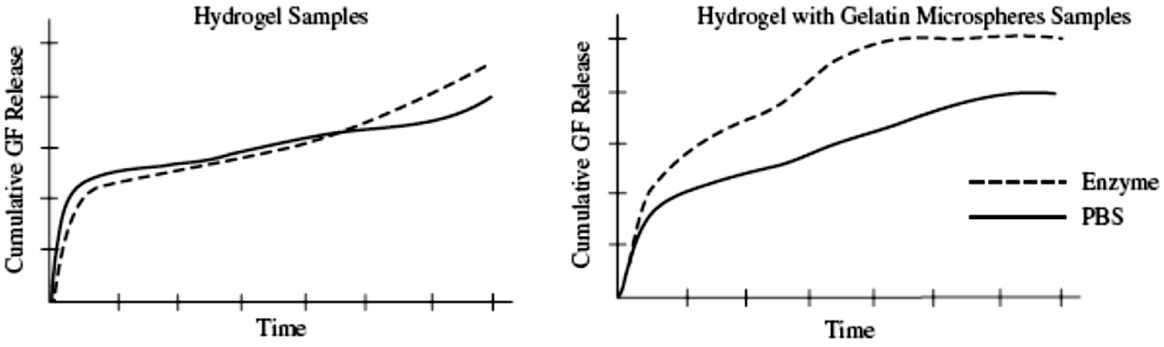
BIOE 476: Tissue Engineering, Fall 2018

In-class exercises, November 13, 2018

1. You have developed two hydrogel systems for controlled release of a therapeutic growth factor (GF). In one system, the GF is dispersed throughout a synthetic polymer hydrogel material that degrades by hydrolysis (left below). In the second system (right below), the GF is loaded into collagen gelatin microspheres, which are subsequently dispersed throughout the same synthetic hydrogel material used in the first system (in this case the GF is only loaded into the microspheres).

   You measure the release *in vitro* of the GF from each system in both (1) phosphate buffered saline (PBS) and (2) PBS containing collagenase. The graphs below illustrate the results of the study.



   (a) Looking at the early time points, the steep release profiles for both systems and the similarity of PBS and enzyme treated conditions suggests that the initial release is mediated by diffusion (TRUE or FALSE).

   TRUE

   (b) Briefly describe <u>one potential advantage</u> that the microspheres could provide.

   The microspheres would allow for an inducible increase in the rate of release, in the presence of the collagenase (MMP) enzyme. Collagenase is produced by cells and in tissues.

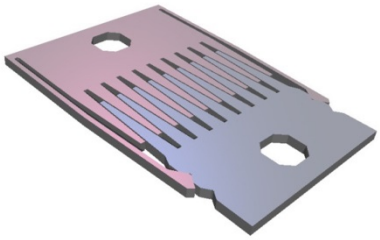
   (c) You are trying to modify the synthetic polymer system on the left so that you can achieve a SLOWER release profile. A colleague suggested that you could covalently link heparin to the polymer backbone. Why would this be a good modification to try?

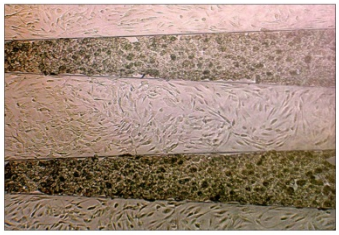
   Heparin binds to growth factors. By covalently linking heparin to the polymer, growth factors would be retained in the scaffold leading to a slower release. This would most significantly affect the initial diffusion phase.

   (d) If you modified the GF resulting in a decreased GF solubility, would you expect a FASTER or SLOWER release from the synthetic polymer system on the left (pick one).

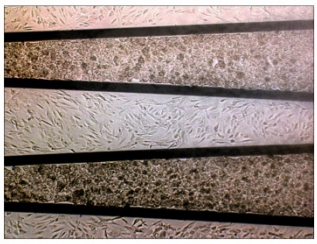
   SLOWER (A decrease in GF solubility would prevent the GF from entering aqueous solutions and decrease diffusion out of the scaffold)

2. In class, we discussed several approaches that could be used to study cell-cell interactions. One platform (shown below) was a microfabricated substrate consisting of interlocking 'combs' that can positioned into 2 configurations: 1) Contact and 2) 80 μm gap.





Contact



Gap

Using this platform you establish a co-culture with endothelial cells and fibroblast cells and make the following experimental observations:

i) Endothelial cells cultured on one comb and paired with a comb containing fibroblast cells exhibit significantly increased proliferation, compared to endothelial cells alone.

ii) Both the contact and gap configurations lead to similar levels of endothelial cell proliferation.

(a) These observations suggest that a particular type of microenvironmental signal is important for inducing endothelial cell proliferation in this culture.
What type of signal is this?

Secreted soluble (paracrine) factor.

(b) In another experiment, you take media from fibroblast cultures in flasks (fibroblast conditioned media) and add to endothelial cells. The fibroblast conditioned media does not increase endothelial cell proliferation.
What is a possible explanation for this result (also considering the observations above)?

A couple of possibilities:

- There is 'reciprocal' signaling. Specifically, factors from endothelial cells signal to fibroblasts which then changes what soluble factors fibroblasts secrete.
- The secreted paracrine factor degrades or is not at a high enough concentration in the bulk conditioned media.