

Stem Cell Delivery to Osteoarthritic Tissue

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INTRODUCTION: Osteoarthritis (OA), or degenerative joint disease, is the most prevalent of the musculoskeletal diseases with characteristic, progressive destruction of cartilage. Adult mesenchymal stem cells (MSCs) contribute to the maintenance of connective tissues and local delivery of MSCs to the injured joint in a goat model of OA retarded the progressive destruction of cartilage (1). However, MSCs did not engraft to either intact or fibrillated cartilage in this model. We are evaluating strategies to promote homing of MSCs to fibrillated cartilage to promote tissue repair using a human osteoarthritic cartilage explant model.

METHODS: Full thickness cartilage explants (1-2 mm thick and 2mm diameter) were taken from the medial tibial plateau or femoral head of tissue obtained after total knee and hip arthroplasty. Human MSCs were obtained from normal donors and all procedures were performed with informed consent and approved by the Clinical Research Ethical Committee at University College Hospital, Galway. Explants were placed in 10% FCS containing media for 48 h at 37°C to recover and subsequently cultured in serum-free chondrogenic media without TGF- β 3 (ICM) for 24 h at 37°C. MSCs were labelled with Cell Tracker™ Red and DAPI and added to the explants at $0.5-2 \times 10^6$ /ml for 1-4 h at 37°C. After stringent washing to remove unattached cells, explants were processed immediately. Cartilage disks incubated without cells were used as controls. Some explants were pre-treated with 0.25% trypsin/EDTA and collagenase (1mg/ml). Formalin-fixed, paraffin-embedded sections were stained with Toluidine blue or processed for fluorescence microscopy. Proteoglycan (GAG) release into the media was determined by DMMB assay over 14 days for explants cultured in ICM and chondrogenic media containing TGF- β 3 (CCM).

RESULTS: The culture model was validated using fibrillated goat cartilage explants. Mean accumulated GAG/DNA was not different in explants cultured in serum-containing media, ICM or CCM over 14 days. Sulphate

incorporation was also similar in serum-containing media and CCM. Sulphate incorporation was significantly lower in explants cultured in ICM. Proteoglycan release by human cartilage explants was constant over a 14 day culture period.

Initial MSC attachment experiments were performed with explants from both the femoral head and tibial plateau embedded in a fibrin gel. Cell attachment to the OA tissue in the fibrin-implanted explants was insignificant at all cell concentrations and times used. Maximum binding of MSCs to the OA cartilage was observed in a free floating system (without fibrin) with cartilage from the medial aspect of the tibial plateau (Figure 1). Binding increased with time, cell concentration and exposure to collagenase and trypsin. Collagenase treatment alone resulted in less cell binding than PBS treatment.

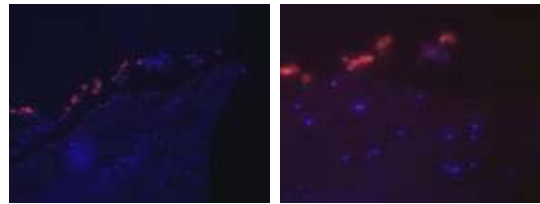


Fig. 1: hMSC attachment to fibrillated cartilage (a) Magnification 100x (b) 200x

DISCUSSION & CONCLUSIONS:

Strategies for stem cell-mediated repair of damaged cartilage as a result of OA will require targeting stem cells to damaged cartilage. We have established a functional culture system to evaluate *in vitro* MSC binding to OA cartilage to allow development of specific targeting strategies. The explants are maintained in culture for up to 14 days in serum-free media and will enable testing of the ability of the targeted stem cells to repair the surface.

REFERENCES: J.M. Murphy, D.J. Fink, E.B. Hunziker and F.P. Barry (2003) *Arthritis Rheum* **48**:3464-74.

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