Multiexponential T2 relaxation analysis to assess the development of engineered cartilage

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Introduction Fabrication of high-quality constructs for cartilage repair remains a major challenge for cartilage tissue engineering. Progress towards this would be greatly facilitated by the development of non-invasive monitoring during construct growth, enabling ongoing application of appropriate growth factors and mechanical input to optimize tissue structure. MRI has emerged as an important potential approach to such monitoring, with correlations being established between several MRI parameters and tissue characteristics[1]. T2 measurements, sensitive to tissue water mobility and macromolecular orientation, are widely used for cartilage assessment both in vitro and in living subjects, but these measurements are a highly nonspecific indicator of matrix status[2]. Conventional monoexponential T2 measurements provide an overall assessment of water mobility, but it is known that distinct water compartments are present in cartilage[3]. Thus, multiexponential analysis of T2 relaxation data has been used to investigate water compartmentalization in control and degraded cartilage[4]. The aim of the present study is to extend this approach to monitor the in vitro growth and development of tissue engineered cartilage.

Materials and Methods Bovine articular chondrocytes were mixed with type I collagen gel and allowed to solidify at 37ºC to yield chondrocyte-collagen constructs. Constructs were cultured in six-well plates and studied after 1, 2, 3 and 4 weeks of culture. *Magnetic Resonance Measurements*: Data were acquired with a 9.4T/105mm Bruker DMX NMR spectrometer. T2 relaxation data were obtained using a non-localized CPMG sequence with TE/TR = 0.6ms/10s, 4096 echoes and an NA of 64. A non-negative least squares (NNLS) method was used for the multiexponential T2 analysis. Extensive simulations were carried out with varying degrees of SNR to confirm the reliability of the components identified by the analysis[4]. *Biochemical Quantification*: Sulfated glycosaminoglycan (sGAG) and total collagen contents were determined from the digested constructs using the colorimetric DMMB dye binding assay and the hydroxyproline assay respectively.

Results and Discussion

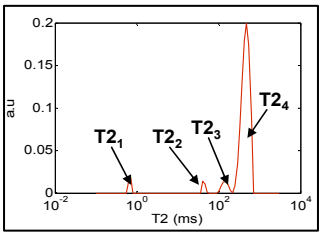

Figure 1: Representative T2 distribution from a 3 week collagen construct

Table 1: Multiexponential T2 analysis of collagen constructs showing T2 and fraction sizes of each water compartment

	T2 component (ms)					Weight fraction of water (%)				
Culture time	$T2_1$	$T2_{1-2}$	$T2_2$	$T2_3$	$T2_4$	w_1	w_{1-2}	w_2	w_3	w_4
week 1	0.405		42.16	251.69	596.04	0.020		0.018	0.052	0.912
week 2	0.610		44.94	174.29	485.27	0.032		0.028	0.033	0.908
week 3	0.543		46.48	160.54	480.33	0.032		0.024	0.039	0.901
week 4	0.538	27.13	54.31	186.40	442.53	0.022	0.022	0.025	0.076	0.868

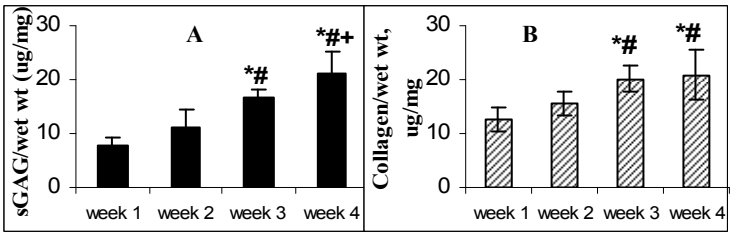

Figure 2 A) sGAG and B) collagen contents of 1-4 week old collagen constructs. *, # and + indicate significantly different from 1, 2, and 3 weeks respectively. p<0.05

Multiexponential T2 analysis (Table 1, Fig. 1) consistently demonstrated four distinct water compartments with fraction sizes w_1, w_2, w_3 and w_4. An additional component with an intermediate value of T2, $T2_{1-2}$ and fraction size w_{1-2} was detected at week 4. sGAG and collagen results (Figs 2A & 2B) show that both of these matrix components increased with culture time. The most slowly-relaxing component, $T2_4$, assigned to the water compartment most loosely associated with macromolecules, showed a decreased T2 and decreased weight fraction over time, as expected from the ongoing macromolecular synthesis in the constructs. The redistribution of relatively bound and free water was further seen by the changes in the relaxation times and fraction sizes of water associated with macromolecules. An initial decrease was observed in both $T2_3$ and w_3 before an increase was seen in both parameters at week 4. The decreased T2 and weight fraction was attributed to increased macromolecular concentration restricting the mobility of the water molecules. It is possible that this water compartment, w_3, is associated with macromolecules of different lengths and structural complexities. Cartilage proteoglycans consist of several distinct subpopulations with varying molecular weights[5]. As expected, each species will have a distinct T2 and there is a T2 distribution associated with each water compartment. Hence, the observed T2s are weighted averages of the T2s of individual species present in any given water compartment. Some of the macromolecular species from the w_3 compartment may have been modified as the construct matured, resulting in more flexible aggregates with higher molecular weights and higher mobility. This could explain the increase seen in both $T2_3$ and w_3. The compartment with associated relaxation time, $T2_2$ and weight fraction, w_2, increased in size as macromolecular concentration increased over time. $T2_2$ may be assigned to water bound to newly synthesized PG monomers or non-aggregating PGs and remains fairly constant until a substantial increase is seen at week 4. This may be due to the synthesis of PGs with core proteins of varying molecular weights or PGs relatively enriched in keratan sulfate (KS) chains, as they are smaller and more mobile than chondroitin sulfate (CS) chains[5]. The ratio of KS to CS in native cartilage increases with age, and this phenomenon may also be reflected in the developing constructs. The appearance of the water compartment with relaxation time $T2_{1-2}$ in week 4 is consistent with the maturation and aggregation of the PG monomers into complex aggregating PGs. A rapidly relaxing component with T2 on the order of 0.5ms was detected in all the samples. This compartment was also detected in day 3 scaffolds, prior to the deposition of significant amounts of matrix. Thus, $T2_1$ could be assigned to rapidly-relaxing water bound to the type I collagen scaffold. The decrease in w_1 at week 4 may be attributed to scaffold degradation.

Conclusions: We have demonstrated that multiexponential T2 analysis can be used to greatly increase the specificity of relaxation time measurements in engineered cartilage. This method may be of significant value for non-invasive monitoring of developing constructs

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