University of Toronto
Faculty of Applied Science and Engineering
Final Examination, April 21, 1999

$\$1.75$

Fourth year – Program 05

BME496S – Cellular Bioengineering

Exam type: C

Examiner – P.W. Zandstra

Instructions: Read the whole exam before attempting any of the questions. Answer only one of questions 3 and 4. Good Luck!

[20%] The rate of accumulation of receptor/ligand complexes in a coated pit for ligand induced endocytosis may be limited by diffusion of the complexes in the cell plasma membrane. Indeed, it is conceivable that the complex endocytic rate constant, k_{eC}, could depend on the receptor/ligand binding affinity because the ligand could dissociate from the complex before the complex is able to diffuse into the coated pit (preventing the receptor from being "recognized" by a coated-pit-associated internalization component). Hence, the maximum value of k_{eC} could be smaller for ligands with a faster dissociation rate constant (k_r) than for ligands with a slower dissociation rate constant. Using the typical values for the necessary parameters given below, estimate the minimum value of k_r at which the measured value of k_{eC} might begin to decrease.

Table A. Typical parameter values:

R (cell radius)	5 μm	Average # of coated pits per cell	10^3
D_C (complex diffusion coefficient)	10^{-9} cm^2/sec		

[20%] A graduate student, not wanting to complicate her experiments, would like to perform equilibrium receptor-ligand binding studies without worrying about ligand depletion. Given the simple monovalent cell surface (i.e., no internalization or receptor synthesis) model depicted below:

$$L + R \Leftrightarrow C$$

derive an equilibrium expression, in terms of the bulk ligand concentration (L_0), the total number of cell surface receptors (R_T), the equilibrium dissociation constant (K_D) and the appropriate conversion constant(s) for the maximum cell density (ρ) that her experiments can be run at before she has to worry about ligand depletion.

1

NOTE: PLEASE CHOOSE EITHER QUESTION 3 OR 4.
DO NOT DO BOTH

3. *[20%]* In attempting to understand how mechanical forces might be transduced into biochemical events via cell adhesion receptors, it is proposed that the disassociation rate constant for reversible protein/protein linkages in an intracellular assembly might be increased by imparted stress according to the following expression:

$$k_r = k_{r0} \exp\left\{\frac{\gamma F}{k_B T N}\right\}$$

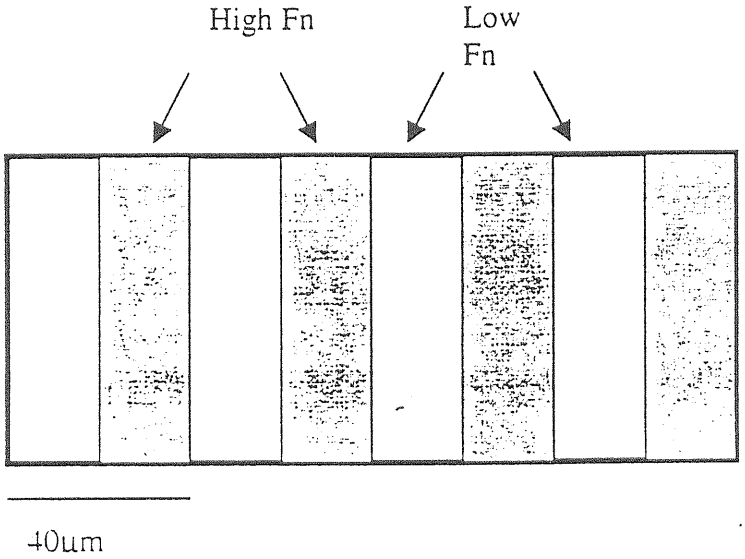
where k_{r0} is the rate constant under stress-free conditions, F is the stress imparted to the assembly of linkages, γ is the linkage interaction distance over which the stress acts, and N is the number of independent linkages present in the assembly.

Describe, in as much conceptual detail as possible within time limits, an experimental approach that could test this expression.

OR

4. *[20%]* Explain, to the most rigorous extent possible within time limits, how ligand-induced signaling information might be encoded in the *frequency* of intracellular ion concentrations generated by the signal transduction pathway.

20%] It has recently been hypothesized that particular substrates can be designed to separate cells based on their migratory properties. Recent experiments in the laboratories of Snail Pace Inc.™ studied the distribution of cells seeded on substrates patterned with alternating stripes of high and low fibronectin (Fn) densities. In these experiments, substrates are prepared by micropatterning the chemistry of glass slides to create 20 μm-wide strips, alternating between hydrophobic and relatively hydrophilic. Hydrophobic strips absorb more fibronectin than hydrophilic strips, and thus by placing the substrate in contact with a solution of fibronectin, simple protein absorption to the substrate creates patterns of high and low fibronectin concentration. In a typical experiment, cells are seeded at a high density of ~20,000/cm^2 to create a uniform initial distribution on the substrate. The cells are examined again after 24 hr and the cell distribution is recorded. The cells employed in these studies are CHO LA cells, which have a maximum migration speed of about 20 μm/hr.



a) Do you think the cell distribution observed at the 24-hr time point is an "equilibrium" distribution (i.e., assuming no cell growth or death, would it change over the subsequent 24-hr). Would your answer change if the strips were 500 mm wide?

b) An experiment is conducted in which the concentration of Fn in the absorption solution is varied and the following results are obtained:

Fn concentration in adsorbing solution (mg/mL)	Cell distribution after 24 hr
10	all cells on "high" strips
5	all cells on "low" strips

i) Interpret these results (i.e., offer a mechanistic hypothesis which could explain the observed distribution) and *ii)* suggest a set of additional experiments which might prove the hypothesis.

Short answer questions

You should spend a maximum of 5 min on each of these questions

A) *[5%]* The degradation rate constant (k_{deg}) can be considered a "lumped" parameter – taking into account several different intracellular molecular processes. Briefly outline what these processes may be and what parameters may affect the measured value of k_{deg}.

B) *[5%]* "Growth factors" can influence cellular responses by acting locally or systemically. Four possible modes of growth factor action were introduced in class. Intracrine ligand binding, where the ligand binds its receptor intracellularly, is one of the four. Briefly sketch and label the other three.

C) *[5%]* Cells use adaptation or desensitization to a constant stimulus to prevent the hyper-stimulation of their responses. In fact, disruption of the processes involved in cellular desensitization has been shown to be at the root of several types of malignant transformation. Briefly describe two mechanisms cells use to "desensitize" themselves to further stimulation by a constant signal.

D) *[5%]* Sketch a single cell (top view) migrating orthokinetically up a gradient. Indicate in your diagram the cell's migration path and the gradient direction. Briefly indicate how linear translation speed (S) and persistence time (T) could be determined from your diagram.