

## Biochemical Markers of the Mechanical Quality of Engineered Hyaline Cartilage

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**INTRODUCTION:** The mechanical properties of articular cartilage are dependent on the composition of the extracellular matrix. However, it is still unclear which extracellular matrix proteins are the most appropriate indicators of the mechanical properties of tissue-engineered cartilage. The aim of this study was to determine which biochemical markers can be used as surrogate measures of the mechanical quality of engineered cartilage. We modified the matrix composition of engineered cartilage by forming constructs from scaffolds seeded with varying numbers of chondrocytes or by seeding with a single cell concentration/scaffold and varying the length of time constructs were cultured. We then compared changes in GAG, total collagen (by measurement of hydroxyproline), collagen I and II concentrations and collagen cross-links with the mechanical properties of the constructs.

**METHODS:** Chondrocytes were isolated as described previously [1]. Non-woven HYAFF<sup>11</sup> scaffolds (2mm depth, 5mm diameter, Fidia Advanced Polymers, Italy) were seeded dynamically with 2, 4, 8, or 16 x 10<sup>6</sup> chondrocytes and cultured for 42 days as described previously [1]. 3mm cores were taken and tested under confined compression. A ramp displacement corresponding to 10% strain at a ramp speed of 0.001 mm/sec was applied. Two subsequent ramp displacements of 5% were then applied to give a total strain of 20%. The cores were analyzed [2] to determine proteoglycan content [measured as glycosaminoglycan (GAG), by calorimetric assay using dimethylmethylene blue) total collagen (measured as hydroxyproline by amino acid analysis), collagens I and II (measured by inhibition ELISA) and collagen cross-links (measured by amino acid analysis). For experiments varying the time in culture, the HYAFF<sup>11</sup> scaffolds (2mm depth, 8mm diameter) were seeded with 16 x 10<sup>6</sup> chondrocytes and cultured for 20, 30, 40 or 80

days [1]. The constructs were tested under non-confined compression using the described loading regimen.

**RESULTS:** All the engineered constructs formed an extensive extracellular matrix with hyaline characteristics. In experiments changing the number of chondrocytes seeded, the aggregate modulus correlated positively with the percentage matrix composition of both GAG ( $P < 0.0001$ ,  $r = 0.5737$  to  $0.9147$ ) and collagen II ( $P < 0.0001$ ,  $r = 0.6215$  to  $0.9261$ ) but not with collagen I content. Varying the length of culture showed that Young's modulus increased over the culture period and correlated positively with GAG ( $P < 0.0001$ ,  $r = 0.3926$  to  $0.7375$ ), collagen II ( $P < 0.0001$ ,  $r = 0.48268$  to  $0.7557$ ), ratio of mature to immature collagen cross-links ( $P = 0.0001$ ,  $r = 0.2802$  to  $0.6739$ ). No correlation was found between Young's modulus and matrix hydroxyproline.

**CONCLUSIONS:** The results suggested that measurement of collagen II and GAG are good predictive markers of the mechanical quality of tissue-engineered hyaline cartilage. The lack of correlation of Young's modulus with hydroxyproline concentrations suggested that collagens other than collagen II were a significant matrix component of these relatively immature engineered cartilage constructs.

**REFERENCES:** 1. Crawford A. & Dickinson S.C. *Chondrocyte isolation, expansion and culture on polymer scaffolds.* Methods Mol. Biol. 238:147-157, 2004.

2. Kaffienah W. & Sims T.J. *Biochemical methods for the analysis of tissue-engineered cartilage.* Methods Mol. Biol. 238:217-230, 2004

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