Combined rotations exert a detrimental stress on nucleus pulposus cells- *ex vivo* simulation of sport overloading on the spine in multiaxial bioreactors

Amra Šećerović[1], Aapo Ristaniemi[1], Mauro Alini[1], Francesco Crivelli[2], Sarah Heub[2], Gilles Weder[2], Diane Ledroit[2], Stephen J Ferguson[3], Sibylle Grad[1]
[1]AO Research Institute Davos, Switzerland, [2]Swiss Center for Electronics and Microtechnology, CSEM, Switzerland,
[3]Institute for Biomechanics, ETH Zurich, Switzerland
amra.secerovic@aofoundation.org

Disclosures: /

INTRODUCTION: Excessive spine loading due to tennis, swimming, golf or contact sports can exert significant stress on the intervertebral disc (IVD), potentially leading to degeneration and pain. We simulated a common sport movement consisting of rotations about all three anatomical axes (i.e. extension, lateral bending and torsion). The simulation was performed in a novel bioreactor that enables testing of *ex vivo* whole organ IVDs under multiaxial loading in a biologically controlled environment [1]. In this study, we assessed the impact of different magnitudes of this complex motion [2] on IVD physiology and the specific contribution of bending motions alone to the observed effects.

METHODS: Based on the previously described procedure [1], bovine caudal whole IVDs were isolated from 10 donors and subjected to 3 different loading protocols: (Group 1), Extension 0-3º, Lateral Bending 0-3º and Torsion 0-2º, (Group 2) Extension 0-6º, Lateral Bending 0-6º and Torsion 0-4º, (Group 3) Extension 0-6º and Lateral Bending 0-6º. All protocols were run daily for 2h at 0.3 Hz for 14 days. During the loading and free swelling recovery, IVDs were kept in the culture medium in a sterile 37ºC environment. The IVD height change was measured daily after loading and free swelling recovery and at the same time the medium was collected for glycosaminoglycan (GAG) and nitric oxide (NO) release analyses, indicators of matrix degradation and inflammation, respectively. IVD tissue was collected at the endpoint for catabolic gene expression, Safranin-O/Fast Green staining and collagen type-2 immunohistostaining, and cell viability assessment using lactate dehydrogenase staining compared to day 1 non-loaded control.

RESULTS: All 3 groups (n=4 per group) lost at the endpoint on average -1.9±2.5% IVD height after loading and recovered the height after swelling to 0.6±2.6% from the previous day of loading. Group 2, which combined the three axial rotations at higher magnitudes, induced the highest GAG and NO release in the medium (Fig.1, one-way ANOVA, p < 0.0001). More GAG was released in the medium during the recovery period than immediately after loading (Fig.1A). This trend was the opposite for NO release until day 10 (Fig.1B), when a shift occurred towards a higher release after swelling, suggesting a change in IVD physiology at a specific time point. More GAG was also released from day 12. GAG release from nucleus pulposus (NP) and inner annulus fibrosus (iAF) was further demonstrated by the reduction or loss of red positive staining on histology sections in groups 1 and 2 that involved torsion (Fig.2A,E). Furthermore, it was accompanied by reduced collagen type-II immunostaining at the interface between AF and NP (Fig.2D) and disintegration of NP structure (Fig.2A). Phenotype analyses indicated that all groups, irrespective of magnitude and omitting torsion, have upregulated collagenase genes *MMP1* and *MMP13* in AF and NP, with particularly high values in group 1 (38- and 316-fold increase for *MMP1* and 69- and 238-fold increase for *MMP13*, respectively). Based on a macroscopical evaluation of transverse sections from the IVD center, all three loading regimes caused high cell death in NP and iAF (Fig.2F) and cell clustering in NP typical of IVD degeneration (Fig.2B,C), aligning with the changes of extracellular matrix (ECM) observed in these regions.

DISCUSSION: We showed that a combination of two or three cyclic rotations about the anatomical axes induces high cell death and catabolic response at the IVD center (i.e. NP) and the interface with AF, possibly caused by detrimental mechanical stresses at these locations. This effect occurred even when the loading was run at a physiological frequency of 0.3 Hz and despite a minimally reduced IVD height. However, data from diurnal analyses suggest that torsion at 4º additionally to backward and lateral bending at 6º induces a stronger GAG and NO release than rotations at lower magnitudes, making the IVD prone to mechanical impairment caused by inflammatory response and ECM degradation and release.

SIGNIFICANCE/CLINICAL RELEVANCE: This pioneering study in a new generation of bioreactors brought a new perspective on the potentially detrimental effect of intensive sport motion on IVD homeostasis, making the spine prone to injury, impairment and/or low back pain. It can further help optimize protocols for spine health maintenance during exercise based on the type of movement and intensity of daily loading.

REFERENCES: [1] Šećerović, A., et al., *Toward the Next Generation of Spine Bioreactors: Validation of an Ex Vivo Intervertebral Disc Organ Model and Customized Specimen Holder for Multiaxial Loading.* ACS Biomater Sci Eng, 2022. [2] Ristaniemi, A., et al., *Physiological and degenerative loading of bovine intervertebral disc in a bioreactor: A finite element study of complex motions.* Journal of the Mechanical Behavior of Biomedical Materials, 2023.

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IMAGES AND TABLES:

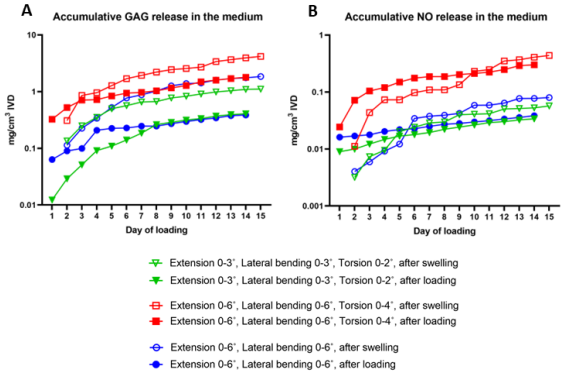

Fig.1. Accumulative GAG (A) and NO (B) release in the IVD cell culture medium from 3 different loading regimes.

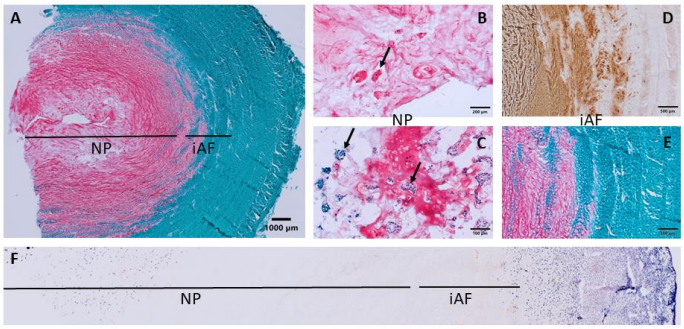

Fig.2. Representative histology sections from group 2: reduction or loss of red positive GAG staining in NP (A) and iAF (A,E), cell clusters (arrow) in NP (B,C), reduced collagen type II immunohistostaining in iAF (D), viable cells stained in blue in outer AF and innermost NP only (F).