

Culture of Meniscal Chondrocytes on Alginate Hydrogel Matrices

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INTRODUCTION: Injury to the fibrocartilaginous tissue of the knee meniscus represents a major challenge clinically and failure to repair serious defects can result in joint degeneration. Tissue engineering of the meniscus represents a potential alternative to the use of artificial materials, autogenous or allograft tissue. In this study we have used cells from ovine meniscal tissue which have demonstrated good similarity, at least mechanically, to human tissue¹. We have investigated the proliferation of ovine meniscal chondrocytes (OMC) to a range of growth factors, and also their attachment to selected matrix proteins, in order to ascertain optimal conditions for *ex vivo* expansion of these cells as a pre-requisite for future tissue engineering studies. Alginate hydrogels, crosslinked ionically (with calcium ions) and covalently (with 1-ethyl-3-(3-dimethylaminopropyl)-N-hydroxy succinimide carbodiimide) have also been investigated for their suitability as scaffolds for OMC cell culture.

METHODS: OMC were isolated using the method of Kuettner et al² and cultured in DMEM supplemented with 10% FCS. Confluent cultures (passage 3) were used for these studies. To assess the effect of growth factor treatment, OMC were seeded into 48-well plates at a density of $2 \times 10^4/\text{cm}^2$. Recombinant human platelet-derived growth factors AB (PDGF-AB) and BB (PDGF-BB), transforming growth factor $\beta 1$ (TGF- $\beta 1$), bone morphogenetic protein 2 (BMP-2), insulin-like growth factors I (IGF-I) and II (IGF-II) and basic fibroblast growth factor (bFGF) were added at final concentrations of 0-100 ng/ml and cells incubated for 72h at 37°C. Proliferation was measured using the MTT assay. Cell attachment to matrix proteins was assessed as follows: Briefly, non cell culture grade 96 well plates were coated with 10 $\mu\text{g}/\text{ml}$ fibronectin, laminin, vitronectin, type I collagen or BSA as control, overnight at 4°C. Plates were then washed once with PBS and blocked with 1% heat-inactivated (Δ)BSA for 2h at 37°C. OMC were suspended in adhesion buffer (HBSS supplemented with 20 mM HEPES, 0.1% Δ BSA, 1 mM CaCl_2 , 1 mM MgCl_2 and 0.2 mM MnCl_2) and plated at 7.2×10^4 cells/well. Attachment at 0-120 min was measured using the MTT assay. For studies using alginate hydrogels, OMC were encapsulated at

$2.6 \pm 0.6 \times 10^6$ cells/ml in (2-4% w/v) alginate beads and also seeded on to the surface of flat alginate hydrogels. Scanning electron microscopy (SEM) was used to provide high resolution surface images of alginate beads. The number and viability of adherent cells was assessed using trypan blue, and histological analysis was carried out using sections through beads embedded in O.C.T. fluid, stained using haematoxylin and eosin.

RESULTS: Treatment of OMC with IGF-I, TGF- $\beta 1$, bFGF, PDGF-AB or PDGF-BB resulted in an increase in proliferation compared to untreated cells. PDGF-AB and -BB had the greatest effect, with maximal proliferation occurring at concentrations of 50 ng/ml. In contrast, BMP-2 and IGF-II had no significant effect over the concentration range tested. The results from matrix protein studies revealed that the greatest cell attachment occurred with fibronectin. Significant increases in attachment were also seen with both vitronectin and type I collagen, compared to BSA controls, with maximal attachment recorded at 60 min. In comparison, OMC adhesion to laminin was minimal. The alginate beads supported cell growth for over 50 days, however, cell numbers and viability in the alginate beads were found to decrease over time. Covalently crosslinked gels were found to disintegrate over a 3 day period, and were generally unsuitable for supporting meniscal chondrocyte growth.

DISCUSSION & CONCLUSIONS: These results demonstrate that short-term (72h) treatment with a range of growth factors is able to increase ovine meniscal fibrochondrocyte proliferation, with the two isomeric forms of PDGF, AB and BB, having significantly greater effects than any other factor tested. We have also shown that these cells attach preferentially to the matrix proteins fibronectin, vitronectin and type I collagen, with the greatest attachment seen consistently with fibronectin. We also show that ionically crosslinked alginate gels will support OMC growth.

REFERENCES: ¹ M.D. Joshi, J.K. Suh, T. Marui and S.L. Woo (1995) *J. Biomed. Mater. Res.* **29**:823. ² K.E. Kuettner, B.U. Pauli, G. Gall et al (1982) *J. Cell Biol.* **93**, 742-750.

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