SUSTAINED HYPOXIA ENHANCES CHONDROCYTE VIABILITY AND MATRIX SYNTHESIS IN 3-DIMENSIONAL CULTURE

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INTRODUCTION: Articular cartilage is an avascular tissue and chondrocytes in the deeper layers of articular cartilage have been reported to exist in oxygen tensions less than 1%. As such, chondrocytes likely possess adaptations for survival in low oxygen tensions. We have previously shown tonic activation of hypoxic inducible factor (HIF-1α) within the deep layers of articular cartilage consistent with a hypoxic environment. Our study also demonstrated alterations to proteoglycan synthesis in cartilage explants cultured in normoxia compared to hypoxia. Reproducing hypoxic conditions in vitro may be important for studies of chondrocyte metabolic responses to injury, disease and potential treatments. This study was performed to test the hypothesis that chondrocyte viability and metabolism in three-dimensional culture is enhanced by sustained hypoxia.

METHODS: Cell culture. Articular chondrocytes grown in alginate beads provide a 3-dimensional (3D) environment for maintaining chondrocyte phenotype while allowing homogeneous oxygen levels throughout the culture. Alginate cultures also permit recovery of individual cells for study. Bovine articular chondrocytes (BAC) were obtained from knee cartilage of freshly slaughtered calves and prepared into alginate bead cultures at a density of 4x10^6 cells per ml as previously described (2). Normoxic cultures were maintained in 21% O_2/5%CO_2 at 37°C. Hypoxic cultures were equilibrated in a sealed glove-box (Coy Laboratories) at 2% O_2/5% CO_2, and maintained at 37°C in a sealed hypoxic chamber (Billups-Rothenberg).

Proteoglycan synthesis: Intact beads were pulsed with ^35S-sulfate for 8 hours to assay proteoglycan synthesis after 1, 3 and 10 days in alginate culture. Six sets of two beads were assayed for each condition.

Histology and Immunofluorescence. Intact beads were fixed in 10% formalin, embedded in paraffin and sectioned. Sections were stained with Safranin O. Indirect immunofluorescence for HIF-1α was performed with anti-HIF-1α antibody (1:500). Nuclei were counterstained with DAPI.

Viability was assessed in alginate recovered cells by trypan blue exclusion, performed in triplicate.

Glycosaminoglycan (GAG) and DNA content: After 17 days, alginate recovered chondrocytes were digested with papain and assayed for GAG content by colorimetric analysis using dimethyl blue. DNA content was measured by using PicoGreen fluorescence.

Statistics: Data was analyzed by ANOVA followed by a Bonferoni t-test, and two-tailed t-test. Significance was set at P<0.05.

RESULTS: Alginate beads were approximately 1.5 to 1.75 mm in diameter (Fig 1A, C). Viability of recovered chondrocytes was higher in hypoxic cultures by 33% (P=0.026) at Day 10 and 21% higher (P=0.04) at Day 17. Histology revealed small cells in normoxia (Figure 1A) while large, icosahedral cells within lacunae were observed throughout beads cultured in hypoxia (Figure 1C). Uniform oxygen tensions were achieved as evidenced by HIF-1α immunostaining. Normoxic beads demonstrated perinuclear HIF-1α throughout the thickness of the bead (Fig 1B). In hypoxia, HIF-1α was translocated to the nucleus in cells throughout the bead (Fig 1D). Abundant type 2 collagen surrounded chondrocytes in beads grown in hypoxia for 17 days (Fig 2).

Proteoglycan synthesis was higher in hypoxia than normoxia by 42% (P<0.001) after 1 day, by 170% after 3 days (P=0.025) and by 101% after 14 days (P<0.001) (Figure 2).

DISCUSSION: Although hypoxia is the physiological condition for deeper zones of articular cartilage, studies of chondrocyte metabolism are frequently performed in normoxic conditions. Differences in chondrocyte viability and matrix synthetic activity were demonstrated in three-dimensional culture between chondrocytes cultured in hypoxia and normoxia. Sustained hypoxia increased viability, proteoglycan synthesis, GAG content and type 2 collagen deposition. Immunolocalization of HIF-1α showed homogenous oxygen distribution throughout the beads and confirmed achievement of intended conditions with perinuclear staining of HIF-1α in normoxia and intranuclear localization in hypoxia. The results of this study show that hypoxic 3-D culture of articular chondrocytes yield different results in studies of chondrocyte viability and metabolism than the more conventionally used normoxic cultures. As such, hypoxic 3-D culture may more accurately model the metabolic responses of chondrocytes to injury, disease and treatments.

References: