Culturing disc endplates

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Introduction

Intervertebral discs (IVDs) consist of a firm, flexible outer layer (*annulus fibrosus*) surrounding a gelatinous, compressible core (*nucleus pulposus*). Endplates (EPs) are cartilaginous layers that lie between the IVD and the vertebral body (Fig. 1). As *nucleus pulposus* is avascular, its nutrient and oxygen supply relies on diffusion from the vertebral body blood vessels through the EPs [1].

Due to aging or some diseases, EPs can calcify, impairing nutrient diffusion to the *nucleus pulposus* [2] and potentially leading to disc degeneration, a major cause of low back pain.

**Overall objective:** To investigate the calcification process of the disc EPs, and a way to reverse it.

**Study objective:** To establish an explant culture system for disc EPs.

Methods

Cartilaginous EPs were harvested from bovine tails (24-30 month old; n=3, each donor in duplicate) and consisted of cores (Ø 10mm) surrounded by thin layers of bone on one side and of *nucleus pulposus* on the other.

EPs were cultured for 3 weeks in hg-DMEM, 10% FBS, 25 µg/ml ascorbic acid, 0.1 mM NEAA, 0.4 mM L-proline. At day 0, 7, and 21 cell viability was evaluated by calcein/propidium iodide staining; tissue phenotype was evaluated by Hoechst dye, DMMB, and chloramin-T assays for DNA, glycoaminoglycan (GAG), and hydroxyproline (representative of collagen) contents respectively; and tissue morphology was assessed with safranin-O/Fast Green staining for proteoglycans and collagens.

Results

**Cell viability**

No significant differences were observed between day 0, 7, and 21 (Fig. 2).

**Tissue morphology**

Proteoglycan staining was less intense at day 21 than at day 1 and 7, with EP layers thinning observed at day 7 and 21 (Fig. 4).

Discussion

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E. Potier, P.H. Wang, K. Ito

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**Introduction**

Intervertebral discs (IVDs) consist of a firm, flexible outer layer (*annulus fibrosus*) surrounding a gelatinous, compressible core (*nucleus pulposus*). Endplates (EPs) are cartilaginous layers that lie between the IVD and the vertebral body (Fig. 1). As *nucleus pulposus* is avascular, its nutrient and oxygen supply relies on diffusion from the vertebral body blood vessels through the EPs [1].

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**Discussion**

Culture conditions were appropriate for EP viability, but not sufficient to maintain EP phenotype (GAG loss, EP thinning).

Will investigate the capacity of high osmotic pressure (similar to native *nucleus pulposus* environment) to impede proteoglycan loss in EP explant culture.

**References**