Molecular Engineering of Arthritic Human Cartilage *Ex Vivo* Using Biomimetic Proteoglycans

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Statement of Purpose: Osteoarthritis (OA) is the most common form of arthritis and one of the leading causes of disabilities, affecting more than 20 million people in the US[1]. Several biomolecules are depleted in OA cartilage including aggrecan. Aggrecan plays a vital role in the hydration and mechanical properties of articular cartilage and is lost during the early stages of degradation. Current conservative treatments for OA aim to slow the progression of the disease and mediate pain with analgesics, anti-inflammatory drugs, physical therapy, and weight loss; however, these treatments do not address the loss of aggrecan. *We propose to molecularly engineer the damaged articular cartilage with novel biomimetic proteoglycans (BPGs) with the eventual goal to restore hydration and mechanical function to the cartilage.* BPGs mimic the 3-D bottle brush structure and properties of naturally occurring aggrecan and consist of a poly(acrylic acid) (PAA) core with chondroitin sulfate (CS) bristles[2]. Here we report the infiltration of BPGs into human osteochondral sections of varying grades of OA and examine the distribution of BPGs through the cartilage extracellular matrix (ECM).

Methods: Human osteochondral fragments were obtained from patients that underwent total knee arthroplasty (IRB #1503003490). The osteochondral fragments from the tibial plateau were cut into 9x9mm sections from both load bearing and non-load bearing regions to obtain samples with varying degrees of osteoarthritis. The Collins' method was utilized to grade the cartilage sections where grade 0 indicates non-osteoarthritic cartilage, grade 1 mild osteoarthritis, grades 2-3 intermediate osteoarthritis, and grade 4 severe osteoarthritis where the cartilage is essentially fully degraded[3]. BPG10 and CS molecules were fluorescently labeled with DCCH (7-Diethylaminocoumarin-3-Carboxylic acid, Hydrazide)[4] and reconstituted in 1X PBS. Briefly, BPG10 consists of a 10kDa PAA core with approximately 8 attached CS chains giving the molecule a molecular weight of approximately 180kDa[2]. The human osteochondral sections were equilibrated in 1X PBS and then immersed in fluorescently labeled 20mg/mL CS and BPG10 solutions for 24 hours with care taken to expose only the articular surface to the solution. The osteochondral sections were then embedded in OCT and cryosectioned. The sections were observed using an Olympus FV1000 confocal microscope through a DAPI filter to detect the DCCH fluorescence.

Results: Both CS (~22kDa) and BPG10 (~180kDa) passively diffused into the human cartilage for grades 0-3 and dispersed throughout the cartilage ECM as seen in the representative images. The BPG10 and CS molecules tend to aggregate around the chondrocytes within the cartilage, as indicated by higher fluorescence intensity in the

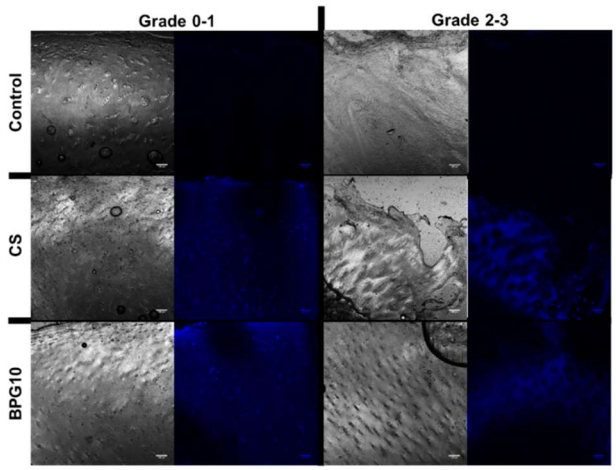

Figure 1: Contrast and fluorescent images for controls, CS, and BPG10 at Collins' grades 0-1 and 2-3, n=3 (scale bar 100μm).

images. OA grades 0-1 showed more BPG10 and CS chondrocyte localization than grades 2-3. The localization of BPGs and CS around chondrocytes and the overall diffusion into the cartilage ECM seems to decrease with more degenerative cartilage. This localization may be due to preferential binding to collagen VI present in the pericellular matrix over collagen II in the rest of the ECM[5]. This localization may also be due to an interaction with chondrocytes, which helps explain why there is less BPG10 and CS aggregation in more degenerative cartilage samples which have fewer cells. Due to the insufficient cartilage in OA grade 4 samples, the diffusion experiments with these samples yielded more variable results (not shown here).

Conclusions: We have demonstrated that BPGs can infiltrate into human articular cartilage with varying degrees of OA, distribute throughout the ECM, and localize around the chondrocytes. Recently, we have also shown BPG diffusion following the same results in normal bovine knee cartilage *ex vivo* and normal rabbit knee cartilage *in vivo*[6]. Biomimetic proteoglycans have the potential to replace lost aggrecan in the cartilage ECM in a minimally invasive manner through intra-articular injections. Infiltration through the cartilage surface may serve as a method to introduce BPGs into arthritic cartilage to molecularly engineer the tissue and restore hydration and mechanical properties.

References: **1.** Lawrence RC. *Arthritis and Rheum.* 2008:58:26-25. **2.** Prudnikova K. SFB. 2013. **3.** Collins DH. *Ann of the Rheum Diseases* 1960:19:31-41. **4.** Stuart K. *Biomacromolecules.* 2009:10:25-31. **5.** Poole CA. *J Anat.* 1997:191:1-13. **6.** Phillips E. WBC. 2016.