Pre-clinical testing of injected biomaterials into the disc – two hydrogels withstand dynamic loading in an in vitro experiment simulating daily activities

Jan Ulrich Jansen[1], Graciosa Quelhas Teixeira[1], Rebecca Williams[2], Ronak Janani[2], Chris Sammon[2], Christine Le Maitre[2], Karin Benz[3], Andrea Vernengo[4], Sibylle Grad[4], Cornelia Neidlinger-Wilke[1], Hans-Joachim Wilke[1]

[1]Institute of Orthopaedic Research and Biomechanics, Ulm University, Germany, [2]Tissue Engineering and Biomechanics Research Group, Sheffield Hallam University, United Kingdom, [3]TETEC Tissue Engineering Technologies AG, Reutlingen, Germany, [4]AO Research Institute, Davos, Switzerland
jan.jansen@uni-ulm.de

Disclosures: Karin Benz (TETEC Tissue Engineering Technologies AG), all other authors (no disclosures)

INTRODUCTION: Biomaterials are playing an increasingly important role in the development of regenerative approaches for the intervertebral disc [1,2], but most implants for the nucleus pulposus are extruded under dynamic loading. Therefore, preclinical testing is essential in the development of new regenerative approaches utilizing biomaterials for nucleus replacement. Bovine tail discs, often used as a model for testing purposes, do not show disc degeneration, but degeneration can be artificially induced with enzymes. This reduces disc height (DH) and increases range of motion (ROM) and allows injection into small defects, cavities, or fissures. Subsequently, biomechanical testing of disc-"repair" and extrusion risk due to dynamic loading is possible. The aim of this study was to test two biomaterials with such a model using dynamic loading of simulated daily activities [3].

METHODS: Fresh bovine motion segments (n=16) were prepared, embedded and artificially degenerated with papain under hypoxic condition with 37° for seven days. Then, either 0.7 ml of Albugel or NPgel was injected with a 27-G needle. One specimen for Albugel was scanned with the µCT beforehand to understand the distribution of the hydrogel in the disc (Figure 1A). Similar as previously shown for human specimen [3], four different daily activities were simulated using a dynamic disc simulator for 10,000 cycles (Figure 2, n=6). Here, DH and ROM were determined four times: in the native state before enzyme treatment, after artificial degeneration, after injection of the hydrogel into the "degenerated" BS, and after the cyclic tests. Possible extrusions were continuously observed by camera. Two additional discs per group were stained with Safranin-O/Fast Green immediately after injection of the hydrogels. Statistics: Wilcoxon, Mann-Whitney-U ($p \leq 0.05$).

RESULTS: After 7 days, digestion with papain led to a void in the disc center (Figure 1B), a decrease of the DH, and an increase of the ROM in all groups ($p<0.001$). For both hydrogels, injection increased DH and decreased ROM ($p<0.001$). During cyclic testing, both hydrogels stayed inside the disc, i.e., no extrusion occurred. However, DH decreased for Albugel (2.3 mm) and NPgel (2.6 mm) without significant differences between the hydrogels ($p=0.818$). ROM increased by 35.3% and 41.9%, respectively ($p=0.746$). Histological examination demonstrated the close interface between hydrogel and disc tissue (Figure 3) as well as the filling of the papain digested void.

DISCUSSION: Our tests with 10,000 cycles could show a low extrusion risk for Albugel and NPgel directly after injection. The adapted testing protocol allowed simulation of complex everyday activities, such as tying shoes, sweeping the floor, lifting boxes, which are often associated with lumbar disc herniations [3]. In combination with papain-digested bovine specimens, this was thought to mimic a worst-case scenario of biomaterial extrusion after implantation but is also limited by the transferability of a bovine tail disc to humans (e.g., lack of facet joints). However, since cultured discs were used for this experiment, cellular interactions (gene expression, matrix composition, immunostaining, etc.) of the hydrogels will also be determined as the next step and the results can be interpreted as a whole.

SIGNIFICANCE/CLINICAL RELEVANCE: Before using new therapies in vivo they should be evaluated using adequate in vitro models. Many new regenerative treatment options for lower back pain are investigated using bovine tail organ cultures. This new method allows improved preclinical evaluation of the biomechanical performance of novel injected biomaterials, and thereby, facilitates faster preselection of biomaterials for animal studies or clinical trials.

REFERENCES:
[1] Teixeira, G.Q. et al. (2016). A Degenerative/Proinflammatory Intervertebral Disc Organ Culture: An Ex Vivo Model for Anti-inflammatory Drug and Cell Therapy.
[2] Zheng, K. et al. (2021). Recent advances of hydrogel-based biomaterials for intervertebral disc tissue treatment: A literature review.
[3] Zengerle, L. et al. (2021). In Vitro Model for Lumbar Disc Herniation to Investigate Regenerative Tissue Repair Approaches.

ACKNOWLEDGEMENTS: Funded by the European Union's Horizon 2020 Research and Innovation Programme (iPSpine project, grant no 825925).

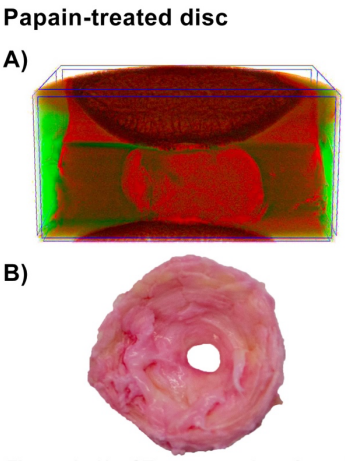

Figure 1: A) µCT reconstruction of papain-treated disc with injected radiopaque Albugel. B) Sagittal view of void created by papain in a bovine disc.

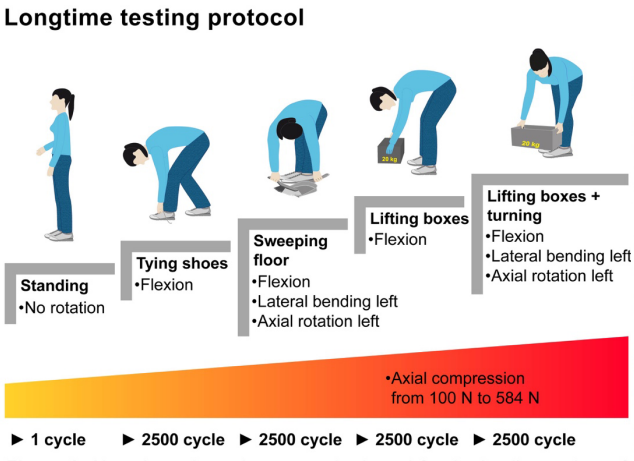

Figure 2: New dynamic testing protocol adapted for the loading regime of bovine tails. Previously published by Zengerle et al. 2021 for human specimens. The testing protocol allows the simulation of daily activities which are often associated with lumbar disc herniations.

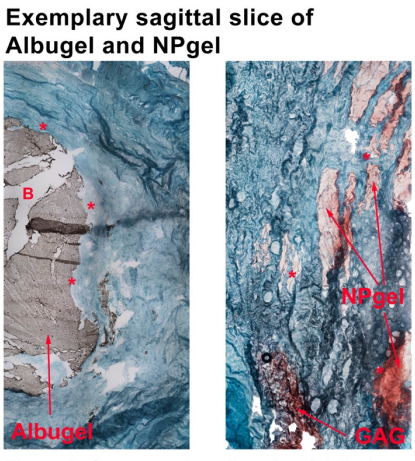

Figure 3: One exemplary slice of artificially degenerated bovine tail disc with injected hydrogel (left = Albugel, right = NPgel), Staining with Safranin-O/Fast-Green, GAG stained in red, collagen in bluish green, asterisks (*) indicate hydrogel-nucleus-tissue-interface. Background labelled with B.