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].

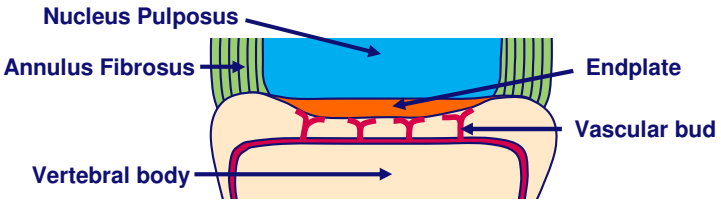

Figure 1. IVD and endplate structure.

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).

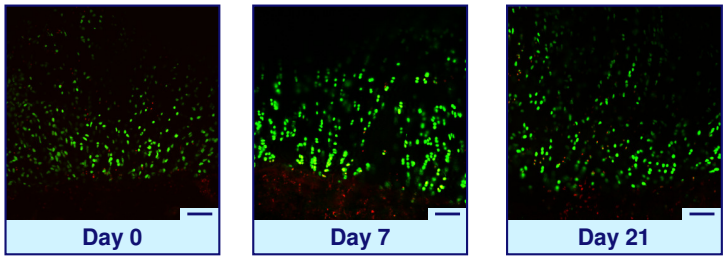

Figure 2. Cell Viability. Calcein/propidium iodide staining. Representative of 6 explants/group. Scale bar: 100 µm.

DNA, GAG, hydroxyproline content

DNA content remained stable up to 21 days (Fig. 3).

GAG and hydroxyproline stayed constant up to day 7, but decreased at day 21 (Fig. 3).

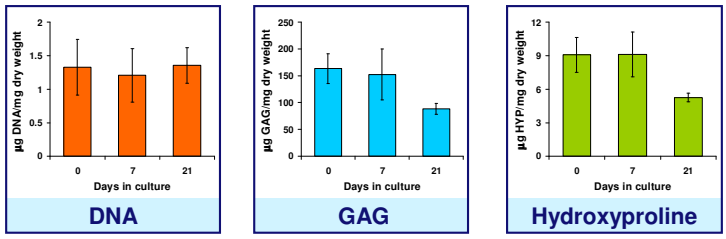

Figure 3. DNA, GAG, hydroxyproline contents. Per mg of dry weight. Values are means +/- St Dev. N=6/group.

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).

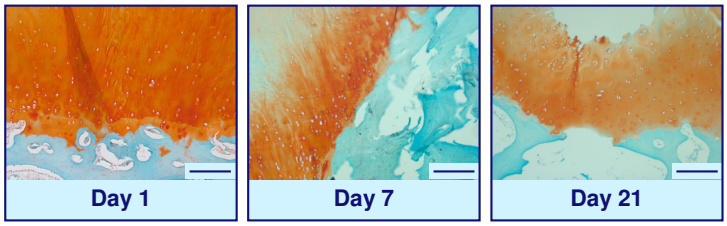

Figure 4. Tissue morphology. Safranin-O/Fast Green staining, Hematoxylin counter-staining. Representative of 6 explants/group. Scale bar: 250 µm.

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

[1] Moore, 2000, Eur Spine J 9 [2] Roberts, 1996, Spine 21