Biomimetic Aggrecan Based on a Polyacrylic Acid (PAA) Core and Chondroitin Sulfate (CS) Bristles

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Statement of Purpose: Intervertebral disc (IVD) degeneration is characterized by the loss of the proteoglycan (PG) aggrecan from the inner nucleus pulposus of the disc, which is responsible for hydration and load bearing[1]. Aggrecan consists of a core protein with covalently attached chondroitin sulfate (CS) chains, containing highly charged anionic groups[1], which provide electrostatic repulsion and draw water into the tissue to resist mechanical deformation during loading. There is a linear reduction of aggrecan with increased degeneration[2] due to enzymatic cleavage of the protein core[1]. *The goal of the project was the fabrication of an enzymatically resistant biomimetic aggrecan brush structure (using a synthetic protein core) to serve as a minimally invasive injection procedure for the alleviation of back pain*. A biomimetic polymer was synthesized with a synthetic core protein of PAA and bio-based bristles of CS via the "grafting-to" polymerization technique.

Methods: *Synthesis and Optimization of Reaction Conditions.* A primary amine on the terminal end of the CS (CS-4, MW 22 kDa, Sigma) was used as a handle[2] to conjugate it with the carboxylic acid groups present on PAA (MW 250 kDa, Sigma). PAA was activated in MES buffer using the zero length crosslinkers EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-Hydroxysuccinimide) and reacted with CS in PBS buffer at pH 7.0. Reaction completeness was measured via the fluorescamine dye assay. The reaction of fluorescamine with free primary amines forms a fluorescent derivative, whose signal can be measured. Percent conjugation was calculated by normalizing the fluorescence of the conjugate against a CS control. The reaction parameters optimized were ionic concentration of buffer, temperature and reactant ratios. *Characterization.* The biomimetic polymer's morphology was characterized by Transmission Electron Microscopy (TEM, uranyl acetate staining). Rheological measurements of solutions of CS, PAA, and polymer with PBS buffer as a control solution were conducted. (AR 2000ex rheometer, 25°C, shear rate 10-500 s^{-1}).

Results: *Synthesis and Optimization of Reaction Conditions.* The ionic concentration of the buffer mixture was varied based on Na^+ concentrations and ~0.69 M Na^+ showed the highest and most consistent percent conjugation over the time of the study, thereby being considered optimal. Three reaction temperatures were investigated: 4°C, 21°C, 37°C. There was no statistically significant difference in the percent conjugation obtained, therefore 21°C was chosen as the optimal reaction temperature for convenience of lab conditions. The reactant ratios (CS:PAA, w/w) tested were 20:1. 10:1, 1:1 and 0.1:1. The 1:1 CS:PAA reached ~98% conjugation and was chosen as the optimal ratio.

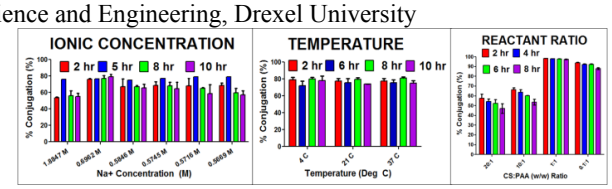

Figure 1: Effects of Reaction Conditions

Characterization. The morphology of the conjugate was characterized by TEM using aggrecan as a control. Morphological similarities were observed between the aggrecan and the biomimetic polymer.

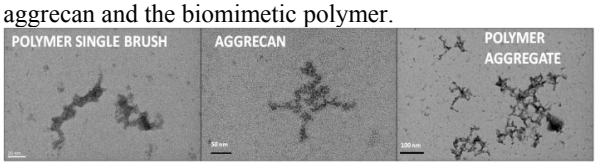

Figure 2: TEM Images of Aggrecan and Polymer

Specific viscosities were calculated by normalizing sample viscosity against PBS viscosity values. The biomimetic polymer showed higher specific viscosity than CS, PAA and the mixture of unreacted CS-PAA. The change in viscosity was indicative of interactions having occurred between the reactants, to form a new molecule with a higher viscosity.

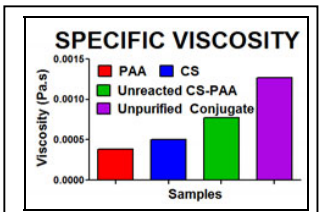

Figure 3: Viscosity Data

Bristle Density Calculations. Taking the molecular weight of acrylic acid (monomer of PAA) as 72 Da, the number of binding sites on 1 mole PAA is ~3470. Theoretically, for a densely grafted brush, we would require at least 1 CS molecule for each binding site. Considering a PAA concentration of 10 mg/ml (Molarity $4x10^{-8}$M), the equivalent molarity of acrylic acid is $1.39x10^{-4}$M. The equivalent CS molarity to achieve a 1:1 molecule ratio is $1.39x10^{-4}$M.

In our study, concentrations of CS and PAA used were 10 mg/ml each (Molarity CS: $4.55x10^{-7}$M, PAA: $4x10^{-8}$M), from which the CS:PAA mole ratio is 0.003, meaning there is 1 CS molecule for every 300 binding sites on PAA. This is a very low bristle density compared to the theoretical value calculated above. However, even a molarity as low as $1.39x10^{-4}$M for CS results in the formation of a very viscous solution, which hinders its conjugation. Further investigation is required to modulate the CS concentration to achieve better bristle densities.

Conclusions: A biomimetic aggrecan polymer was successfully created and partially characterized for morphological and mechanical properties.

References: [1]Raj PP, *Pain Practice,*8, 18-44, 2008, [2]Olczyk et al, Zeitschrift für Rheumatologie 1994;53(1):19-25, [3]Sarkar S et al. *SFB Annual Meeting 2010* Seattle, Washington, USA. **We acknowledge C. Winkler (Drexel University) for TEM imaging and The Coulter Foundation for funding.**