

EXAM 1

ChE 590B: Tissue Engineering

NAME:

SOLUTIONS

ID #:

PEYTON

This is a take-home exam. You may use any CITED, peer-reviewed, sources that you like in your answers, but you cannot collaborate with a partner. Suspicions of dishonesty will be submitted for academic review.

Exam 1 is due IN PERSON, to 154D Goessmann, between 4-5:15p on 2/14/13.

Other Rules

1. All written submissions will be checked with TurnItIn to check for plagiarism. Feel free to pre-check your submissions as well.
2. Box all your final answers. If your final answer is not clear, you will not be given the benefit of the doubt.
3. Write governing equations and illustrations to obtain partial credit if you are running out of time or stumped.
4. You will be penalized for improper use of significant digits and missing units.
5. Do not work with a partner.
6. The only acceptable citations are from peer-reviewed literature.
7. Questions created from *Tissue Engineering* and other sources. Use any source you want to help you answer questions, so long as you cite.
8. Put your name on every page!!!

PROBLEM 1 (40 POINTS POSSIBLE):

40

PROBLEM 2 (40 POINTS POSSIBLE):

40

PROBLEM 3 (20 POINTS POSSIBLE):

20

TOTAL (100 POINTS POSSIBLE):

100

1. Literature research (40 points)

From exercise 2.2 in *Tissue Engineering*, and written by Prof. Song Li.

Artificial skin may or may not contain living cells. What are the advantages and disadvantages of having cells in the artificial skin? Are keratinocytes immunogenic? Why? If you are going to make artificial skin with cells, where are you going to get the cells?

Attach additional pages if necessary. Cite your references used. **Limit your response to no more than 2 total pages.**

GRADING CRITERIA:

GRAMMAR/SPELLING: 5/5 pts

PROS & CONS OF LIVE CELLS: 10/10 pts.

IMMUNOGENIC? : 5 pts.

WHAT IS THE CELL SOURCE OR ARTIFICIAL SOURCE?
5/10 pts

"Page length: should be 1.5-2 pgs. 5/10 pts.

References: 5 pts. (3+, peer reviewed)

Turn it in: 5 pts

2. Literature Research/Editorial, Stem Cell Therapies (40 points)

In class, we have discussed several sources of human stem cells, each of which is accompanied by certain advantages and disadvantages.

Imagine a scenario where you are the owner of a new start-up company, which is founded on using stem cells to repair or replace pancreatic tissue that has been lost in patients with pancreatic cancer (a debilitating disease with a nearly 100% fatality rate due to metastasis and pancreatic failure). You hope that by catching the disease very early in patients, before the cancer has spread (metastasized), you will be able to work with surgeons to completely remove the pancreas, and replace the pancreatic tissue by using a stem-cell generated engineered tissue.

In 5,000 characters (including spaces) or less, describe how you would go about doing this. Some considerations are:

- A) What your stem cell source is.
- B) What biomaterial scaffold you will use to culture the cells in the lab, if any.
- C) What structure and function your engineered tissue must satisfy to be functional.
- D) How long you think it will take from stem cell harvesting to implanting a functional tissue in the body.

Conclude your editorial with 1-2 sentences stating whether you think this is a viable strategy for future research. Make sure to include citations for all peer-reviewed literature.

GRADING CRITERIA:

GRAMMAR/SPELLING: 5pts.

What is cell source: 5pt.

~~Is~~ Biomaterial? Why or why not, what is mat'l: 5pt

What is key structure/function of pancreas?: 5pts

Timeline for stem cells → implant: 5pts.

Editorial conclusion: 5pts.

length: 4 - 5K characters: 5pts

References: 5pts.

2 Turn it in

3. Kinetics. (20 points)

Exercise 4.21 from *Tissue Engineering*. Written by Professor Linda Griffith.

EPO is a growth hormone secreted by the pancreas that influences red blood cell (RBC) development. The number of RBCs secreted roughly corresponds to the equilibrium number of signaling complexes. For this reason, Amgen sells a recombinant form of EPO for administration to patients who are anemic or suffering extreme blood loss.

- A) Describe, briefly, how you would make a recombinant form of EPO.
 B) In order to supply an appropriate amount of the drug, the equilibrium-binding behavior needs to be established. So a cell biology researcher measured equilibrium binding versus ligand concentration. Using the given data, calculate K_d and R_T .

Data: equilibrium binding of EPO to 1×10^5 cells in culture [ref. 33 in text].

L_0 (nM)	0.025	0.05	0.075	0.15	0.25	0.33	0.66	0.75	1.25	1.75	3	10	12	16	33
C (pM)	1.05	2.2	3	5.5	8	10.5	14	16	20	22	26	35	30	32	33

- C) Plot
- C_{eq}
- versus
- $\log(L_0)$
- using the equilibrium binding equation:

$$K_d = \left(\frac{RL}{C} \right)_{eq} \approx \frac{(R_T - C_{eq})L_0}{C_{eq}}$$

In what range of ligand concentrations is the response curve linear? Where does the curve start to plateau?

- D) For both cost and safety purposes, hormones are usually administered at the lowest effective concentration. Does it make sense that a "unit/mL" of EPO is defined as 0.2 nM?

Attach additional pages as necessary.

A → D 5pts. each

A) Make in bacteria or yeast. Bacteria is easiest to transfect, so try this first. If not possible, use a transformed yeast line.
~~Isolate EPO~~ isolate, or, better, purchase EPO gene & insert into bacteria with a primer, make line by selection against Pen or Strep.
 Induce expression with IPTG or other inducible primer.
 Produce, purify plasmid.

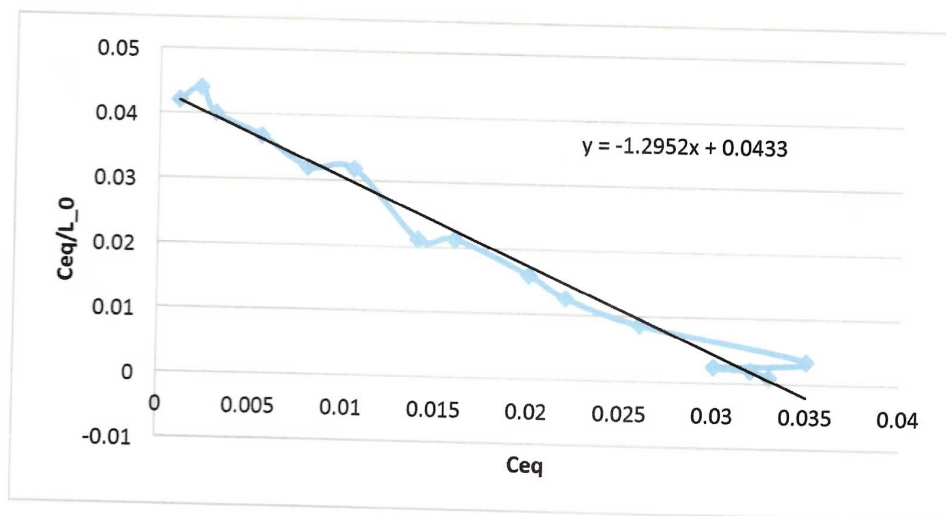
B) Attached $K_d = \frac{RL}{C} \bigg|_{eq} = \frac{(R_T - C_{eq})L_0}{C_{eq}}$ ~~$\frac{C_{eq}}{L_0}$~~

→

f_n (B)

L_0 (nM)	Ceq (pM)	Ceq (nM)	Ceq/L_0
0.025	1.05	0.00105	0.042
0.05	2.2	0.0022	0.044
0.075	3	0.003	0.04
0.15	5.5	0.0055	0.036667
0.25	8	0.008	0.032
0.33	10.5	0.0105	0.031818
0.66	14	0.014	0.021212
0.75	16	0.016	0.021333
1.25	20	0.02	0.016
1.75	22	0.022	0.012571
3	26	0.026	0.008667
10	35	0.035	0.0035
12	30	0.03	0.0025
16	32	0.032	0.002
33	33	0.033	0.001

$1/K_d = 1.2952$
 $K_d = 0.772082 \text{ nM}$
 $R_T/K_d = 0.0433$
 $R_T = 0.033431 \text{ nM}$



Egn 4-8 from Tiss. Egn.

$$\frac{C_{ef}}{L_0} = -\frac{1}{K_d} C_{ef} + \frac{R_T}{K_d} \Rightarrow \text{plot}$$

$$K_d = 0.77 \text{ nM}$$

$$R_T = 0.03 \text{ nM}$$

c)

for (c)

$$C_{eq} = R_T \cdot L_0 / (K_d + L_0)$$

L ₀ (nM)	C _{eq} (nM)	log (L ₀)
0.025	0.001049	-1.60206
0.05	0.002033	-1.30103
0.075	0.00296	-1.124939
0.15	0.005438	-0.823909
0.25	0.008177	-0.60206
0.33	0.01001	-0.481486
0.66	0.015407	-0.180456
0.75	0.016473	-0.124939
1.25	0.020666	0.09691
1.75	0.023197	0.243038
* 3	0.026588	0.477121
10	0.031035	1
12	0.03141	1.079181
16	0.031892	1.20412
33	0.032667	1.518514

linear from 1.75 nM
 $L_0 = 0.33 - 3 \text{ nM}$

plateaus @ $L_0 = 10 \text{ nM}$

D)

Ligand = EPO

Effective dose appears to be ~ 3 nM,
 lowest effective concentration is ~ 0.33 nM
 which is ~ 1 unit/ml, so yes, this
 makes sense.

Linear range: 0.25-1.75 nM

The curve start to plateau at $L_0 = 10 \text{ nM}$

