

Characterization of ECM Embedded Biomimetic Scaffolds for Cartilage Tissue Engineering using Sodium Triple-Quantum-Coherence Spectroscopy

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Introduction: Biomimetic scaffolds have been shown to be effective for bone regeneration and similar strategies are under active investigation for cartilage tissue regeneration[1]. The principal components for cartilage tissue regeneration are proteoglycans and collagen, II. We have investigated application of triple quantum (TQ) sodium spectroscopy for characterization of biomimetic scaffolds at the early growth stage for cartilage tissue regeneration. Triple quantum sodium NMR is insensitive to the fast motion limit ($\omega_0\tau_c \ll 1$, where τ_c is rotational correlation time) conditions; therefore it filters out all contribution from free sodium ions. Relaxation is biexponential in this case with a fast and a slow relaxation components. Although, the fast relaxation component has been shown to be sensitive to tissue changes for patients with osteoarthritis, we found that both components are equally sensitive to the early stage of the growth [3]. We calculated motional parameter $\omega_0\tau_c$ from derived relaxation rates and found that it increases during the first two weeks and then suddenly drops before increasing again. We hypothesize that during the early stage of the growth, proteoglycan production dominates the relaxation rates, whereas during the later stage of growth, random collagen network formation dominates the relaxation rates. Further investigation is underway to analyze and understand this behavior of engineered tissue and correlate it with the biochemical data.

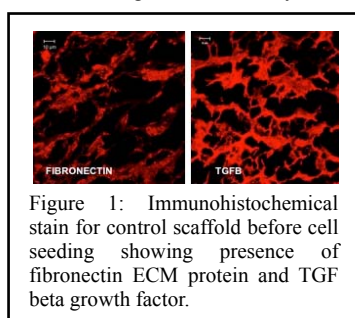


Figure 1: Immunohistochemical stain for control scaffold before cell seeding showing presence of fibronectin ECM protein and TGF beta growth factor.

Materials and methods: Biomimetic scaffolds were prepared using a technique similar to our published protocol [1]. Briefly, human marrow stromal cells (HMSCs) were subjected to chondrogenic differentiation in a collagen/chitosan scaffold for a period of 2 weeks. The scaffolds were then decellularized as published previously leaving behind the cell-secreted extracellular matrix (ECM). This procedure resulted in a scaffold that contained within, the ECM of HMSCs undergoing chondrogenic differentiation. We used these scaffolds to induce chondrogenic differentiation of undifferentiated HMSCs without the use of differentiating agents. Figure 1 shows

Immunohistochemical staining of the scaffold sections showing the presence of fibronectin ECM protein and TGF beta growth factor. Each week, three scaffolds (about 1-2 mm in diameter) were taken out of the incubator and placed into a 5mm NMR tube with growth media for sodium NMR study. All NMR experiments were performed on Bruker 9.4T and 11.7T spectrometers equipped with broadband probes capable for multinuclear NMR measurements. Single quantum spectra were acquired using standard single pulse sequence in order to compute the line broadening of the central transition as compared to the standard 150mM NaCl solution. Triple quantum signal were acquired using standard TQ filter pulse sequence by varying the delay τ in logarithmic steps from 50 μ s to 100ms[4]. The 90° pulse was 9 μ s for 9.4T and 5 μ s for 11.7T spectrometers for standard 150mM NaCl solution and it was calibrated for each sample. The relaxation rates, T_f and T_s , average quadrupolar coupling ω_Q and motional parameter $\omega_0\tau_c$ were computed using custom written Matlab™ program.

Results and discussion: Single quantum spectra of these scaffolds showed very small (few Hz) increase in line width as compared to the standard 150mM NaCl solution, therefore it is more likely that at the early stage of tissue growth, motion of sodium ion is isotropic. However, since we observed strong triple quantum signal, the sodium ions are expected to be in the regime of slow motion ($\omega_0\tau_c \geq 1$) and the average quadrupolar coupling is non-zero [2, 4]. The triple quantum signal intensity in this case can be written as; $S(\tau, t) \sim M_0 C \left[\left\{ e^{-(R_1 - i\omega_Q)\tau} - 2e^{-R_2\tau} + e^{-(R_1 + i\omega_Q)\tau} \right\} \cdot \left\{ e^{-(R_1 - i\omega_Q)t} - 2e^{-R_2t} + e^{-(R_1 + i\omega_Q)t} \right\} \right]$, where R_1 and R_2 are the fast and the slow relaxation rates and ω_Q is the residual quadrupolar coupling. Figure 2 shows one such example of a fit for one day old scaffold at 11.7 T field strength. The calculated relaxation rates and residual quadrupolar couplings from best fit of experimental data for 4 week period are given in Table 1. For comparison, similar parameters are also given for human and bovine cartilage explants. As HMSC cells differentiate into chondrocytes, they generate chondrogenic ECM components, collagen and proteoglycans primarily. Sodium ion primarily binds to proteoglycans because of the negative charges on them. The growth of these macromolecules changes the immediate environment around sodium ions and therefore, its relaxation behavior is representative of the growth of these macromolecules. The motional averaging parameter $\omega_0\tau_c$ represents how fast (or slow) sodium ion can tumble depending upon the anisotropy in its immediate environment. It seems that in the beginning, there is synthesis of proteoglycans, which correlates with increasing $\omega_0\tau_c$, and by the week 3, there may be increase in type II collagen with random orientations, which reduces value of $\omega_0\tau_c$. Further increase is observed towards the end of fourth week with increasing amount of ECM macromolecules. Further studies are underway to correlate these data with biochemical data.

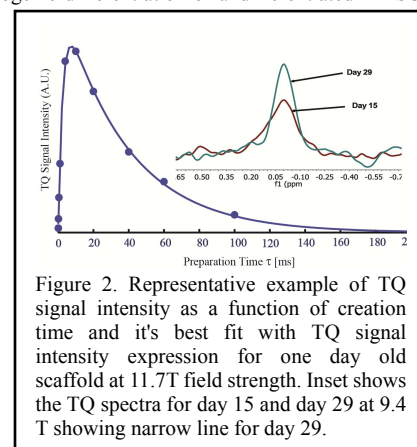


Figure 2. Representative example of TQ signal intensity as a function of creation time and its best fit with TQ signal intensity expression for one day old scaffold at 11.7T field strength. Inset shows the TQ spectra for day 15 and day 29 at 9.4 T showing narrow line for day 29.

ECM embedded scaffolds	9.4T				11.7T			
	Ts (ms)	Tf (ms)	ω_Q (Hz)	$\omega_0\tau_c$	Ts (ms)	Tf (ms)	ω_Q (Hz)	$\omega_0\tau_c$
Day1	44.5	2.1	212	4.98	38.4	2.6	156	4.06
Day8	55.0	1.7	211	6.30	44.3	1.2	1712	6.8
Day15	54.0	1.5	408	6.69	-	-	-	-
Day22	38.2	2.1	0.06	4.63	37.6	2.8	2.6	3.91
Day29	46.9	2.3	84	4.87	48.6	2.6	271	4.62
Human explants	18.6	0.3	2304	8.42	-	-	-	-
Bovine explants	15.8	1.1	1719	4.09	-	-	-	-

Table 1. The fast and slow relaxation times and residual quadrupolar couplings for sodium ion in ECM embedded scaffold seeded with HMSC cells as a function of growth time for 4 week period at 9.4T and 11.7T. For comparison, similar parameters from native bovine cartilage and human cartilage (1mm thick, 3mm diameter) explants at 9.4T field are included in the table. The last column in each section gives estimated motional parameter calculated using eq. 58 of ref [2].

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Conclusions: We show that the sodium triple quantum relaxation rates can be used to predict the type and amount of ECM components generated by HMSC differentiating into chondrocytes.

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References: 1. Ravindran, S. *et al*, Tissue Engineering Part A, 2011 in press. 2. Jaccard, G. *et al*, Journal of Chemical Physics, 1986 **85**(11): 6282. 3.

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