Enzymatic Stability of Novel Biomimetic Aggrecan for Treatment of Tissue Degeneration

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Statement of Purpose: Proteoglycans are a major component of the cartilage extracellular matrix, and are essential for tissue hydration, integrity and resist compression stress. (1) Cartilage contains 10% of the proteoglycan aggrecan. (2) It has been shown that early in tissue degeneration the extracellular matrix is depleted of aggrecan, resulting in loss of hydration, osmotic pressure and associated reduction in mechanical stability which leads to pain. (3, 4) We propose a new strategy to restore the extracellular matrix of cartilage and mitigate pain by molecularly engineering the degenerated tissue with biomimetic proteoglycans (BPGs).

We have designed, synthesized and characterized Biomimetic Aggrecan (BA) which mimics the three-dimensional bottle brush architecture of the chondroitin sulfate proteoglycan aggrecan using a hybrid natural/synthetic polymer approach composed of natural glycosaminoglycan (chondroitin sulfate, CS) bristles attached end-on to a synthetic polymer core. This study will focus on enzymatic stability of our novel BA molecules

Methods: Biomimetic aggrecan was synthesized through conjugation of the terminary amine of CS (Sigma Aldrich) to PAA (Sigma Aldrich: 250 kDaltons and 100 kDaltons) and poly(acryloyl chloride) (PAC) (Sigma Aldrich, 10 kDaltons) via EDC/sulfo-NHS activation of the carboxylic acid of PAA and the chlorine of PAC. Samples were **p**urified via dialysis.

Biomimetic aggrecan was analyzed chemically, by confirming that CS can conjugate to reactive certain monomers (i.e. carboxylic acid and acyl chloride) via its terminal amine through flurorescamine assays, proton Nuclear Magnetic Resonance (^{1}H-NMR), and Fourier Transform Infrared Spectroscopy (FTIR).

The enzymatic stability of BA bristles was explored by digestion with native mammalian hyaluronidase (Sigma Aldrich) and non-native chondroitinase ABC (Sigma Aldrich) enzymes. Glycosaminoglycan content after digestion was determined by a Blyscan Assay (Accurate Chemicals) and a 1,9-dimethylmethylene (DMMB) assay.

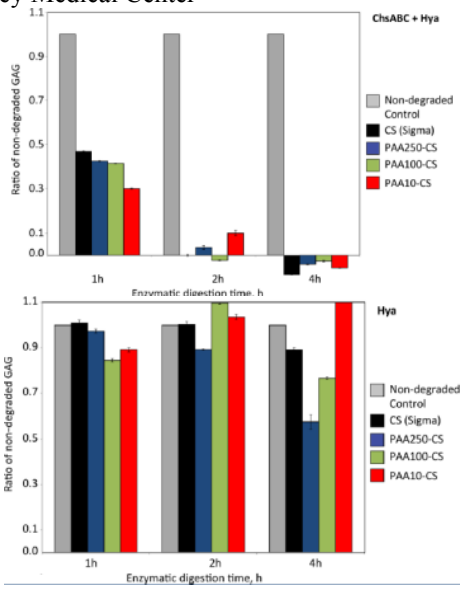

Figure 1. *Enzymatic Degradation of BA bristles over time with chondroitinase and hyaluronidase (top) and hylauronidase (bottom)*

Results: CS bristles were successfully conjugated. No degradation of CS bristles after the synthesis and purification was observed as confirmed with ^{1}H-NMR. Enzymatic digestion of three types of BA and a non-reacted CS with chondroitinase ABC and hyaluronidase depleted GAG content almost completely within 2 hours of incubation. However, enzymatic digestion with only hyaluronidase did not show as strong an effect within the same incubation period. Further, the CS bristles in BA behave similarly to unreacted CS, suggesting that chemical processing and assembly into a larger macromolecule does not affect enzymatic stability.

Conclusions: A family of BA was synthesized via a "grafting-to" strategy by coupling CS to polymer backbones (PAA and PAC) to mimic structure of natural aggrecan. Our studies on enzymatic stability of novel macromolcules suggest that the CS bristles of various types of BA are susceptible to degradation by non-native enzymes (chondroitinase ABC) but not by native enzymes (hyaluronidase), suggesting CS of biomimetic aggrecan would not degrade *in vivo*.

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