Controlling Degradation and Protein Release in Heparin-containing Hydrogels with Varying Levels of Sulfation

Karthik Nathan[1], Tobias Miller[1], Johnna S. Temenoff[1,2].
[1]Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA
[2]Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA

Statement of Purpose: The current use of growth factors in tissue engineering is often hindered by drug delivery systems that inadequately 1) control growth factor release, 2) stabilize protein bioactivity, and 3) degrade upon completion of release. Negatively charged glycosaminoglycans (GAGs) can be integrated into polymer-based cross-linked hydrogels to electrostatically sequester positively charged growth factors that can be released over time in a controlled manner without loss of bioactivity (Cai 2005). In order to achieve a more tunable system for protein delivery, modification of sulfation levels of the integrated GAGs, along with introduction of hydrolytically cleavable bonds will allow for controlled growth factor sequestration and release and hydrogel degradation kinetics. Using a model protein (histone) that has similar charge and size to many growth factors, the objective of this study was to characterize the control of protein release from heparin-containing hydrogel systems. We hypothesize that reducing heparin's sulfation level will lead to faster and more complete protein release due to less electrostatic interactions between the protein and hydrogel. Additionally, to achieve a high degree of release from hydrogels with natively-sulfated heparin, hydrolytic degradation of the polymer matrix (through the addition of dithiothreitol [DTT]) will be required.

Methods: 4-arm poly(ethylene) glycol (PEG) was modified with acrylate functional groups, while 6O,N-desulfated (6O,N-dhep) and fully desulfated (dHep) heparin derivatives were prepared through solvolytic desulfation. The sulfation levels were quantified using a 1,9-dimethylmethylene blue assay. Thiol functional groups were added to all heparin derivatives through cystamine/hydroxybenzotriazole/1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide reactions. Quantification of heparin thiol functionalization was achieved through ^{1}H nuclear magnetic resonance spectroscopy. Hydrogels were prepared by dissolving 4-arm PEG-acrylate and thiolated heparin in a solution of 50 mM tris(2-carboxyethyl)phosphine (TCEP) and water at pH 10. Four hydrogel types were formed: heparin-free, dHep, 6O,N-dHep, and natively-sulfated heparin (Hep). Hydrogels were cross-linked by varying DTT concentrations- 0 mM, 15 mM, and 25 mM- and by free radical polymerization (Irgacure D2959, 5 min UV light at 365 nm). The hydrogels were allowed to swell in phosphate buffered saline solution (PBS) for 1 h, loaded with a 40 ug/mL histone protein solution, and re-immersed in 1 mL PBS. After 3 h, the supernatant was removed and assayed with o-pthalaldehyde to determine non-sequestered protein quantities (loading). Protein release was measured with the Bradford assay for all subsequent time points.

Results: Sulfation levels for Hep, 60,N-dHep, and dHep were 100.0±0.6%, 20.0±1.6%, and 0.0±0.0%, respectively. Thiolation degrees of heparin derivatives were similar with 1 thiol group per 10 heparin disaccharide units. Swelling of hydrogels (Fig. 1A) demonstrated that 15 and 25 mM DTT formulations with heparin swelled the most, followed by the 6O,N-dHep formulations. Protein loading in hydrogels (Fig. 1B) was highly dependent on the sulfation level of heparin. Heparin-free and dHep hydrogels sequestered less than 5% of histone whereas 6O,N-dHep and Hep hydrogels had loadings between 25%-60% and 55%-90%, respectively. Due to the low loading capacity of heparin-free and dHep-containing hydrogels, both systems were not included in the histone release study.

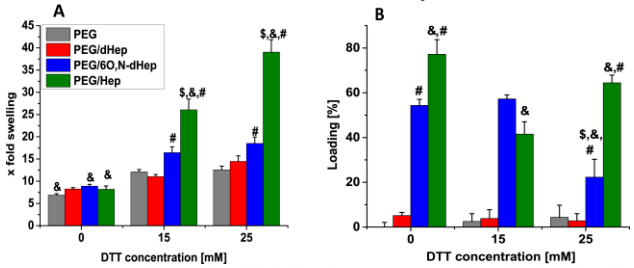

Figure 1: A. Swelling study with hydrogels (n=4) B. Loading of hydrogels with histone (n=4-6). Statistical analysis was done with ANOVA ($p<0.05$) and Tukey's Post-hoc test, significant differences between: $ all other groups, & same formulations with different DTT concentrations, # other groups with same DTT concentration.

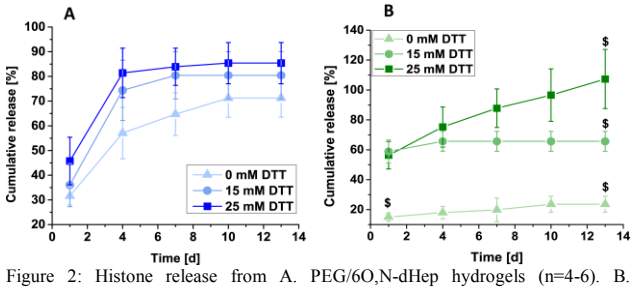

Figure 2: Histone release from A. PEG/6O,N-dHep hydrogels (n=4-6). B. PEG/Hep hydrogels (n=4-6). Statistical analysis as before, significant differences between: $ all other groups

The 15 and 25 mM DTT 6O,N-dHep-containing hydrogels (Fig. 2A) released 75% and 81% of their histone payload, respectively, within 4 d, indicating early release due to reduced affinity resulting from lower sulfation levels, whereas the DTT-free hydrogels of this group released histone more gradually over 13 d. In contrast, Hep hydrogels (Fig. 2B) without DTT released only 20% of loaded histone over 13 d. Introducing hydrolytic degradation with 15 mM DTT (degradation > day 13) and 25 mM DTT (degradation between day 10-13) led to a more complete release (60% and 100%, respectively) of the protein. Moreover, 25 mM Hep hydrogels released histone in a linear fashion over 13 d.

Conclusions: These results demonstrate that protein release can be controlled via either altering GAG sulfation pattern, hydrogel degradation, or a combination of the two. Such systems with orthogonal means of altering release kinetics provide greater tunability and therefore make these GAG-based systems very attractive for many regenerative medicine applications where a high degree of temporal control over protein delivery is needed.

References: Cai SS Biomaterials 2005 (26:6054-67)