Macromolecular Architecture of Biomimetic Proteoglycans Directs Tissue Micromechanics and Cellular Mechanotransduction

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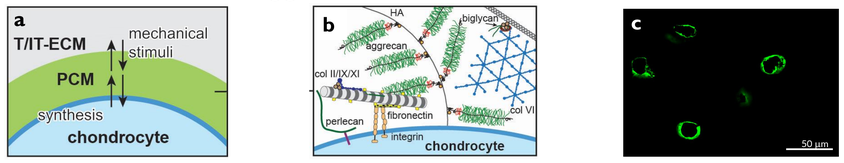
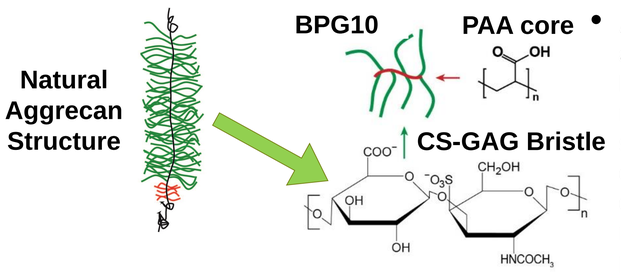
Introduction

Osteoarthritis (OA) is a degenerative disease of articular cartilage affecting more than 30 million Americans[1]. Perturbation of the pericellular matrix (PCM) portends extracellular matrix (EMC) degradation and is partially driven by perturbed cell mechanotransduction, contributing to irreversible cartilage breakdown and proteoglycan (PG) loss[2]. A reduction in PG content leads to a decrease in the biomechanics of both the PCM and the ECM contributing to pathogenesis[3]. Novel biomimetic proteoglycans (BPGs), enzymatically resistant synthetic versions of PGs, localize to the PCM and molecularly engineer tissue in joints that have been damaged by OA[4,5]. BPGs' molecular architecture facilitates increased interactions between aggrecan and increases the PCM micromodulus.



A) The PCM helps translate biochemical and biophysical signals to cells B) PCM has a unique molecular composition with C) collagen VI exclusive to the PCM

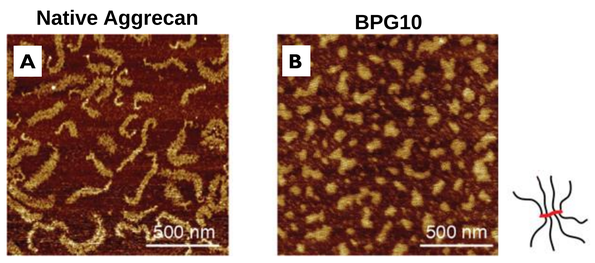
Biomimetic Proteoglycans (BPGs)



- A) Structure of aggrecan vs. B) BPG10 with its natural chondroitin sulfate (CS) bristles coupled to an enzymatically resistant synthetic polymer core (PAA)[6,7]

	Core length (kDa)	CS (22kDa)	Bristle-to-bristle spacing	Total MW (kDa)
Natural Aggrecan	~200-250	~100	3-5 nm	~2,500
BPG10	10	~7-8	~3-4 nm	~160-180

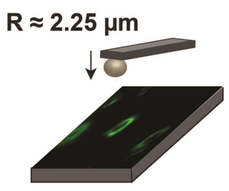
- BPG10 has CS-GAG bristle-to-bristle spacing very similar to what occurs on native aggrecan



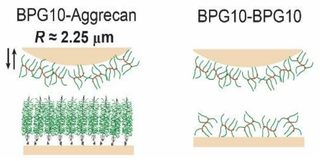
- Via AFM imaging, BPG10 has more of a star-shaped structure compared to native aggrecan's bottle-brush architecture

AFM imaging of A) native aggrecan B) BPG10, a star-like mimic

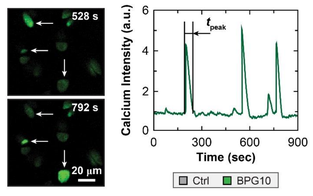
Experimental Methods



IF-guided AFM



MFS Set-up



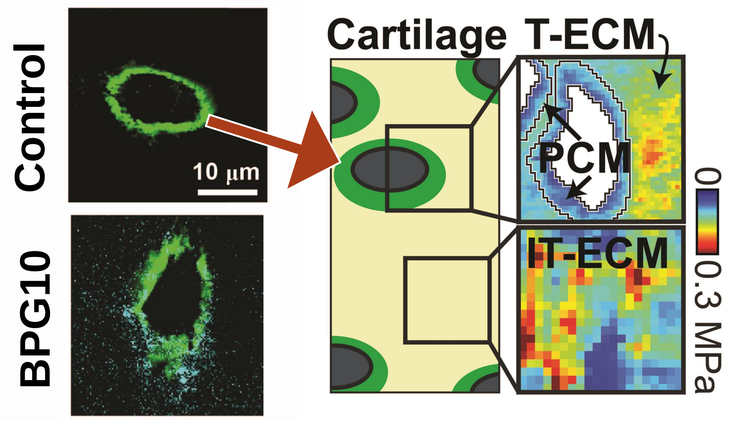
Calcium Signaling ($[Ca^{2+}]_i$)

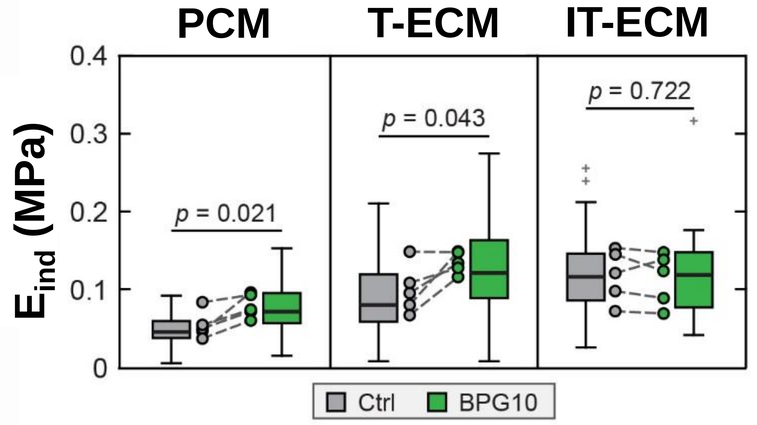
- Osteochondral plugs from the femoral condyles of bovine knee joints were suspended in fluorescently labeled-BPG10 or 1×PBS (as control) for 24 hours and sectioned by Kawamoto's film-assisted cryosectioning[8]
- 8 μm sections were stained with collagen VI antibody to differentiate the PCM from the territorial (T-) or interterritorial (IT-) ECM for IF-guided AFM[9]

For molecular force spectroscopy (MFS), surfaces were coated with thiolated BPG10 or thiolated aggrecan. The surfaces were compressed for 0 or 20 seconds dwell times in 1 × PBS and the maximum adhesion force (F_{ad}) and adhesion energy (E_{ad}) were extracted from the force-distance (*F-D*) curve[10]

4 mm x 2 mm fresh, adult bovine cartilage explants were cultured for 24 hrs with or without BPG10. Explants were assessed for cell viability and intracellular calcium signaling ($[Ca^{2+}]_i$), a measure of the mechanosensing of chondrocytes *in situ*.

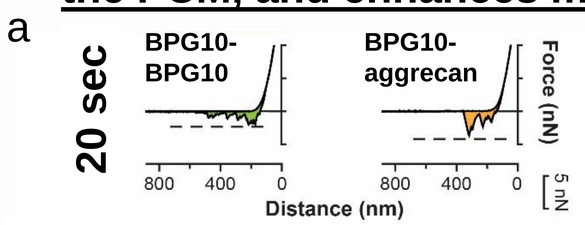
BPG10 increases the micromodulus of the PCM in normal bovine tissue

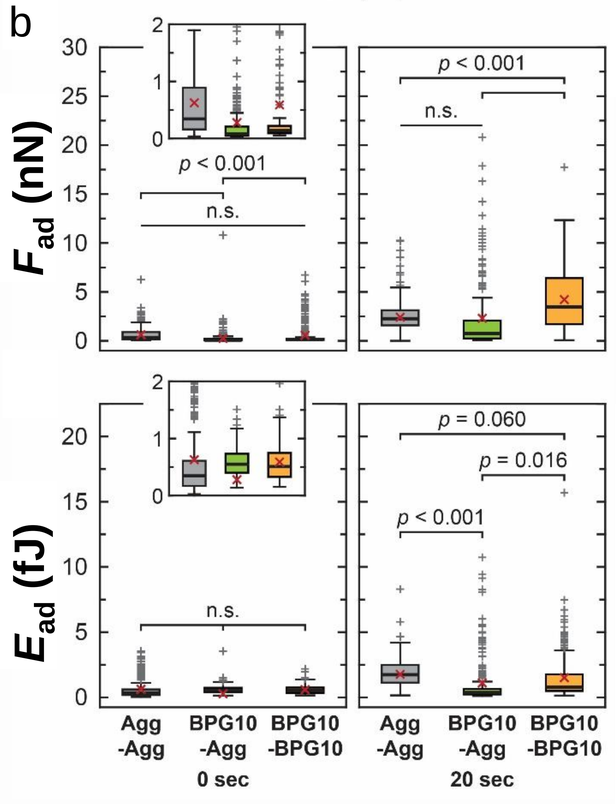




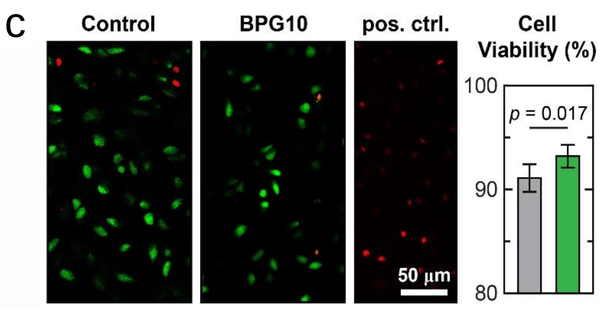
- Box-and-whisker plot of mean PCM and ECM values with matched pair averages of each of *n* = 5 animals
- BPG10 caused a significant 53±32% increase in the PCM modulus and a 44±32% increase in the T-ECM
- IF images show the localization of BPG10 (blue) to the PCM (green). Preferential, adhesive interactions of BPG10 with PCM predominant molecules, such as aggrecan, could account for the observed localization and increased mechanics

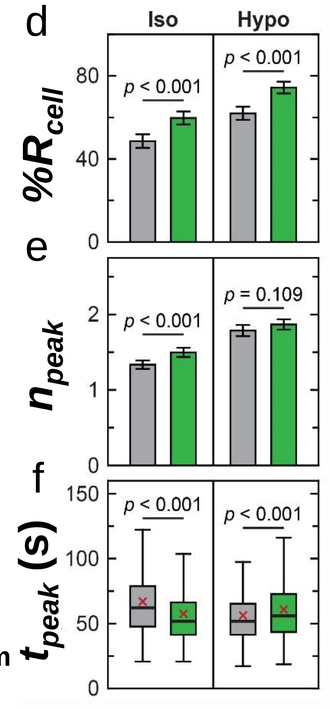
BPG10 demonstrates direct adhesion with aggrecan, a major proteoglycan of the PCM, and enhances mechanotransduction of the cellular microniche





- a) representative *F-D* curves of BPG10 against itself or against aggrecan
- b) BPG10 demonstrates comparable F_{ad} and E_{ad} to itself as to aggrecan with slightly elevated adhesion to itself at 20 sec dwell time. These adhesive interactions directly with aggrecan may enable BPG10 to localize to and integrate within the aggrecan-rich pericellular domain These molecular adhesions may also increase the local fixed charge density (via the highly negatively charged CS bristles) and provide additional physical bonding with existing matrix molecules thus contributing to an increase in the osmotic environment of the PCM





- c) 24 hr incubation of explant cartilage with BPG10 does not negatively impact cell viability
- d-f) BPG10 increases $\%R_{cell}$ under both physiologic (isotonic) and hypotonic (osmotically-simulated mechanical compression) conditions and alters other spatiotemporal characteristics of $[Ca^{2+}]_i$
- Spontaneous $[Ca^{2+}]_i$ is positively correlated with chondrocyte anabolism[11], indicating a potential for BPG10 in mediating downstream chondrocyte metabolic activities

Conclusions

- We have demonstrated molecular engineering of cartilage PCM resulting in biomechanical and mechanobiological augmentation of the cellular microniche
- Localization of BPG10 to the immediate cellular microenvironment, and integration therein, significantly increases PCM and T-ECM micromodulus through enhanced molecular adhesion with aggrecan, facilitated by BPG10's macromolecular architecture[12]
- Anionic BPG10 also directly interacts with the aggrecan-rich PCM thereby contributing to augmentation of osmotic environment and enhancing $[Ca^{2+}]_i$
- Collectively, BPG10 could potentially improve cartilage regeneration in OA

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