

CHARACTERIZING mRNA PROFILES IN BOVINE INTERVERTEBRAL DISC CELLS

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INTRODUCTION: To date there is no marker specific for intervertebral disc, as the matrix components are very similar to those of articular cartilage. Since this is an important deficiency in terms of tissue engineering, the aim of this study is to isolate mRNA from intervertebral disc cells in the nucleus pulposus in order to determine potential markers. These will be compared with expression of the same markers in low passage (<3) cultured primary intervertebral disc cells and other primary bovine cell lines including skin dermal fibroblasts, adipose cells, chondrocytes and bone marrow stromal cells. We have primers available to examine a panel of mRNA profiles of ECM proteins, proteases (MMPs and ADAM-TS) / protease inhibitors (TIMPs), cytokines / cytokine receptors and transcription factors that can be compared to a house keeping gene. Measurement of these markers in primary cell lines, together with observations on cell morphology, may enable identification of a candidate cell phenotype for introduction into damaged intervertebral disc tissue to facilitate repair; this could be considered to treat some patients with back pain or spinal deformities.

METHODS: Bovine nucleus pulposus tissue was dissected from the intervertebral disc, snap frozen for RNA extraction and ground to powder in N_(L). Sample temperature was elevated to room temperature and mRNA extracted in tri reagent and chloroform, before running on Qiagen RNA mini-prep columns. RNA concentration from disc tissue is low due to low cell number in the nucleus pulposus. Therefore, use of global PCR amplification to create a library of 3' biased cDNAs, whose relative quantities are representative of the original mRNA prep, has been investigated. Bovine nucleus pulposus tissue was dissected, cells extracted via collagenase digestion and filtration, and cells were cultured in DMEM: F12-Hams with L-glutamine, 10% foetal calf serum, amphotericin B, gentamicin and ascorbic acid. Dermal tissue, articular cartilage and subcutaneous adipose tissue were exposed to an identical cell extraction regime and culture

conditions. Cell populations were assessed for morphology in addition to mRNA profile.

RESULTS: RNA has been extracted from disc tissue samples and the global PCR amplification methodology optimized to allow measurement of the aforementioned panel of markers. Distinct cell phenotypes have been photographed (Fig.1) for all established primary cells lines and RNA extractions are being performed to build a library of mRNA to compare with extracted disc tissue mRNA.

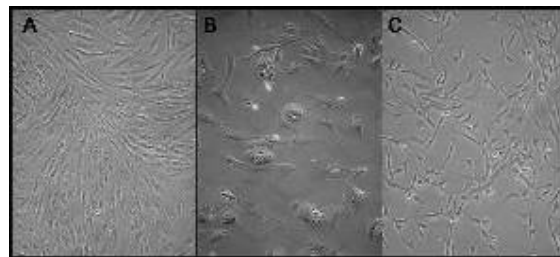


Fig.1. Images of primary bovine cell cultures established from **A:** dermal layer **B:** subcutaneous adipose **C:** nucleus pulposus.

DISCUSSION & CONCLUSIONS: Established primary bovine cell lines have shown distinct phenotypes when cultured *in vitro*. Both adherent dermal cells (Fig. 1A) and nucleus pulposus cells (Fig. 1C) have a fibroblastic-like phenotype, whereas subcutaneous fat cells (Fig 1B) have two distinct populations of cells *in vitro*: mesenchymal-like cells surrounding granular cells with a rounded phenotype, recapitulating a similar cell orientation to their *in vivo* environment. It is anticipated that our primary cells may possess similar RNA profiles to RNA extracted from disc tissue and that these cells may display plasticity in their phenotype and genotype. Primary cells may be induced to revert into disc-like cells when placed into a nucleus pulposus ECM environment and these may be used to regenerate damaged disc tissue.

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