

## Perfusion-Enhancement of Molecular Transport within 3D Scaffolds

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**INTRODUCTION:** Hydrogels have demonstrated their usefulness in studying the response of isolated chondrocytes to a range of stimuli, while excluding the influence of other factors present in native cartilage. Examples of such studies involve the effects of mechanical stimulation on metabolism<sup>[2]</sup> and cell deformation<sup>[1,3]</sup>. It is clear, however, that the diffusion of oxygen and soluble nutrients to cells centrally-located within the 3D constructs is inadequate to maintain both cell viability and proliferation. Indeed, GAG deposition by chondrocytes and mineralised matrix deposition by osteoblasts have both been reported to curtail, approximately 400 $\mu$ m and 240 $\mu$ m from the surface of the 3D constructs, respectively<sup>[4]</sup>.

In the present study, bioreactors have been designed to investigate the influences of two strategies of medium perfusion on cell proliferation and matrix synthesis within agarose constructs. Solute movement within the 3D gels was profiled using confocal microscopy, to test the hypothesis that perfusion-increased fluid flow will enhance molecular transport and dispersion.

**METHODS:** Two bioreactors, using confined and unconfined configurations, were used to perfuse fluid into 4%(wt/vol) agarose cylinders, of 10mmx3mm cylinders. 0.001% (wt/vol) FITC-Dextran dissolved in PBS was perfused for 24 hours and scanned, using a confocal microscope. Control samples in the free-swelling state were immersed in the same FITC-Dextran solutions for 24 hours.

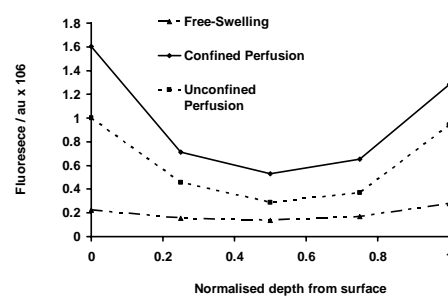
The agarose cylinders were seeded with 20x10<sup>6</sup>/ml of bovine articular chondrocytes and cultured for 20 days using both perfusion strategies, with the medium refreshed every 3 days. Biochemical assays were used to measure cell proliferation and matrix synthesis and were compared to control groups that had been cultured under free-swelling condition.

**RESULTS:** Solute concentration gradients, from peripheral regions of the constructs to their central regions, were less significant following perfusion with either bioreactor compared to those that were incubated in the free-swelling condition. Using the perfusion bioreactors, an increased Dextran (figure 1a) and essential nutrient

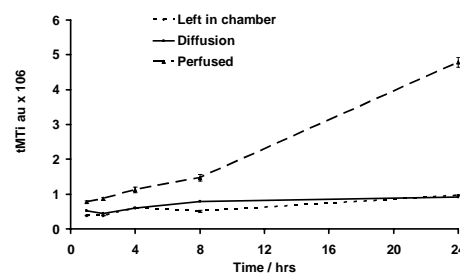
concentration were achieved at the central regions of the scaffolds.

The perfusion-aided increased media convection resulted in improved cell proliferation (figure 1b) and matrix synthesis (data not shown).

a



b



**Figure 1:** Perfusion a) improves molecular transport into the agarose constructs and b) improves cell proliferation over 20 days

**DISCUSSION & CONCLUSIONS:** Perfusion enhanced the transport of solutes into agarose constructs, increasing their concentration in the central regions. The improved solute delivery to cells centrally located within the 3D structures was maintained even after the cells began to produce their extracellular matrix, averting the temporal decline of cellular viability at the construct centres (data not shown).

**REFERENCES:** <sup>1</sup>Buschmann *et al.*, (1995) *J. Cell Sci.* **108** 1497-508 <sup>2</sup>Chowdhury *et al.*, (2003) *Arch Biochem Biophys* **417** 105-111 <sup>3</sup>Knight *et al.*, (1998) *Biochim et Biophys Acta* **1405** 67-77 <sup>4</sup>Martin *et al.*, (1999) *Ann. Biomed. Eng.* **27** 656-662

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