

Assignment 6: Cell Numbers, Growth and Kinetics

Cell and Tissue Engineering

1. Exercise 4.8 From Tissue Engineering, Saltzman

A cell culture is initially composed of 100 cells. After 12 hours 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.

The rate of growth being proportional to the number of cells present:

$$dN / dt = K \times N$$

- Separating variables: $dN/N = K \times dt$.
- Integrating between time zero when $N = N_0$ and time t , when $N = N$:
 $\ln N - \ln N_0 = Kt - 0$, or $\ln(N/N_0) = Kt$, or $N = N_0 \times e^{Kt}$

Plugging the numbers: $100 \times 1.5 = 100 \times e^{12K}$ or $150 = 100 \times e^{12K}$

$$e^{12K} = 150 / 100 = 1.5$$

Taking the \ln on each side of the previous equation: $12 K = \ln(1.5)$

$$K = \ln(1.5)/12 \sim 0.033$$

The time for the number of cells to triple: $N = 3 \times N_0 = N_0 e^{Kt}$ or $e^{Kt} = 3$

Taking the \ln on each side of the previous equation: $K \times t = \ln(3)$

$$\Rightarrow t = \ln(3) / K = \ln(3) / 0.033 \sim 32.51 \text{ hours}$$

$$t = 32 \text{ hours } 30 \text{ min } 36 \text{ s}$$

- b) What is the time required for a culture with 1×10^6 of the same cells to triple?

Explain your results.

It will take the same time to triple for the culture with 1×10^6 of the same cells as the culture with 100 cells since the constant K is constant for the same cell type and independent of the initial number of cells in the culture.

- c) Under what conditions would the answers obtained in part b) be invalid?

In practice, cell growth is density-dependent, it is constrained not only by cell density, but substrates, nutrient availability, waste product accumulation, sensitivity to enzymes, hormones concentrations, growth factor, cytokines, receptor-ligand interactions and other environmental factors. The answer in part b) does not consider these different constraints and will be invalid. If these constraints are included; more complex mathematical models, like the Monod model which describes the influence of the substrate on growth rate, are required.

2. Exercise 4.9 From Tissue Engineering, Saltzman

For a specific type of cell after 3 hours, the concentration of cells per milliliter of solution is about 400/mL. After 10 hours the concentration has gone up to 2000/mL. Determine the initial concentration of cells.

Assuming no constraints to their growth, the rate of growth is proportional to the number of cells and we have the equation: $N = N_0 \times e^{Kt}$.

Per mL:

$$400 = N_0 \times e^{3K}$$

$$2000 = N_0 \times e^{10K}$$

$$N_0 = 400 / e^{3K} = 2000 / e^{10K} \Rightarrow e^{(10-3)K} = 2000/400$$

$$\Rightarrow e^{7K} = 2000/400 = 5$$

Taking the ln on each side: $K = \ln(5)/7 \sim 0.2299$

Substituting back, we obtain the initial concentration of cells:

$$N_0 = 2000 / e^{10K} = 2000 / e^{10 \times (\ln(5)/7)} = 400 / e^{3 \times (\ln(5)/7)} = 200.67 \sim 200$$

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 to 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.

There are a variety of factors we can control in tissue culture which can have undesired impact on cell viability (Table 1 below).

Table 1: Factors with actions and impact on cell cultures

Factors	Action	Negative impact
<ul style="list-style-type: none"> ▪ Nutrients ▪ Vitamins ▪ Ammino acids ▪ Glucose ▪ Salt ▪ Growth factors ▪ sHormones 	<ul style="list-style-type: none"> ▪ Culture medium is added to nourish the cells. Growth factors or cytokines improve cell viability, and growth and keep cells healthier. ▪ Ex.: Activin A regulates cell proliferation¹ 	<ul style="list-style-type: none"> ▪ If cells do not have proper nutrients and sufficient abundance they cannot function and fulfill their functions. They are eventually going in apoptosis. ▪ Quality of the serum used in term of nutrients (vitamins, glucose, salts, etc.) can affect cell differentiation.
Media volume	<ul style="list-style-type: none"> ▪ Sized accordingly to desired growth and pattern proliferation. ▪ To continue to grow we may need to subculture the cells. 	With reduced area, once cells reach confluence, proliferation stops. Density also affects cell growth.
Waste accumulation	Medium needs to be exchanged regularly to maintain nutrients, and growth factors consumed by the cells and to eliminate waste produced by the cells.	Waste accumulation can contaminate the cells and leads to apoptosis.
pH	Maintain appropriate pH for the types of cells to grow. Average pH for mammalian cells is pH 7.4.	Too low and high pH can be toxic for the cells.
Temperature	<p>Like pH, it depends on body temperature from which the tissue was extracted. Maintain appropriate temperature:</p> <ul style="list-style-type: none"> ▪ Mammalian cell lines 36-36°C ▪ Insert cell lines: 27-30 °C. 	Too high temperatures increase the fluidity or permeability of cell membrane allowing potential harmful proteins to enter the cell or damage integral or peripheral proteins. Similarly, too low temperatures stiffen the cell membrane preventing essential molecules like oxygen or glucose into the cell.

¹ For an example of list of supplements: <https://www.ptglab.com/support/cell-culture-protocol/introduction-to-cell-culture/>

Factors	Action (Positive impact)	Negative impact
Cell contamination	<ul style="list-style-type: none"> Follow safety and sterilization guidance for laboratory practices. 	<ul style="list-style-type: none"> Cell could be contaminated by bacteria, fungi, yeast or virus. Chemical contamination which may originate from endotoxins, detergents, impurities of the media, or water used in media or buffers, or from equipment and supplies, can affect cell culture. Antivirus could be used to prevent virus contamination, although antibiotics could be toxic to cell cultures.

