Introduction Hydrogels (gels) are playing an increasingly important role in the development of regenerative approaches for the intervertebral disc (IVD) [1]. Since there is limited availability of native human discs for research and often animal models do not mimic human disc degeneration, testing the biomechanical performance of a gel after implantation remains difficult. The objective of the present study was to adapt and optimize an in vitro organ culture model of bovine tail discs for biomechanical testing. Discs were artificially degenerated with different enzymes. The distribution and biomechanical effects of a gel after injection were investigated. Methods Four groups of motion segments from 24 fresh bovine tails (CY3/4, n=6/group) were prepared and embedded. In three groups either 5 U/ml chondroitinase ABC (ChABC), 65 U/ml papain, or PBS was injected into the discs. After culturing (7 days, 6% O2, 37°C), complex loading was applied to diminish disc swelling. The maximum possible volume of gel was injected and measured into all four groups. µCT scans were performed after injection to view the material distribution within the discs. Before and after enzyme treatment, after complex loading, and after injection, the range of motion (ROM) and disc height were determined. Statistics: Mann-Whitney-U, Friedman, Benjamini-step-up ($p \leq 0.05$). Results At day 7, papain-digested discs developed a cavity in the nucleus, whereas all other groups seemed macroscopically intact. After incubation and subsequent loading, the disc height significantly decreased and ROM increased in all groups ($p \leq 0.026$, Fig 1B). Significantly more gel could be injected into ChABC- and papain-digested discs than into the controls ($p=0.002$). µCT reconstructions and dissections of the IVDs showed one large sphere for papain and a more inhomogeneous “fluffy-cloud-like” distribution of the gel for ChABC (Fig. 1A/C). The injection of the gel restored the initial disc height in all digested groups, and also increased the height of non-treated discs ($p \leq 0.015$). For ChABC and papain, the ROM decreased to the intact state ($p \leq 0.037$). Discussion Using enzymes, we were able to artificially degenerate IVDs. With cavities, ROM increase, and loss of disc height, papain simulates herniation or nucleotomy [2], whereas ChABC more closely mimics human degeneration occurring without cavities. Both enzymes lead to similar changes of ROM and disc height and allow standardized gel injections and testing. However, differences in the gel distribution could be noticed. We hypothesize that the specific digestion of glycosaminoglycans by ChABC may lead to different structural defects than papain. These results have improved our overall understanding of the biomechanical effects of IVD tissue digestion with ChABC and papain, and indicate that hydrogels can be investigated with both models depending on the research question. References 1. Zheng et al, 2021 2. Wilke et al, 2013 Acknowledgements iPSpine (825925)

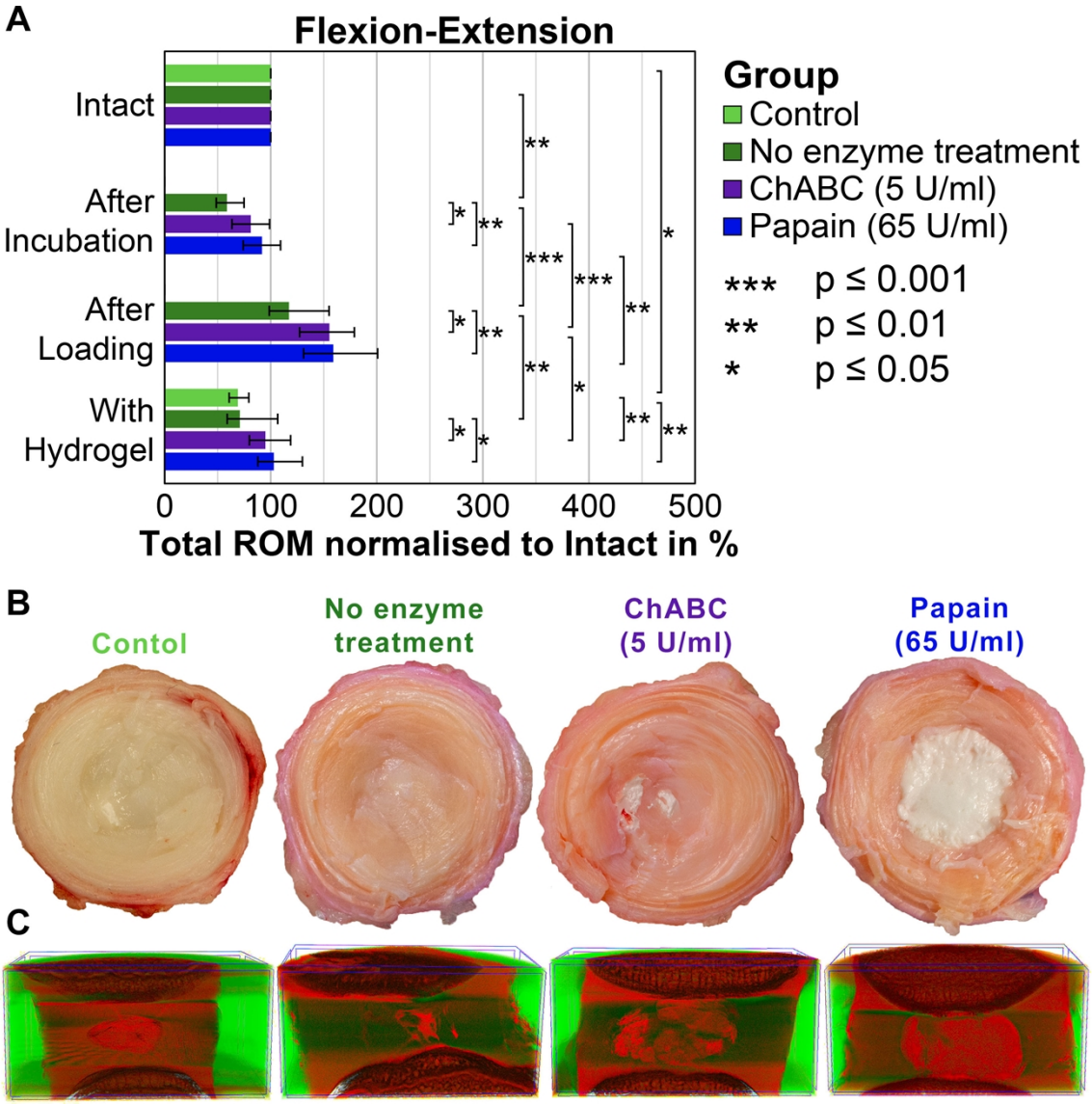

Fig. 1 A) Total ROM in Flexion-Extension normalized to the Intact condition B) Transversal, macroscopic view of one exemplary disc per group C) Reconstruction of µCTs of disc with injected radiopaque hydrogel (one example per group)