

Topographical Guidance of Intervertebral Disc Cell Growth *In Vitro*: Towards the Development of Tissue Repair Strategies for The Anulus Fibrosus.

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INTRODUCTION: The anulus fibrosus of the intervertebral disc (IVD) consists of an aligned extracellular matrix, which is synthesised during development by similarly aligned IVD cells¹. This highly organised structure may be severely disrupted during the onset or treatment of IVD prolapse. Of those prolapse cases that require surgical intervention, approximately 10% of patients re-herniate, most often at the original site of injury. Topographical guidance has been used previously to promote the alignment of tenocytes and of the extracellular matrix they synthesise in experimental models of tendon repair. Therefore, we have investigated the growth of IVD cells on micro-grooved polycaprolactone (PCL) *in vitro* to determine if topography can be used to promote IVD cell alignment and matrix synthesis.

METHODS: Cells isolated from the anulus fibrosus (AF) or nucleus pulposus (NP) of bovine IVD and expanded in monolayer to passage II were subsequently cultured on micro-grooved PCL for 1-4 weeks. Their growth pattern and the expression of extracellular matrix molecules were examined by scanning electron microscopy, video microscopy, immunohistology and RT-PCR (for type I collagen). IVD cells were cultured also on non-grooved PCL as a control.

RESULTS: AF and NP cells adhered to the PCL membranes, became fibroblast-like and proliferated to confluence (Figure 1).

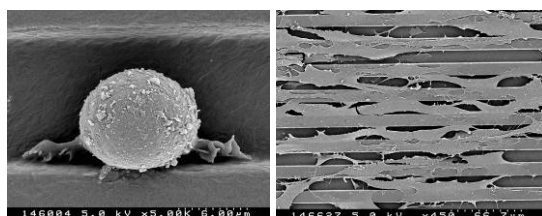


Fig. 1: An AF cell at 1 hour post-seeding (left panel), which has settled into the micro-groove of the PCL membranes and begun to extend filopodial and lamellipodial attachments onto the substratum. By day 14 (right panel) the

majority of AF cells became elongated and aligned to the microgrooves/ridges.

Both cell types aligned to and migrated along the micro-grooves/ridges of the substrata, but AF cells were significantly more closely aligned than NP cells. In addition, AF cells were markedly more immunopositive for type I collagen, but less immunopositive for chondroitin-6-sulphated proteoglycans, than NP cells (Table I). There was no evidence of extracellular matrix deposition. Disc cells cultured on non-grooved PCL did not show any preferential alignment at sub-confluence and did not differ in their pattern of immunopositivity to those on grooved PCL.

Table 1. Immunoreactivity for Extracellular Matrix Molecules (ECM) on Disc Cell-Seeded Membranes of Polycaprolactone.

ECM Molecule	AF Cells	NP Cells
Collagen I	+++	+
Collagen II	-	-/+
Collagen III	-	-
KS PGs	++	++
C-4-S PGs	++	++
C-6-S PGs	+/-	++

DISCUSSION & CONCLUSIONS: Substrate topography was effective in aligning IVD cell growth. However, the differential response of AF and NP cells to topographical cues, and the fact that neither cell type deposited an extracellular matrix, suggests a need to optimise cell sources and / or environmental conditions further to promote the synthesis of an aligned repair AF tissue.

REFERENCES: ¹Rufai A, Benjamin M, Ralphs JR (1995) The development of fibrocartilage in the rat intervertebral disc. *Anat. Embryol.* **192**: 53-62.

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