Homework #5 Answers

1. Your company developed a scaffold which incorporates microspheres containing the growth factor, vascular endothelial growth factor (VEGF). In laboratory studies it was found that the VEGF is released over a period of 3 weeks. The CEO has been so excited with this demonstration that she wants to immediately proceed with the development of a product, even though there is some question regarding the concentration of the VEGF that would be expected in the defect implanted with a scaffold containing these microspheres. Do you agree with her? If not, on what data would you base your case on the importance of the concentration of VEGF?
   
   Several assays in vitro demonstrate the variation in the response of endothelial cells to the dose of VEGF (e.g., in R.R. Chen, et al., FASEB J 20:1;2007).

2. In an animal study it was found that at the end of the 3-week period of implantation there was an increase in the number of vessels in the ulcers implanted with the scaffold containing a formulation of the 3-week microspheres containing VEGF. However, in follow-up studies at 6 weeks the new vessels were no longer present. Is there a second microsphere formulation (i.e., another growth factor in a second type of microsphere) that you would recommend adding to this implant to maintain the new blood vessels that had formed?
   
   The VEGF treatment needs to be followed by another regulator (viz., PDGF; in C. Fischbach, et al., Biomat 28:2069;2007), that will recruit support cells for the growth and maintenance of the vessels.

3. For many tissue engineering applications it is important that the appropriate growth factor be present in the implant site for periods of 8 weeks or longer. While microspheres can be prepared to delivery growth factors over a period of 8 weeks in vivo, there are several problems in this approach. When using microspheres that can provide a 3-week release of a growth factor, for what reasons may the growth factor no longer be present in the implant site at 8 weeks?
   
   Degradation and diffusion from the site.

4. For the reasons in 3, above, you propose to incorporate genes for growth factors, instead of the growth factors themselves, into the microspheres.

   - Sustained endogenous synthesis of the growth factor
   - Potentially greater biological activity
   - Multiple genes can be transferred and independently regulated
   - Cost and efficiency
Would you propose using the DNA for the growth factors or viral vectors containing the genes? Give one advantage and one disadvantage of each approach.

Non-viral
- Advantage: safe; Disadvantage: low transfection efficiency (i.e., % of cells transfected)

Viral
- Advantages: high transfection efficiency, persistence of gene expression;
- Disadvantages: safety, complexity

If you were instructed to use a viral vector which one would you choose? Explain.

Prof. Evans explained the advantages of Adeno-Associated Virus

5. Describe an implant that might be used to treat the ulcer in Fig. 1, using the genes for specific growth factor(s), instead of the growth factor(s). In addition to being poorly vascularized, the dermis adjacent to the ulcer in Fig. 1 is generally less cellular than normal dermis. Your implant should not require the use of culture cells.

- VEGF and PDGF genes