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COMPARING THE RESPONSIVENESS OF CHONDROCYTES ISOLATED FROM YOUNG AND MATURE ARTICULAR CARTILAGE TO IGF-1 AND TGFβ1

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INTRODUCTION: Injury to articular cartilage does not result in a spontaneous repair process due to the avascular and aneural properties of the tissue. Surgical strategies to enhance this repair process result in filling of the defect but with poor integration between the endogenous and repair tissue. This study investigates whether exposure to IGF-1 and TGFβ1 may enhance the repair process by increasing chondrocyte migration and matrix biosynthesis in chondrocytes isolated from young and mature articular cartilage.

METHODS: Cell Migration/Chemotaxis – Isolated chondrocytes were allowed to adhere to Boyden chambers coated with fibronectin, aggrecan, type II collagen or BSA as a control. After 48 hours IGF-1 and/or TGFβ1 (both at 10ng/ml) or BSA were added to the bottom of the wells. After 24 hours, cells remaining on the top of the chamber were removed and the migrated cells stained with crystal violet and counted.

Matrix Biosynthesis – Chondrocytes were plated at 5 x 10^5 cells/well and allowed to adhere to the plate. After 24 hours the cells were supplemented with IGF-1 and/or TGFβ1 (both at 10ng/ml). Media served as control. All treatment groups were supplemented with 10µCi/ml ^{35}Sulphate and 20µCi/ml ^{3}H-proline and incubated for 1, 3 or 7 days. Radiolabel was counted in both the media and cell lysates (and associated material) giving biosynthesis values for both sulphated glycosaminoglycans (sGAGs) and collagen.

Additional 'cold' experiments were set up as described above for protein and RNA extraction. PCR was used to confirm the chondrocytic phenotype using Sox 9 and Col 2 as markers. Western blotting using the monoclonal antibody AVT-6E3 was performed to confirm that the collagen synthesised was the characterisitic type II collagen expressed in articular cartilage.

RESULTS: Cell Migration/Chemotaxis. Chondrocytes from both age groups were seen to migrate on all the substrates however aggrecan significantly inhibited the migration of chondrocytes isolated from young cartilage when compared to BSA controls (P=0.012). The addition of growth factors only enhanced migration of the young chondrocytes if coated onto fibronectin, with TGFβ1 having an additive effect on IGF-1 (P=0.028). Only combined growth factor treatment enhanced the migration of the mature chondrocytes (P=0.025).

Matrix Biosynthesis. Increases in both sGAG and collagen biosynthesis were seen in both age groups of chondrocytes with growth factor treatment. Treatment with TGFβ1 increased biosynthesis at 24 hours and then within the cell associated material by 3 days, suggesting deposition of the newly synthesised matrix. No significant differences in biosynthesis levels were seen in either the conditioned media or cell associated material by day 7, suggesting a possible up-regulation of matrix degradation.

PCR and Western blotting confirmed maintenance of the chondrocytic phenotype through time in culture with Sox 9 and Col 2 present within RNA samples and type II pro-collagen found in protein extracts from the treated cells.

DISCUSSION & CONCLUSIONS: Both IGF-1 and TGFβ1 can enhance chondrocyte migration in a substrate dependent manner, only occurring on fibronectin. IGF-1 and TGFβ1 also exert an anabolic effect on both age groups of cells up-regulating their sGAG and collagen biosynthesis whilst maintaining the chondrocytic phenotype. These results suggest that IGF-1 and TGFβ1 may have an application in cartilage repair where a combination of increased chondrocyte migration and matrix biosynthesis are required for the generation and integration of a biomechanically stable repair tissue.

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