Sodium triple-quantum coherence characterization of scaffolds used in cartilage tissue engineering

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Target Audience: Members of the MR community involved in the characterization of engineered tissue using spectroscopy, imaging or elastography. The talk will also be interesting for tissue engineers who would like to use MR spectroscopy for non-invasive tracking of tissue growth.

Purpose: The purpose of this work is to establish the role of sodium triple quantum coherence spectroscopy (and imaging) for understanding tissue growth dynamics in cartilage tissue engineering. Sodium ion motion reflects the tissue matrix microstructure and characterizing this motion provides insight into the how the tissues are formed. We selected three different models of engineered cartilage and monitored sodium motion as the proto-cartilage developed during four weeks of incubation.

Introduction: Cartilage damage is a major cause of disability in adults and current treatment approaches are inadequate, therefore cartilage tissue engineering is being pursued as a possible alternative. The goal of cartilage tissue engineering is to generate native-like cartilage in its biochemical composition and mechanical strength to replace damaged or diseased tissue. In order to mimic native cartilage, tissue engineers use a variety of growth strategies and focus on the production of the principle extracellular matrix components, proteoglycan and collagen, as a measure of success [1]. Some commonly used tissue growth strategies are: i) scaffold free chondrocyte pellets; ii) chondrocytes seeded in a synthetic or natural hydrogel; and iii) mesenchymal stem cells seeded in a biocompatible scaffold with chondrogenic growth factors [2]. Each of these methods has advantages and disadvantages, but their overall effectiveness depends on the ability of the scaffold to produce proteoglycans and collagen in a matrix that matches the mechanical properties of native cartilage. In the present study, we investigated the application of sodium triple quantum (TQ) coherence NMR spectroscopy for monitoring tissue growth dynamics for tissue-engineered cartilage at the early growth stage after cell seeding. For comparison, we have also examined normal human cartilage tissue. The sodium triple-quantum coherence filter is insensitive to the sodium ions undergoing fast isotropic motion, therefore, it filters out any signal from free sodium ions [3, 4]. This allows us to observe the interaction of sodium ions with the tissue macromolecules and to gauge the anisotropy and dynamics in the growing engineered tissue.

Materials and methods: **1. Chondrocytes seeded in alginate beads:** Bovine chondrocytes (4 millions cells/ml) were cultured using chondrogenic growth media in alginate beads using the established protocol [5]. Typical size of the beads was 2-3 mm in diameter at the start of the experiments (~0.5 millions cells/bead). **2. Scaffold-free pellets:** Scaffold-free chondrocytes pellets were formed by centrifuging 5×10^5 bovine chondrocytes in tissue culture medium at 1000×g for 10 min. The NMR measurements on these pellets were performed from weekly, starting one week after cell seeding to allow the pellets to gain mechanical strength and cartilage macromolecules. **3. Mesenchymal stem cells seeded in ECM integrated biomimetic scaffolds:** 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. This procedure resulted in extra cellular matrix with integrated biomimetic chemical signals [6]. Undifferentiated HMSCs (1million cells/ml) were seeded in these scaffolds. The chondrogenic differentiation of HMSCs at the end of four-week period was confirmed by up-regulation of chondrogenic marker genes using gene expression analysis [7]. **4. Human articular cartilage explants:** The human articular cartilage explants were purchased from Articular Engineering (http://articular.com/) and cultured in the incubator with growth media. The explants were about 3 mm in diameter and 1 mm in thickness. Therefore, they do not represent the complete articular cartilage with all three zones intact. Each week, three or four samples were removed from the incubator and placed into a 5 mm NMR tube with the growth media for sodium NMR study. All NMR experiments were performed on a Bruker 9.4 T spectrometer equipped with a broadband probe. Triple quantum signal were acquired using standard TQ filter pulse sequence by varying the delay τ in logarithmic steps from 50 µs to 100 ms [4]. 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.

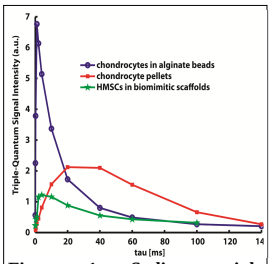

Figure 1: Sodium triple quantum coherence signal intensity at week 2 as a function of preparation delay τ. The markers are the experimental data points and the line is for guide to eyes only.

Results and discussion: We observed a strong triple quantum signal that indicates that the sodium ions are undergoing through motion without motional averaging. The triple quantum filtered signal strongly depended on the scaffold used for tissue engineering purpose and an example of this behavior is shown in figure 1. The biexponential relaxation times and average dipolar couplings were calculated using the equation [4]; $S(\tau,t)\sim M_0C\left[\left\{e^{-(R_1-i\omega_Q)\tau}-2e^{-R_2\tau}+e^{-(R_1+i\omega_Q)\tau}\right\}\cdot\left\{e^{-(R_1-i\omega_Q)t}-2e^{-R_2t}+e^{-(R_1+i\omega_Q)t}\right\}\right]$, where R_1 and R_2 are the fast and the slow relaxation rates and ω_Q is the residual quadrupolar coupling. The motional parameter, $\omega_0\tau_c$, was calculated using the equation [8]; $\omega_0\tau_c = sqrt\{(1/8)\ [5\ R_1/R_2\ -9+((5\ R_1/R_2\ )\text{^}2-58\ R_1/R_2\ +49)\text{^}(1\ /\ 2)\}$. The calculated parameters are tabulated in Table 1.

Conclusions: At the early stages of tissue growth, cells generate collagen and proteoglycan, primarily. The deposition of these macromolecules change the immediate environment around the sodium ions and therefore, sodium relaxation behavior post-cell seeding is representative of the accumulation of these macromolecules. Although sodium ions bind primarily to negatively charged proteoglycan, our triple-quantum coherence data indicate, that the sodium quadrupolar properties may be affected by both proteoglycan and collagen. We found that the sodium triple quantum coherence spectroscopy can differentiate between different tissue-engineering constructs and the native tissues based on the fast and the slow relaxation rates as well as based on the average quadrupolar coupling. Both the fast (T_f) and the slow (T_s) relaxation times were found to be longer in the chondrocyte pellets and the biomimetic scaffolds as compared to the chondrocytes suspended in alginate beads and the human articular cartilage tissues. In all cases, it was found that the relaxation rates and the motion of sodium ions, as measured from correlation time, were dependent on the amount of macromolecules, high cell density and the anisotropy of the cartilage tissue engineering constructs. Average quadrupolar couplings were found to be lower in the engineered tissue as compared to the native tissues, presumably due to the lack of order in collagen accumulated in the engineered tissue. These results indicate the use of sodium triple quantum coherence spectroscopy as a tool to investigate anisotropy and growth dynamics of cartilage tissue engineered constructs in a simple and reliable way.

Time points	Biomimetic scaffolds (Human HMSC) (n=3)				Alginate Beads (Bovine Chondrocytes) (n=4)				Scaffold free chondrocyte pellets (Bovine Chondrocytes) (n=3)			
	Ts	Tf	ω_Q	$\omega_0\tau_c$	Ts	Tf	ω_Q	$\omega_0\tau_c$	Ts	Tf	ω_Q	$\omega_0\tau_c$
Week 0	44.5 ± 5.6	2.09 ± 0.32	211 ± 190	4.98 ± 0.26	16.3 ± 1.0	0.51 ± 0.04	829 ± 459	6.15 ± 0.16	-	-	-	-
Week 1	55.0 ±14.8	1.65 ±0.55	*	6.30 ± 0.71	15.2 ± 0.7	0.60 ± 0.04	*	5.45 ± 0.12	73 ± 35.6	12.0 ± 4.6	*	2.42 ± 0.49
Week 2	51.1 ± 11.1	1.4 ± 0.37	*	6.69 ± 0.58	14.8 ± 1.2	0.29 ± 0.05	2601 ±1357	7.84 ± 0.39	49 ± 1.33	15.5 ± 0.33	9.6 ± 13.2	1.26 ± 0.03
Week 3	36.0 ± 10.6	2.2 ± 0.68	*	4.63 ±0.47	16.4 ± 1.2	0.44 ± 0.04	*	6.75 ± 0.20	-	-	-	-
Week 4	41.0 ± 7.0	2.37 ± 0.43	*	4.65 ±0.29	17.8 ± 1.0	0.50 ± 0.03	628 ±458	6.5 ± 0.14	-	-	-	-
Human Cartilage	18.66 ± 1.08	0.32 ± 0.03	2463 ± 798	8.42 ± 0.24								

Table 1: The fast (T_f) and the slow (T_s) relaxation times (ms), residual quadrupolar coupling ω_Q (Hz), and motional parameter, $\omega_0\tau_c$, for native and engineered cartilage tissues. * -Values not included because of high standard error.

Acknowledgements: This study was supported by the NIH/NIBIB grant EB007537. We acknowledge the use of UIC's Chemistry department's NMR facility and the technical support provided by its director Dr. Dan McElheny. We thank Dr. Sriram Ravindran, Dr. Anne George and Dr. Thomas Schmid for their contribution of engineered tissue samples. We thank Charis Merrihew at Articular Engineering (http://articular.com/) for assistance in processing the human explants.

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