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.

1. True/False (2 points each) If your answer is false, justify your answer.

   a) During collagen synthesis, the pro-peptides are cleaved inside the cells before secretion.
   False. Pro-peptides are cleaved outside the cell by procollagen peptidases.

   b) 10% deformation of tissue = 9% stretching of bonds + 1% changes due to sliding
   False. 10% deformation = 1% stretching of bonds + 9% changes due to sliding

   c) In a parallel plate flow chamber, the shear stress is proportional to the fluid viscosity.
   False. Shear stress is proportional to y and dp/dx.
   (We'll accept also TRUE because of error in class notes.)

   d) Adult stem cells can only be found in bone marrow and blood.
   False. Adult stem cells can be found in a variety of tissues including skin, liver and cornea.

   e) BrdU is a quantitative method to measure the apoptosis rate.
   False. BrdU measures proliferation rate.

   f) Inhibition of cell spreading increases apoptosis rate.
   True.

   g) Integrins are secreted ECM signaling molecules.
   False. Integrins are ECM receptors.

   h) Cells can communicate via direct cell-cell contact.
   True.

2. (a) You have decided to use a cell-seeded matrix to make a tissue engineered equivalent of cartilage, and there are two main types of scaffold materials available, biological and synthetic. Name four properties of an ideal scaffold and describe how each property is important. (12 points)

biocompatible (1): if a material is not biocompatible, it will cause the device to fail and even put a person's life in danger (2)

bioactive (1): a scaffold needs to recruit host cells to help populate the scaffold (2)

bioconductive (1): cells should be able to integrate the material into the host tissue so the material should allow cell penetration (2)

bioinductive (1): adding growth factors to stimulate host cell differentiation and proliferation will help the scaffold be more influential on the surrounding tissue (2)

bioabsorbable in tune with healing (1): scaffolds that degrade without toxic residues will not incite encapsulation of the device and allow complete integration of the tissue engineered product, also the scaffold should not degrade too fast or the device may fail (2)

mechanical compliance (1): if the scaffold is in a area of mechanical stress, the scaffold must be as elastic as the surrounding tissue so it is does not break under repeated stress (2)

(b) Name the two main classes of macromolecules in ECM. For each class, name one specific macromolecule and give an example its function in a tissue. (8 points)

Fibrous proteins (2)

Structural – collagen (1), elastin (1) (elastin provides extensibility while collagen regulates the limits of extension) (1)
Adhesive – fibronectin (1), laminin (1) (cell adhesion and ECM component) (1)

Glycosaminoglycans and proteoglycans (2) - highly negatively charged polysaccharide chains, resist compressive force (1)

Hyaluronan (1)
Chondroitin sulfate and dermantan sulfate (1)
Heparin sulfate and heparin (1)
Keratin sulfate (1)

3. (a) What are telomeres? Why are they important? Name 3 cells where telomerase is found. (10 points)

Telomeres are at the end of chromosomes and after each cell division and are shortened by 50-200 base pairs per cell division. (2) When the telomere gets too short, the cell will stop replicating and cell death occurs. (2) Telomerase is the enzyme that rebuilds telomeres. (0) It is found in yeast and germ cells and may also be active in stem cells. (6)

(b) Liver failure affects millions of patients each year. Describe one treatment strategy using stem cell therapy (list at least 5 steps). What are two challenges that must be overcome? (10 points)

(1.5 points for each reasonable step, max 7.5)

1. Isolate adult stem cells from the patient or a donor using FACS or magnetic bead sorting. Use markers like Stro-1 to look for stem cells of the mesenchymal line. Test donor cells to be sure there are no viruses. For donor cells, modify genetic response by removing MHC markers.
2. Culture cells *in vitro* and expand the cell population.
3. Add appropriate growth factors and ECM molecules to help stem cell differentiation into liver cells.

4. Seed differentiated cells into a scaffold using an appropriate bioreactor. Culture cells in scaffold to form a liver lobule structure.
5. Implant the cells and matrix into the damaged liver.

Challenges may include getting a large supply of suitable mescenchymal stem cells. It is also hard to direct the differentiation because you need to add the correct combination of growth factors and seed the cells on the right ECM. You also need to determine the best scaffold for liver application and make sure cells spread out evenly in the scaffold. It is also necessary to have cell proliferation in the scaffold, and the scaffold should allow for matrix remodeling and easy handling for implantation. (2.5)

4. (a) Suppose you have a cell culture exhibiting unconstrained growth rate where dx/dt=u*X. Given the doubling time (t_d), derive the equation for cell number X as a function of time t. Given: at time t=0, X=C_o. (12 points)

$dx/dt = \mu x$

x: cell #

μ = rate of proliferation (proportional to cell #)

need initial conditions to solve ...

- $t=0,\ x=x_0$
- $t=t_0,\ X=2x_0$

(t_0: cell doubling time)

Solve ...

$dx/x = \mu\, dt$

$\int_0^x \frac{1}{x}\, dx = \int_0^t \mu\, dt$

$\ln x = \mu t + C_0$

$x = e^{\mu t + C_0} = e^{\mu t} \cdot e^{C_0} = C_1 e^{\mu t}$

@ $t=0$, $e^{\mu t} = 1 \rightarrow C_1 = x_0$

@ $t=t_0$, $x_0 e^{\mu t_0} = 2x_0 \rightarrow \mu = \ln(2)/t_0$

$x(t) = C_0 e^{(\ln 2/t_0)t}$

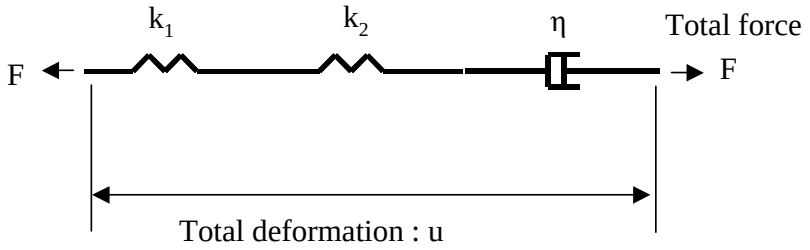
(b) For the same cell culture, suppose now you introduce a toxin that kills cells where the apoptosis rate is α. What is the new equation for cell number X as a function of t? What are three factors might affect apoptosis rate? (8 points)

$dx/dt = \mu x - \alpha x$

$x(t) = C_0 e^{(\mu-\alpha)t}$ (4)

Factors that might affect apoptosis rate include cell-cell contact inhibition, secreted soluble factors between cells, lack of growth factors or proper nutrition, oxygen deprivation, inability of cells to bind to substrate and lack of waste removal system. (4)

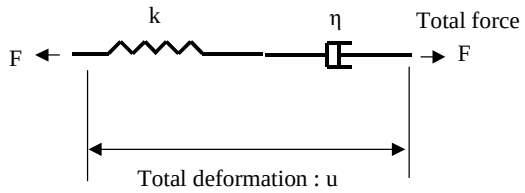
5. (a) To model the viscoelastic behavior of different components (collagen, elastin and proteoglycans) in native extracellular matrix, a researcher decided to use the model drawn below, including 2 linear elastic components and a linear dashpot in series.(1) Derive the constitutive equation for the model (the relationship between F and u). (10 points)



$F_1=k_1u_1$
$F_2=k_2u_2$
$F_3 = \eta*(du_3/dt)$
$F_1=F_2=F_3=F$

$u=u_1+u_2+u_3$
$du/dt=du_1/dt+du_2/dt+du_3/dt$
$du/dt=(dF/dt)/k_1+(dF/dt)/k_2+F/\eta$
or $du/dt=(dF/dt)(1/k_1+1/k_2)+F/\eta$

(b) You argue that same type of components in series can always be combined. To simplify the model, you want to combine the 2 elastic components into one, what's the "apparent" Young's modulus k for the new component? Express k as a function of k_1 and k_2. Draw the stress vs. time and strain vs. time plots for a creep response and label the graph with what is happening to the model at each significant change in the plots. (14 points)



Similarly, we can obtain the constitutive equation:
$du/dt=(dF/dt)/k + F/\eta$ (2)
thus $1/k = 1/k_1 + 1/k_2$ or $k=k_1k_2/(k_1+k_2)$ (2)

Creep plots (10)