

Bioreactor Culture of Cartilage from Mesenchymal Populations

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INTRODUCTION: Cartilage degeneration results in severe pain or disability for millions of individuals worldwide. However, the potential for cartilage to self-regenerate is limited. Cartilage is composed of only one cell type, is avascular and has a relatively simple composition and structure, thus cartilage tissue engineering has tremendous potential. Therefore to address this clinical need, we have adopted a tissue engineering approach to the generation of cartilage *ex vivo* from mesenchymal cell populations encapsulated in alginate, a natural polysaccharide that favours chondrogenesis, and cultured within a rotating-wall bioreactor and a perfused bioreactor system.

METHODS: Alginate (2% solution, phosphate 300mM) and chitosan (Ca²⁺ 50mM) semi-permeable polysaccharide capsules were generated by a one-step method as described by Leveque *et al*¹. Human femoral head and bone marrow samples were obtained from haematologically normal patients undergoing routine total hip replacement surgery. Marrow aspirates were washed in α -MEM and centrifuged at 1100rpm. Marrow cells were resuspended in α -MEM with 10% FCS. Primary chondrocytes were isolated from articular cartilage by enzymatic digestion and grown in 10% α -MEM supplemented with 100uM Ascorbate-2-Phosphate. Cells were grown in monolayer and when confluent 2-4 x 10⁵ cells were encapsulated within the polysaccharide capsules with the addition of TGF- β 3. Marrow cells and chondrocytes were also co-cultured within polysaccharide capsules in a ratio of 2:1 respectively². Capsules were subsequently placed in either a Synthecon rotating-wall bioreactor, perfused at a flow rate of 1ml/hour or held in static conditions for 28 days at which point they were harvested for biochemistry and histology.

RESULTS: Alcian Blue and Sirius Red staining indicated a more ordered, structured and even cell distribution within capsules from the rotating bioreactor system in comparison with perfused and static conditions. In addition, only alginate beads that were cultured in static conditions with mixed cell populations revealed positive staining for both collagen and proteoglycan, with areas that closely resembled the formation of osteoid. Cell viability, assessed using the fluorescent dye Cell Tracker

Green, indicated a higher proportion of metabolically active cells in capsules from rotating-wall conditions in comparison with perfused or static. Immunohistochemistry indicated the expression of type II collagen, SOX9 and C-MYC in samples from all conditions after 28 days. C-MYC is implicated in cell proliferation and differentiation and type II collagen and SOX9 are cartilage-specific markers. Biochemical analysis revealed significantly increased ($p < 0.05$) protein synthesis in samples encapsulated with mixed cell populations compared with alginate samples that were encapsulated with single cell populations. There was also a significant increase in protein synthesis in samples that were cultured in the rotating-wall bioreactor in comparison with perfused or static conditions. DNA and cell proliferation was significantly increased in the rotating-wall compared with perfused or static for the bone marrow cultures. Interestingly in chondrocyte cultures perfused conditions were found to result in significantly higher DNA than rotating-wall and static. Increased DNA and cell proliferation was observed in static conditions for mixed cell population samples.

DISCUSSION & CONCLUSIONS: The current studies outline a tissue engineering approach utilising progenitor populations, bioreactors and appropriate stimuli to promote the formation of cartilage within a unique innovative polysaccharide capsule structure. These studies indicate the potential of rotating-wall bioreactor systems to promote cartilage formation and also the potential of the encapsulation of mixed cell populations. Understanding the conditions required for the generation of functional 3D cartilage constructs using such bioreactor systems carries significant clinical potential.

REFERENCES: ¹ Leveque I, Rhodes R, Mann S. J.Mater. Chem, 2002 12, 2178-2180. ² Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. PNAS, 2002 99(19), 12025-12030. ³ Green DW, Leveque I, Walsh D *et al*. Adv. Func.Mat, (2005) 15: in press

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