

Human Osteoarthritic Chondrocytes are Capable of Forming Cartilage Matrix on Hyalograft C under Hypoxic Conditions

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INTRODUCTION: Articular cartilage consists of chondrocytes surrounded by an extracellular matrix (ECM) made of collagens (mainly collagen type II) and proteoglycans. Chondrocytes isolated from tissue can be expanded in culture, but rapidly lose their round morphology and chondrogenic capacity. They stop synthesizing collagen type II and dedifferentiate into fibroblastic-like cells with high collagen type I expression. Culture conditions such as 3-D culture systems, low oxygen tension and anabolic growth factors are parameters that have been shown to partially restore the chondrogenic phenotype of dedifferentiated cells. This study examined the potential of expanded aged human osteoarthritic chondrocytes to produce a cartilage ECM by combining some of the above parameters. Hyalograft-C (FIDIA Advanced Biopolymers, Italy) was used as a culture template for the chondrocytes in the presence of growth factors in either normal or hypoxic conditions.

METHODS: Chondrocytes were enzymatically isolated from osteoarthritic knee cartilage from joint replacement surgery. Cells were plated at a density of 20,000 cells/cm² and expanded for 2 passages (DMEM supplemented with 10% FCS) before seeding on 1cm² Hyalograft C at densities of 2.5x10⁵, 5x10⁵, 1x10⁶ and 2x10⁶. Constructs were cultured under normal (21% O₂) or hypoxic (5% O₂) conditions in chondrogenic medium (DMEM, 10% FCS, ascorbic acid, dexamethasone ITS+1 and TGFb-3). Samples were analysed at 7, 14 and 21 days for gene expression by quantitative RT-PCR and at 21 days for histology (safranin-O staining) and immunohistochemistry (collagen type I and type II).

RESULTS: Histological evaluation of the Hyalograft/cell constructs at day 21 revealed evenly spread, attached chondrocytes at all densities both in hypoxia and in normoxia. Constructs seeded at 2x10⁶ cells/cm² cultured in hypoxia had a hyaline-like morphology, whilst normoxic cultures appeared more fibrous (*Fig. 1*). Furthermore, hypoxic cultures exhibited higher collagen type II (*Fig. 1*) and lower collagen type I immunostaining in their ECM. High seeding

density combined with hypoxic culture for 21 days maximised the expression of the col2a1 gene, while levels of col1a1 were significantly suppressed. SOX9 mRNA expression levels were not altered under any of the conditions.

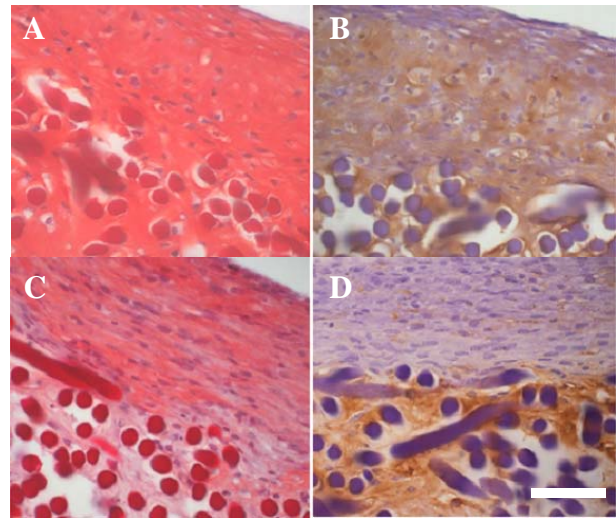


Fig. 1: Hyalograft/chondrocyte constructs (Day 21) stained with safranin-O (A, C) or immunolabelled for collagen type II (brown) (B, D) in hypoxic (A, B) and normoxic (C, D) conditions. All sections were counterstained with haematoxylin. Scale bar = 100µm

DISCUSSION: Previous studies have shown that embryonic chondrocytes expanded on biomaterials and bovine chondrocytes cultured using bioreactors were capable of regaining their chondrogenic phenotype and produced a cartilagenous ECM. In this study the results show that the potential of expanded primary aged human osteoarthritic chondrocytes to produce hyaline-like ECM is greatly enhanced by hypoxia. Together with high cell seeding density, hypoxia played an important role in controlling the gene expression and ECM production of the primary osteoarthritic chondrocytes. The ECM produced was rich in glycosaminoglycan and collagen type II and this appeared to occur independently of any change in SOX9 gene expression.

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