

IDENTIFYING PROGENITOR CELLS WITHIN ARTICULAR CARTILAGE

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INTRODUCTION: Previous studies have shown that articular cartilage grows by apposition from the articular surface driven by proliferation of a progenitor cell sub-population that resides in the surface zone. This study concentrates on identifying markers for this progenitor cell population. Cell surface receptors CD105 and CD166 are known markers of progenitor/stem cells in endothelial cells, bone marrow constituents as well as other tissues and have recently been reported within normal and osteoarthritic human articular cartilage^{1,2}. Notch 1, Delta and Jagged 1 and 2 are known to be expressed by stem/progenitor cells during various stages of limb development³. Localisation of these markers to progenitor cells in articular cartilage would enable selective isolation facilitating further characterisation of the chondroprogenitor.

METHODS: Full depth cartilage explants were taken from 7-day-old bovine metacarpal-phalangeal joints. The tissue was then snap frozen and cryosectioned. Immunofluorescence was carried out using antibodies for the cell surface markers CD105, CD166 (Ansell, USA), Notch 1, Delta, Jagged 1 and Jagged 2 (Santa Cruz, USA). Surface zone explants were also enzymatically digested and the resulting cell suspension, filtered and counted. Cells were plated in monolayer at 30,000 cells/cm² and cultured for 4 days. Cells were lifted using accutase (Sigma,UK), then immunolabelled using directly conjugated CD105RPE and CD166FITC (Ansell). Cells were analysed using flow cytometry (BD FACSCanto).

RESULTS: Positive immunolabelling was observed for all markers. Jagged 2 occurred throughout the thickness of the cartilage, Delta was also present throughout the tissue at low levels but highly expressed at the articular surface. CD105, CD166 and Jagged 1 were localised to the superficial zone cells. Notch 1 labelling was found within the superficial layer and also in the deep zone. Labelling of CD105, CD166, Jagged 1 and Notch 1 were predominately present as clusters within the superficial layer (Figure 1).

Using flow cytometry surface zone cells were labelled for all the previous mentioned markers. CD105 labelled 10% of the surface population and CD166 labelled 45% of the surface population.

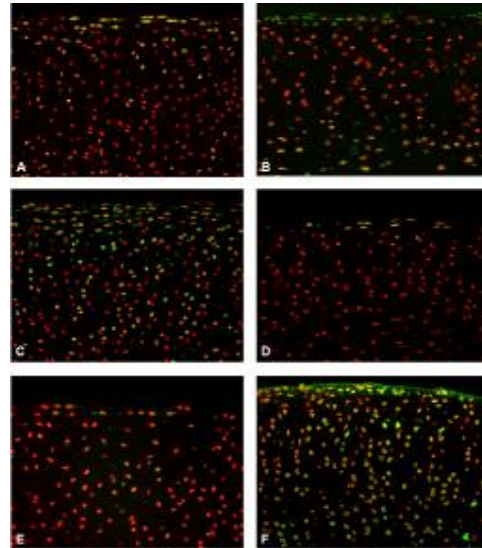


Fig.1: Immunofluorescence showing superficial zone labelling of CD105 (A), CD166 (B) Notch 1(C), Delta (D) and Jagged 1 (E) and uniform labeling throughout the tissue for Jagged 2 (F)

DISCUSSION & CONCLUSIONS: The above results demonstrate that CD105, CD166, Jagged 1, Delta and Notch 1 are promising markers for chondroprogenitor cells. At present, studies are concentrating on sorting labelled cells using flow cytometry (BD FACS Aria) to select the progenitor cells that can be expanded for use in monolayer and pellet systems.

REFERENCES: ¹ S. Alsalameh *et al.* (2004) *Arthritis & Rheumatism* **50**: 1522-1532. ² J. Diaz-Romero *et al.* (2005) *J Cell Phys* **202**: 731-742. ³ A.J. Hayes *et al.* (2003) *J Anat* **202**: 495-502.

ACKNOWLEDGEMENTS: With thanks to The Department of Health for funding this research.