1. (38 pts) Amyotrophic lateral sclerosis, ALS, also known as Lou Gherig's disease, is a neuromuscular disorder in which patients gradually lose peripheral motor activity. One approach under investigation for treatment of ALS combines gene therapy with cell encapsulation therapy. In the ALS treatment under consideration, rodent cell lines are modified genetically to release ciliary derived neurotrophic factor, CNTF, an agent which has shown promise for slowing the deterioration of neurons in more conventional preclinical drug tests. In conventional cell culture studies, Hamster-BHK cells transfected with the gene for CNTF, showed promisingly high secretion rates of 1 ng/10^6cells/day.

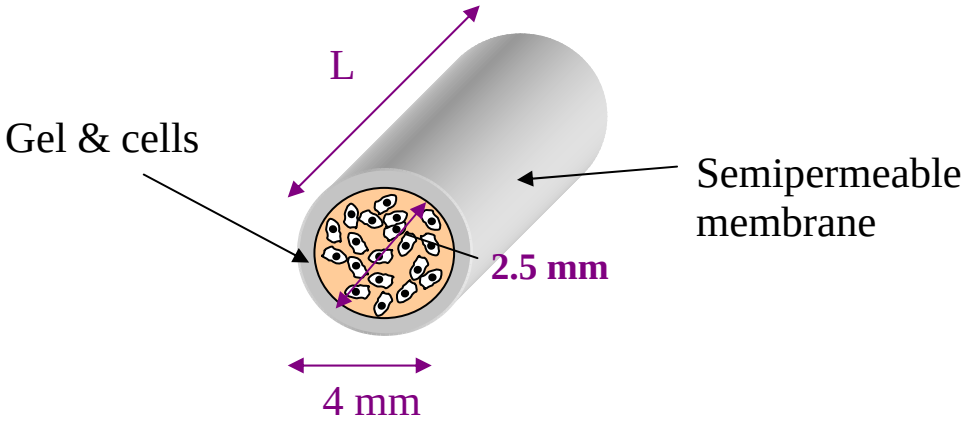
You are working for a company that is developing a cell encapsulation device to house the Hamster-BHK cells in vivo. Prototype membranes having the porosity characteristics given in the table below have been developed.

(a) What functions does the membrane serve in this device? (3 pts)

(b) What processing route would be used to make the membrane for this device? What would be a typical material for such a membrane? (4 pts)

(c) Which prototype membrane will exhibit the highest CNTF delivery rates, assuming the same number of cells is contained in each device? Justify your response quantitatively, stating any assumptions you make. (6 pts)

Membrane Prototype	1	2	3
Pore size (Å)	16±7	27±7	46±20
Surface Porosity (%)	0.044±0.008	0.045±0.006	0.053±0.006
Thickness (μm)	670±25	810±14	819±22



(d) Approximately how long should this device be (L) to hold 10^6 cells? State any assumptions you make in arriving at your value. (4 pts)

(e) Based on pore size considerations, would any of the prototype membranes be expected to exclude the antibody IgG (molecular weight 160,000 g/mol)? Justify your response as quantitatively as possible, stating any assumptions you make. (5 pts)

(f) In prototype devices prepared by your company, cells were seeded in a polyethylene oxide (PEO) hydrogel within the membrane, and cultured in nutrient-rich media to test for CNTF release. To your management's dismay, the release rate was found to be much lower than anticipated from studies of the same cells seeded on conventional culture plates. Moreover, CNTF secretion was seen to decrease monotonically over several days in culture. Provide <u>two</u> likely explanations for these observations. (4 pts)

(g) Suggest design changes to the device to rectify the problems noted in (f). (4 pts)

(h) Would you expect this device to be more resistant to crushing in the longitudinal or transverse direction? Justify your answer quantitatively. (5 pts)

(i) Your company is also considering development of cell encapsulation therapies for type I diabetes based on these membranes. Would you recommend pursuing this application? Explain your answer. (3 pts)

2. (34 pts) Expanded polytetrafluoroethylene (ePTFE) is a commonly used material for synthetic vascular grafts. One mode of failure of such devices is via bacterial infection initiated during implantation. Upon the introduction of a foreign material into the body, a competition for the surface ensues between bacteria and leukocytes that serve to fight infection. One hypothesis for elevated infection rates for PTFE graft implantations is that leukocyte migration is impeded on ePTFE surfaces, allowing bacteria to become established in the early stages following implantation.

(a) Describe the process by which leukocytes are attracted and migrate to the site of a vascular graft implantation. (4 pts)

To investigate which integrin subunits play a significant role in leukocyte migration on ePTFE, Chang and coworkers performed random migration studies on populations of fluorescently labeled polymorphonuclear leukoocytes (PMN's or neutrophils) exposed to antibodies for different integrin subunits. Confocal microscopy images depicting PMN migration on ePTFE after 3 h of incubation with IgG (A) or anti-CD18 (C) are shown below.

(b) Explain how one would obtain a motility coefficient for the PMN's from such data. (4 pts)

(c) Provide a molecular level explanation for the observed differences in migration between PMN's exposed to anti-CD18 vs. those exposed to IgG. (3 pts)

Images removed for copyright reasons.

See Fig. 4(a) and (c) in Chang, Charlie C., Rene S. Rosenson-Schloss, Tanuja D. Bhoj, and Prabhas V. Moghe. "Leukocyte Chemosensory Migration on Vascular Prosthetic Biomaterial is Mediated by an Integrin ß2 Receptor Chain." *Biomaterials* 21 (2000): 2305-2313.

As a potential surface modification method for ePTFE, Kidd and Williams investigated temporarily seeding epithelial cells onto ePTFE to deposit extracellular matrix proteins and thus potentially improve endothelial cell adhesion and vascularization of new tissues. In their study, 6 different cell types were seeded on ePTFE: human microvessel endothelial cells (HMVEC), rat microvessel endothelial cells (RMVEC), human squamous epithelial cell line (HaCaT), a tumorigenic variant of HaCaT (II-4), rat bladder squamous cell carcinoma cell line (804-G), and lung carcinomatous epithelial cell line (A549). Cells were seeded at equivalent densities and left in culture for 8 days, after which they were removed using ammonium hydroxide, which allowed deposited ECM proteins to remain on the surface. Subsequently, ECM was collected from the surface of the ePTFE with a rubber scraper and SDS polyacrylamide gel electrophoresis was performed to determine the proteins present. Western blot analysis was performed employing rabbit or mouse antibodies for collagen I, collagen IV, fibronectin, laminin-1 and laminin-5 in different lanes of the gel, followed by a secondary antibody, rat antimouse IgG or goat antirabbit IgG, conjugated to horseradish peroxidase. Results from the SDS-PAGE study are shown below.

(d) Explain the function of the two antibodies used in the Western blot analysis. (4 pts)

(e) Based on the SDS-PAGE results, which protein has a higher molecular weight, fibronectin or laminin-5? Explain. (2 pts)

Images removed for copyright reasons.

See Fig. 1 in Kidd, Kameha R., and Stuart K. Williams. "Laminin-5-enriched Extracellular Matrix Accelerates Angiogenesis and Neovascularization in Association with ePTFE." *J Biomed Mater Res* 69A (2004): 294-304.

To ascertain the effects of ECM-modification on vascularization near the ePTFE grafts, discs of ePTFE modified as described above were implanted into the fatty tissue of rats. After 5 weeks, the devices were explanted and tissues in the vicinity of the implant examined for new vessel growth. Data quantifying angiogenesis and neovascularization are given in the figure below, reported as means and standard deviations of n=4 samples for RMVEC-, HMVEC, A549-, and II-4-modified samples and n=8 samples for 804-G, HaCaT and nonmodified samples.

(f) Describe the expected tissue morphology in the vicinity of the ePTFE implant after (i) five days; (ii) five weeks. (6 pts)

(g) Which of the ECM-modified samples exhibit a statistically significant increase in angiogenesis compared with the unmodified ePTFE control? (6 pts)

(h) From the data provided, give a possible explanation for your findings in (g). (2 pts)

(i) How might the results of this study be relevant to cancer therapies? (3 pts)

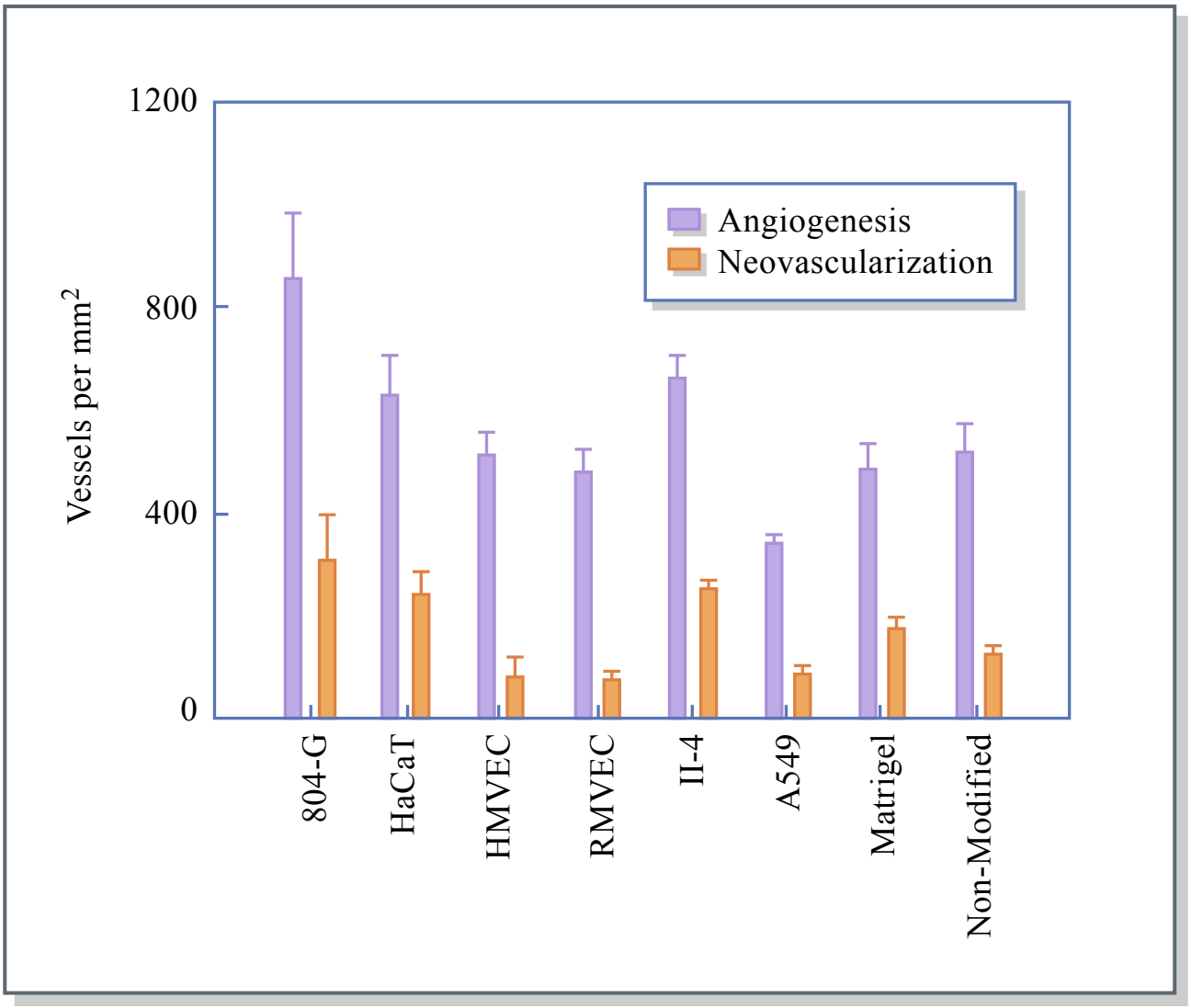


Figure by MIT OCW.

3. (39 pts) Over 15M people in the United States alone are afflicted with type I diabetes, a disease in which the pancreas fails to generate adequate insulin necessary for metabolizing glucose. The management of blood glucose is currently achieved by active monitoring of blood glucose concentration, typically ranging from 1-30 mM, along with daily insulin injections to replace or supplement the patient's natural hormone production.

(a) Describe the operation of commercial biosensor devices used for glucose monitoring. (4 pts)

As a potential new method for glucose monitoring, Hsieh et al. developed a surface plasmon resonance device. For this device, bacterially-derived glucose/galactose-binding protein (GGBP), a known receptor for glucose, was covalently attached to the SPR surface by replacing specific amino acids in the structure by cysteine (R group= CH_2SH) through genetic mutation. Below is a ribbon diagram of GGBP showing its binding pocket for glucose and locations of mutation sites.

(b) Sketch the general reaction employed to bind the GGBP to the SPR surface. (2 pts)

(c) Is this device a biosensor? What constitutes the detection element, analyte and transducer? (4 pts)

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See Fig. 1 in Hsieh, H. V., Z. A. Pfeiffer, T. J. Amiss, D. B. Sherman, J. B. Pitner. "Direct Detection of Glucose by Surface Plasmon Resonance with Bacterial Glucose/galactose-binding Protein." *Biosensors and Bioelectronics* 19 (2004): 653-660.

The figure below shows the SPR signal (RU= response units, 1RU ~1 pg/mm^2) generated upon the injection of 100 μM glucose in a running buffer solution flowing at 5 μl/min over a surface coated with GGBP mutated at the E149 site (solid line), and a second surface prepared with E149-mutated GGBP subsequently denatured by overnight exposure to 1M guanidine HCl (dashed line).

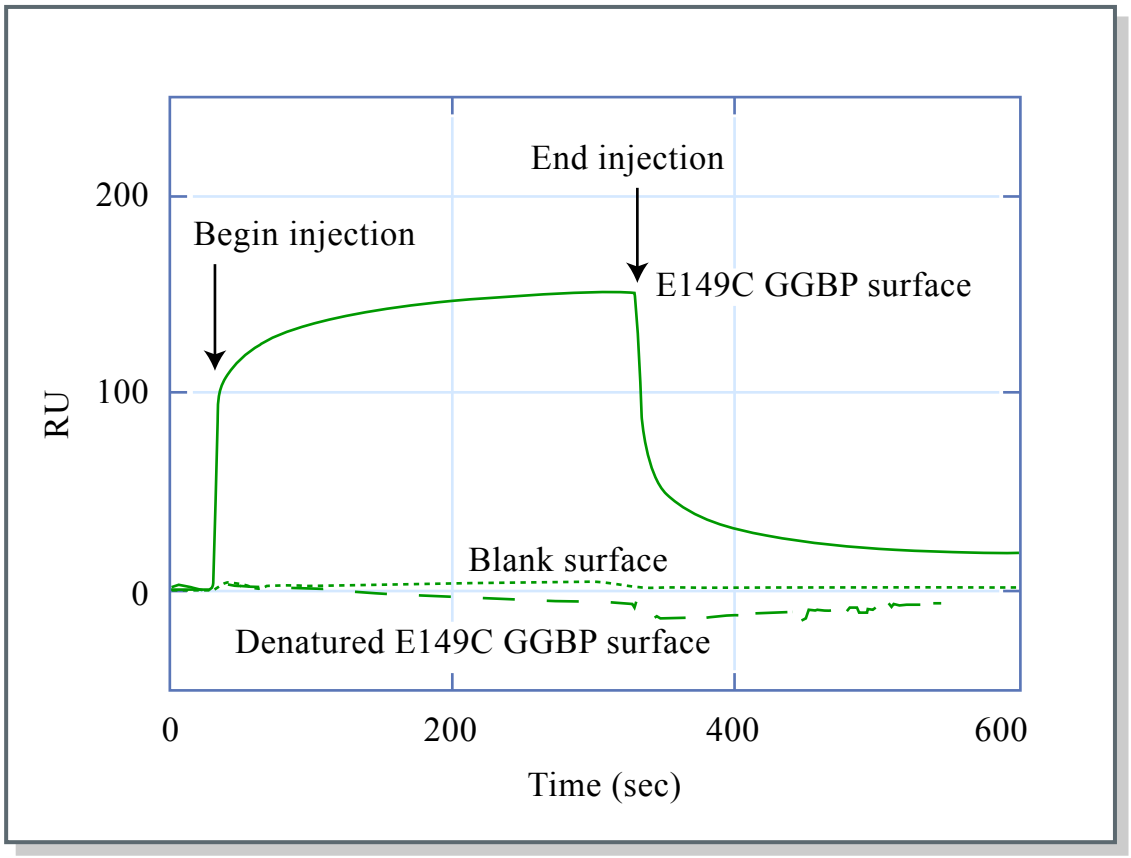

Figure by MIT OCW.

(d) Explain the observed features in the SPR signal as a function of time for the E149C mutant GGBP surface (solid line). (3 pts)

(e) Why does the SPR signal differ when E149C GGBP is denatured prior to glucose exposure? (2 pts)

(f) What techniques could you use to characterize changes in the: (i) secondary structure content, (ii) surface chemistry, (iii) surface morphology and (iv) thickness of the GGBP layer after exposure to guanidine HCl? (8 pts)

In a series of experiments, the SPR signal change was recorded as a function of glucose concentration for a E149C mutant GGBP surface and a GGBP with cysteine mutations at E149C, A213S and L238S, as shown below.

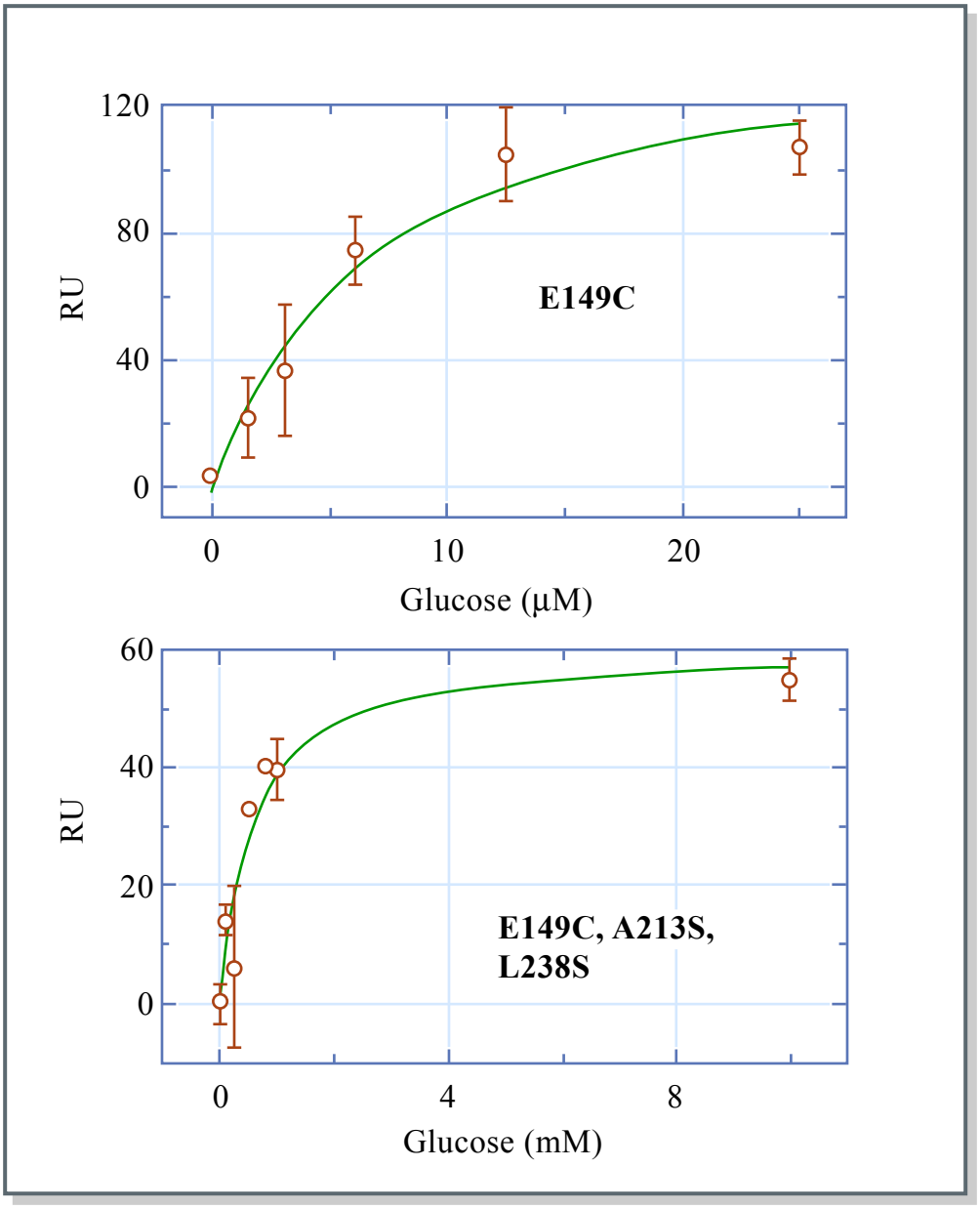

Figure by MIT OCW.

(g) From the data provided, calculate the equilibrium dissociation constant for glucose-GGBP binding in each case. (6 pts)

(h) Explain the observed differences in K_d for the two surfaces. (3 pts)

(i) How would you expect the value of K_d to change if the cysteine mutation was performed at the K137 site? (2 pts)

(j) Would either of the sensors above be useful for monitoring glucose levels typical in humans? Explain. (3 pts)

(k) Provide one key drawback to the proposed SPR sensor for blood glucose monitoring. (2 pts)