Matrix Proteins as Predictive Markers of the Mechanical Strength of Engineered Cartilage.

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INTRODUCTION: The mechanical properties of articular cartilage are dependent on the extracellular matrix composition. However, it is not clearly defined which matrix components are the most appropriate markers for predicting mechanical ability of tissue-engineered cartilage. The aim of this study was to investigate which matrix proteins correlate with mechanical quality of engineered cartilage. Engineered cartilage constructs were formed from scaffolds seeded with varying numbers of hyaline chondrocytes or; by seeding with a single cell concentration/scaffold and varying the length of time constructs were cultured. to yield varying matrix compositions. Changes in proteoglycan content [determined as glycosaminoglycan content (GAG)], total collagen (determined as hydroxyproline), collagen I and II concentrations and collagen cross-links were correlated with the mechanical properties of the constructs.

METHODS: Non-woven HYAFF 11® scaffolds (2mm depth, 5mm diameter, Fidia Advanced Polymers, Italy) were seeded dynamically with 2, 4, 8, or 16 x 10^6 bovine hyaline chondrocytes and cultured for 42 days as described previously [1]. 3mm cores were taken and tested under confined compression and analyzed [2] to determine proteoglycan content [by calorimetric assay with dimethylmethylene blue) total collagen (by amino acid analysis), collagens I and II (measured by inhibition ELISA) and collagen cross-links (measured by amino acid analysis). For varying the time in culture, scaffolds (2mm depth, 8mm diameter) were seeded with 16 x 10^6 chondrocytes and cultured for 20, 30, 40 or 80 days [1]. The constructs were tested under non-confined compression and analyzed as described.

RESULTS: In experiments changing the number of cells seeded, the aggregate modulus correlated with the of both GAG *(P*<0.0001) and collagen II (*P*<0.0001) but not collagen I expressed as a percentage of the matrix composition. Varying the length of culture showed that Young's modulus increased over the culture period and correlated with GAG (*P*<0.0001*)*, collagen II (*P*<0.0001), and ratio of mature to immature collagen cross-links (*P*=0.0001). No correlation was found between Young's modulus and matrix hydroxyproline.

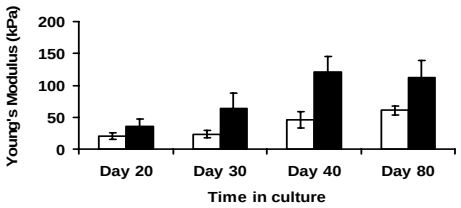

Fig. 1: The Effect of Time in Culture on Young's Modulus of Engineered Hyaline Cartilage.

DISCUSSION & CONCLUSIONS: The results indicated that measurement of collagen II and GAG are good predictive markers of the mechanical quality of tissue-engineered hyaline cartilage. However, there was no significant correlation between total collagen content and mechanical quality. Therefore, collagens other than type II probably compose a significant percentage of the matrix of immature engineered cartilage constructs

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