Proteoglycan Mimetic Graft Copolymers for Growth Factor Stabilization and Delivery

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Statement of Purpose: Proteoglycans are important molecules in the extracellular matrix (ECM) of tissues. They have a bottlebrush structure made up of a core protein with glycosaminoglycan (GAG) side chains. GAGs carry negative charges that make them hydrophilic and create electrostatic repulsion between the molecules. These properties provide lubrication and compressive strength to tissues. In addition to mechanical properties, proteoglycans also provide biochemical function. They participate in cell signaling and bind and stabilize growth factors. Growth factors are important proteins for maintaining tissue homeostasis. In disease and aging, homeostasis is out of balance and proteoglycans are degraded, leading to a loss of mechanical strength and biochemical function. Therapies using growth factors to restore homeostasis hold much promise. However, these proteins are unstable in solution and are prohibitively expensive. Including a proteoglycan mimetic molecule with growth factor treatments can reduce the amount of protein required, control release of the protein, and provide mechanical function until the native tissue is restored. We have created proteoglycan mimetic graft copolymers using GAGs (hyaluronan and chondroitin sulfate). We can alter the size and composition to tailor these to mimic specific proteoglycans for different applications.

Methods: Hyaluronan (743 kDa) was obtained from Fluka; chondroitin sulfate (84 kDa), cysteamine, and sodium triacetoxyborohydride (STAB) were obtained from Sigma Aldrich; 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrocholoride (EDC), and N-β-maleimidopropionic acid hydrazide trifluoroacetic acid (BMPH) were obtained from Thermo Scientific. The first step in the synthesis is to thiolate the carboxylic acid groups on the hyaluronan with cysteamine using EDAC coupling chemistry. The thiolation is confirmed and quantified through an Ellman's reagent test. The coupling agent, BMPH, has a maleimide activated end and a hydrazide functional group on the other end. The maleimide is then reacted with the thiols along the hyaluronan backbone. Hydrazide-functionalized hyaluronan is then combined with the desired amount of chondroitin sulfate with a reducing agent, STAB. The end group on the chondroitin sulfate side chain opens to form a carbonyl which then reacts with the hydrazide through reductive amination. Chondroitin sulfate side chains are then grafted onto the hyaluronan backbone forming a proteoglycan mimetic bottlebrush structure. Ellman's reagent test was used to quantitate the degree of hyaluronan thiolation. This data was then used to determine the amount of chondroitin sulfate to react with thiolated hyaluronan. A 1:1 and a 1:3 ratio of chondroitin sulfate chains to thiol activated sites were used. These were then characterized with dynamic light scattering, zeta potential, and ^{1}H NMR.

Results: The Ellman's reagent test determined that 50% of the carboxylic acid groups are thiolated. Each of these reactions was performed and examined using dynamic light scattering and zeta potential to characterize them. The results are displayed in Table 1.

Table 1. Dynamic light scattering results

	Mean D_H (nm)	ζ-potential (mV)
Aggecan	400[1]	-
Chondroitin Sulfate	120	-60
Hyaluronan	-	-30
1:3 Graft Density	270	-5
1:1 Graft Density	430	-70

It is important to note that the hydrodynamic diameter of aggrecan, an important proteoglycan in articular cartilage, is 400 nm.[1] The hydrodynamic diameters of the lower and higher grafting densities are 270 nm and 430 nm, respectively. The zeta potential for the 1:1 grafting density is higher than chondroitin sulfate alone. This indicates a higher negative charge density at this pH for the complex. The zeta potential for the lower grafting density is lower than both chondroitin sulfate alone and unmodified hyaluronan. Previously, our group has shown that nanoparticles containing a high density of chondroitin sulfate were able to preserve the activity of fibroblast growth factor 2 (FGF-2) for at least 14 days (figure 1). These performed better than FGF-2 delivered in solution and similarly to aggrecan. These results suggest that these graft copolymers would also be capable of preserving FGF-2 activity under similar conditions.

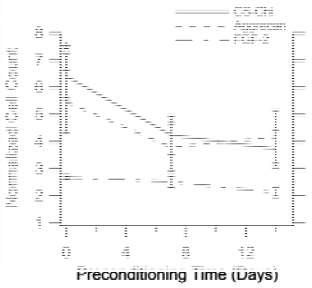

Figure 1. FGF-2 activity after 0, 3, 7, and 14 days of destabilization when bound to chondroitin sulfate containing nanoparticles, aggrecan, or in solution.

Conclusions: Hyaluronan was successfully modified with thiols attached to 50% of the available carboxylic acid groups. This allows for a large range of possible grafting densities. Two different grafting densities were created. They resulted in two different sizes and two different zeta potentials. The size and high negative zeta potential of the highest grafting density shows promise for mimicking the proteoglycan aggrecan. This work can be expanded to a variety of grafting densities and to other GAGs such as heparin to mimic other proteoglycans such as versican and perlecan. In future experiments these will be tested for solution dynamics and growth factor stabilization and delivery.

References: 1. (Papagiannopoulos A. Biomacromolecules, 2006; **7**: 2162-2172.)