

Growth Characteristics of Chondroprogenitor Cells *In Vitro*

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INTRODUCTION: Cell transplantation therapy has shown promise in augmenting the inadequate repair mechanisms within damaged articular cartilage. It has been reported that the proliferative potential of autologous chondrocytes decreases with increasing age (1) posing a major problem in providing adequate cell numbers for repairing osteoarthritic lesions. Hence, alternative sources of donor cells have been sought in order to make cell based therapies more readily available for patients. The isolation of a putative chondroprogenitor (CP) cell from the surface zone of articular cartilage by differential adhesion to fibronectin (2) broadens the horizon for articular cartilage repair strategies. Some of the problems associated with the use of MSCs such as the lack of integration and unstable phenotypes and reduced proliferation capacity may potentially be overcome using CP cells.

METHODS: Chondroprogenitor cells isolated by differential adhesion and expanded approximately 20 population doublings were resuspended in the experimental culture medium at a concentration of 2×10^5 cells per 10 μ L medium. Aliquots of cells were placed at the centre of either 12 well culture dishes or 8 well chamber slides (Labtek, Nunc International, IL). Micromass cultures were incubated in a humidified 37°C, 5% CO₂ air incubator for 5 hours prior to being flooded very carefully with matching medium. The base medium used in micromass culture studies was DMEM (containing 4500 mg/L D-glucose, 4 mM L-glutamine), 1 mM sodium pyruvate, 0.1% Gentamycin, 50 μ g mL⁻¹ ascorbate-2-phosphate, 10 mM HEPES. To the latter medium was added either insulin-transferrin-selenium (ITS; 10 mg mL⁻¹, 5.5 μ g mL⁻¹, 6.6 ng mL⁻¹, respectively; Gibco) and/or TGF β 1 (5 ng mL⁻¹; Peprotech).

RESULTS: Chondroprogenitor clones were isolated and expanded for a minimum of 20 population doublings. Clones were grown as micromasses and formed spheroid aggregates, a typical stem cell behavioural characteristic. All spheroids were alcian blue positive. When grown in the presence of TGF β 1 and ITS we observed that a homogenous cell mass was formed (Figure 1), whereas individual growth factor addition

enhanced spheroid formation. RT-PCR analysis showed that TGF β 1 immediately activates Sox9 expression on day 0 following micromass formation but that expression falls below detectable levels after 3 days. In the presence of ITS, Sox9 transcripts appear after day 1 and are maintained over 7 days of culture.

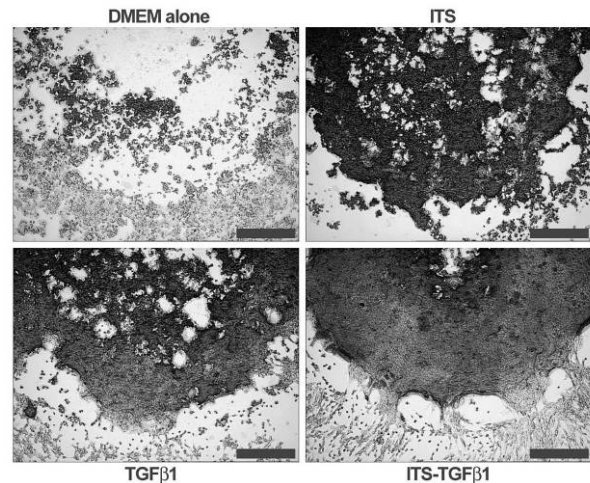


Figure. 1: Alcian Blue (pH 1) staining of chondroprogenitor clones grown as micromass cultures. Bar 500 μ m.

DISCUSSION & CONCLUSIONS: Our work shows chondroprogenitors grown in micromass culture recapitulate aspects of early development in that they form condensations or spheroid bodies. Addition of growth factors to the basal medium led to an increase in sulphated glycosaminoglycans (GAG) being deposited in micromasses following the order; DMEM alone < plus TGF β 1 < plus ITS < TGF β 1 + ITS. Synergistic activity of TGF β 1 and ITS may account for increases in GAG deposition and increased metabolic activity and this link is being investigated further.

REFERENCES: 1. Dozin B *et al. Matrix Biol.* 2002;21(5):449-59. 2. Dowthwaite GP *et al. J. Cell Sci.* 2004;117:889-97.

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