# Durability and Impact of Annulus Fibrosus Repair with Functionalized Hydrogels Under Loading

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**INTRODUCTION:** During intervertebral disc (IVD) herniation, the nucleus pulposus (NP) extrudes beyond the boundaries of the annulus fibrosus (AF), compressing neighboring spinal elements and inducing pain. Microdiscectomy can relieve the immediate symptoms of herniation but fails to promote long-term healing as it does not restore mechanical integrity of the AF or address the associated inflammation.[1] While a variety of repair materials have been developed to improve IVD mechanical stability following microdiscectomy,[2,3] few utilize localized therapeutic delivery to combat these adverse biologic changes. We previously developed a hyaluronic acid (HA)-based hydrogel capable of spatiotemporal controlled delivery via secondary reactions between the streptavidin-modified hydrogel backbone and biotin-modified therapeutics.[4] Here, we investigated the *ex vivo* retention of these functionalized hydrogels in injured AF in living discs that were subjected to dynamic uniaxial compression over an extended duration.

**METHODS:** Hydrogel Fabrication: HA synthesis and hydrogel fabrication were accomplished using established protocols.[5] Streptavidin modification was achieved by incorporating 1% thiol-streptavidin (TS) into the hydrogel precursor solution and was covalently attached via reaction with norbornene residues on the HA backbone. Unless otherwise specified, hydrogels were 3 wt% with a crosslinking density of 50% using MMP-degradable crosslinks.[6] 30 µM biotin-modified Cy5 was also incorporated to visualize the hydrogel via the biotin-streptavidin secondary reaction. IVD Tissue Preparation: IVD motion segments were isolated from bovine tails. The vertebral bodies were carefully removed to enhance nutrient diffusion through the endplates and prepared for sterile organ culture.[6] AF Injury and Repair: A 5 mm x 2.5 mm cruciate laceration was made in the AF (Depth = 2 mm) followed by a 7 mm deep puncture with a 14G needle into the NP. Hydrogel repair was performed immediately following injury by injecting 25 µL of the sterile hydrogel precursor solution and photocrosslinking for 10 minutes at 5 mW/cm$^2$. Samples were incubated overnight prior to loading. Uniaxial Compressive Loading: Physiologic loading (0.02–0.2 MPa, 0.2 Hz) was applied for 2 hours each day for 3 consecutive days.[7] After the final loading cycle, samples were incubated overnight prior to processing. Outcomes: Disc height was measured before and after loading (n=8 per group). Stress-strain data were collected during each loading cycle (n=8 per group). Half of the samples were processed for PCR (n=4 per group) while the remaining were processed for histology (n=4 per group). Statistical Analysis: Significant differences ($p<0.05$) were detected using two-way ANOVA (Disc Height Reduction, Mechanics) or unpaired t-tests (PCR) in GraphPad Prism.

**RESULTS:** The cruciate-puncture injury substantially disrupted AF structure without significant injury to the NP (1A). Injury coupled with physiologic loading resulted in a clear increase in expression of matrix remodeling (ADAMTS5, MMP-1, and MMP-13) and inflammatory (IL-6) genes (1B-E). There were no differences in disc height reduction with loading between intact and injured discs (1F). On the first day of loading, the linear modulus for injured samples increased with increasing cycles of compression while intact samples remained constant (1G, p=0.078). The maximum strain for injured samples decreased with increasing cycles of compression to eventually reach the same level as intact controls (1H, Day 1 p=0.0614, Day 2 p=0.0318). On all three days of loading, the amount of energy dissipated was significantly higher at every cycle for injured discs compared to intact controls (1I, p<0.0001). The hydrogel remained in place over three days of loading (2A), with histological analysis confirming that the hydrogel penetrated through the depth of the AF injury and remained resident over this time course (2B). At this early timepoint, there were trends towards increasing ECM deposition markers expression (COL1, ACAN) with hydrogel repair (2C-D). ADAMTS5, IL-6, and MMP-1 gene expression were unchanged compared to injured samples, while MMP-13 was significantly decreased with hydrogel repair (2E-H).

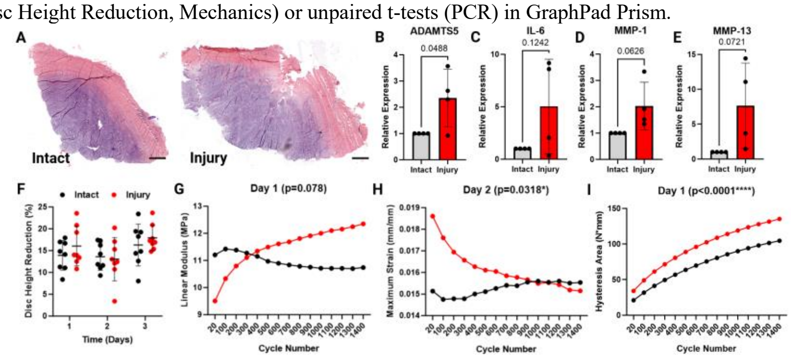


*Figure 1: A) H&E histology of intact and injured discs. Scale = 2.5 mm B-E) Gene expression in the injured AF normalized to the intact AF. F) Disc height reduction during loading. G-I) Mean mechanical changes with injury (n=8/group).*

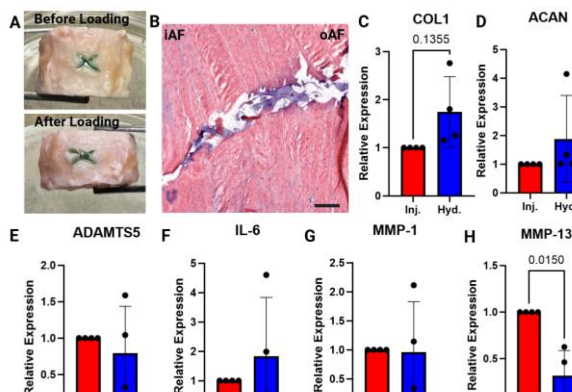


*Figure 2: A) Hydrogel repair before and after loading. B) H&E histology of hydrogel retention. Scale= 0.5 mm C-H) Gene expression in the repaired AF normalized to the injured AF.*

**DISCUSSION:** Our data supports prior findings that acute AF injury increases matrix remodeling and initiates an inflammatory cascade that hinders endogenous repair.[8] At the onset of loading, injured discs were softer and experienced higher strain, which normalized at later cycles (likely due to compaction of the injured disc). Injured discs also dissipated more energy than intact discs across all cycles. Altogether, these differences highlight how acute AF injury impacts the mechanical integrity of the disc even at early timepoints. These changes to the mechanical stability of the disc could compound with time and further contribute to inferior tissue repair – motivating the need for novel AF repair biomaterials. Importantly, we demonstrated that TS hydrogels can effectively seal the AF injury and are retained with physiologic loading.

The introduction of this hydrogel did not worsen inflammation and matrix remodeling following injury. Instead, hydrogel repair decreased MMP-13 expression, suggesting less collagen degradation, and exhibited trends towards increased ECM deposition that may promote tissue repair. Future studies will further probe the mechanical and biologic response of the disc to injury and repair via immunofluorescence and RNAScope to better understand the consequence of rapid and sustained delivery of biologics from functionalized hydrogels. We also plan to utilize this methodology to evaluate the efficacy of localized therapeutic delivery using the secondary reactions between our streptavidin-modified hydrogels and a biotin-modified therapeutic.

**SIGNIFICANCE:** In addition to characterizing the effects of acute AF injury, this study demonstrates that functionalized hydrogels are a promising repair material for the AF as well as other dense connective tissues. The ability of this hydrogel to penetrate and remain in place under loading suggests that such materials are promising for providing spatiotemporal controlled delivery of a therapeutic throughout the tissue to promote endogenous healing. This development could transform clinical practice by creating superior surgical alternatives and outcomes for patients.

**REFERENCES:** 1. Peredo, AP *et. al.* JOR Spine (2021) 2. Sloan, SR *et. al.*, Sci. Trans. Med. (2020) 3. Panebianco, CJ *et. al.* Euro. Cells & Mat. (2020) 4. Sharp, EE *et. al.* ORS (2025) 5. Gramlich, WM, *et. al*., Biomaterials (2013) 6. Loebel, C *et. al.* Nat. Mater. (2019) 7. Saravi, B *et. al.* Jove (2021) 8. Bonnevie, ED *et. al.* Nat. Biomed. Eng. (2019)