Quantitative Magnetization Transfer Imaging for Evaluating the Tissue-engineered Cartilage

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INTRODUCTION

Arthritis and degenerative diseases of cartilage affect millions of Americans [1]. Tissue-engineered cartilage is a promising approach for cartilage regeneration and repair [2]. Magnetization transfer imaging (MTI) has been used for the evaluation of tissue-engineered cartilage [3,4]. However, the parameter most often used in these studies - magnetization transfer ratio (MTR) - is highly dependent on the imaging conditions. The Bound Proton Fraction (BPF), a parameter derived from quantitative magnetization transfer imaging (QMTI) [5], directly reflects the percentage of macromolecules in the total extracellular matrix (ECM), and hence is a more objective parameter than MTR. For example, BPF was used recently in the diagnosis of Multiple Sclerosis [6]. To our knowledge, however, there has been no report on the use of BPF to monitor the development of tissue-engineered cartilage, e.g., Glycosaminoglycans (GAGs); therefore, we performed a series of experiments on tissue-engineered cartilage to seek a correlation between BPF and cross-relaxation rate (k), and other conventional MRI parameters: T1, T2, and ADC, as well as direct biochemical methods.

MATERIALS AND METHODS

Sample Preparation Tissue-engineered constructs were generated by seeding gelatin cubes with mesenchymal stem cells (MSCs) [7]. The constructs then were divided into two groups: treatment and control. One group was cultured in chondrogenic differentiation medium, while the other group was cultured only in basic DMEM medium. The constructs were examined using MRI every seven days over a three-week period.

MRI Experiments All MR experiments were conducted using a 56-mm vertical bore 11.74 T Bruker Avance imaging spectrometer with a micro-imaging gradient insert with a maximum gradient strength of 200 G/cm and a 5-mm diameter saddle coil (Bruker Instruments). MTI was performed using the spoiled 3D MT-GRE pulse sequence with the following parameters: TR/TE/α=36ms/1.9ms/10°; FOV = 8.0×8.0mm; slab thickness = 2.0mm; 3D matrix = 128×128×16; NEX = 1. A Gaussian RF pulse with peak power of 25 μT was used as the MT pulse. The MT-weighted images were acquired for offset frequencies Δ = 1, 1.5, 2, 3, 4, 6, 8, 12, 15, 20, 25, 31, 37, 43, 50 kHz. A water cooling system was used to keep the gradient temperature between 25 and 30 ℃. The T1, T2, and ADC for the samples were also measured [8].

Biochemical Analysis After MRI analysis, the GAG content of the matrices at each time period was assessed by a modification of the dimethylmethylene blue method [9]. The matrices were digested in 1 ml of protease (2.5 mg/ml; Type XIV Bacterial from Streptomyces Griseus, Sigma) in TBS solution in a 55 ºC water bath overnight. A 100 μl aliquot of the digest was assayed for total GAG content by adding 2 ml of 1,9-dimethyl methylene blue dye solution (Polyscience Inc., Northampton, UK). Absorbance at 535 nm was determined with a spectrophotometer (LKB Biochrom Ultraspec).

Data Processing A specific region of interest was localized in the treatment and control samples. T1, T2, and ADC were calculated using a least squares single exponential fitting model implemented in MATLAB 7.1. BPF and k were extracted with a program written using Henkelman's two-pool model [5]. The results of MRI data and biochemical data are reported as the mean ± std. A student *t* test was performed between data from the control and the treatment groups. Statistical significance was defined as $p = 0.05$.

RESULTS

Fig.1. shows MT-weighted MR images of the constructs cultured in the three-week period. The difference between the control and treatment construct at each stage is shown. The treatment group has a lower intensity in the images after the first week. Also, after one week in the culture, there are significant differences ($p<0.05$, $n=6$) between control and treatment groups for the MRI parameters: T1, T2, ADC, BPF, and k, as well as the GAG content determined by biochemical analysis (Fig. 2.). The BPF and k of the constructs cultured in the chondrogenic differentiation medium (treatment) showed a significant difference ($p<0.05$, $n=6$) at all stages of development (Fig.2. B & C). The BPF and k of the constructs cultured in the chondrogenic differentiation medium (treatment) were highly correlated (0.98 and 0.93, respectively) with the increase of GAG content in the constructs.

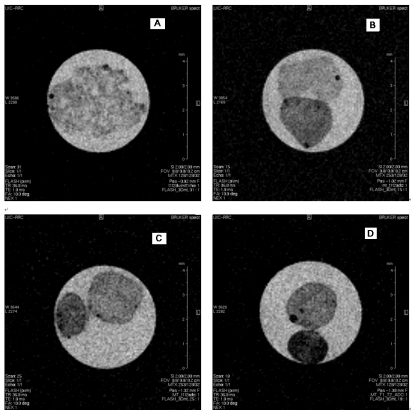

Fig.1. MT-weighted images of the constructs in the three-week period. The offset frequency of MT pulse is 2000 Hz. A. MR Image of one construct after MSCs were seeded and before being cultured in different media (week 0). B. C. and D are MR images of constructs after one week, two weeks, and three weeks in culture, respectively.

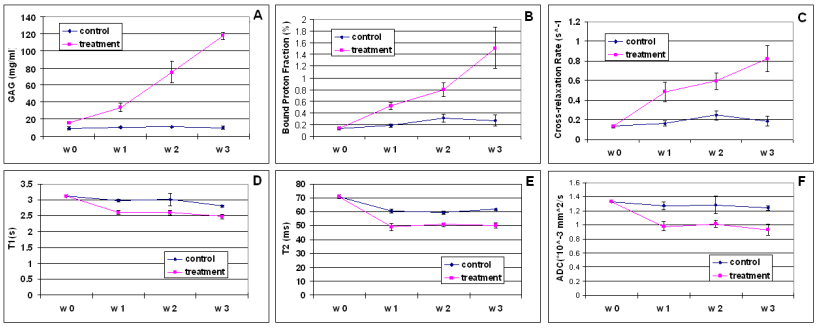

Fig.2. Changes of GAG, BPF, k, T1, T2, and ADC during the three-week growth period. A. GAG content measured by biochemical analysis. B. bound proton fraction (BPF) from MTI; C. cross-relaxation rate (k) from MTI; D. longitudinal relaxation time (T1); E. transverse relaxation time (T2); F. apparent diffusion coefficient (ADC);

DISCUSSION AND CONCLUSIONS

The small changes observed in the MRI parameters measured during the growth of tissue-engineered cartilage (T1, T2, and ADC), indicate that these parameters will not be effective for monitoring engineered cartilage development. On the other hand, both bound proton fraction (BPF) and the cross-relaxation rate (k) increased substantially during the study (factors 9 and 4, respectively). In addition, the high correlation between BPF and construct GAG content suggests that extracellular matrix (ECM) development can be observed by quantitative magnetization transfer imaging. Therefore, we conclude that BPF can be used as a marker for evaluating the cartilage development process.

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