

Establishment of an In-vitro whole Organ Intervertebral Disc / Endplate Culture Model

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INTRODUCTION: Primary or posttraumatic degeneration of the intervertebral disc and endplates are of major orthopaedic and socioeconomic importance. To study disc degeneration, generally, investigators use either animal models or *in-vitro* assays. However, it remains difficult to mimic the complex pattern of the disease *in vivo* and with current *in-vitro* systems. Moreover, cell or organ cultures exhibit often cell dedifferentiation due to profound changes of the intercellular matrix and the micro-environment.

Aim of the study was therefore to establish a whole organ disc / endplate culture system as a model for studying primary and posttraumatic disc and endplate degeneration

METHODS: Thoracolumbar and lumbar intervertebral discs including adjacent endplates were harvested from female 6 months old New Zealand White Rabbits and cultured in 6 well plates containing supplemented medium for up to 49 days. Concurrent changes of cell viability (Live/Dead®, Molecular Probes), total proteoglycan content (Alcian blue binding assay) as well as collagen I/II and aggrecan gene expression (RT-QPCR) were determined.

RESULTS: Whole organ disc / endplate cultures retained their viability over 49 days (from $81\% \pm 7\%$ to $78\% \pm 2\%$, Fig. 1). Total proteoglycan content was stable over 28 days ($23,8 \pm 1,04 \mu\text{g} / \text{mg}$ disc to $20,83 \pm 3,26 \mu\text{g} / \text{mg}$, $m \pm \text{SD}$ from two separate experiments). Quantitative PCR demonstrated a significant down-regulation of the aggrecan gene (decrease of $88\% \pm 10\%$ for annulus (A) and of $44\% \pm 18\%$ for nucleus pulposus (N) cells after 42 days, $m \pm \text{SD}$, from three separate PCR experiments, normalized to the expression of GAPDH) as well as of collagen type II mRNA (decrease of A: $96\% \pm 2\%$, N: $25\% \pm 1\%$). In contrast collagen type I gene expression was initially up-regulated until day 28 (A: $30,31 \pm 12$ fold, N: 193 ± 71 fold) and subsequently dropped by day 42 (A: $3,08 \pm 1,3$ fold, N: 56 ± 19 fold), still remaining significantly up-regulated compared to day 0.

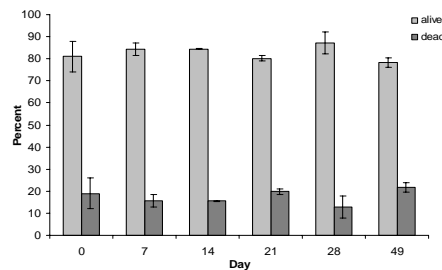


Fig. 1: Cell viability of disc cells after culture as a whole organ system. Live/Dead® viability assay. Mean \pm SD of three separated experiments

DISCUSSION & CONCLUSIONS: We have demonstrated that constrained intervertebral disc / endplate cultures from rabbits remain viable for 49 days. This ongoing viability without administration of growth factors exceeds most current disc culture systems other than primary cultures¹. Moreover, disc proteoglycan content did not change significantly over 28 days despite a marked down-regulation of aggrecan gene expression. In contrast, the collagen gene expression profile showed significant alteration, with up-regulation of type I and down-regulation of type II collagen. Diminished aggrecan gene expression and a switch in collagen type gene expression are commonly observed with degenerative disc disease².

The described disc / endplate culture system is a promising model to induce and analyse disc degeneration and study the interplay between intervertebral disc and vertebral endplates.

REFERENCES: ¹ Risbud M, Izzo M, Adams C et al (2003) *Spine* **28**:2652-2659. ² Antoniou J, Steffen T, Nelson F et al (1996) *J Clin Invest* **98**:996-1003.

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