BIOE 476: Tissue Engineering, Fall 2018

Homework 3- due Tuesday, October 16th

- Read the following article: DeMuth et al. Polymer multilayer tattooing for enhanced DNA vaccination. *Nature Materials* (2013). For some questions, you may need to look into referenced papers or outside material for additional information. http://www.nature.com/nmat/journal/vaop/ncurrent/full/nmat3550.html

3 points each, 30 total points.

a) Polyelectrolyte multilayers (PEMs) are films of alternately charge polymers. What are the positively charged polymers used in this work? What are the negatively charged polymers used in this work?

3 points

(1.5 points for positive, 1.5 points for negative)

The positive polymers are poly-b-amino-esters (PBAEs) including polymer 1 and polymer 2, and the negative polymers are plasmid DNA (pDNA) and poly (I:C).

Would also accept the following which are part of the initial deposition layer: positive charged polymer is protamine-sulfate (PS), negative charged polymer is poly(4-styrene-sulphonate) (PSS).

b) What is the composition of the 'release layer'? What purpose does this layer serve, and what are the key characteristics of this layer?

3 points

(1.5 points) The release layer is made of PNMP. The purpose of the release layer is to rapidly deposit the PEM films upon microneedle exposure. This occurs due to solubility of the release layer in skin, causing the release of the PEM films from the microneedle substrate.

(1.5 points) The key characteristics are the solubility in water above pH 6.5, and the switch from organic soluble to water soluble upon UV exposure.

c) What are the 2 signals that can be detected using Cy5 pLUC and what do they measure?

3 points

(1.5 points) Cy5 fluorescence can be detected and this measures the delivery of the plasmid DNA pLUC from the microneedles into the tissue.

(1.5 points) A luciferase signal can also be detected from cells transfected with pLUC. This measures the amount of transfection resulting from pLUC delivery.

d) Why is the co-localization of pLUC and poly(I:C) with MHC-II+ cells a positive finding (i.e. a good thing)?

3 points

MHC-II+ cells are antigen presenting cells. The transfection of MHC-II+ cells with pLUC, and activation with poly(I:C), would lead to antigen presentation and activation of immune cells leading to immunity based on the vaccine treatment.

e) What is poly(I:C), and why is it co-delivered with plasmid DNA in these studies?

3 points

Poly(I:C) is a synthetic mimic of double stranded RNA, which acts as a vaccine adjuvant. It enhances the activation of immune cells, and therefore, enhances the efficacy of the vaccine treatment.

f) What benefit does the poly-2 PBAE formulation exhibit compared to the poly-1 PBAE formulation?

3 points

Poly-2 PBAE exhibits a slower release profile compared to poly-1. This sustained release over a longer time resulted in a more prolonged expression of the delivered DNA.

g) What do the in vivo luminol experiments (Figure 4b) demonstrate?

3 points

In vivo luminol experiments measure the presence of oxidative bursts (reactive oxygen species, ROS), which are due to localized phagocytes as part of the immune response.

Intradermal injection leads to a short increase in luminol signal, suggesting a brief immune activation. Microneedle delivery of PEM films containing poly-2 leads to sustained immune response with sustained activation of local phagocytes.

h) What advantage does the described technology have compared to in vivo electroporation?

3 points

Electroporation, although effective, is not practical for widespread prophylactic vaccination. The microneedle approach does not require specialized equipment and would lead to less pain/discomfort.

i) What advantage does the described technology have compared to intradermal injection of polymer-pDNA complexes?

3 points

Due to the sustained release, the described microneedle technology leads to much improved transfection (i.e. gene expression), leading to enhanced immunity and effectiveness of the vaccine.

j) What are 2 advantages that the described technology has compared to needle-based administration of a liquid vaccine?

3 points

(1.5 points) The microneedle approach can be administered safely with minimal intervention. Needle-based vaccines have associated safety issues (re-use and injuries), which require trained personnel.

(1.5 points) The microneedle approach enables storage without refrigeration. Needle-based vaccines also require refrigeration, which raises cost and complexity of distribution.