Combination of an Annulus Fibrosus Repair with a Mechanically Interlocked Patch and a Nucleus Pulposus Augmentation with Acid-Tyramine Hydrogel in an *Ex Vivo* Model

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INTRODUCTION: Lumbar disc herniation stands as a dominant cause for radicular pain among middle-aged patients. This condition arises from the protrusion or extrusion of the nucleus pulposus (NP) through a defect in the annulus fibrosus (AF), subsequently exerting pressure on the adjacent nerve structures and causing pain. The gold standard treatment for this condition is discectomy, which removes the protruded or extruded material, leading to symptomatic alleviation. Nonetheless, this intervention does not prevent re-herniation, which has an incidence of 5 to 26%. Biomaterial-based treatments showed promising potential; however, no clinically approved intervention is currently able to completely restore mechanical function of the intervertebral disc (IVD). Our approach is a combination of (i) AF repair by sealing the annular defect with a mechanically interlocked patch (iPatch) comprising a non-woven medical textile of polyethylene terephthalate (PET) fibers[1], and a (ii) NP augmentation with hyaluronic acid-tyramine hydrogel (HA-Tyr). The aim of the study was to test the influence of the viscosity of the HA-Tyr on the mechanical function of the repaired disc after experimental herniation. We used an established bovine tail explant model.

METHODS: Bovine caudal IVD segments from levels cc1/2 and cc3/4 were prepared and subjected to biomechanical evaluation (N=23). The study consisted of three distinct treatment groups (n=6 each) and one control group (n=5). Each single sample underwent the entire protocol without being removed from the mechanical testing machine. The protocol encompassed two parts: the first part assessed compressive-tensile cycles and determined biomechanical parameters. The same number of cycles and loads were tested in three different IVD conditions for each sample: intact, injured, and repaired. The second part of the protocol measured the load capacity until failure at a flexion angle of 13° with the defect size exposed, wherein failure was defined as either NP herniation (control) or hydrogel extrusion (treatment groups). This test was conducted through visual analysis of recorded videos and was constrained to a maximum of 10 MPa, with the purpose of avoiding setup or endplate failures. The injury was induced through a cruciate annulus defect combined with nucleus detachment and nucleotomy (NP volume removal of ~20%). As treatment, HA-Tyr at varying viscosities (low viscosity, medium viscosity, high viscosity) was injected into the IVD and the iPatch was subsequently anchored over it by mechanical interpenetration of fibers to seal the defect. The last compressive-tensile cycle of every condition was recorded, and the related pressure-displacement curve was plotted. The distance from maximum tension to maximum compression was expressed as range of motion (ROM), whereas the slope of the linear regression of the top 20% values of the compression curve was identified as compressive stiffness. Matched mixed-effects model analysis with Tuckey's post-hoc was used to statistically compare the groups. Significance level was $\alpha = 0.05$.

RESULTS: All results were always normalized to the related intact values. ROM for injury in all three treated groups was significantly increased compared to intact ($p<0.001$)(Fig. 1). Evaluated as a group, ROM values in the high viscosity group were closest to the intact state (mean diff. to injury = 13.1%; $p<0.001$), followed by the medium viscosity group (mean diff. to injury = 7.3%; $p=0.01$), and then the low viscosity group which was mechanically equivalent to the unrepaired disc (mean diff. to injury = 3.4%; $p=0.48$)(Fig. 1). None of the treatment groups could fully recover compressive stiffness, although improved compressive stiffness was observed for the low viscosity repair (mean diff. to injury = 8.0%; $p<0.001$), medium viscosity repair (mean diff. to injury = 7.8%; $p=0.001$), and high viscosity repair (mean diff. to injury = 16.0%; $p<0.001$) to the unrepaired injury group.
When comparing the herniation rate during the failure strength test, the group treated with low viscosity hydrogel exhibited the most favorable outcome with 33% of the samples experiencing failure in contrast to the injury group that was remarkably higher (80%) (Fig. 2). The medium viscosity group displayed an intermediate herniation frequency of 50%, while the rate seen in the high viscosity group (83%) was similar to the unrepaired group (Fig. 2). Both the low viscosity and medium viscosity groups reported either herniation over the physiological stress level or no herniation. No herniation occurred in 67% of the specimens tested in the high viscosity group and 60% of the specimens tested in the injury group. In all treatment groups and the non-repaired injury group, the mean pressure at herniation was greater than the human physiological stress level for IVDs of 2.3 MPa (Fig. 3). All treated groups showed a greater failure strength compared to the injured group, although none of the differences reached statistical significance (Fig. 3). The highest mean difference in failure strength was seen in the low viscosity group (mean diff.=4.1 MPa; $p=0.256$).

DISCUSSION: This biomechanical study clearly demonstrates a lower risk of herniation when the defect is repaired with the iPatch, as, among all treated groups, 8 out of 18 specimens resisted 10 MPa of pressure without showing any herniation. Notably, all samples repaired with the iPatch and either low or medium viscosity hydrogel, successfully withstood the supraphysiological stress of 2.3 MPa. This finding is encouraging for addressing the challenge of re-herniation, which exhibits a recurrence rate of 25.3% within a 2-year timeframe. Furthermore, it was shown the importance of an optimized viscosity of an injectable hydrogel for the restoration of NP biomechanical parameters, which are altered through injury. An evident pattern of increasing mechanical recovery with increasing viscosity was observed. Nevertheless, the increasing viscosity predisposes re-herniation risk. A limitation of this study is that our test is an approximation of the *in vivo* mechanical functions excluding for example lateral bending or flexion or extension. In conclusion, the utilization of the HA-Tyr hydrogel as a NP augmentation in combination with the iPatch has the potential to be a carrier for cells or drugs and may therefore be applied to promote healing in the IVD, while simultaneously restoring biomechanical properties of an injured IVD.

SIGNIFICANCE: We propose a potential repair strategy capable of at least partially restoring mechanical function after IVD herniation, effectively sealing the annulus, replacing the NP with a drug-loadable hydrogel, and ultimately exhibiting resilience against re-herniation under supraphysiological loads.

REFERENCES:
1. Meyer and Bachmann et al., Am. J. Sports Med., in press.

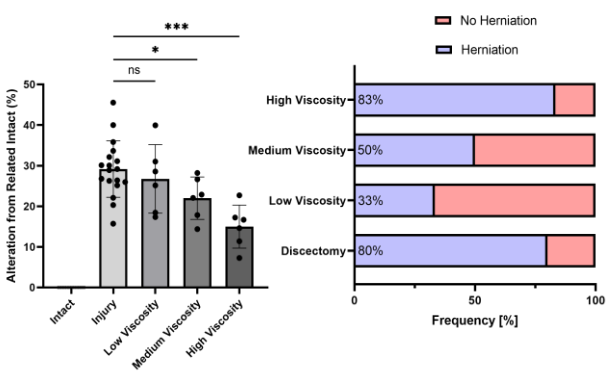

Fig. 1: Calculated range of motion from strength-displacement curve. Treatment values were normalized to intact of the same sample. *: $p<0.05$, ***: $p<0.001$.

Fig. 2: Frequency of herniation illustrated in each treated group and in the discectomy group and expressed in percentage.

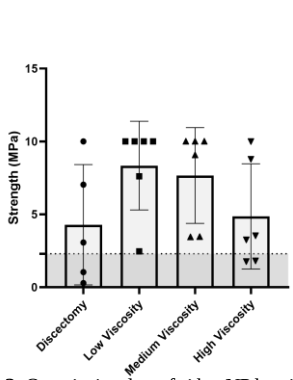

Fig. 3: Quantitative data of either NP herniation or hydrogel herniation or no herniation until 10 MPa. The gray area represents the physiological stress levels up to 2.3 MPa.