Mechanically Tunable Gelatin Hydrogels for Use in Meniscal Tissue Engineering

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INTRODUCTION: Meniscus tissue injury is the most prevalent orthopedic injury requiring surgical intervention with over 750,000 procedures performed annually in the United States alone. As the meniscus is a multilayered fibrocartilaginous tissue, recapitulating its' dynamic load bearing behavior while maintaining structural integrity has proven challenging for researchers. As such the goal of this research is to evaluate Glutaraldehyde cross-linked Gelatin A based hydrogel compositions and their stress-relaxation behavior and mechanical properties. By characterizing hydrogels of varying compositions based on stress relaxation behavior, polymer content, cross-linking concentration, and relative water content, we can begin to develop and optimize the mechanical properties of meniscal tissue engineered (MTE) constructs, which could be used for repair of injured tissues.

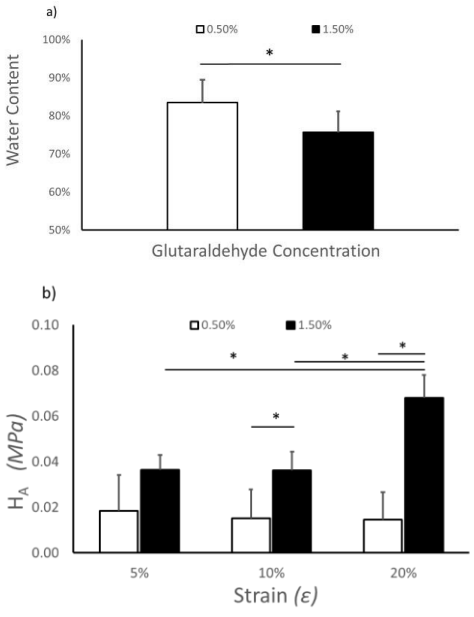
METHODS: ***Hydrogel Preparation:*** Hydrogels were prepared by dissolving 3g of GelA (Gelatin Type A from Porcine skin, 300 bloom, Sigma-Aldrich) in 20 mL of PBS (Phosphate Buffered Saline) to a 15% wt./vol. concentration. GelA solution heated in a water bath at 65ºC. Glutaraldehyde stock solution (25% stock solution, Sigma Aldrich) was diluted in PBS at concentrations 0.5% and 1.5%, and immediately added to the heated GelA solution in a volume-to-volume ratio of 3ml GelA: 0.3ml Glutaraldehyde to achieve a hydrogel thickness of approximately 3 mm. The gel was then crosslinked for one hour and then immersed in PBS to arrest cross-linking. Samples were allowed to swell to osmotic equilibrium for 12 hours at 4ºC. ***Water Content and Swelling Equilibrium:*** To determine the Water Content and swelling equilibrium point, hydrogel samples were rinsed in PBS for a period of 1 hour to remove uncross-linked gelatin and dehydrated under a fume hood for a period of 24 hours to complete dehydration Completely dehydrated samples were weighed, and initial dry weights were recorded. Samples were placed in individual wells and rehydrated with 5 mL of PBS solution. Following swelling, samples were weighed at 1,2,3,4,5,6, 12, and 24 hrs. At each time point, samples were removed from solution, patted dry to remove excess solution on surfaces and weighed. Water Content was then calculated by equation (1), using the fully dehydrated sample weight for dry weight (Wt_{dry}) and full osmotic equilibrium weight for wet weight (Wt_{wet}). All samples were determined to reach osmotic equilibration following 12 hours in PBS. For each cross-linking condition, a sample size of (n=3) was used. ***Confined Compression Stress Relaxation Procedure:*** Hydrogels were punched using a 12.7mm diameter trephine punch immediately upon removal from refrigeration. Prior to testing, samples were preloaded with ~0.15 N using a Univert Cell Scale Uniaxial Testing system in a custom transparent 3D printed compression chamber with compressing plug and compressed for 1 minute to ensure proper contact reduction, prior to introduction of room temperature (~22 ºC) PBS into the testing well. Samples were compressed at a rate of 3.12 μm */sec* to strains of 5%, 10%, and 20% based on measured sample thickness, and then held for 30 min, 30 min, and 60 min, respectively, to allow for stress relaxation to occur. Data was then processed using a custom Python 3.9 script to determine Aggregate Modulus H_A (MPa) at each strain level. H_A was calculated using equation (2), where F_{Eq} is the equilibrium force, A is the sample area, and ε is the strain level. A total of n=5 samples were tested for each group. ***Statistical Analysis:*** A two sample t-test was used to assess any difference in *Water Content* between hydrogel concentrations. A two-way ANOVA with post-hoc was used to assess differences in H_A across strain levels (5, 10 ,20%) and concentrations (0.5%, 1.5%). A significance level of α=0.05 was used for all statistical analyses.

$$Water\ Content = \frac{Wt_{wet} - Wt_{dry}}{Wt_{wet}} x100 \qquad (1)$$

$$H_A = \frac{F_{Eq}}{(A)(\varepsilon)} \qquad (2)$$

RESULTS SECTION: The results of this study are shown in Figure 1. Our results indicate that glutaraldehyde cross-linker concentration significantly alters the water content ($p < 0.05$) of the gelatin gels, with the water content of the 0.5% glutaraldehyde gel being higher than the 1.5% gel. Two Way ANOVA showed that the Aggregate Modulus of the gels is significantly affected by the glutaraldehyde concentration and the strain level ($p<0.001$). Significant differences between individual groups revealed in post hoc analysis are shown in Figure 1b.

DISCUSSION: Our results show variation in composition relative to mechanical properties and viscoelastic material behavior for the gels tested. The 0.5% and 1.5% concentration hydrogels exhibiting water contents of 83.48% ± 5.99 and 75.62% ± 5.49, which is larger than that of native meniscus tissue (~70%). This difference likely accounts of the measured aggregate modulus values observed during confined compression testing as literature values place native meniscus tissue in axially loaded orientation between ~100-150 kPa. Our hydrogel values range from ~27 kPa at the 0.5% concentration and 5% strain to ~68 kPa for the 1.5% concentration at 20% strain. Given that our hydrogels are lower in polymer content and lack relative fiber orientation compared to native tissue, the lower aggregate modulus values were expected. Nonetheless, this study serves as a starting point for future work in meniscal tissue engineering (MTE). The utilization of other fabrication methods can allow for further optimization of biomaterials to be used in MTE. As researchers continue to characterize native meniscus mechanical properties and biological composition, the target criteria for a successful tissue engineered alternative becomes clearer. Future MTE initiatives will likely draw upon various fabrication methods to develop a successful tissue engineered alternative. As such our lab will continue to investigate varied compositions along with fabrication methods for utilization in the field of meniscal tissue engineering.



SIGNIFICANCE/CLINICAL RELEVANCE: Gelatin A based hydrogels serve as a promising foundation in the field of meniscal tissue engineering to expand therapies related to cartilage tissue healing and restoration of healthy articular joint dynamics, while mitigating long term degeneration.

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Figure 1: (a) Water content for 0.5% and 1.5% Glutaraldehyde cross-linking solution concentrations with statistical significance ($p<0.05$) between groups denoted by (*). (b) Aggregate Modulus values for each cross-linking concentration across various compressive strain rates. Statistical significance ($p<0.05$) for each strain rate and cross-linker concentrations denoted by (*)