BIOE 476: Tissue Engineering, Fall 2018

Homework 2- due Thursday, September 27th (in class)

- Read the following article: Bencherif et al. Injectable preformed scaffolds with shape-memory properties. *PNAS* (2012), and answer the following questions (a-j). http://www.pnas.org/content/109/48/19590.long

33 total points.

a) (3 total points) What chemical modification is made to the alginate that enables free-radical polymerization?

3 points

Methacrylate groups are added to the alginate by reacting with 2-aminoethyl methacrylate (AEMA).

b) (3 total points) What method did the authors use to evaluate the chemical modification of alginate, and what did they observe with this method?

3 points

H NMR was used. They observed the presence of characteristic vinylic peaks (5.3-5.8 ppm) which indicated that the methacrylate groups were present within the alginate.

c) (3 total points) What methods did the authors use to observe/measure the pore size of the gels? How do cryogels and conventional gels compare?

3 points

(1 point) Scanning electron microscopy was used to measure pore size. Micro-CT was used to examine pore characteristics such as the degree of interconnections.

(1 point) Compared to conventional gels, cryogels are more highly macroporous with more interconnected and better distributed pores.

(1 point) Cryogels form an opaque hydrogel while conventional gels are transparent.

d) (3 total points) In regards to mechanical properties including Young's modulus and % strain, how do the cryogels compare to conventional gels?

3 points

(1.5 points) Conventional gels exhibited a Young's modulus of approx. 42 kPa. Cryogels exhibited a significantly lower modulus of approx.. 4 kPa.

(1.5 points) Cryogels could withstand 90% strain without permanent deformation or mechanical failure while conventional gels could only support approx. 16% strain.

e) (3 total points) What do the authors suggest is the mechanism leading to the shape-memory properties?

3 points

They suggest that the shape-memory properties are due to the reabsorption of water into the gels. When the cryogels are forced through the needles, the interconnected pores reversibly collapse, forcing water out. Upon relaxation, the gels resume their original structure as the water is reabsorbed.

f) (3 total points) How did they incorporate an RGD peptide into the gel?

3 points

The RGD peptide was coupled to the alginate through the covalent binding of the peptide to the ACRL-PEG-NHS linker.

g) (3 total points) As a comparison to the RGD (Arginine, Glycine, Aspartic acid) peptide, these authors also fabricated gels incorporating the RGE (Arginine, Glycine, Glutamic acid) peptide instead of RGD. What is the reasoning for that additional condition?

3 points

The RGE peptide is used as a non-adhesive control peptide. Cells do not attach to RGE, so this tests if there are any non-specific effects of adding a peptide. Glutamic acid is substituted because it exhibits similar charge and polarity to Aspartic acid.

h) (3 total points) What do the release profiles of crosslinked BSA and encapsulated BSA suggest is the mechanism of release in vivo? Why?

3 points

The release profiles are similar which suggests that the release is due to matrix degradation. If release was mediated primarily by diffusion, the encapsulated BSA would be released while the crosslinked BSA would be retained.

i) (3 total points) What is the effect of injection on cells bound to the gel scaffolds (retention and viability)?

3 points

Injection had a minimal effect.

Cell retention efficiency was 80% and the viability of cell was approx. 92% (comparable to the control group).

j) (6 total points) Briefly list the benefits of the cryogel approach compared to the following alternative approaches: (1) the injection of a cell suspension, (2) the surgical implantation of a 3D scaffold, (3) an in situ crosslinked hydrogel scaffold.

6 total points

(1) 2 points

The cryogel delivery approach is advantageous because it allows for localized delivery and retention at the site of injection.

(2) 2 points

The cryogel approach is less invasive, decreases scarring, and lessens infection risks.

(3) 2 points

The cryogel approach could help to mitigate several issues with in situ crosslinked systems including difficulties in controlling the gelling kinetics, an inability to pre-define the gel architecture, and the possibility of leakage into neighboring sites before polymerization.