Porcine notochordal cell injected into a bovine organ culture model of intervertebral disc degeneration

Andrea Vernengo1, Helen Bumman1, Nadine Kluser2, Jan Ulrich Jansen2, Cornelia Neidlinger-Wilke2, Hans-Joachim Wilke3, Mauro Alini1, Zhen Li4, Sibylle Grad1

1 AO Research Institute Davos, Switzerland, 2 Institute of Orthopaedic Research and Biomechanics, Ulm University, Germany
Andrea.Vernengo@aofoundation.org

Disclosures: None.

INTRODUCTION: Low back pain affects nearly 75% of the human population at some point in their lives [1] and is a major world-wide economic burden [2]. Low back pain is closely associated with degeneration of the intervertebral disc (IVD), a pathophysiologic process characterized by changes in the nucleus pulposus (NP) such as decreased cell viability, a transition in cell phenotype from notochordal to chondrocytic, and a loss of glycosaminoglycan (GAG) content. These cellular and biomolecular changes eventually lead to the hallmark clinical manifestations of IVD degeneration, such as decreased tissue hydration, loss of disc height, and other structural irreversibilities. Notochordal cells (NCs) are a promising candidate for cell-based therapies for IVD degeneration. A multitude of in vitro studies in 2D monolayers and 3D hydrogels indicate beneficial molecular effects of NCs on resident IVD cells, like increased protection from apoptosis [3] and stimulation of GAG production [4,5,6]. However, there is still a need to elucidate the therapeutic effects of NC-based therapies in terms of clinically-driven outcomes, like tissue structure and biomechanics, without a reliance on costly in vivo studies and ensuring accordance with European Commission 3R guidelines.

We have previously established an ex vivo organ culture system of whole bovine IVDs. Bovine IVD tissue is a close match to that of the adult human in terms of cellular and ECM composition [7]. We have shown that injection of the enzyme collagenase II, after 7 days of dynamically-loaded culture, induces a loss of cell viability, disc height, and tissue matrix [8]. Towards developing a cost-effective preclinical platform for screening NC cell-based treatments, the objective of this study was to examine the stimulatory effects of porcine-derived NCs in this collagenase-induced ex vivo model of IVD degeneration.

METHODS: Bovine caudal IVDs were harvested from two donors (less than 24 months old) and 100 µL of 0.5 U/mL collagenase II was injected into the center of each explant. IVDs were cultured according to established protocols with daily physiological loading applied [9] for the duration of the study. At day 7 after collagenase injection, the IVDs were separated into two groups, either receiving 500 µL of 4x10^6 cells/mL PKH67-labeled porcine notochordal cells (+NC, n=4) or 500 µL of media (-NC, n=4). The IVDs were maintained under dynamic culture for an additional 14 days. At the end of the study (day 21), unfixed IVDs were snap frozen, cryosectioned, and stained with Safranin-O and Fast Green to visualize GAG and collagen, respectively.

RESULTS SECTION: Gross observations of collagenase-digested specimens at day 21 of culture (with bone removed) revealed greater structural integrity for the +NC group (Figure 1A). IVDs in both study groups exhibited similar rates of height loss over 21 days (p=0.34, Figure 1B). During the period of NC culture (days 7-21), height loss tended to slow for the +NC compared to the -NC group (Figure 1C), though the differences were not statistically significant (p=0.2). Histology results (Figure 2A) revealed that ECM disruption by collagenase II resulted in loss of GAG in both +NC and -NC groups relative to day 0. Overall, however, the +NC group exhibited areas of concentrated GAG staining in the NP at day 21 (black arrows, Figure 2A), which were not observed in the -NC group. Collagenase treatment induced similar levels of annulus fibrosus (AF) disorganization for both groups (yellow arrows, Figure 2A). Annulus fibrosus tissue in the +NC group trended toward higher water retention relative to day 0 compared to the –NC group, though differences were not significant (p=0.2). Figure 2B). Finally, while both groups exhibited significant losses in overall cell viability in the NP region relative to day 0 (p <0.003, Figure 2C), loss of viability was significantly greater for the -NC than the +NC group (67.6 ± 31.1% for -NC versus 34.19 ± 19.9% for +NC, p=0.009). Green fluorescent labeled NCs could not be located within the tissues at the end of the study.

DISCUSSION: Although the fate of the porcine NCs in this study could not be determined, collagenase-digested IVDs exhibited reduced degenerative changes with NC injection (+NC) compared to the untreated group (-NC) after 21 days of culture. Ongoing studies are focusing on quantitative biochemical and biomolecular output measures to elaborate our understanding of the therapeutic benefits of the porcine NCs in this model.

SIGNIFICANCE/CLINICAL RELEVANCE: Recent research in the field of IVD repair focuses on the generation of NC-like cells from human induced pluripotent stem cells. A promising approach that can potentially transform the clinical treatment of low back pain. Results from this study point towards the benefits of NCs, and we propose this ex vivo IVD degeneration model as a translational, cost effective screening tool for such therapies.

REFERENCES:

ACKNOWLEDGEMENTS: The work described is part of the iPSpine project, which has received funding from the European Union’s Horizon 2020 Research and Innovation Programme (Grant No 825925).

Figure 1. Macroscopic characteristics of collagenase-digested bovine IVDs with or without notochordal cell treatment (+NC or -NC, respectively). (A) Gross morphology of representative specimens from day 0 (immediately after isolation) and day 21 of culture. (B) IVD height loss per day averaged over the entire 21-day study period. (C) IVD height loss per day averaged over the period after notochordal cell injection (days 7-21).

Figure 2. Microscopic and tissue-scale characteristics of collagenase-degenerated bovine IVDs with or without notochordal cell treatment (+NC or -NC, respectively). (A) Representative Safranin O/Fast Green results. The black arrows highlight areas of concentrated GAG staining observed in the +NC group and yellow arrows highlight disorganization in the annulus fibrosus observed in both the +NC and -NC groups. (B) Average percent change in water content for the sample groups over the 21-day study. (C) Average percent change in cell viability over the 21-day study. Hash symbol (#) indicates significant change relative to day 0 (p<0.003). Asterisks (***) indicates significant difference between sample groups (p<0.009). Scale bars = 5 mm (overviews) or 200 µm (NP, AF).