Monitoring the Formation of Tissue-Engineered Cartilage in Scaffold-Free Pellet Culture Using Magnetic Resonance Imaging

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Introduction: Proteoglycan (PG) and collagen are the major biochemical markers for tissue-engineered cartilage [1]. In order to noninvasively optimize the production of engineered tissue with appropriate properties for implantation, MRI is under active investigation for monitoring the development of these markers in tissue regeneration [2-4]. One approach to regenerating cartilage involves *in vitro* culturing chondrocytes in a scaffold-free pellet culture system [5]. In this study, we used MRI to evaluate the scaffold-free chondrocyte pellets over a 4-week period, and observed the distinct changes in histograms of T_2, T_1, $T_{1\rho}$, and apparent diffusion coefficient (ADC). Single-peaked distributions of the MR properties developed in the first week, which became clearly double-peaked starting at week 3. These results were correlated with the biochemical determination of PG and collagen accumulated in the pellets. Our findings suggest that MRI could be used to monitor the accumulation of PG and collagen in regenerated cartilage.

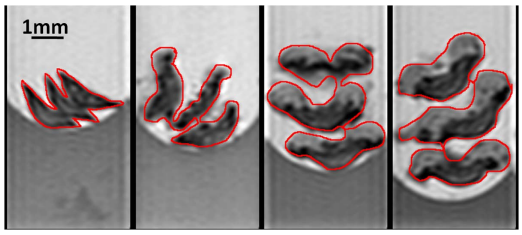

Fig. 1. T2W images of pellets cultured at week 1, 2, 3 and 4; the in-plane resolution was 125 μm, and slice thickness was 1 mm. Red lines indicate ROIs selected. The MR parameters (T_2, $T_{1\rho}$, T_1, and ADC) were calculated for each pixel in ROIs.

Materials and methods: Bovine chondrocytes were harvested according to a well-established protocol [6]. Each chondrocyte pellet was formed by centrifuging 5×10^5 chondrocytes in tissue culture medium at 1000*g* for 10 min. The pellets were cultured for 4 weeks. Each week, three pellets were stacked in layers and placed into a 5 mm NMR tube with media for MRI study (Fig. 1). All MRI experiments were performed on a Bruker DRX-500MHz Avance Spectrometer/56-mm vertical bore magnet/5-mm RF saddle coil system. T_2 was acquired using a CPMG imaging sequence modified to eliminate the diffusion losses by placing the bipolar read-refocusing gradient pair after the 180º pulse [7]. $T_{1\rho}$was obtained using a preparatory pulse cluster with self compensation (90_x-(spin-lock)$_y$-(spin-lock)$_{-y}$-90_{-x}) followed by a readout fast spin-echo (SE) sequence to minimize B_1 and B_0 field imperfections [7]. T_1 was acquired using a saturation recovery sequence. ADC was obtained using a diffusion-weighted SE sequence. The PG and collagen accumulation in pellets was determined using DMB (dimethylmethylene blue) dye binding and hydroxyproline assays, respectively [6].

Results: Tissue heterogeneity was observed in the pellets during the 4-week culture period (Fig. 1). In the first week of growth, the pellets had conical shapes with smooth boundaries, and appeared relatively homogenous. Subsequently, the pellets grew into amorphous shapes at week 2. After three weeks of growth, the pellets appeared involuted with regions by hyper- and hypo- signal intensities in T2W images. Tissue volume increased greatly from week 1 to week 4. In order to quantify the changes in tissue composition and components, histograms of T_2, $T_{1\rho}$, T_1, and ADC were derived from quantitative MR measurements (Fig. 2). The area, position and width of the peaks represent tissue volume, averaged MR values (see Table 1) and variability in the MR parameters. The tissue heterogeneity is reflected in the distributions of all MR parameters: a single peak at week 1, a right-skewed peak at week 2, and two peaks at week 3. From week 1 to week 4, the area of the peaks increased, representing an increase in tissue volume for the developed components. The results of biochemical measurements are presented in Fig. 3. The ratio of collagen/PG content accumulated in pellets increased from 0.21 at week 1 to 0.46 at week 3, and to 0.47 at week 4.

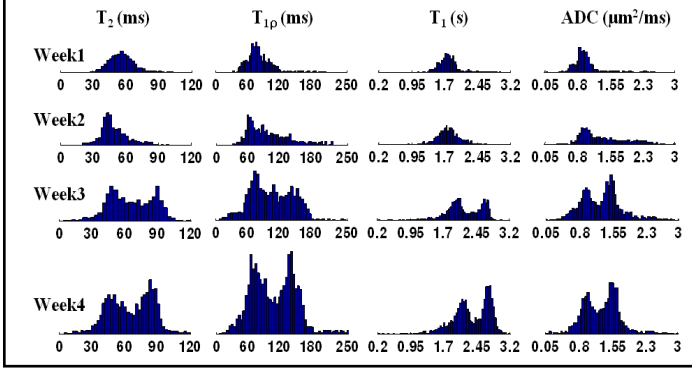

Fig. 2. Histograms of T_2, $T_{1\rho}$, T_1 and ADC during development. Acquisition parameters: T_2 (TR = 5 s, TE = 5.8 – 371 ms, 64 echoes), $T_{1\rho}$ (TE/TR = 7.5 ms/ 5 s, TSL = 10, 20, 40, 80, 120, 240 ms, spin-lock frequency = 500 Hz), T_1 (TE = 11.45 ms, TR = 104 ms – 10 s, 16 steps), and ADC (TE/TR = 25 ms/ 6 s, δ/Δ = 3/18 ms, b = 0, 91, 234, 443, 717, 1057, 1463 s/mm^2).

Table 1. The averaged MR parameters (n = 3)

	T_2 (ms)		$T_{1\rho}$ (ms)		T_1 (s)		ADC (μm^2/ms)	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
Week 1	61 ± 3	N/A	93 ± 4	N/A	1.85 ± 0.06	N/A	0.979 ± 0.014	N/A
Week 2	47 ± 2	N/A	79 ± 4	N/A	1.80 ± 0.04	N/A	0.975 ± 0.050	N/A
Week 3	50 ± 1	89 ± 2	79 ± 4	154 ± 6	1.93 ± 0.08	2.59 ± 0.06	0.963 ± 0.025	1.523 ± 0.022
Week 4	50 ± 1	84 ± 3	77 ± 2	140 ± 3	2.14 ± 0.02	2.72 ± 0.05	0.966 ± 0.050	1.565 ± 0.031

Discussion and conclusions: We show that the second peak developed at week 3 in the MR data corresponds to a time when biochemical assays revealed a large increase in collagen. The peak shifting observed in the MR data is also consistent with the increase of the collagen/PG ratio in the pellets during culturing. Further analysis will focus on determining whether the PG and collagen contents accumulated in the pellets are representative of those present in native articular cartilage. Although more biochemical assays are needed, this study suggests that MRI could be used to assess specific biochemical properties of engineered cartilage.

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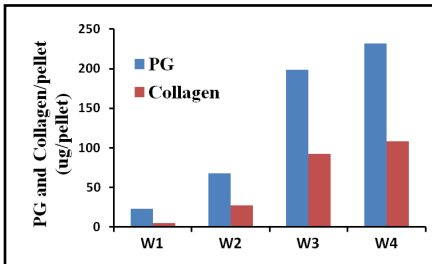

Fig. 3. Accumulation of proteoglycan (PG) and collagen in one pellet (n = 1) over 4 weeks