Bi-exponential Characterization of T2 Relaxation Decay in an Engineered Osteogenic Tissue Model

H. Xu[1], S. F. Othman[1], L. hong[1,2], R. L. Magin[1]

[1]Bioengineering, University of Illinois at Chicago, Chicago, IL, United States, [2]Orthodontics, University of Illinois at Chicago, Chicago, IL, United States

Introduction

Each year millions of patients confront musculoskeletal tissue loss due to surgery, disease, or trauma. Tissue engineering utilizing autologous progenitor cells promises to address this need by the development of biological substitutes to repair damaged tissues. Magnetic resonance imaging (MRI) is widely used to analyze and evaluate tissue degeneration and regeneration in clinical settings. Magnetic resonance microscopy (MRM), typically with spatial resolution of less than 100 microns, makes it feasible to visualize and quantify microscopic changes in biophysical/biochemical properties associated with tissue regeneration. In this work, 11.74 T magnet was used to characterize the T_2 relaxation in engineered osteogenic tissues over a 4-week period of *in vitro* incubation. Mono-exponential and bi-exponential characteristics of T2 relaxation time were investigated during the development of engineered osteogenic tissues. We found that mono-exponential T_2 decay is not sufficient to describe the fundamental change inside the engineered osteogenic tissue since the fitting error increases with the development of osteogenesis. Comparison between mono- and bi-exponential models indicates the separation of the T_2 relaxation time into a fast and a slow component is more representative for studying osteogenesis.

Materials and Methods

Mesenchymal stem cells isolated from fresh adult human bone marrow were provided commercially. Nucleated cells were incubated in a basic culture medium composed of Dulbecco's Modified Eagle's Medium supplemented with 10 % fetal bovine serum, 1 % antibiotics at 37 °C and 5 % CO_2. A biodegradable and biocompatible sterile gelatin sponge (Pharmacia & Upjohn, Kalamazoo, MI) was trimmed into 4 mm cubes for use as the biological scaffold. Tissue engineered constructs were generated by seeding the gelatin cubes with MSCs at a density of 10^6 cells/ml. The cell-seeded constructs were subsequently transferred to a petri dish and divided into two groups: experimental and control. The experimental group was cultured in an osteogenic culture medium to stimulate the osteogenic differentiation. The osteogenic culture medium was created by adding 100 nM dexamethasone, 10 mM β-glycerophosphate, and 0.05 mM ascorbic acid-2-phosphate to the basic culture medium. The control group was cultured in only the basic culture medium. Both groups were allowed to grow *in vitro* for 4 weeks. MRI experiments were conducted at 11.74 T (500 MHz for protons) using a 56 mm vertical bore magnet (Oxford Instruments, Oxford, UK) and a Bruker DRX Avance Spectrometer (Bruker Instruments, Billerica, MA) controlled by a Silicon Graphics SGI O2 workstation (Mountain View, CA). MR images were acquired using a Bruker Micro 5 imaging probe with triple axis gradients (maximum strength 200 G/cm) and a 5 mm diameter RF saddle coil was used. Osteogenic stimulated and control tissue constructs (sample size n = 7, respectively) were studied at five growth stages (i.e., week 0, 1, 2, 3, and 4). The spin-spin relaxation time (T_2) was measured by applying a spin echo imaging sequence to acquire 32 echoes with a 7 msec echo spacing (TE) from the chosen axial slice (repetition time TR = 4 sec, TE = 7 msec, matrix = 128 x 128, and number of averaged experiment = 1). The experimental data were collected from a specific region of interest localized in the center of each sample. The T_2 were modeled using both a mono-exponential fitting and a bi-exponential fitting of SNR-TE for osteogenic and control constructs. The mono-exponential decay was fit by $SNR = SNR_0 e^{-TE/T_2}$ while the bi-exponential decay by $SNR = A_1 e^{-TE/T_{2f}} + A_2 e^{-TE/T_{2s}}$, where T_{2f} and T_{2s} are the extracted "fast" and "slow" T_2 relaxation components while A_1 and A_2 indicate the amplitude coefficients of two compartments [1].

Results

Figure 1 shows bi-exponential fitting graphs of T_2 for both control and osteogenic constructs from week 0 to week 4. Notice that the mono-exponential fitting error increases with the development the osteogenic tissue from week 0 to week 4. The quantitative mono - exponential T_2 relaxation times as well as the bi-exponential parameters of "fast" compartment T_{2f} and "slow" compartment T_{2s} are shown in Table 1.

Mono- and Bi-exponential Fitting			Week 1		Week 2		Week 3		Week 4	
Control	Bi-exp	T_{2f}/T_{2s} (msec)	70	138	80	121	72	136	80	138
		Amplitude (%)	86	14	94	6	79	21	92	8
	Mono-exp		78		82		84		83	
Osteogenic	Bi-exp	T_{2f}/T_{2s} (msec)	52	158	43	165	30	175	28	178
		Amplitude (%)	83	17	85	15	84	16	85	15
	Mono-exp		67		58		49		44	

Table 1. The mono-exponential T_2 relaxation times and bi-exponential parameters for "fast" compartment T_{2f}, "slow" compartment T_{2s}, and the corresponding amplitude for each compartment from week 1 to week 4.

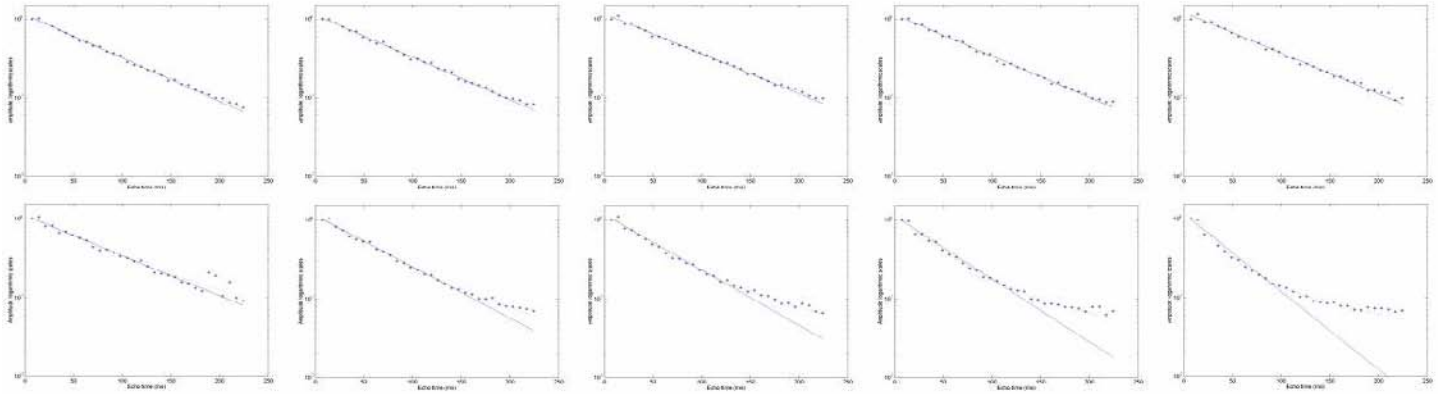

Figure 1. T_2 mono-exponential and bi-exponential fitting for control (top) and osteogenic (bottom) tissues at week 0, 1, 2, 3, 4 (left to right). In each figure, stars indicate the experimental data, dashed line denotes bi-exponential fitting while real line denotes mono-exponential fitting result.

Discussion and Conclusion

This study examined the T_2 relaxation time accompanying with osteogenic differentiation. The bi-exponential analysis shows the dependence of the T_2 relaxation time on both fast and slow compartments for the osteogenic constructs. In contrast, a relatively large fraction of the signal decays with a short T_2 relaxation time, with only a small fraction decaying with a long T_2 relaxation time, which results in a good approximation of using mono-exponential model to examine the T_2 relaxation characteristic for control constructs. Above result indicates the separation of the T_2 relaxation time into a fast and a slow component (bi-exponential) is more representative for studying engineered osteogenic tissues.

References: [1] Ababneh Z *et al* 2005 Magnetic Resonance in Medicine 54: 524-531