

The in vivo Fate of Implanted Chondrogenitor Cells in SCID Mice

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INTRODUCTION: Articular cartilage is a complex tissue comprising phenotypically distinct zones with a low capacity for repair. Recent research has identified the presence of a progenitor cell population found in the surface zone of articular cartilage based on differential adhesion to fibronectin¹. These cells were shown to possess an extended cell cycle time, form colonies from a single cell and shared many of the properties of mesenchymal stem cells.

The aim of the present study was to determine the in vivo plasticity of articular cartilage progenitor cells isolated from the surface of 7 day old bovine metacarpal-phalangeal joints.

METHODS: Chondrocytes were collected from the bovine metacarpal-phalangeal joints by fine dissection and progenitor cells were isolated by differential adhesion to fibronectin and expanded in supplemented DMEM-F12 with 10% fetal calf serum¹. To determine progenitor cell plasticity in vivo, 2×10^7 cells ml^{-1} were labelled with PKH26 and injected into the rear thigh muscle of 8-week-old, skeletally mature severe-combined immunodeficient (SCID) mice. After 2 weeks, the muscles were dissected to determine the presence of a cartilage pellet. If no pellet was present, the muscle fibres from each sample were pooled and frozen for cryosectioning. Sections were then examined under a fluorescent microscope and stained with safranin O. Immunohistochemical analysis was carried out looking for sox9 and collagen type II.

RESULTS: Fluorescent PKH26 labelled cells were detected after two weeks in all the samples analysed. A cartilage pellet was present after intramuscular injection of freshly isolated full depth chondrocytes. After injection with a clonal population of chondrogenitors, a distinct pellet was not present although regions of intense safranin O stain and sox9 expression corresponded to areas containing fluorescently labelled cells. Low levels of collagen type II were also detected in these areas. The intensity

of the staining was weaker than the staining seen after injection of full depth chondrocytes.

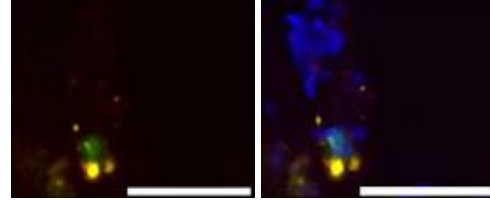


Fig. 1: Immunohistochemical analysis of chondrogenitors in vivo labelled with PKH26 (red) and sox9 (green) (left image) and the same image with a nuclear stain (blue) (right).

DISCUSSION & CONCLUSIONS: The plasticity of bovine chondrogenitors isolated from the surface zone of articular cartilage has already been demonstrated in vitro and in ovo¹. Here we demonstrate that full depth chondrocytes maintain the chondrocytic phenotype when injected intramuscularly into the SCID mouse as shown by the presence of an intensely stained safranin O pellet and the expression of sox9 after 2 weeks in vivo. The injection of pure chondrogenitor populations resulted in the absence of a distinct pellet but the presence of sox9 and PKH26 positive cells distributed throughout the muscle fibres. These results suggest that these progenitor cells can express and maintain a chondrogenic-like phenotype within a foreign tissue albeit at a low level. Current work is concentrated on analysing the expression of homotypic genes such as MyoD.

REFERENCES: ¹ G. P. Dowthwaite, et al. (2004) The surface of articular cartilage contains a progenitor cell population *J Cell Sci* **117**:889-97 ² W. Bi, et al. (1999) Sox9 is required for cartilage development *Nat Genet* **22**:85-9

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