Problem Set 2 Solution **BE.462J/3.962J**
Spring 2003

Issued: Day 4
Due: Day 5
(20 pts total)

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1. (4 pts) An early study in the development of peptide-presenting biomaterials showed that polymer surfaces bearing RGD peptides at a density equivalent to ~10^5 peptides per each cell was sufficient to promote cell attachment, spreading, and subsequent cell growth. In contrast, when whole fibronectin protein was adsorbed to polymer surfaces, it was found that many more copies of the intact protein were needed per unit area to obtain similar responses from cells. Explain this result. How should the binding of cell integrin receptors to a short RGD peptide compare with binding to native fibronectin?

These experiments were discussed in the reading assignment: Two factors leading to the need for more copies of FN adsorbed to a *surface vs. RGD* are *denaturation of the protein on adsorption (binding sites* are *destroyed by unfolding) and masking of binding sites, e.g. the RGD site of FN arranged 'face down' on the substrate where receptors cannot access it. This result is striking since short peptides taken from adhesion proteins typically show much lower affinities (by up to 100-fold) for their receptors than the intact whole protein, due to the presentation of additional residues that are spatially local to the binding site but distant along the folded length of the protein chain and the relative immobilization of the binding sequence in the optimal configuration within the protein backbone.*

2. (3 pts) In the article by Schense et al., Figures 1 and 2 show that different adhesion peptides incorporated into a natural biopolymer matrix (a fibrin fiber network) induce different degrees of neurite outgrowth from neuronal cells, even when conjugated to the matrix at the same total densities. Provide 2 physico-chemical reasons to explain how different peptides can provide different responses.

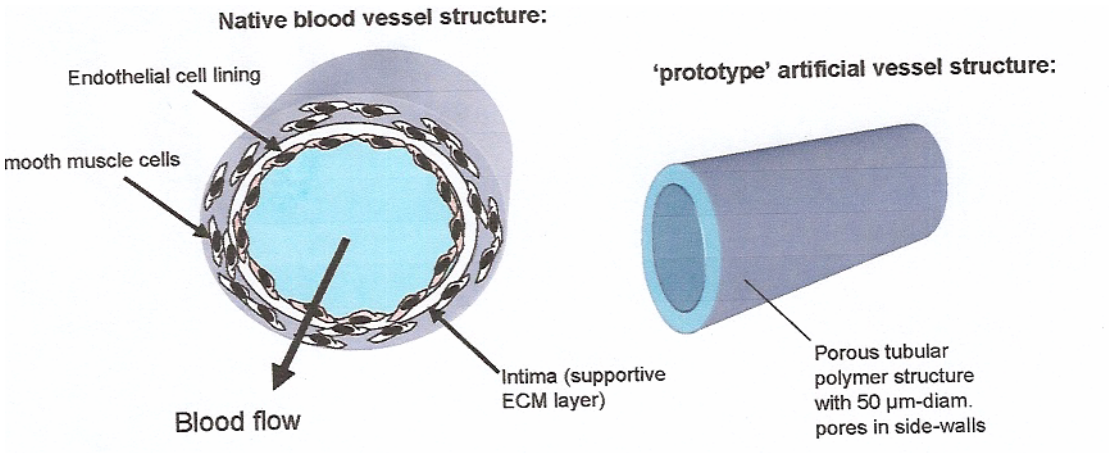
Each peptide may target a different integrin receptor on the cell, and different integrins may be expressed at different total densities on the cell surface. Second, each peptide may be bound with a different affinity even if it binds the same integrin- together, these two factors allow different peptides to provide very different results.

3. (3 pts) Compare and contrast the erosion characteristics expected for a device to be used *in vivo* that is fabricated from a polyesteramide vs. the characteristics of the same device fabricated using a polyanhydride.

Polyesteramides are degradable by proteolytic enzymes in addition to being sensitive to hydrolysis. This enzymatic sensitivity will cause both the polyesteramide and the polyanhydride device to undergo surface erosion if suitable enzymes are present at the implant site.

4. ***Design* of *an artificial artery***. (10 pts) Shown below is a simplified schematic of the structure of an artery .The two primary features of an artery are the endothelial cell lining on the interior lumen of the vessel (contacting blood flow) and the smooth muscle cell layer surrounding the vessel, which

can provide contractile force to support the mechanical integrity of vessels under blood pressure. Artificial blood vessels (vascular grafts) have been studied for many years and are in extensive clinical use, however, the simple synthetic grafts typically used (e.g. Dacron or poly(tetrafluoroethylene) (Teflon) polymer tubes) are not safe for small-diameter blood vessel replacement (vessel diameters < 6 mm) and show loss of patentcy, due to rapid occlusion of the grafts by depositing platelets. (Patentcy is the term used to describe an open, viable vessel.) Read the attached brief discussion of approaches to blood vessel engineering, and then answer the design questions below for an improved small-diameter artificial vessel based on the biomaterials design principles discussed in class so far and the prototype
design sketched below: The 'prototype' is to be sutured to the ends of natural blood vessels to replace diseased/blocked sections of vasculature.



a. Describe the first events that will occur at the surface of a hydrophobic polymer like poly(tetrafluoroethylene) (structure shown below) when it is implanted as a vascular graft, and how these events could lead to graft occlusion.

Upon exposure to blood, protein adsorption will occur within seconds, as proteins from serum make hydrophobic contacts with the surface. Depending on the quantity and physical arrangement of adsorbed protein, mononuclear cells in the blood or platelets may be triggered to adhere to the vessel surface.

b. Would you choose a degradable or non-degradable polymer for the artificial vessel scaffolding? Explain your choice. (5 pt.s extra credit: describe what polymer in particular you would choose and motivate why you'd make that choice.)

One might choose a degradable scaffold, in hopes of achieving entirely native tissue in place of the initially artificial implant. If the degradation rate of the scaffold is tuned to the rate at which new natural extracellular matrix can be produced by cells from the surrounding tissue, this approach could lead to a blood vessel with no artificial components after some time.

c. One strategy that could be applied to this artificial vessel would be to implant the polymer and seek to have native endothelial cells and smooth muscle cells from the adjacent native vessels migrate into the polymer scaffold to recreate the native inner and outer cellular lining of the graft. Describe a strategy to modify the 'prototype' scaffold in order to promote this process, and outline a preliminary experiment you could use to test the utility of your approach.

One approach to this problem would be to modify the inner surface of the scaffold with adhesion peptides specific for endothelial cells and adhesion peptides specific for smooth muscle cells on the outside of the scaffold. By 'specific', we mean choose peptides that are recognized by integrin adhesion receptors expressed on the target cells and not other cells in the surrounding environment. (For example, endothelial cells selectively adhere to the amino acid sequence REDV – you weren't expected to know the particular sequence in this case, just the idea that it can be done). Presentation of adhesion peptides could promote the migration of endothelial cells and smooth muscle cells into the graft, in order to line the artificial vessel appropriately. An additional modification might be to immobilize growth factors that could stimulate these cells to proliferate as they enter the graft, in order to more rapidly 'coat' the artificial vessel with a natural confluent layer of cells.

d. Inclusion of adhesion peptides in the inner surface of the artificial vessel could have both positive and negative effects on patentcy early after implantation- describe one positive and one negative outcome that could occur, depending on the choice of peptides utilized.

Presenting adhesion peptides that are recognized by integrins on lymphocytes or platelets in blood in addition to endothelial cells could lead to problems- if platelets adhere on the vessel inner surface they may initiate formation of a blood clot, that can lead to a stroke (a stroke occurs when a blood clot breaks free and travels downstream to lodge in a smaller vessel in a critical location). Alternatively, choosing a peptide which is specific only for endothelial cells could speed up migration of these cells into the graft, and improve patencty by providing a living cellular lining that looks to platelets and immune cells like the 'right' surface in the blood vessel.