In Situ Swelling and Transport of Synthetic Polyelectrolytes in Articular Cartilage Explants

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INTRODUCTION: The structural function of articular cartilage is derived primarily from two sources: collagen, which provides stiffness, and negatively charged proteoglycans, which create an osmotic pressure to resist loads. Structural interventions to restore damaged cartilage, like in osteoarthritis, typically focus on stiffness and far fewer approaches target proteoglycan loss. While cartilage stiffness is routinely measured, there are far fewer methods to measure the functional incorporation of proteoglycans, even *ex vivo*. Here we report a new platform based on graphene strain sensors to evaluate proteoglycan loss during common enzymatic degradation of equine articular cartilage explants. To measure proteoglycan loss, we use a swelling assay where equine explants are placed into a hypotonic solution and the degree of swelling is measured continuously and *in situ*. Time-permitting, we also discuss our ongoing areas in testing new proteoglycan mimics through our platform, with the goal of providing new tissue restoration compounds for osteoarthritis.

METHODS: *Strain Sensor Fabrication.* Strain sensors were made by evaporating palladium on a single-layer of graphene and the film was supported by copper foil. To measure cartilage swelling mechanics *in situ*, we embedded the thin-film graphene strain sensors inside a poly(dimethylsiloxane) (PDMS) elastomeric bulk and in close proximity to the superficial zone of cartilage explants (**Figure 1**). The strain sensor is then read by simple DC resistance. *Cartilage Explant Testing and Enzymatic Degradation to Simulate Damage.* Cartilage explants were superglued (Scotch, 3M) to fabricated devices with the superficial zone side facing down and the bone side facing up. Explants were then incubated, following a 6 h timeline, at 37°C and in a temperature and humidity-controlled chamber. During the first 2 h period, the explants were incubated in media. During the second 2 h period, the explants were incubated in one of the following three treatments: 1) media as the control, 2) 0.1% type II bacterial collagenase in media or 3) 0.25 U/mL chondroitinase ABC in media. During the third 2 h period, the explants were incubated in 0.01M NaCl.

RESULTS SECTION: During our hypotonic challenge, the control group showed the largest swelling strain, indicated by the largest increase in resistance of 19.3%. Comparatively, the chondroitinase treated group had a lower 2.1% and the collagenase treated group showed a resistance change of -10.9%. (**Figure 2**) All groups were determined to be statistically significant from the control samples through a Tukey's HSD *post-hoc* test.

DISCUSSION: We have developed a platform that rapidly and conveniently resolves swelling strains in full thickness articular cartilage explants *in situ*. Our devices were able to discriminate between healthy cartilage swelling, reduced swelling in the chondroitinase digested group and loss of superficial collagen integrity in collagenase digested group. Justifying this platform, we demonstrated that bulk stiffness measurements, from indentation testing, are not sufficiently sensitive to resolve such behaviors alone.

SIGNIFICANCE/CLINICAL RELEVANCE: (1-2 sentences): The significance of this work is that it enables the development of compounds which target proteoglycan replacements in damaged cartilage by directly evaluating compound efficacy in full thickness equine explants. Approaches to replace or restore fixed charge density or proteoglycans (GAGS) are more rare than cartilage stiffening or lubricating approaches because a lack of methods to evaluate them.
REFERENCES: 1. Sundar, Shalini, Dhong, Charles* et al. "Optics-Free, In Situ Swelling Monitoring of Articular Cartilage with Graphene Strain Sensors." ACS Biomaterials Science & Engineering 9.2 (2023): 1011-1019.
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IMAGES AND TABLES:

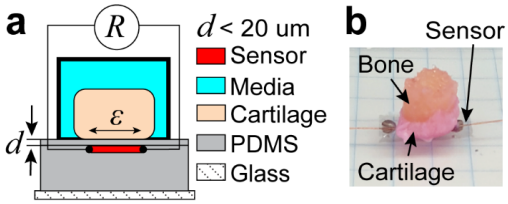

Figure 1. Platform to measure swelling in equine plugs. (a) The sensing device is made from graphene strain sensors embedded into PDMS. The swelling is then measured by changes in DC resistance. (b) Full thickness equine plugs are placed onto the sensing device, with the superficial zone side down, *i.e.*, glued onto the sensor surface.

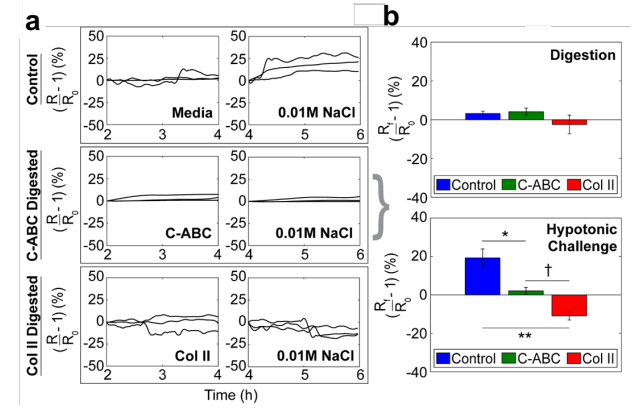

Figure 2. Swelling in healthy versus enzymatically-digested cartilage explants. (a) Raw swelling of cartilage explants under media (healthy), C-ABC (chondroitinase, which selectively digests proteoglycans), and Col II (Bacterial collagenase, which broadly digests proteoglycans and collagen). On the left time plot is the swelling data during the enzymatic digestion phase. On the right time plot is the hypotonic challenge phase, which assesses how much the cartilage will swell under hypotonic conditions of 0.01 M NaCl. (b) The final equilibrated swelling in all samples. During the digestion, the explants in all groups are statistically similar. During the hypotonic challenge, all groups show distinctive swelling behavior, suggesting that the platform is able to resolve differences in explants arising from proteoglycan levels.