

Problem Set 5 Model Solutions

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100 points total

20.462J/3.962J

Spring 2006

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1. (50 points) Compare and contrast a natural extracellular matrix based on collagen fibers with a synthetic matrix formed by a covalent PEG-based hydrogel containing –APGL- peptide linkages sensitive to the enzyme collagenase—answer in brief paragraphs:
- a. Structurally, do these gels present the same barrier to cell movement/migration?

A homogeneous covalent synthetic hydrogel will differ significantly in structure from a collagen fiber matrix. Collagen gels are typically formed via temperature-driven self-assembly of collagen triple helices into fibrils, and these fibrils can in turn assemble into fibers, which have cross-sections ranging from several hundred nanometers to 1 or 2 microns. These fibers, which can extend tens to hundreds of microns in the axial length, then form a gel by physical entanglement, with a mesh size which is often 100's of nanometers and can be microns. In contrast, covalent gels form with the mesh size determined by the molecular weight between crosslinks, and because the mesh size is determined by such a molecular length scale, the mesh size rarely exceeds ~10 nm (the molecular weights required for larger mesh sizes are typically difficult to achieve in practice). Thus, a very different physical environment is presented to cells by these two structures. In addition, as a barrier to migration, collagen, because it is a physical network formed by entanglement of fibers, can be re-arranged/disrupted by cells themselves, while a covalent gel can only be disrupted by the action of cell-derived enzymes on the protease-sensitive peptide linkages of the network. Altogether, cells will be able to migrate more quickly through a native collagen matrix than the synthetic ECM.

- b. Which type of gel would you predict to be more rapidly degraded *in vivo*? Why?

It is highly likely that a native collagen gel will degrade more quickly than a synthetic protease-sensitive gel. First, the collagen gel will contain multiple degradation sites within each fibril/triple helix, which may be recognized by a broader array of degradative enzymes than the synthetic gel with its minimal peptide epitopes. Second, the small mesh size of the covalent gel will slow diffusion of remodeling enzymes through the matrix, further slowing its degradation.

- c. *In vivo*, cells in either of these matrices will also be surrounded by other cells and ECM components. In what ways would you expect these matrices to differ in the way they mediate the interactions of cells with their surroundings?

As discussed in class, native ECM proteins, and especially collagen, possess binding sites for numerous extracellular molecules. Thus, a collagen gel will itself serve as a site for binding/sequestration of various factors in the cells' environment. In contrast, the minimal synthetic gel will not have this capability. As mentioned in part (b), transport through the synthetic gel will also be retarded relative to the collagen matrix (in fact, for most proteins, diffusion rates through a collagen gel are close to the molecules' diffusion rates in pure water, if the factor does not bind to collagen). Thus, the transfer of soluble factors between cells in the synthetic matrix may occur with different kinetics and outcomes relative to the collagen matrix.

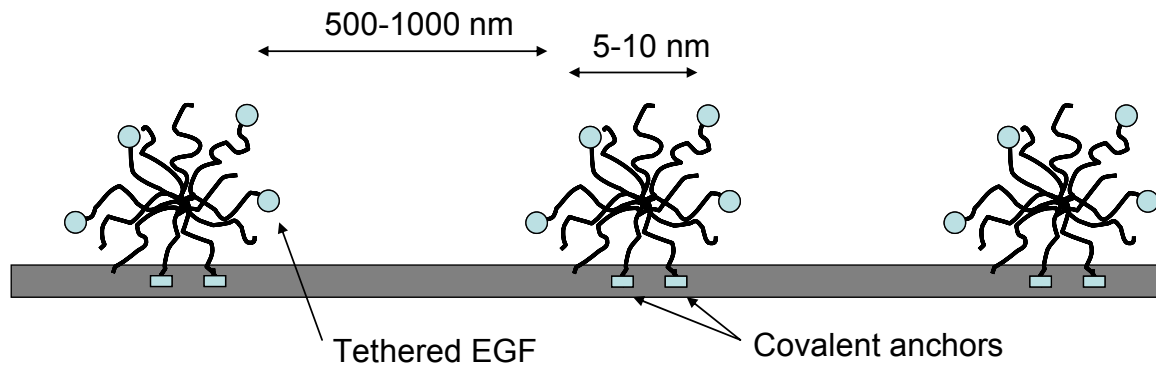
1. (50 points) One of the first studies of immobilized growth factor signaling utilized poly(ethylene oxide) (PEO) star polymers as anchors for the presentation of epidermal growth factor (EGF) on a solid surface (see schematic below). EGF receptor-expressing cells seeded on such a surface can bind the surface-bound EGF and receive stimulation to grow or migrate. EGFR is a receptor tyrosine kinase that begins signaling by homodimerization- two EGF receptors bound to their ligand dimerize and phosphorylate one another to initiate the biochemical signaling chain. The covalent linkages linking EGF to the star polymer and the star polymer to the surface are stable and not enzyme-sensitive. Let's analyze differences between signaling cells receive from a star-PEO-EGF surface compared to signaling received if the cells are simply incubated with soluble EGF:

- a. Identify two ways in which the physical chemistry of the EGF-EGF receptor signaling will be affected by presentation by star PEO relative to soluble EGF, and discuss how these differences could **augment** signal transduction from the star-PEO surface.

First, immobilization to a solid substrate by a permanent linkage will prevent the ligand from being internalized by responding cells (and subsequent recycling of the ligand with its receptor back to the surface or degradation). If the biochemical signaling cascade induced by the ligand binding to its receptor on a given cell type occurs primarily at the cell surface (prior to internalization of the ligand-receptor complex), then such internalization-resistant ligands may provide more prolonged signaling. Second, presentation of the immobilized ligands tethered to star polymers induces nanometer scale clustering of the ligand. This will favor multimerization of the receptor- two receptors that bind to ligands immobilized on the same star molecule will be in close proximity, favoring dimerization-dependent signaling.

- b. Identify and explain one potential mechanism by which star-PEO presentation of EGF could **reduce** EGF signaling relative to soluble EGF.

If an important part of the signaling cascade for a particular receptor-ligand system occurs after receptor internalization (as is true for some of the signaling pathways activated by EGF-EGFR signaling), then such internalization-dependent signaling will be blocked by surface immobilization of the ligand.



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