Novel Biomimetic Proteoglycans for Molecular Engineering of Degenerated Tissue.

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Statement of Purpose: Proteoglycans (PGs) are natural nano-scale biomacromolecules which are essential for hydration and structural integrity of soft and connective tissues[1,2]. PGs consist of glycosaminoglycan (GAG) bristles that are attached to a protein core in a three-dimensional bottle brush architecture with core length and GAG bristle density varying based on tissue origin. During degeneration, the enzymatic digestion of proteoglycans outpaces the cellular synthesis, leading to loss of these matrix macromolecules what can result in a host of mechanical, hydration and nutritional deficits to tissue function. Restoration of the proteoglycan content within normal levels with natural molecules may help to restore tissue functionality but it is cost prohibitive.

Here, we report synthesis and characterization of a family of biomimetic proteoglycans (BPGs) composed of a synthetic backbone and natural GAG side chains to mimic the 3D architecture of the natural molecules.

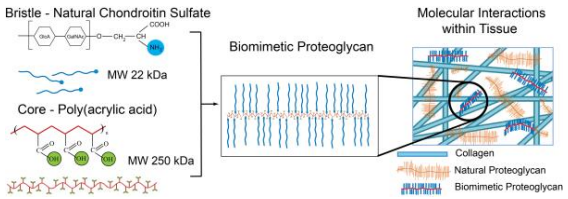

Fig 1. Molecular engineering of degenerated tissue with biomimetic proteoglycans.

Methods: BPGs were synthesized using a "grafting to" strategy, in which linear chondroitin sulfate (CS) molecules (Sigma-Aldrich) with primary amine end groups were covalently coupled to functional groups along polymer backbones poly(acrylic acid) (PAA) (MW 250kDa and 100kDa, Sigma-Aldrich; 1:15 mol:mol) and poly(acryloyl chloride) (PAC) (MW 10kDa, Polysciences; 1:10 mol:mol). Reaction kinetics was monitored with the fluorescamine assay. Macromolecules were purified via dialysis and chemical structures were confirmed with ^{1}H-NMR. Bottle brush configuration was confirmed with AFM (image obtained in Dr Ortiz lab, MIT). Cytocompatibility of our biomimetic PGS was assessed with L929 fibroblasts.

Osmotic pressure and water uptake of the BPGs solutions were measured via gel osmometry (Sephadex G-50) and TGA, respectively.

For a preliminary study on tissue engineering, 1ml of PAA250-CS (200 mg/ml, 1X PBS) was injected into a human lumbar cadaveric disc (T12-L1, 76 yo old male) through a 25 gauge needle. The injected disc was tested in diurnal compressive creep and recovery (16h 400N and 8h 50N, respectively) prior to and after injection.

Results:

CS molecules were successfully incorporated onto all polymer backbones, and the largest BPG was estimated to have ~ 85 bristles on the 250kDa PAA core with bristles spaced at ~ 4-8 nm, similar to natural aggrecan. BPGs were shown to be cytocompatible. AFM image of PAA250-CS sample showed bottle brush structure with a size of a molecule of at least 160 nm (Fig 2). This compares to ~100-300 nm for natural aggrecan.

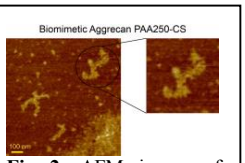

Fig 2. AFM image of BPG PAA250-CS.

BPG solutions had a statistically higher osmotic pressure than CS alone ($p<0.001$ at 50mg/ml) through the range of physiologically relevant concentrations and were in the range of osmotic pressure of natural aggrecan.

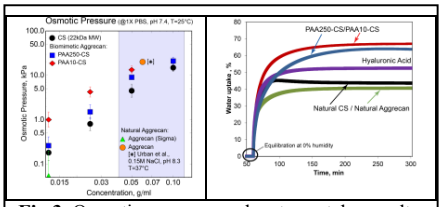

Fig 3. Osmotic pressure and water uptake results.

From water uptake studies, CS and natural aggrecan were shown to have comparable water uptake at ~40%, while BPGs demonstrated ~60% uptake.

Water retention of the intervertebral disc was examined before and after introduction of biomimetic aggrecan as inferred from creep displacement, which correlates to water loss from the disc. As expected, the disc showed a lower degree of creep displacement after injection with biomimetic PG molecule than before what implies that the disc had a higher osmotic pressure after the injection retaining more water. This also suggests that the injected sample was able to remain in the tissue after 16h of compressive loading.

Conclusions: "Grafting to" strategy was used to fabricate a family of cytocompatible BPGs with varied core length and bristle density. Our biomimetic aggrecan was shown to have comparable osmotic pressure of natural aggrecan and statistically higher pressure than natural CS alone, indicating an importance of bottle brush architecture of the molecule. Our BPG molecules were also demonstrated to have an increased water uptake as compared to CS and natural aggrecan. Injections of biomimetic aggrecan (PAA250-CS) into a disc stabilized the disc and reduced creep displacement. Our approach allows fabrication of a family of biomimetic proteoglycans of different molecular weights that mimic natural PGs and can be potentially used to molecular engineer and stabilize degenerated tissue by increasing hydration and osmotic pressure.

References:

1. Roughley PJ. Euro Cells Mater. 2006; 12:92-101.
2. Olczyk K. Zeitschrift für Rheumatologie. 1994; 53(1):19-25.