European Cells and Materials Vol. 10 Suppl. 3, 2005 (page 77) ISSN 1473-2262

Cells from the distinct regions of the intervertebral disc differ in terms of their mechanosensitivity

K. Würtz[1], J. PG. Urban[2], A. Ignatius[1], H.-J. Wilke[1], L. Claes[1], C. Neidlinger-Wilke[1]

[1]Institute of Orthopaedic Research and Biomechanics, University of Ulm, Germany

[2] *University Laboratory of Physiology, University of Oxford, UK*

INTRODUCTION: The intervertebral disc (IVD) as a spinal joint that shall allow movement and absorb impulsive load has to be regarded as a tissue of complex structure, expressed in its division into nucleus (N), transition zone (TZ) and annulus (A). These distinct regions differ not only in terms of their histological structure, constituent parts and cell types, but also in terms of their nutrient status, pH and representative mechanical load. As the three distinct zones of the IVD differ in so many respects, one may expect that the application of the two main mechanical loads, cyclic strain and hydrostatic pressure, will result in different cellular responses depending on the local origin of the cells.

METHODS: In order to determine if cellular responses to mechanical load are spatially-diverse, bovine disc cells (n=12) were isolated from the distinct regions (N, TZ, A) by collagenase-pronase digestion, expanded in monolayer and seeded in a three-dimensional collagen type-I gel. Cell-seeded gels were either stimulated by application of cyclic strain (1%, 2%, 4%, 8%; 1 Hz; 24 h) or by application of hydrostatic pressure (0.25 MPa; 0.1 Hz; 30 min) as previously described[1]. Load-induced changes in mRNA-expression of anabolic, catabolic and anti-catabolic genes relevant in the maintenance and degradation of the IVD-matrix were determined by real-time RT-PCR (collagen-I, collagen-II, aggrecan, MMP-2, MMP-3, TIMP-2). A Wilcoxon signed-rank Test was performed to check for differences between stimulated samples and control samples. Furthermore, a nonparametric Wilcoxon 2-sample Test was added to check for differences between the distinct regions of the IVD. Multiple testing was considered by adjusting the p-value to 0.008.

RESULTS:
Cyclic strain as well as hydrostatic pressure caused changes in gene expression in cells from each zone, but sign and extent differed. Nucleus and annulus as the gap-including zones showed deviant or even opposite responses in many cases. Effects of the transition zone mostly ranged in-between, confirming thus its intermediate position in the IVD. In nucleus cells, cyclic strain resulted in a significant increase of collagen-II ($p<0.001$), aggrecan ($p<0.001$) and MMP-2 ($p=0.002$) and there was a strong trend towards an increase of collagen-I ($p=0.009$). Additionally, cyclic strain tended to inhibit expression of MMP3 ($p=0.034$) and significantly inhibited expression of TIMP-2 ($p=0.001$) in nucleus cells. Effects tended to be contrariwise in annulus cells for collagen-I ($p=0.016$), collagen-II ($p=0.016$), MMP-2 ($p=0.031$) and TIMP-2 ($p=0.016$). Hydrostatic pressure tended to result in an inhibited expression of aggrecan, MMP-2, MMP-3 and TIMP-2 in nucleus cells but in an increased expression in annulus cells. Significant differences between N and A could be observed for collagen-I, collagen-II, MMP-2 and TIMP-2 (all $p<0.0007$) after the application of cyclic strain. Hydrostatic pressure tended to result in different effects between N and A for aggrecan, MMP-2 and MMP-3 (all $p<0.05$).

DISCUSSION & CONCLUSIONS: Findings indicate that cells from the three distinct regions of the IVD differ in their mechanosensitivity relating to cyclic strain and hydrostatic pressure. These regional differences concerning gene expression may be ascribed to intradiscal variations of the mechanical environment, cellular species, ECM and cellular morphology. Taking the results into account, subclassification of IVD cells is one of the primary preconditions for the better understanding of the biomechanical situation in the IVD.

REFERENCES: [1]Neidlinger-Wilke C, Würtz K, Liedert A, et al., J Neurosurg 2: 457-465, 2005

ACKNOWLEDGEMENTS: Supported by the European Union EURODISC (QLK6-CT-2002-02582)