

Alginate Scaffolds and Perfusion Bioreactors: A Promising System For Cartilage Engineering With Stro-1+ Progenitors From Human Bone Marrow

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INTRODUCTION: The use of robust three dimensional (3D) constructs to produce viable hyaline cartilage models is vital in developing clinical approaches to cartilage regeneration. Previous work which examined the use of polyglycolic acid (PGA) fleece seeded with the Stro-1+ progenitor population of human bone marrow, showed production of fibrocartilage¹. This study, therefore, examines the use of alginate/chitosan scaffolds, a widely used system in tissue engineering², using both perfused and static conditions to engineer 3D hyaline cartilage with Stro-1+ progenitors and ATDC-5 as a positive control.

METHODS: Stro-1+ cells were expanded in monolayer cultures, to confluence, in 10% FCS, α -MEM, while ATDC-5 cells were expanded as monolayer cultures in DMEM, 5% FCS, 1X ITS, before being harvested for encapsulation. Approximately 4×10^6 Stro-1+/ ATDC-5 cells were resuspended in 1ml of 2% alginate containing 10ng/ml TGF- β 3. Alginate/chitosan cell beads were formed by carefully placing droplets into a 1.5% chitosan solution using a 1ml syringe and 25G needle. Beads were washed in media, placed either into a perfusion bioreactor (4 reactors/n=5) or a 6 well plate (n=10) and cultured for 21 days (ATDC5) and 28 days (Stro-1+). Bioreactors were set with a perfusion flow rate of 1ml/day. Samples of media were taken at regular time points for both bioreactor and static systems and frozen (-20°C) for later analysis. Following culture, the beads were analysed for cell viability (Cell tracker green/Ethidium homodimer staining and media analysis) and chondrogenesis (histology; Alcian blue/Sirius red [A/S] staining).

RESULTS: Cell survival was evident in alginate/chitosan beads in both static and perfused cultures for both cell types. This was observed by confocal imaging of cells stained with Cell tracker green throughout the constructs. Confocal imaging showed that cell distribution was even in both static and perfused cultures of both cell types. In addition, cell metabolism was monitored by measuring changes in metabolites over time, as determined through analysis of stored media

samples using a culture media analyzer (Bioprofile 400).

Histological analysis of A/S-stained sections of ATDC5 capsules cultured in the perfusion bioreactors revealed a distinctly hyaline cartilage-like morphology composed of chondrocytic cells lodged in lacunae. In comparison, in A/S-stained sections of ATDC5 capsules cultured under static conditions, the chondrocytic cells appeared to be in the initial stages of organising themselves in a 'cell within lacuna' morphology. Alginate capsules with Stro-1+ cells cultured under perfused and static conditions exhibited a cartilaginous morphology in which cells were lodged in lacunae.

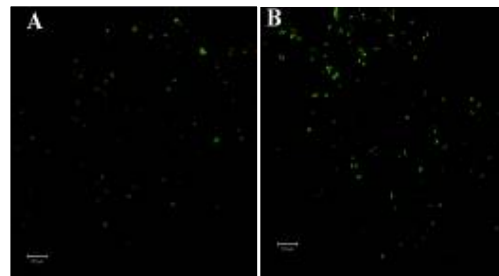


Fig. 1: Cell tracker green staining of Stro-1+ cells in alginate/chitosan beads A) Static B) Perfused cultures. Scale bar = 100 μ m

DISCUSSION & CONCLUSIONS: This study has shown the suitability of the alginate/chitosan system as a potential 3D environment for chondrogenic differentiation of Stro-1+ progenitors from human bone marrow. The formation of hyaline cartilage-like structures with both cell types and the good dispersal of viable cells throughout the beads reinforces the use of alginate as a scaffold for tissue engineering. The observed hyaline cartilage-like morphology highlights the benefits of using a perfused bioreactor and underscores the potential of such systems within cartilage engineering.

REFERENCES: ¹ R. Tare *et al.* (2005), Annual TCES Conference, London p38 ²D W Green *et al.* Adv Func Mater 15, 917, 2005

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