3-sulfopropylmethacrylate forms an interpenetrating polymer network that augments GAG-depleted cartilage

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INTRODUCTION: Hyaline cartilage is a porous, viscoelastic, biphasic composite material comprised of a type-II collagen (COLII) fibril network (5-20% wet weight) that affords structure and tensile strength, complemented by a negatively charged, sulfated glycosaminoglycan (GAG) matrix (5-15% wet weight) that retains interstitial water. These components act synergistically, bestowing the rheological and tribological material properties essential to cartilage function. GAGs are highly sulfated, anionic, carbohydrates that decorate the core aggrecan protein, conferring its characteristic bottlebrush structure, and ability to coordinate water molecules through negative charge. Greater than 90% of the applied joint load is supported by pressurization of entrapped interstitial fluid, i.e., interstitial fluid load support (*IFLS*).[1] Osteoarthritis (OA) is characterized by cartilage degeneration. Early in the disease process, GAGs are depleted from the cartilage as a result of cytokine-mediated upregulation of matrix metalloproteinases and downregulation of GAG production. Depletion of cartilage GAG reduces *IFLS*, transferring load to the COLII fibril network, which subsequently breaks down, culminating in increased hydraulic permeability, and decreased cartilage stiffness.[2] We propose to restore the material properties of damaged cartilage critical to diarthrodial joint function by forming an interpenetrating polymer network (IPN) with the native collagen using, a synthetic, hydrophilic, and charged GAG-mimetic polymer to restore *IFLS* and reestablish the integrity of the collagen fibril network. This is accomplished by using the monomer 3-sulfopropylmethacrylate (SPM) to form a sulfated and anionic IPN that entangles within the existing collagen matrix by crosslinking with polyethylene glycol diacrylate (PEGDA). The o*bjective* of this work is to identify the mechanism by which the IPN augments GAG-depleted cartilage by examining the change in water weight percent before and after IPN installation to determine whether the polymer is filling the pores or re-enforcing the degraded hydrogel matrix.

METHODS: Using a biopsy punch, 2 mm diam. cylindrical cartilage plugs were harvested from the stifle joints of immature bovine specimens and cut to a thickness of 1 mm, excising the superficial zone cartilage layer (simulating Outerbridge 3 changes). To simulate OA compositional changes, the tissue was depleted of its intrinsic GAG content by incubating each plug in a solution of chondroitinase-ABC (C-ABC) at a concentration of 0.1 U/mL for 24 hours at 37ºC. IPN treatment was accomplished by incubating the GAG-depleted cartilage plugs in solutions with 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 irradiation of the samples using high intensity white light to initiate polymerization of the network. The material properties of the cartilage were determined using a 4-step stress-relaxation procedure conducted in unconfined compression, where the equilibrium modulus was calculated from the best fit linear slope to the equilibrium stress-vs-strain for each incremental deformation step. The equilibrium modulus for each group: healthy (n=8), GAG-depleted (C-ABC, n=8), and IPN treated (60% SPM IPN, n=8), was compared using one-way analysis of variance (ANOVA), with a post-hoc Tukey multiple comparisons test, at a significance α=0.05. Next, the water content of each group, surrogate metric for tissue porosity, was measured to determine the mechanism by which the SPM IPN restored the mechanical properties of GAG-depleted tissue. The water content of each cartilage plug (n=5) was derived from the weight difference of each sample *before* (wet weight) and *after* lyophilization (dry weight), divided by the wet weight. The water content of each group was compared using a one-way, unpaired ANOVA, with a post-hoc Tukey multiple comparisons test at significance α=0.05. The IPN content of the treated plugs (n=4) was measured by digesting the proteinaceous tissue in a 1 mg/mL solution of papain at 60ºC for 24 hours, dialyzing the resulting solution for 24 hours through dialysis tubing with a 50 kDa M_W cut off to remove non-IPN components, lyophilization and weighing the final dried product. The relationship between the IPN content vs water content (porosity) was evaluated by bivariate linear regression.

RESULTS: GAG-depletion using C-ABC significantly decreased the equilibrium modulus from 0.34 MPa (healthy bovine) to 0.099 MPa (Fig. 1A). IPN treatment of the GAG-depleted tissue augmented the equilibrium modulus to 0.84 MPa, significantly greater than either the healthy or GAG depleted cartilage (Fig. 1A), demonstrating that the SPM IPN reconstituted the tissue stiffness beyond that of healthy cartilage. Depleting GAG from the cartilage increased the tissue water content to 86.1% from 82% in healthy tissue (presumably representing an increase in tissue porosity). Infiltrating the degraded tissue with sulfated and anionic SPM to form an IPN within the existing collagen network reduced the water content of the tissue to 79.1%, equivalent to the water content of healthy cartilage (Fig. 1B). Following treatment, the IPN content of the GAG depleted tissue was 5.74%±4.12% (mean ± SD) by wet weight %. For treated cartilage, the tissue water content was inversely related to and explained 74% of the variation of the IPN content (Fig. 1C).

DISCUSSION: Crosslinking SPM with PEGDA formed a polymer that restored the stiffness of degraded cartilage. Mechanistically there are two possible explanations for this observation: 1) owing to the increased permeability and tissue porosity of the GAG depleted tissue, the IPN polymer filled the pores of the cartilage ECM with a dense hydrogel that directly augmented tissue stiffness; or 2) SPM formed a sulfated and anionic IPN that entangled within the existing collagen fibril network, increasing tissue stiffness by restoring IFLS. If the polymer filled the tissue pores, the water content of the treated cartilage would be reduced by the extent that the hydrogel displaced the entrapped fluid, while if the IPN augmented the tissue IFLS, the water content of the treated cartilage would remain relatively unchanged. The IPN-treated samples have a reduced water content compared to GAG-depleted tissue, but not significantly different from healthy tissue. At 60% w/v, while the SPM IPN did not completely displace all the entrapped fluid present within the tissue pores, the inverse relationship between water content (porosity) and IPN content, suggests that the IPN (when higher) did tend to displace entrapped fluid contained within the pores (lower water content) and that the mechanism of action is by directly filling the tissue pores with a dense hydrogel that augments tissue stiffness.

SIGNIFICANCE/CLINICAL RELEVANCE: This work suggests that the SPM IPN restores the material properties of degraded cartilage by filling the increasingly porous OA cartilage with a dense hydrogel that can bind water and restore the tissue IFLS. Augmenting the existing degraded cartilage with a GAG-mimetic monomer that can be instilled into the joint and polymerized with white light using conventional arthroscopy represents a novel, minimally invasive, immediate clinical treatment approach.

REFERENCES: [1] Park, S., et al. *Journal of biomechanics* 36.12 (**2003**): 1785-1796. [2] Akizuki, K., et al., *Journal of Orthopedic Research,* 5.2 (**1987**): 173-186.

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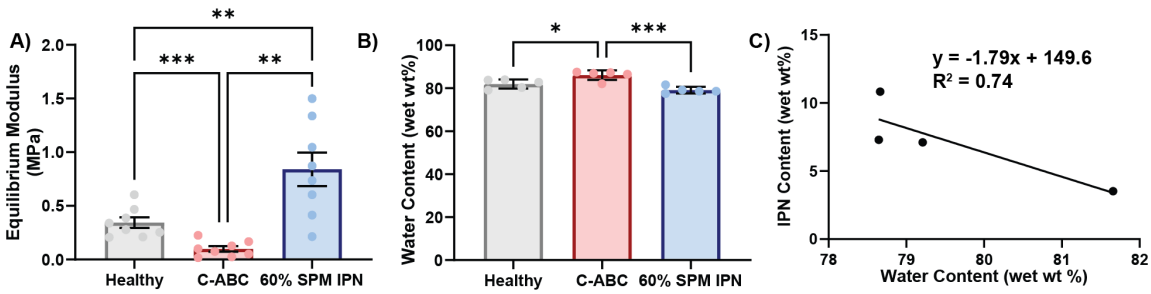

Figure 1. A) GAG depletion significantly reduces the equilibrium modulus compared to healthy cartilage; IPN-treated samples have an equilibrium modulus significantly greater than both healthy and degraded samples. **B)** Water content of GAG-depleted tissue significantly greater than both healthy and IPN-treated samples. **C)** IPN content was measured following removal of proteinaceous content of each plug. IPN content was inversely related to water content. *=p<0.05, **=p<0.01, ***=p<0.001.