Module 06: Cell Numbers, Growth and Kinetics
Assignment
Total Point Value = 30

Due by midnight on Day 7 of Module 6
This should be submitted to Blackboard as a pdf.

1. Exercise 4.8 From *Tissue Engineering*, Saltzman

A cell culture is initially composed of 100 cells. After 12 hrs the number of cells is 1.5 times the number in the initial population.

A) If the rate of growth is proportional to the number of cells present determine the time necessary for the number of cells to triple?

First we need to solve for the rate constant, k_p

$$N = N_0 * e^{k_p \cdot t}$$
$$150 = 100 * e^{k_p \cdot 12hrs}$$
$$1.5 = e^{k_p \cdot 12hrs}$$
$$\ln(1.5) = k_p \cdot 12hrs$$
$$\frac{\ln(1.5)}{12} = k_p = 0.034$$

Next we use the value of k_p to solve for the time to triple (when N = 300).

$$N = N_0 * e^{k_p \cdot t}$$
$$300 = 100 * e^{0.034 \cdot t}$$
$$3 = e^{0.034 \cdot t}$$
$$\ln(3) = 0.034 \cdot t$$
$$\frac{\ln(3)}{0.034} = t = \mathbf{32.5hrs}$$

B) What is the time required for a culture with $1x10^6$ of the same cells to triple. Explain your results

We were told that the growth rate was proportional to the number of cells – therefore we know growth is governed by the equation used in part 1, with an unchanging k_p. With the ratio of N/N_0 remaining 3, the time to triple will remain 32.5 hr. We can do that math and show this as follows:

$$N = N_0 * e^{k_p \cdot t}$$
$$3{,}000{,}000 = 1{,}000{,}000 * e^{0.034 \cdot t}$$
$$3 = e^{0.034 \cdot t}$$
$$\ln(3) = 0.034 \cdot t$$
$$\frac{\ln(3)}{0.034} = t = \mathbf{32.5hrs}$$

C) Under what conditions would the answers obtained in part B be invalid.

The estimate for time to triple in part B may be invalid if the growth rate was hindered or accelerated so that it was no longer proportional to the starting number. Some of the things that hinder cell growth are nutrient depletion, contact inhibition, and the Hayflick limit. One way that growth could be accelerated is through paracrine signaling. In this event, as the cell density increases proliferation factors

produced by neighboring cells become more abundant and the rate of cell growth increases.

2. Exercise 4.9 From *Tissue Engineering*, Saltzman
   For a specific type of cell after 3hrs the concentration of cells per milliliter of solution is about 400/mL. After 10hrs the concentration has gone up to 2000/mL. Determine the initial concentration of cells.

   To begin we need to solve for k_p. One way to do this is using the equation for doubling time.

   $$t_D = \frac{\ln(2)}{k_p}$$

   $$3hrs = \frac{\ln(2)}{k_p}$$

   $$k_p = \frac{\ln(2)}{3} = 0.23$$

   Now we can assume 1ml (or any volume will do!) and solve for the initial cell number.

   $$N = N_0 * e^{k_p \cdot t}$$

   $$400 = N_0 * e^{0.23 \cdot 3hrs} \quad or \quad 2000 = N_0 * e^{0.23 \cdot 10hrs}$$

   $$N_0 = \frac{e^{0.23 \cdot 3hrs}}{400} = \frac{e^{0.23 \cdot 10hrs}}{2000} = \mathbf{200cells/ml}$$

3. You're excited when you hear that you've been given a new pre-clinical research project where you'll be culturing liver cells and testing new drug compounds for toxicity. You know that the liver has great regenerative properties so you attempt cell and explant cultures from your rodent liver biopsies. To your dismay the cells aren't growing in culture – in fact they are dying. Please explain what factors you can control in tissue culture and how these might negatively affect your cell viability.
   Liver is notoriously a difficult cell type to culture, which has been a source of frustration for tissue engineers since this tissue does so well growing in the body. Out of the body we have almost complete control of factors to optimize growth conditions. These factors include media components and supplements (and how often media is changed), plating density (initial and splitting), substrate composition, pH, temperature, oxygen concentration and applied mechanical forces. The latter includes culturing cells in a more native 3D format rather than a 2D plate. Another tool in the tissue culture repertoire is to use a feeder or support cell layer. These are cells that supply a complex cocktail of factors needed to maintain the cells you are interested in.

Assignment Rubric

Question	Component	Total Point Value
1	A	4
	B	4
	C	4
2		8
3		10

Total Point Value = 30

References: