Interactions of Complexes of Heparin and a Positively-Charged Protein with GAG-based Hydrogels

Song P. Seto[1], Johnna S. Temenoff[1,2]
[1]Coulter Dept. of Biomedical Engineering, Georgia Institute of Technology and Emory University, [2]Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA

Introduction: While the use of growth factors are attractive in regenerative medicine, growth factor delivery remains challenging due to rapid loss of activity during loading as well as release. Heparin-binding growth factors have been stipulated to have prolonged bioactivity when complexed with sulfated heparin[1]. Therefore, release of growth factors associated with heparin, rather than "naked" growth factors may be a promising means to improve clinical efficacy. However, the stability of these complexes, particularly in the presence of charged hydrogel materials, are not well understood. In order to study these interactions, a positively-charged model protein, histone, was co-delivered with soluble heparin and evaluated with glycosaminoglycan (GAG)-based hydrogels. The hydrogel consisted of covalently immobilized heparin, capable of competing off histone from soluble heparin. Interactions of complexed histone and hydrogel materials were evaluated by release studies and sequestration studies. Our hypothesis is that complexes will have greater mobility over free histone in hydrogels until the threshold where large amounts of immobilized heparin attract histone out of the complexes.

Materials and Methods: A heparin:histone ratio of 25 molar excess of heparin to histone was chosen for release studies. Hydrogels (90% wt. H_2O) were formulated with either 0% or 10% heparin methacrylamide (hep MAm), with the remaining amount composed of poly(ethylene glycol)-diacrylate (MW~3,400). Free or co-delivered histone was mixed into macromer solutions prior to crosslinking by ammonium persulfate/TEMED. Release of histone was tracked up to 8d and protein concentration was quantified by the bicinchoninic acid (BCA) assay. For sequestration studies, a complex ratio of 35 molar excess of heparin to histone was chosen. Hydrogels of 0%, 10%, or 100% hep MAm were fabricated and allowed to swell for 24h prior to placement in a free or complexed histone solution containing fluorescein-tagged histone for 24h. Thereafter, hydrogels were imaged by confocal microscopy ($n \geq 3$) and the average fluorescence across the depth for each hydrogel was computed. The supernatants from the pull-down study were analyzed for remaining protein concentration. Data from supernatants were analyzed by one-way ANOVA and data from the sequestration study by t-tests ($p<0.05$).

Results: The cumulative release of histone at 8d from 0% hep MAm hydrogels was significantly higher (135.8 ±15.5µg/mL) than the amount released from 10% hep MAm hydrogels (103.8±8.3µg/mL). The cumulative release of complexes from 0% hep MAm hydrogels (122.1±37.0µg/mL) and from 10% hep MAm hydrogels (127.7±4.6µg/mL) were not significantly different from each other or histone-only samples.

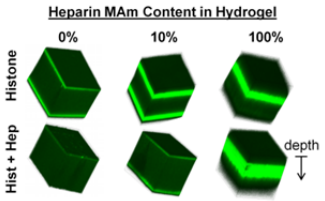

Fig. 1. Representative 3D renders of pull-down study. Green indicates labeled histone and y-direction represents depth through hydrogel.

Histone and complexes were relatively well distributed across the depth of a hydrogel containing 0% hep MAm (Fig. 1). However, when hydrogels contained 10% hep MAm, the maximum intensity of histone was 1.9 times higher than complexes within the same hydrogel formulation ($p<0.05$) and the histone-only samples appeared to distribute primarily to the surfaces of the hydrogels. The maximum intensity of complex was not significantly different from histone pulled into 100% hep MAm hydrogels.

From analysis of the remaining supernatant, while similar levels of protein were observed for histone incubated with 0% and 10% hep MAm gels, a significantly less level was detected for histone incubated with 100% hep MAm gels (Fig. 2). In comparison, the amount of protein in the wells of the complexes were not statistically different across all gel formulations.

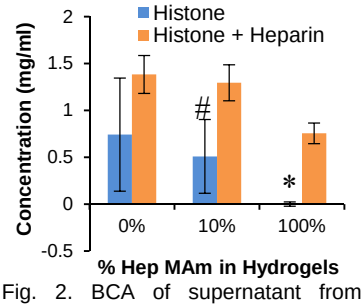

Fig. 2. BCA of supernatant from sequestration. * Lower than all other samples, # lower than 0% hep MAm hydrogels incubated in complexed histone, $p<0.05$, n=3± SD.

Discussion and Conclusions:

Results indicate that histone release and sequestration is modulated when co-delivered with soluble heparin. When complexed, histone can diffuse more uniformly into 10% hep MAm hydrogels, possibly due to charge shielding. At 100% hep MAm amounts, the distribution of histone alone and in complex begin to resemble each other, suggesting that the immobilized heparin in the hydrogel competes for histone out of the complexes. These results suggest that complexes can be delivered intact to a certain level. Furthermore, there were no significant differences between the amount of complexed histone left from sequestration studies across hydrogel types, indicating that these complexes may have less affinity for the hydrogels. Such results are important when designing delivery devices that rely on electrostatic interactions to release bioactive growth factors at an injury site and are applicable to a variety of GAG-based hydrogel systems.

References: [1]Schlessinger, *et al*. *Molecular Cell*. 2000; 6: 743–750. **Acknowledgements:** NSF DMR 1207045