + F_2$$

$$F_1 = \eta_1 \times \frac{du_1}{dt} = \mu \times u_2$$

$$F_2 = \eta_2 \times \frac{du}{dt}$$

$$u = u_1 + u_2$$

$$F = F_1 + F_2 = \eta_1 \times \frac{du_1}{dt} + \eta_2 \times \frac{du}{dt}$$

$$; F = \eta_1 \times \frac{du}{dt} + \eta_2 \times \frac{du}{dt} - \eta_1 \times \frac{du_2}{dt} \quad ;$$

$$\frac{du_2}{dt} = \frac{1}{\mu}\frac{d(F - F_2)}{dt} = \frac{1}{\mu}\frac{dF}{dt} - \frac{\eta_2}{\mu}\frac{d^2u}{dt^2}$$

$$F + \frac{\eta_1}{\mu}\frac{dF}{dt} = \frac{\eta_1\eta_2}{\mu}\frac{d^2u}{dt^2} + (\eta_1 + \eta_2)\frac{du}{dt}$$

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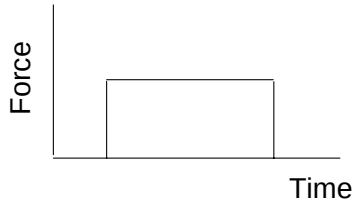
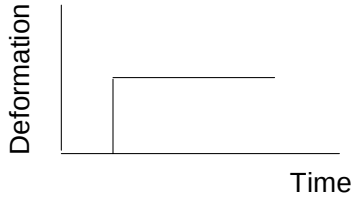
B. Graph the creep responses for the following loading and unloading processes. Label the graph at points of interest (e.g., the points with loading or unloading, the long-term trend) and explain briefly how spring and dashpots contribute to these changes in deformation.



C. Graph the force relaxation responses for the following loading process. Label the graph at points of interest (e.g., the points with loading or unloading, the long-term trend) and explain briefly how spring and dashpots contribute to these changes in force.



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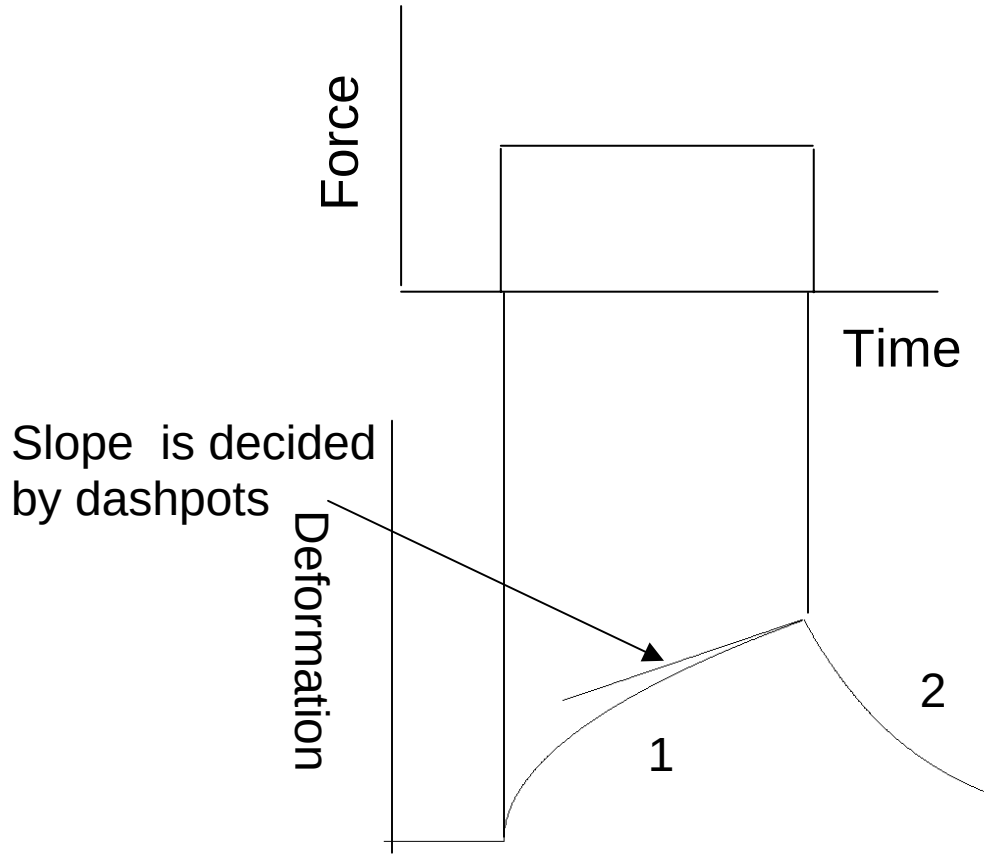

The force is applied at time 0 and the whole system start deformation gradually due to all the parts. In the long term the slope of the deformation is F/ $(\eta_1 + \eta_2)$ *as the spring is stabilized and the change of deformation are all from dashpots. At the time release the force, the spring is going back while the dashpot 2 resists a sudden deformation. The whole system won't go back to the original position as there is left deformation in the dashpots.*

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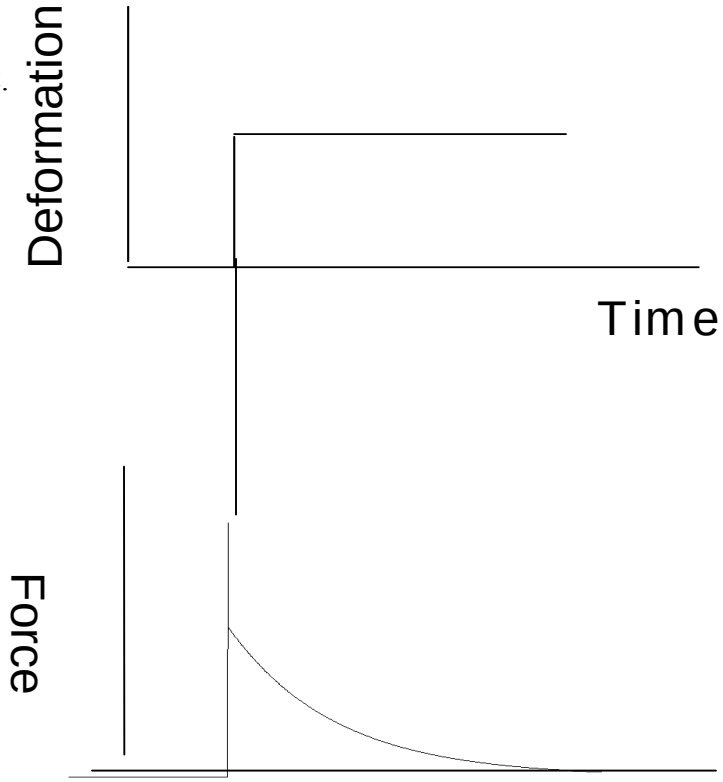
C.



The force is infinite at time zero at the rate of change in deformation is infinite. After that, the model is the same to the Maxwell model and the force will decay to zero. At the end the deformation is only from dashpots.