MR Characterization of Osteogenic Tissue Engineered Constructs

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INTRODUCTION: Bone defects often result in disfiguration and disabilities. Large size bone defects that cannot self-heal require clinical procedures. Tissue engineering combined with life science, medicine, and engineering techniques provides a new strategy to restore bone defects. Magnetic resonance microscopy (MRM) refers to the application of magnetic resonance in the microscopic domain (< 100 microns). Although MRM continues to be used to study transgenic animal models, it is clear that it also has potential to study engineered tissues both *in vitro* and *in vivo* since the MR signal intensity depends directly on the physical and chemical characteristics of the tissue being imaged. For example, it is well established that the MR parameters (spin-lattice relaxation time T_1, spin-spin relaxation time T_2, and apparent diffusion coefficient ADC) can be spatially localized to provide information from living systems that is not available from other techniques without staining or sacrifice. These biologically relevant parameters can be used by tissue engineers to characterize and optimize cell and tissue growth, and differentiation [Hartman 02].

MATERIALS & METHODS: After isolation from fresh bone marrow (AllCells, Berkeley, CA), human bone mesenchymal stem cells (MSCs) were expanded in a basic DMEM culture medium with 10 % FBS and 1 % antibiotics. Upon reaching 80 % confluence, the cells were trypsinized and sub-cultured with an initial density of 2 x 10^5 cells per 100 mm petri dish as first passage. The human bone MSCs of passage-6 to 8 were used in all preliminary studies [Hong 04]. Gelatins sponges (Pharmacia & Upjohn, Kalamazoo, MI), characterized by high porosity and excellent biocompatibility, were utilized as scaffolds simulating extracellular matrices. Engineered constructs were fabricated by seeding human mesenchymal stem cells onto gelatin (denaturized collagen) sponges. Tissue engineered constructs received either continued exposure to the basic medium or osteogenic differentiation medium, prepared by basic medium supplemented with osteogenic reagents (Dexamethasone, β-glycerophosphate and ascorbic acid).

All MR experiments were conducted at 500 MHz in a 56-mm vertical bore 11.74 T magnet equipped with Bruker DRX Avance spectrometer. Engineered tissue samples were loaded into a 5-mm diameter saddle coil and inserted into the Bruker Micro5 imaging probe (linear triple axes gradients with maximum of 200 Gauss/cm). MR parameters, i.e., T_1 relaxation time, T_2 relaxation time, and ADC were measured in two cases. The first is progressive monitor over a 4-week period tissue development for both the control and osteogenic constructs. The other one is the osteogenic constructs with three different cell-seeding densities. T_1 values were calculated by fitting saturation recovery curve, utilizing a series of spin echo sequences with minimal TE value and variable TR values: 50 ms, 100 ms, 200 ms, 500 ms, 1000 ms, 2000 ms, and 4000 ms. T_2 values were measured using spin echo sequence with 32 echoes ranging from 7 ms to 224 ms and TR 1000 ms. ADC values were measured using a spin echo diffusion weighted imaging pulse sequence with variable "b" values corresponding to 0 to 30 Gauss/cm. Histochemical changes were examined in histological section for selected tissue engineered constructs. Alkaline phosphatase (ALP) is one of the routinely morphological expressions of cell and tissue specific proteins during osteogenesis. Upon Day-5, ALP activities were measured for three osteogenic constructs prepared with different densities (5 x 10^5, 10^6, 5 x 10^6 cells/ml) of bone MSCs seeded on gelatin sponges by catalyzing ALP reagent p-nitrophenyl-phosphate [Hong 04].

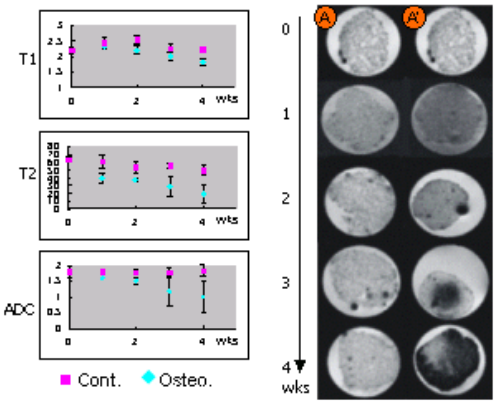

Figure 1: Left is the bar chart description of variations of MR parameters, T_1 (s), T_2 (ms), and ADC ($10^{-3}mm^2/s$). Right is the time course of high resolution MR axial images for control (A) and osteogenic (A') constructs with TR / TE = 1000 / 60 ms; FOV = 0.8 cm; slice thickness = 0.5 mm; in-plane resolution = 62.5 µm x 62.5 µm; NEX = 8.

RESULTS: The consecutive changes of tissue engineered constructs over 4-week tissue development were examined through both MR images and variations of MR parameters, as shown in Figure 1. Compared to the control group, MR images of osteogenic constructs become denser and denser at each time period. At the same time, the MR parameters, including T_2 and ADC, significantly decrease. Upon 4-week differentiation, the histological examination was conducted and the construct exposed to osteogenic medium was stimulated to initiate the osteogenesis, evidenced by black-stained mineral nodules observed in histological section by von Kossa staining, as shown in Figure 2. ALP activities were measured for three osteogenic constructs prepared with different densities of bone MSCs seeded on gelatin sponges. Good correlation of MR parameters and ALP activities was found, as shown in Figure 3.

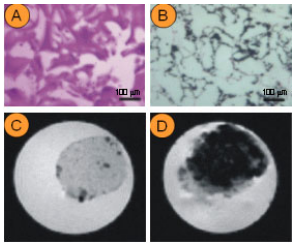

Figure 2: MR images and histological examinations for tissue engineered constructs upon 4-week differentiation. The control (A and C) and osteogenic constructs (B and D) show no specific changes and significant osteogenesis, respectively.

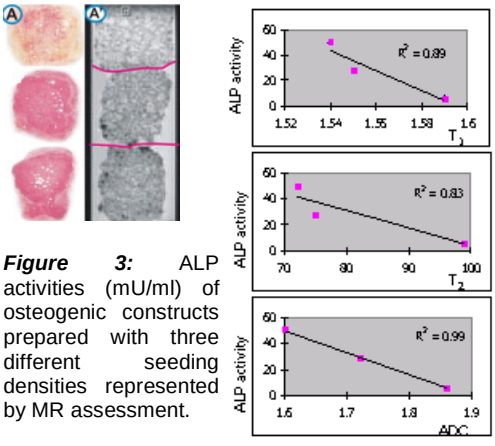

Figure 3: ALP activities (mU/ml) of osteogenic constructs prepared with three different seeding densities represented by MR assessment.

CONCLUSION & DISCUSSION: Our preliminary data demonstrate progressive reduction in both the relaxation times and ADC with time of incubation for MSC-derived osteogenic constructs, correlating well with MRI image contrasts. Good correlation between the histochemical observation and MR parameters suggests that MRM can effectively evaluate and visualize *in vitro* osteogenic constructs. In addition, MRM is capable of identifying difference in cell density of tissue engineered constructs, which correlates well to the ALP activity measured by biochemistry technique. This work strongly supports the hypothesis that biochemical cascades during osteogenesis of human bone MSCs could be indirectly reflected by MR images and assessment, indicating that MR technique could be a non-invasive monitoring tool for osteogenic differentiation of tissue engineered construct *in vitro.* This hypothesis still needs to be confirmed through more extensive studies both *in vitro* and *in vivo* for future clinical applications.

REFERENCES: Hartman et al. Tissue Engineering, 2002 (8): 1029. Hong et al. IADR, 2004

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