An in-vitro model to test regenerative therapies for disc degeneration

Orthopaedic Biomechanics

An in-vitro model to test regenerative therapies for disc degeneration

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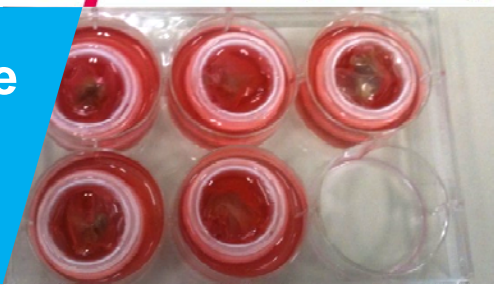

Background

Regenerative therapies are promising treatments for intervertebral disc degeneration and a long-term *in vitro* model would be valuable to screen for their efficacy.

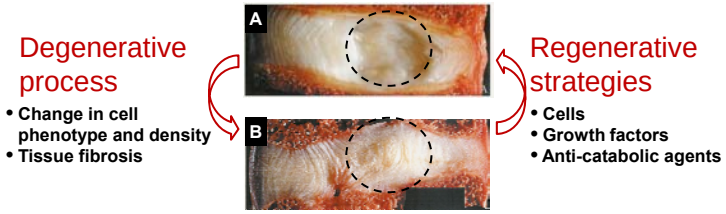

Figure 1. Healthy (A) and degenerated (B) intervertebral discs. Dashed circles; NP.

As the most substantial degenerative changes occur in the nucleus pulposus (NP), NP explant culture may be a suitable model. The aim of this study was to reproduce and maintain *in vitro* the unique NP environment which includes low oxygen, low glucose, and high osmolarity levels in long-term culture.

Methods

- NP explants from caudal discs of 24 month-old cows
- High tissue osmolarity balanced by an artificial annulus (physical constraint) or by increased medium osmolarity (raised to iso-tonic and hyper-tonic levels compared to native tissue) (Figure 2)
- Glucose and O_2 levels adjusted to standard (4.5 g/l and 21%) or physiological (1 g/l and 5%) conditions

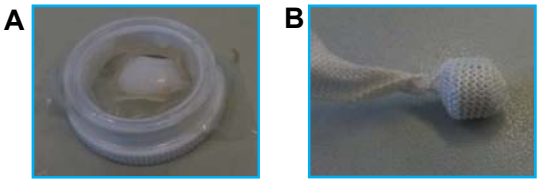

Figure 2. Explants in increased osmolarity (A) or artificial annulus (B) systems.

After 42 days measured and compared to day 0:

- Water content
- Glycosaminoglycan (GAG) content with DMMB assay
- Fixed Charge Density (FCD) calculated from GAG
- Cellular phenotype with gene expression of aggrecan, collagen type II and MMP-13
- Tissue morphology with Safranin-O /Fast Green staining

Results

Hyper-tonic PEG and artificial annulus prevented GAG loss and kept tissue FCD stable (Figure 3). However, matrix protein expression for PEG cultures was different from day 0 (Figure 4A, B). Only aggrecan expression was different for artificial annulus culture (Figure 4A). Physiological conditions were beneficial for MMP-13 gene expression (Figure 4C), but did not affect any other outcome measures.

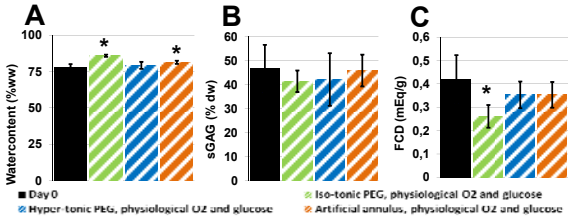

Figure 3. Water (A), sGAG (B) content, and FCD (C). Values are mean ± St Dev, n = 5, *p < 0.05 compared to day 0.

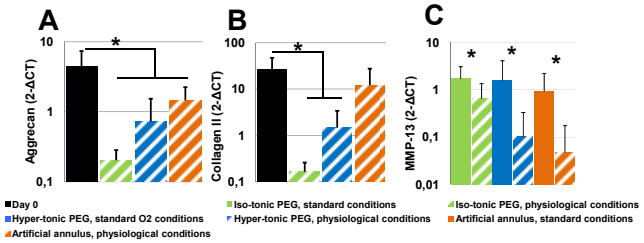

Figure 4. Aggrecan (A), collagen type II (B), and MMP-13 (C) gene expression. Values are mean ± St Dev, n = 5, *p < 0.05 compared to day 0.

Tissue composition of both approaches resembled day 0 in physiological conditions (Figure 5).

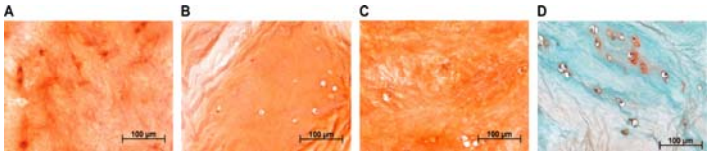

Figure 5. Safranin O/ Fast Green stained sections: Day 0 (A), hyper-tonic PEG (B), and artificial annulus (C) with physiological conditions. (D) NP explants cultured for 21 days in hypo-osmotic media when osmolarity is not balanced [1].

Discussion

Both approaches could maintain the matrix composition specific to the NP. However, artificial annulus culture kept cellular phenotype closer to native tissue and thus constitutes a more promising long-term model to test regenerative therapies. Human degenerated NP tissues are currently being cultured using this system.

[1] B.G.M. van Dijk, E. Potier, K. Ito. Culturing bovine nucleus pulposus explants by balancing medium osmolarity, Tissue Eng Part C, 2011.

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