3-sulfopropylmethacrylate interpenetrating polymer network reconstitutes the fixed negative charge density in GAG-depleted cartilage critical for interstitial fluid load support

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INTRODUCTION: Cartilage is comprised of an anionic proteoglycan matrix (5-15% wet weight) that retains interstitial water, intertwined with a type-II collagen (COLII) fibril network (5-20% wet weight) that affords structure and tensile strength. The proteoglycan matrix coordinates water because of the high fixed negative charge provided by the highly sulfated glycosaminoglycans (GAGs) that decorate the core aggrecan protein. These components harmonize to bestow cartilage with the load bearing and tribological tissue properties essential to diarthrodial joint function. According to Van Mow's triphasic theory, cartilage material properties depend on the fixed negative charge density within the cartilage matrix, the stiffness of the collagen and proteoglycan fibril matrix, and the ionic concentration of the bathing solution.[1] Osteoarthritis (OA) mediated cartilage degeneration is a result of cytokine-mediated upregulation of matrix metalloproteinases and downregulation of GAG production, leading to GAG depletion which reduces the fixed negative charge density of the cartilage matrix, thereby decreasing cartilage stiffness.[2] We propose to restore the fixed negative charge density of GAG-depleted cartilage critical to tissue function by forming an interpenetrating polymer network (IPN) with the native collagen network using a synthetic anionic GAG-mimetic polymer. This is accomplished by using the monomer 3-sulfopropylmethacrylate (SPM) to form a sulfonated IPN that entangles the existing collagen fibril network by crosslinking with polyethylene glycol diacrylate (PEGDA). The o*bjective* of this work is to verify that the SPM-IPN restores the fixed negative charge density of GAG-depleted cartilage by testing tissue mechanical properties in solutions of varying ionic strength. Specifically, cartilage was tested in an ion free condition (0 mOsm water) to identify Donnan osmotic swelling pressure, a high ionic strength solution (2000 mOsm saline) to determine the non-electrostatic contribution of the IPN to the tissue stiffness, and a non-charged solution (400 mM maltose) to demonstrate that the mechanical property changes are because of the rejuvenated fixed negative charge. The mechanical properties in these solutions were normalized to those measured in 400 mOsm saline, the tonicity of synovial fluid.

METHODS: Two mm diameter cylindrical cartilage plugs were harvested from the femoral groove of immature bovine stifle joints and cut to a 1mm thickness. To simulate OA-associated GAG depletion, the tissue was depleted of its native GAG content by incubating in a 0.1 U/mL solution of chondroitinase-ABC for 24-hours at 37ºC. The tissue fixed negative charge density was reconstituted by instilling the IPN into the GAG-depleted cartilage by incubating in a solution of 60% w/v SPM, 1% PEGDA (mol/mol SPM, M_W=3.5 kDa), 115 mM triethanolamine, 94 mM n-vinylpyrrolidone, and 0.1 mM eosin Y for 24-hours at room temperature followed by polymerization of the network by irradiation of the tissue using high intensity white light. Cartilage samples were equilibrated in testing solutions (0 mOsm water, 400 mOsm saline, 2000 mOsm saline, and 400 mM maltose) for ≥24-hours prior to mechanical testing. While submerged in the testing solution, the samples were subjected to unconfined compression (5% strain/step at a strain rate of 8 µm/s) using a 4-step incremental stress-relaxation procedure to determine the effect of ionic strength on the mechanical properties of healthy and IPN-treated degraded cartilage. Equilibrium modulus (E_{eq}) was determined from the slope of the linear best fit to the equilibrium stress-vs-strain for each incremental strain step. Donnan osmotic swelling pressure was derived from the ratio of E_{eq} in 0 mOsm water to that in 400 mOsm saline while the non-electrostatic contribution of the GAG network and IPN were derived from the ratio of E_{eq} in 2000 mOsm saline to that in 400 mOsm saline. To confirm that the augmented cartilage stiffness was the result of enhancing the fixed negative charge density of the depleted tissue, the effect of mobile ion transport was evaluated by comparing the ratio of E_{eq} in 400 mM maltose (a noncharged sugar) solution to that in 400 mOsm saline relative to the ratio of E_{eq} in 0 mOsm water to that in 400 mOsm saline. E_{eq} ratios for healthy and IPN-treated cartilage in each testing solution are represented as mean±S.D.; significant Δ determined by two-way ANOVA with post-hoc Tukey test or t-test at a significance α=0.05.

RESULTS: E_{eq} for both healthy and IPN-treated cartilage doubled after equilibration in 0 mOsm water compared to 400 mOsm saline (Fig. 1A): ratio 0 mOsm/400 mOsm = 2.38±0.42 and 2.01±0.38, respectively. In contrast, E_{eq} of cartilage decreased when tested in 2000 mOsm saline compared to 400 mOsm saline (Fig. 1A): 2000 mOsm/400 mOsm ratio = 0.45±0.047 for healthy cartilage and 0.83±0.17 for IPN-treated cartilage. E_{eq} for both healthy and IPN-treated cartilage increased in 400 mM maltose compared to E_{eq} in 400 mOsm saline, reaching an E_{eq} equivalent to cartilage in 0 mOsm water (Fig. 1B & C): for healthy cartilage, E_{eq} ratio in 0 mOsm/400 mOsm = 2.42±0.41 which is not-significantly different from the E_{eq} ratio in 400 mM maltose/400 mOsm = 2.15±0.32; for IPN-treated cartilage, the E_{eq} ratio in 0 mOsm/400 mOsm = 2.53±1.41 which while greater was not-significantly different from the E_{eq} ratio in 400 mM maltose/400 mOsm =1.72±0.93.

DISCUSSION: E_{eq} represents the non-fibrillar component of cartilage stiffness that is derived from the fixed negative charge density provided by GAGs in healthy cartilage and is supplemented by SPM-IPN in chondroitinase-ABC depleted cartilage. The doubling of E_{eq} in 0 mOsm water for both healthy and SPM-IPN-treated cartilage indicates the presence of fixed negative charge within the collagen-proteoglycan fibril matrix, which in accordance with Donnan osmotic swelling drives water into the tissue. In contrast, the 2000 mOsm saline solution effectively shields the fixed negative charge within the matrix, allowing for assessment of the non-electrostatic contribution of GAG and SPM-IPN to tissue stiffness, which are 45% for healthy cartilage and 83% for SPM-IPN rejuvenated cartilage. We validated that Donnan osmotic swelling is influenced in both healthy and SPM-IPN-treated tissues by the transport of mobile ions and not uncharged solutes by measuring tissue stiffness in 400 mM maltose, a noncharged carbohydrate. Equivalent Donnan osmotic swelling pressure as demonstrated by a similar increase in E_{eq} for both healthy and SPM-IPN-treated cartilage tested in 0 mOsm water and 400 mM maltose solution was observed. Therefore SPM-IPN augmented the depleted fixed negative charge of chondroitinase-ABC depleted cartilage like native GAGs in healthy cartilage as demonstrated by Donnan osmotic swelling pressure.

SIGNIFICANCE/CLINICAL RELEVANCE: This work suggests that the high fixed negative charge density produced by native GAG, essential for interstitial fluid load support in healthy cartilage, can be reconstituted using the monomer 3-sulfopropylmethacrylate (SPM) to form a sulfonated IPN that entangles the existing collagen fibril network by crosslinking with polyethylene glycol diacrylate (PEGDA) to rejuvenate degraded cartilage in early OA.

REFERENCES: [1] Lai, W.M., et al. *J Biomech Eng* 113(3) (**1991**) 245-258. [2] Basalo, R.L., et al., *J Biomech Eng* 126(6) (**2004**) 779-786.

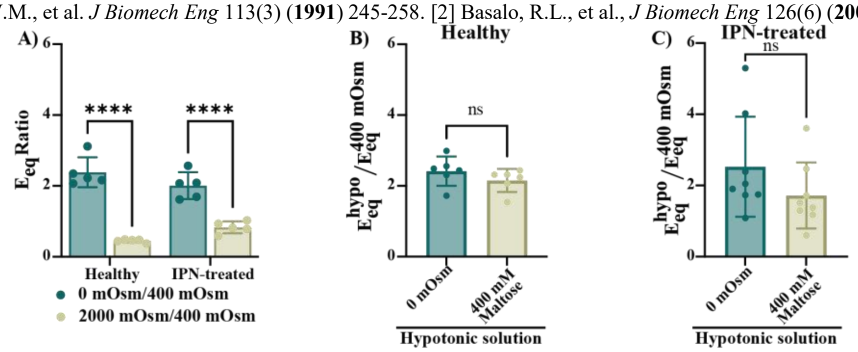

Figure 1. A) Low ionic strength (0 mOsm) increases while high ionic strength (2000 mOsm) decreases E_{eq} of healthy and IPN treated cartilage relative to the E_{eq} in 400 mOsm saline. **** p<0.0001. A 400 mM solution of maltose increase the E_{eq} in a similar manner to 0 mOsm water for **B)** Healthy and **C)** IPN-treated cartilage compared to the E_{eq} in 400 mOsm saline, indicating that noncharged solutes do not influence the E_{eq}. ns=non-significant.