Introduction:
The turnover and metabolic homeostasis of articular cartilage depends on the interaction between the chondrocytes and their surrounding extracellular matrix. Recent studies reported that the receptors on chondrocytes that mediate these interactions are members of a family of glycoproteins called integrins (1). These proteins consist of heterodimeric, non-covalent complexes of alpha and beta subunits that act as transmembrane receptors which link the cytoskeleton and the extracellular matrix. The linkage, mediated by integrins, seems to play a critical role in the elaboration and maintenance of the extracellular matrix.

We have previously discovered that human articular chondrocytes propagated in microcarrier spinner culture more actively produce the extracellular matrix components collagen type II and proteoglycans compared to monolayer culture (2). The mechanisms by which the microcarrier spinner culture promotes expression of the chondrocytic phenotype is not clear. The present study investigates the possible involvement of integrins in the synthesis of extracellular matrix components by chondrocyte spinner culture. The present study tests the hypothesis that propagation of chondrocytes in microcarrier spinner culture promotes expression of integrins. Synthesis of integrins that act as binding sites for extracellular matrix components may help to maintain normal chondrocyte phenotype.

Methods:
Non-fibrillated articular cartilage was obtained from three osteoarthritic patients during knee surgery. Chondrocytes isolated by collagenase digestion were directly seeded onto Cellagen microcarriers (100-400 µm derived from bovine corium, ICN, Cleveland, OH) or as monolayer culture as previously described. Microcarrier and spinner cultures were incubated at 37°C, 5% CO₂ (Fisher Scientific); and for proteoglycans using monospecific antibodies to collagen types I and II (400 µCi/5 x 10⁶ cells) for 24 hrs. Radiolabeled collagen was autoradiographed. Proteoglycan synthesis was determined by a 24 hr. incorporation of ³⁵SO₄.

Discussion/Conclusion:
Increased proteoglycan synthesis in microcarrier spinner culture was verified by incorporation of ³⁵SO₄.

Chondrocytes harvested from microcarrier spinner culture stained intensely for type I collagen and less intensely for proteoglycans. Both microcarrier spinner and monolayer cultures did not stain for ³⁵SO₄ when the primary and secondary antibodies were omitted or an irrelevant anti-human immunoglobulin antibody was used.

Results:
Chondrocytes proliferated from 5 to 20 fold in microcarrier culture and 2-5 fold in monolayer culture. Cells from microcarrier spinner culture stained more intensely for collagen type II, proteoglycans and integrins α5, α6 and β₁ than chondrocytes from monolayer culture. In contrast, monolayer cultures stained more intensely for type I collagen and less intensely for proteoglycans. Enhanced de novo synthesis of collagen type II was confirmed by immunoprecipitation. Increased proteoglycan synthesis in microcarrier spinner culture was verified by incorporation of ³⁵SO₄.

The principal finding of the present study is that the integrins α₁, α₅, and β₁ are prominently expressed in articular chondrocytes propagated in microcarrier spinner culture. The observation that these proteins are detectable at higher levels in chondrocyte microcarrier spinner cultures suggest that they may be involved in enhancing the synthesis of the extracellular matrix components collagen type II and high molecular weight proteoglycans. Integrins are documented to mediate inside-out signaling function and in the regulation of extracellular matrix metabolism (3). Propagating chondrocytes in microcarrier spinner culture, a biomechanically active environment, may help favor the participation of integrins in extracellular matrix formation, thus promoting the chondrocyte phenotype.

References:

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INTEGRIN EXPRESSION BY HUMAN ARTICULAR CHONDROCYTES IN MICROCARRIER SPINNER CULTURE

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