

An *in vitro* tissue culture system for ovine caudal intervertebral discs with endplates

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INTRODUCTION: Arising from the avascular nature of intervertebral discs (IVDs), limited nutrient supply, in addition to mechanical loading, has become one of the main working hypotheses for induction of degenerative disc disease [1]. Because investigating these etiologies and their interaction *in vivo* is challenging, *in vitro* culturing systems are appealing. However, culturing disc explants has had limited success, e.g. culturing with adjacent endplates and static loads avoided ‘disc swelling’, but caused dramatic reduction of cell viability [2]. In this study, a new preparation and loading technique is evaluated for *in vitro* culturing of intact ovine caudal IVD explants with endplates.

METHODS: 12 Swiss alpine sheep (*Ovis aries*) were systemically anticoagulated and the caudal vasculature evacuated post-mortem as previously described [3]. The four most proximal of the caudal discs with adjacent endplates were then prepared with a precision histology band saw [3]. Discs were kept under standard culturing conditions (37° C, 5% CO₂) under diurnal loading (0.2 MPa for 8 h and 0.8 MPa for 16 h) and perfused with DMEM and 10% FBS. 2 discs were analyzed on day 0 (fresh) and 2 discs after culturing for 7 days.

Cell viability was assessed using the LIVE/DEAD® staining kit (Molecular Probes). Samples of disc tissue (~ 5 mm³ of annulus or nucleus) were incubated under “free-swelling” conditions in serum-free DMEM for 3 hours [2]. Stained samples were visualized on an inverted confocal LSM (Zeiss). Total cell number and cell viability was assessed from 3 randomly chosen stacks of nucleus or annulus. Each stack represents a 100µm projection. The number of alive and dead cells was quantified with a custom image analysis macro (Zeiss).

RT-PCR was performed according to standard protocols in order to measure expression of 3 anabolic (*aggrecan*, *col I* and *col II*) and 2 catabolic genes (*ADAMTS-4*, *MMP-13*) [4]. Gene expression was quantified by ΔC_t values using the relative quantification method, which normalises C_t values relative to the gene expression of the ribosomal 18S gene. Relative mRNA values were then calculated:

$$\text{Relative mRNA} = 2^{-\Delta C_t} \quad (1)$$

Statistical significance was assessed by the Wilcoxon-Signed rank test pairing mean relative mRNA values (2 discs per sheep per group) for day 0 and day 7, per sheep.

RESULTS: LSM results showed a small but non-significant decrease in mean cell viability in both tissues (90% at day 0, 84% at day 7, Figure 1). In the nucleus, only *col II* was down-regulated and *MMP-13* ($p = 0.04$) was up-regulated after 7 days. In the annulus, both *aggrecan* and *col II* were down-regulated and *ADAMTS-4* was up-regulated after 7 days. All of these differences were of borderline significance, i.e. $p = 0.05$, except for where noted.

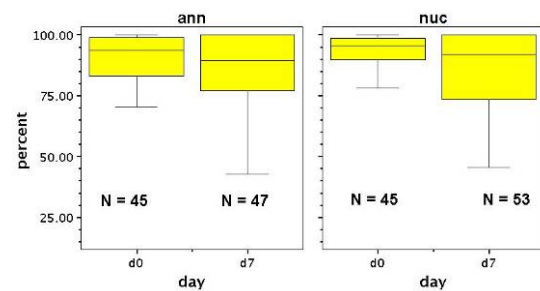


Fig. 1: Percentage of viable cells at day 0 and after 7 days of tissue culture in the annulus (ann) and nucleus (nuc).

DISCUSSION & CONCLUSIONS: Anti-coagulation and vascular evacuation enabled culturing of entire discs with intact endplates for up to 7 days without significant loss in cell viability. Although there was some borderline significant down and up regulation of anabolic and catabolic genes, respectively, over 7 days of culturing, this was not for all genes and less in the nucleus than annulus. These results may be further improved by application of higher frequency physiological loads.

REFERENCES: ¹J. Urban, et al (2004) Spine **29**: 2700-2709. ²C.R. Lee, et al (in press) Spine. ³T. Grünhagen, et al (2004) EORS. ⁴J. MacLean, et al (2004) J Orthop Res **22**: 1193-1200.

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