Magnetization Transfer Imaging Provides a Quantitative Measure of Chondrogenic Differentiation and Tissue Development

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The goal of the present investigation was to test whether quantitative magnetization transfer imaging can be used as a noninvasive evaluation method for engineered cartilage. In this work, we used magnetic resonance imaging (MRI) to monitor the chondrogenesis of stem-cell-based engineered tissue over a 3-week period by measuring on a pixel-by-pixel basis the relaxation times (T_1 and T_2), the apparent diffusion coefficient, and the magnetization transfer parameters: bound proton fraction and cross-relaxation rate (k). Tissue-engineered constructs for generating cartilage were created by seeding mesenchymal stem cells in a gelatin sponge. Every 7 days, tissue samples were analyzed using MRI, histological, and biochemical methods. The MRI measurements were verified by histological analysis, and the imaging data were correlated with biochemical analysis of the developing cartilage matrix for glycosaminoglycan content. The MRI analysis for bound proton fraction and k showed a statistically significant increase that was correlated with the increase of glycosaminoglycan ($R = 0.96$ and 0.87 , respectively, $p < 0.05$), whereas T_1 , T_2 , and apparent diffusion coefficient results did not show any significant changes over the 3-week measurement period.

Introduction

QUANTITATIVE EVALUATION of the growth of tissue-engineered constructs *in vitro* and *in vivo* is one of the unsolved problems in regenerative medicine.^{1,2} Histological and biochemical assessments are the gold standards for *ex vivo* evaluation, but they are destructive to cells and living tissues. In addition, microscopic techniques, such as scanning electron microscopy and confocal microscopy, are dependent on light penetration into the material; therefore, they can only provide information from the uppermost regions of tissue constructs. Magnetic resonance imaging (MRI), on the other hand, can noninvasively provide information about central regions of tissue constructs with high spatial resolution ($<100\mu\text{m}$).³ Further, the wide range of contrast mechanisms available in MRI (i.e., relaxation, diffusion, and magnetization exchange) makes it the modality of choice for many soft tissue imaging applications.

Magnetization transfer (MT) is a dynamic physical process that can probe the subpopulations of water protons in cells and tissues that are bound to macromolecules. Normally, such water protons have very short T_2 values ($<1\text{ ms}$), which are out of reach of the shortest echo times (TE) available in most MRI sequences. Nevertheless, MT imaging (MTI) can

provide estimates of the relative sizes of subvoxel water compartments. However, the traditional MT ratio (MTR) measurements depend on many factors (e.g., the details of the pulse sequence, and assumptions about the compartments) that are not always under the control of the investigator, as discussed in a recent literature.⁴ In addition, from the early literatures, while it was noted that MTR can be used to calculate the pseudo-first order exchange rate, k^* , this calculation is based on the condition that the semisolid pool is fully saturated.^{5,6}

The MT parameters determined by the quantitative MTI (qMTI) model used in this study are not limited by this assumption. qMTI is based on a two-pool model for the bound and free populations of water in tissue. In qMTI, all of the relaxation and exchange rates of the free water and macromolecular protons are individually characterized. Thus, qMTI provides a more direct model of the underlying properties involved in the MT process. In qMTI, the exchange of spin polarization between the pools of water allows the calculation of MT parameters, such as the bound proton fraction (BPF), the rate of MT exchange (k), and the transverse relaxation time of macromolecular spins (T_{2b}). These parameters change in concordance with the intrinsic properties of tissue (hydration, cellularity, porosity, tortuosity,

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etc.) that undergo remodeling during growth and with the onset of disease. One of the qMTI parameters, the BPF (the ratio of the number of bound macromolecular protons to the free water protons), is independent of pulse sequence details.⁴ The qMTI technique has been applied recently in the diagnosis of multiple sclerosis^{7,8} and Alzheimer disease,⁹ and is now being explored for the characterization of engineered tissues, such as cartilage.¹⁰

The two major macromolecular components of cartilage are collagen (CG) and proteoglycan (PG). Therefore, changes in the absolute or relative amounts of CG and PG in different layers of cartilage or in each layer with time can be expected to reflect the biochemical and biomechanical status of engineered cartilage. Over the past decade, MRI has been increasingly used to monitor tissue-engineered constructs,^{11–17} with a great deal of research directed at correlating the CG and PG content with the T_1 and T_2 relaxation times,¹⁸ the apparent diffusion coefficient (ADC),¹⁸ the fixed charge density,^{15,19} and the MTR.^{3,18} However, to our knowledge, there has not yet been a report applying the qMTI technique to assess the early growth and development of tissue-engineered cartilage.

In this study, we monitored the growth over a 3-week period of tissue-engineered cartilage constructs using MRI to test the feasibility of applying the qMTI technique to evaluate the early growth and development of tissue-engineered cartilage by seeking a correlation between the qMTI parameters (BPF and k) and established histological and biochemical markers of chondrogenic tissue development.

Materials and Methods

Sample preparation

Mesenchymal stem cells (MSCs), isolated from fresh adult human bone marrow obtained from AllCells LLC (Berkeley, CA), were plated at a density of 10^7 nucleated cells per 100-mm-diameter Petri dish. The cells were incubated in a basic medium of Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 100 U/mL penicillin, 100 mg/mL streptomycin, and 0.25 μ g/mL amphotericin B (Sigma) at 37°C and 5% CO₂. MSCs were isolated by removing unattached cells at the first medium exchange. The MSCs were incubated and proliferated until 80% confluence, and then trypsinized and sub-cultured as passage 1 with an initial density of 2×10^5 cells per dish. A biodegradable and biocompatible sterile gelatin sponge (Pharmacia & Upjohn, Kalamazoo, MI) was trimmed into $4 \times 4 \times 4$ mm cubes for use as the biological scaffold. Tissue-engineered constructs were generated by seeding gelatin cubes with MSCs at a density of 10^6 cells/mL with the help of a slight vacuum created using a 20 mL syringe.²⁰ One day after cell seeding, the seeded constructs were divided into two groups: stimulated and control. The medium for control group was DMEM (Sigma) with 10% fetal bovine serum and 1% antibiotic-antimycotic. The medium for stimulated group was HG-DMEM (Sigma), 1% antibiotic-antimycotic, 10 ng/mL transforming growth factor- β 1, 1% insulin-transferrin-sodium selenite (ITS) supplement, 50 μ g/mL ascorbate 2-phosphate, and 100 nM of dexamethasone.^{21,22} Both groups were allowed to grow *in vitro* in the incubator for 3 weeks during this study and samples were taken weekly for histological and MRI analysis.

MRI experiments

One day after cell seeding (before grouping), one of the tissue constructs was scanned to produce the week 0 MR images. Subsequently, the tissue-engineered constructs (stimulated and control groups of six each) were examined using MRI every 7 days over the 3-week period of tissue growth. After MRI scanning, each sample was fixed for further analysis.

All MR experiments were conducted in a 56-mm-diameter vertical bore 11.74 T magnet using a Bruker Avance imaging spectrometer with a microimaging gradient insert (maximum gradient strength of 200 G/cm) and a 5-mm-diameter saddle coil (Bruker BioSpin, Billerica, MA). For the qMTI measurements, a spoiled 3D MT-GRE pulse sequence²³ was applied with the following parameters: repetition time (TR)/TE/flip angle (FA) = 36 ms/1.9 ms/10°; field of view (FOV) = 8.0 $\times$ 8.0 mm; slab thickness = 2.0 mm; 3D matrix = 128 $\times$ 128 $\times$ 16; number of excitation (NEX) = 1. A Gaussian RF pulse (peak power = 25 μ T, pulse length = 20 ms, and bandwidth = 137.0 Hz) was used as the MT presaturation pulse. The MT-weighted images were acquired at offset frequencies of 1, 1.5, 2, 3, 4, 6, 8, 12, 15, 20, 25, 31, 37, 43, 50 kHz. A water cooling system was used to maintain the temperature of sample and gradient coils between 25°C and 30°C. The conventional MR parameters (T_1 , T_2 , and ADC) for each sample were measured on a single slice of 0.5 mm thickness, with field of view of 8.0 $\times$ 8.0 mm and in-plane resolution of 62.5 $\times$ 62.5 μ m. For the T_2 measurements, a Carr-Purcell-Meiboom-Gill (CPMG) sequence²⁴ of 16 echoes was used with TR = 4 s and TE = 7.2–115.2 ms with an echo interval of 7.2 ms. For the T_1 measurements, a saturation recovery spin echo sequence was used with TE = 10 ms and 16 TRs from 100 ms to 10 s. The ADC was measured using a diffusion-weighted spin echo (DW-SE) sequence²⁵ with a single diffusion gradient on readout direction. The DW-SE parameters were TR/TE = 1500 ms/30 ms, Δ = 18 ms, δ = 3 ms, and 16 b values (b = 201–4914 s/mm²).

Biochemical analysis of glycosaminoglycan

The glycosaminoglycan (GAG) content in each of the constructs was assessed by a modification of the dimethylmethylene blue method.^{26–29} For each sample, the entire construct was digested in 1 mL of protease (2.5 mg/mL; Type XIV Bacterial from *Streptomyces griseus*; Sigma) in tris-buffered saline in a 55°C water bath overnight. A 100 μ L aliquot of the digest was assayed for total GAG content by addition of 2 mL of 1,9-dimethyl methylene blue dye solution (Polyscience, Northampton, United Kingdom). Absorbance at 535 nm was determined with a spectrophotometer (LKB Biochrom Ultraspec; LabX, Kent City, MI).

Histology for GAG and immunohistochemistry for type II CG

Samples at each growth stage were fixed in 10% formalin for 4 h at 4°C, dehydrated, cleared, embedded in paraffin, and sectioned at 5 μ m. Sections were stained with Safranin O (ScyTek Laboratories, Logan, UT) to identify GAG in the extracellular matrix (ECM), and then with hematoxylin (Biogenex, San Ramon, CA) to label the nuclei of cells in the constructs. To stain type II CG, the sectioned samples were incubated with rabbit anti-CG type II antibody (Cedarlane

Labs, Burlington, NC). The secondary antibody (anti-rabbit IgG, Vectastain ABC kit) was applied, and color was developed using the Vectastain ABC reagent and diaminobenzidine (Vector Laboratories).

Image analysis

Image postprocessing was carried out using Matlab 7.1 (MathWorks, Natick, MA). A specific region of interest (ROI) was drawn in each image of the stimulated and control samples. The T_1 , T_2 , and ADC of the ROIs were calculated using a least squares single exponential fitting model in pixel by pixel and then averaging over the ROIs. The qMTI parameters, BPF and k , were determined using a program written assuming the two-pool model with exchange.³⁰

Statistical analysis

Both the MRI data and biochemical data are reported as the mean $\pm$ standard deviation. A Student's t -test was performed comparing data from the control and stimulated groups. A one-way analysis of variance was performed to

compare the means measured at each week from stimulated and control groups, respectively, over the 3-week period of chondrogenic differentiation. Pearson's correlation analysis was applied to assess the linear relationship between each set of MRI parameters and the corresponding measurements of GAG content. Statistical significance was defined as $p < 0.05$.

Results

MRI measurements

Representative examples of MT-weighted MR images for the constructs cultured over the 3-week period are shown in Figure 1. Differences in intensity are clearly seen between the constructs grown in the differentiation medium (stimulated) and those cultured in the basic medium (control) at each stage. The stimulated group has a lower intensity across the images after the first week when compared with the corresponding control group. For the growth-stimulated group, the signal intensity of the MT-weighted images decreased uniformly over the 3-week experiment, whereas less decrease was found in the control group. The overall size of the

FIG. 1. Magnetization transfer-weighted images of the constructs cultured over a 3-week period, with an in-plane resolution of $62.5 \times 62.5 \mu\text{m}$ and a slice thickness of $125 \mu\text{m}$. The offset frequency of the magnetization transfer pulse is 2 kHz. (A) Magnetic resonance image of a single construct after seeding with mesenchymal stem cells but before culture (week 0). (B–D) Magnetic resonance images of pairs of constructs after 1, 2, and 3 weeks in culture, respectively. The growth-stimulated constructs (arrows) have lower overall image intensities compared with the control.

constructs in the stimulated group was typically smaller than the size observed in the control group throughout the 3-week culture period (Fig. 1B–D).

Histological and biochemical analysis of GAG and immunohistochemistry for type II CG

The stimulated group stained positive for both GAG and CG at each sample time point over the 3-week culture period (Figs. 2 and 3). The intensity of the stain was uniform over the entire construct. In addition, a continuous increase in the GAG density was observed in the stimulated group (Fig. 2B, D, F), but not in the control group (Fig. 2A, C, E). As shown in Figure 3, at both weeks 1 and 3, CG type II exhibited a higher staining intensity in the stimulated group compared with the control group. The GAG content, determined by biochemical assay, was also observed to undergo a progressive increase in the stimulated group over the 3-week

period, whereas no significant changes were found in the control group, as shown in Figure 4A.

Comparison of MRI parameters with biochemical measurements

After 1 week in culture, significant differences (Student's *t*-test, $p < 0.05$, $n = 6$) were found between control and stimulated groups for all the MRI parameters (Fig. 4B–F). However, between weeks 1 and 3, only the MT parameters continued to change in a pattern correlated with cartilage tissue development. For example, the BPF of the constructs cultured in the chondrogenic differentiation medium (stimulated) increased from $0.50\% \pm 0.09\%$ at week 1 to $1.06\% \pm 0.57\%$ at week 3 and k increased from 0.46 ± 0.12 to $0.65 \pm 0.24 \text{ s}^{-1}$, whereas no significant changes were found between weeks 1 and 3 for T_1 (from 2.46 ± 0.27 to $2.37 \pm 0.17 \text{ s}$), T_2 (from 54.18 ± 5.07 to $53.04 \pm 3.57 \text{ ms}$), and

FIG. 2. Safranin-O staining for GAG and hematoxylin staining for cell nuclei. (A, C, E) Sections of control samples for weeks 1, 2, and 3, respectively. (B, D, F) Sections of the corresponding stimulated samples. The tissue blocks were sectioned with a slice thickness of 5 µm, and photographed under a light microscope (magnification, 40×). GAG, glycosaminoglycan. Color images available online at www.liebertonline.com/ten.

FIG. 3. Collagen type II immunohistochemistry at weeks 1 and 3. (A, C) Sections of control samples at weeks 1 and 3, respectively. (B, D) Sections of the corresponding stimulated construct samples. The tissue blocks were sectioned with a slice thickness of 5 μm and photographed under a light microscope (magnification, 20 $\times$). Color images available online at www.liebertonline.com/ten.

ADC (from 1.06 ± 0.10 to $0.99 \pm 0.13 \times 10^{-3} \text{ mm}^2/\text{s}$). The BPF and k , as well as the GAG content, of the stimulated constructs showed a significant difference (one-way analysis of variance, $p < 0.05$, $n = 6$) at all stages of development (Fig. 4B, C), whereas no significant changes were found in the control group. The BPF and k of the constructs cultured in the chondrogenic differentiation medium (stimulated) were highly correlated ($R = 0.96$ and 0.87 , respectively) with the increase of GAG content in the constructs over the entire time course of 3 weeks (Fig. 5A, B).

Discussion

Several previous studies have used MRI to assess the growth and development of tissue-engineered cartilage. Potter *et al.*, for example, found a good correlation between MRI parameters (e.g., T_1 , T_2 , ADC, and MT) and the biochemical composition of cartilage constructs grown in a bioreactor.^{18,31} In addition, Miyata *et al.* showed a strong correlation for T_1 and ADC, but not for T_2 , with the equilibrium Young's modulus of engineered cartilage using articular chondrocytes seeded in an agarose gel scaffold.³² Similarly, Neves *et al.* found that the effects of perfusion and nutrient diffusion on cell growth and distribution and matrix production in meniscal cartilage constructs could be assessed using MRI and spectroscopy.^{13,17} Other investigators have used MRI to estimate the matrix fixed charge density of engineered cartilage using gadolinium exclusion. This measurement was found to be highly correlated with the GAG content of the construct.^{15,33} Recently, to improve contrast in MRI, a Food and Drug Administration-approved super-

paramagnetic iron oxide contrast agent (Feridex) was used to label chondrocytes and observe the presence of individual cells in tissue-engineered cartilage constructs.³⁴ Such information is vital to staging and assessing tissue development.

The GAG content of engineered cartilage is a determinant of biochemical and mechanical quality.^{35,36} Kelly *et al.*, for example, found that the aggregate elastic modulus correlated positively with both GAG and CG II content, but not with CG I.³⁵ These investigators concluded that a measure of CG II and GAG content is needed to predict the biomechanical quality of tissue-engineered cartilage. To confirm the development of GAG in our cartilage tissue constructs, we stained samples with safranin O. The staining was positive for GAG in the ECM of the growth stimulated construct, with the intensity of the staining increasing with incubation time (Fig. 2). An identical trend was observed for GAG content as determined by biochemical measurements in the growth-stimulated group (Fig. 4A). Additionally, the measured GAG content increase over the 3-week incubation period was highly correlated with increases in the qMTI parameters (BPF and k) measured in this study (Fig. 5A, B). Therefore, our results demonstrate that normal cartilaginous matrix is being synthesized in the gelatin scaffold seeded with MSCs during the *in vitro* culture.

The correlation of the MT parameters (BPF and k) with GAG content in this study is consistent with reports of others for cartilage using MT techniques.^{37,38} However, this finding is in contrast with the tissue engineering results of Potter with cartilage grown in a hollow-fiber bioreactor.¹⁸ The inconsistency may be due to the scaffold matrixes used in this study, as well as the methods used to extract the MT

FIG. 4. Changes in GAG, BPF, k , T_1 , T_2 , and ADC during the 3-week growth period ($n = 6$ for each group). (A) GAG content measured by biochemical analysis. (B) BPF measured by magnetization transfer imaging. (C) Cross-relaxation rate (k) measured by magnetization transfer imaging. (D) Longitudinal relaxation time measured using MRI (T_1). (E) Transverse relaxation time measured using MRI (T_2). (F) ADC measured using MRI. The error bars indicate ± 1 standard deviation. BPF, bound proton fraction; ADC, apparent diffusion coefficient; MRI, magnetic resonance imaging.

parameters. In our study, a gelatin sponge is used as the scaffold. Gelatin is a denatured CG and a biodegradable scaffold highly suitable for the growth of cells.^{39,40} The contraction of the gelatin constructs with time for both the growth-stimulated and control groups (Fig. 1) demonstrates that the scaffold is experiencing the normal cell-mediated contraction due to the deposition of ECM macromolecules within the scaffold.⁴¹ It has been reported that MT is sensitive to macromolecular structure as well as to

the concentration of the CG II and GAG.³⁷ Further, in mature cartilage, the dependence of MT on GAG content in cartilage might be explained through the modulation of GAG on the intrafibrillar volume of CG.¹⁸ Our results show that the GAG content increased for the stimulated constructs over the culture period, and that the quantity of GAG was always higher in the stimulated group than in the control group (Fig. 4A). Relatively higher GAG content as well as higher type II CG content (shown in Fig. 3) in smaller constructs lead to

FIG. 5. Graphs showing the relationship between qMTI parameters and GAG content of constructs cultured in the chondrogenic differentiation medium (stimulated group) during the 3-week growth period. (A) BPF plotted versus GAG content. (B) Cross-relaxation rate (k) plotted versus GAG content. The correlation coefficient and its p -value are shown in each graph. The error bars indicate ± 1 standard deviation.

smaller intrafibrillar volume, which force more macromolecules interacting with water in a unit volume, resulting in a higher cross-relaxation rate (k), which is what occurs in mature cartilage and appears to be developing in our experiments. It should be noted, however, that the reduced size of the constructs leads to a relatively higher density of CG in the scaffold, which also contributes to the increase in measured MT parameters.^{18,37} It was reported that CG content contributes more than GAG content to MT parameters.³⁷ Whereas the content of type II CG is not quantified in the present study, an increase of type II CG was qualitatively found in the stimulated group from our immunohistochemistry analysis. Therefore, the high correlations of GAG content with BPF and k cannot lead to a conclusion of the unique dependence of qMTI parameters on GAG content. Further studies are needed to quantify the relation of qMTI parameters and CG content in the process of chondrogenic differentiation and cartilage tissue development in gelatin sponge matrix. On the other hand, this higher density of CG and GAG and the high correlation of GAG content with BPF and k proved the sensitivity of qMTI parameters to ECM changes in engineered constructs. Further investigation of qMTI parameters with different scaffolds is ongoing.

In a previous study by Portnoy and Stanis,⁴² it was shown that BPF can be accurately estimated, even with a high degree of approximation, and could be compared directly, regardless of the model that was used to obtain them. It was reported that BPF is an MT parameter that has a true biological meaning (i.e., the relative number of hydrogen protons that are bound to macromolecules) and hence may directly reflect tissue composition.⁴³ On the other hand, the cross-relaxation rate is poorly constrained by the data and depends on the experimental protocol, as well as the model used to estimate it.⁴² Therefore, we would emphasize the parameter BPF rather than k , even though a high correlation between k and GAG was also found in this study.

According to the model for chondrogenesis proposed by O'Driscoll,⁴⁴ after the undifferentiated chondrocyte precursor has been seeded, differentiation occurs over the period from day 7 to day 28, and matrix synthesis develops between days 10 and 42. In the present study, histological and biochemical analysis of GAG (Figs. 2 and 4A) and the type II CG immunohistochemistry (Fig. 3) have shown that ECM synthesis begins around 7 days after cell seeding in the growth-stimulated group and continues during the 3-week culture period. The qMTI results show ECM changes beginning early during the tissue development (Fig. 4B, C), whereas the conventional MRI parameters (T_1 , T_2 , and ADC, as shown in Fig. 4D–F) are not sensitive to these developmental alterations in the ECM in this study. Therefore, qMTI methods appear to provide an earlier and more sensitive marker for ECM changes compared with the conventional MR parameters. In addition, as shown in Figure 1, the inhomogeneity of the MT-weighted images might reflect the cell distribution and the changing ECM properties, providing information about the central region of the tissue-engineered constructs, which is not easily observed by light microscopy or confocal microscopy. Finally, the BPF measured in this study at week 3 was only about 1.06%, whereas a previous study showed that the BPF can reach 18% for normal mature articular cartilage.⁴⁵ This implies that qMTI can be used to assess tissue-engineered construct growth

beyond the 3-week period examined in this study and may, in fact, be useful in the clinic to monitor the development of engineered cartilage in patients after implantation to repair a cartilage defect.

A limitation of the MRI used in this study is that the work was conducted at a very high field strength (11.7 T), a static magnetic field approximately eight times higher than the more typical 1.5 T used in clinical MRI. The goal of this work was to detect early changes in developing cartilage and to try to quantify them. Given our positive results, we will now begin *in vivo* animal experiments at 9.4 T. The high signal-to-noise ratio and high resolution needed to study cartilage and chondrogenesis will require high-field MRI, special radio frequency (RF) coils, and high performance gradients, but, fortunately, the advent of 7 T human MRI systems will bring such technology to the service of tissue engineers in the near future. Another limitation of the qMTI technique used in this study lies in the relative long imaging time (~ 40 min to finish the qMTI scanning for all the offset frequencies used in this study), which is an ongoing challenge in MR imaging. In this study, multiple scans with a range of offset frequencies for the MT pulses are required to extract the qMTI parameters. The long imaging time will also lead to image artifacts due to patient motion; thus, some form of image tagging will be needed so that a motion correction algorithm can be employed. In addition, the off resonant MT pulses lead to a higher specific absorption rate compared with conventional MRI techniques. In preparation for our planned animal studies, efforts therefore are underway to reduce the scan time and the power deposition needed for qMTI.

Conclusion

This study demonstrates that qMTI analysis, based on the two-pool model, can permit evaluation of tissue-engineered cartilage development by monitoring ECM deposition in a tissue-engineered cartilage construct.

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Disclosure Statement

No competing financial interests exist

References

1. Kuo, C.K., Li, W.J., Mauck, R.L., and Tuan, R.S. Cartilage tissue engineering: its potential and uses. *Curr Opin Rheumatol* **18**, 64, 2006.
2. Williams, G.M., Klisch, S.M., and Sah, R.L. Bioengineering cartilage growth, maturation, and form. *Pediatr Res* **63**, 527, 2008.
3. Burg, K.J., Delnomdedieu, M., Beiler, R.J., Culbertson, C.R., Greene, K.G., Halberstadt, C.R., Holder, W.D., Jr., Loebbeck, A.B., Roland, W.D., and Johnson, G.A. Application of magnetic resonance microscopy to tissue engineering: a polylactide model. *J Biomed Mater Res* **61**, 380, 2002.
4. Ou, X., Sun, S.W., Liang, H.F., Song, S.K., and Gochberg, D.F. Quantitative magnetization transfer measured pool-size

- ratio reflects optic nerve myelin content in *ex vivo* mice. *Magn Reson Med* **61**, 364, 2009.
5. Wolff, S.D., and Balaban, R.S. Magnetization transfer contrast (MTC) and tissue water proton relaxation *in vivo*. *Magn Reson Med* **10**, 135, 1989.
 6. Henkelman, R.M., Huang, X., Xiang, Q.S., Stanisz, G.J., Swanson, S.D., and Bronskill, M.J. Quantitative interpretation of magnetization transfer. *Magn Reson Med* **29**, 759, 1993.
 7. Schmierer, K., Tozer, D.J., Scaravilli, F., Altmann, D.R., Barker, G.J., Tofts, P.S., and Miller, D.H. Quantitative magnetization transfer imaging in postmortem multiple sclerosis brain. *J Magn Reson Imaging* **26**, 41, 2007.
 8. Schmierer, K., Wheeler-Kingshott, C.A., Tozer, D.J., Boulby, P.A., Parkes, H.G., Yousry, T.A., Scaravilli, F., Barker, G.J., Tofts, P.S., and Miller, D.H. Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation. *Magn Reson Med* **59**, 268, 2008.
 9. Ridha, B.H., Tozer, D.J., Symms, M.R., Stockton, K.C., Lewis, E.B., Siddique, M.M., MacManus, D.G., Rossor, M.N., Fox, N.C., and Tofts, P.S. Quantitative magnetization transfer imaging in Alzheimer disease. *Radiology* **244**, 832, 2007.
 10. Li, W., Hong, L., Zhang, G., and Magin, R.L. Quantitative magnetization transfer imaging for evaluating tissue-engineered cartilage. Abstract presented at the International Society of Magnetic Resonance in Medicine (ISMRM), Sixteenth Scientific Meeting. Toronto, Canada, 2008. Abstract no. 1716.
 11. Xu, H., Othman, S.F., and Magin, R.L. Monitoring tissue engineering using magnetic resonance imaging. *J Biosci Bioeng* **106**, 515, 2008.
 12. Xu, H., Othman, S.F., Hong, L., Peptan, I.A., and Magin, R.L. Magnetic resonance microscopy for monitoring osteogenesis in tissue-engineered constructs *in vitro*. *Phys Med Biol* **51**, 719, 2006.
 13. Neves, A.A., Medcalf, N., and Brindle, K. Functional assessment of tissue-engineered meniscal cartilage by magnetic resonance imaging and spectroscopy. *Tissue Eng* **9**, 51, 2003.
 14. Washburn, N.R., Weir, M., Anderson, P., and Potter, K. Bone formation in polymeric scaffolds evaluated by proton magnetic resonance microscopy and X-ray microtomography. *J Biomed Mater Res A* **69**, 738, 2004.
 15. Ramaswamy, S., Uluer, M.C., Leen, S., Bajaj, P., Fishbein, K.W., and Spencer, R.G. Noninvasive assessment of glycosaminoglycan production in injectable tissue-engineered cartilage constructs using magnetic resonance imaging. *Tissue Eng Part C Methods* **14**, 243, 2008.
 16. Ramaswamy, S., Wang, D.A., Fishbein, K.W., Elisseff, J.H., and Spencer, R.G. An analysis of the integration between articular cartilage and nondegradable hydrogel using magnetic resonance imaging. *J Biomed Mater Res B Appl Biomater* **77**, 144, 2006.
 17. Neves, A.A., Medcalf, N., Smith, M., and Brindle, K.M. Evaluation of engineered meniscal cartilage constructs based on different scaffold geometries using magnetic resonance imaging and spectroscopy. *Tissue Eng* **12**, 53, 2006.
 18. Potter, K., Butler, J.J., Horton, W.E., and Spencer, R.G. Response of engineered cartilage tissue to biochemical agents as studied by proton magnetic resonance microscopy. *Arthritis Rheum* **43**, 1580, 2000.
 19. Chen, C.T., Fishbein, K.W., Torzilli, P.A., Hilger, A., Spencer, R.G., and Horton, W.E., Jr. Matrix fixed-charge density as determined by magnetic resonance microscopy of bioreactor-derived hyaline cartilage correlates with biochemical and biomechanical properties. *Arthritis Rheum* **48**, 1047, 2003.
 20. Hong, L., Peptan, I., Clark, P., and Mao, J.J. *Ex vivo* adipose tissue engineering by human marrow stromal cell seeded gelatin sponge. *Ann Biomed Eng* **33**, 511, 2005.
 21. Peptan, I.A., Hong, L., and Evans, C.A. Multiple differentiation potentials of neonatal dura mater-derived cells. *Neurosurgery* **60**, 346, 2007.
 22. Mehlhorn, A.T., Niemeyer, P., Kaiser, S., Finkenzeller, G., Stark, G.B., Sudkamp, N.P., and Schmal, H. Differential expression pattern of extracellular matrix molecules during chondrogenesis of mesenchymal stem cells from bone marrow and adipose tissue. *Tissue Eng* **12**, 2853, 2006.
 23. Sled, J.G., and Pike, G.B. Quantitative interpretation of magnetization transfer in spoiled gradient echo MRI sequences. *J Magn Reson* **145**, 24, 2000.
 24. Meiboom, S., and Gill, D. Modified spin-echo method for measuring nuclear relaxation times. *Rev Sci Instrum* **29**, 688, 1958.
 25. Stejskal, E.O., and Tanner, J.E. Spin diffusion measurements: spin echoes in the presence of a time-dependent field gradient. *J Chem Phys* **42**, 288, 1965.
 26. Farndale, R.W., Buttle, D.J., and Barrett, A.J. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim Biophys Acta* **883**, 173, 1986.
 27. Henning, T.D., Sutton, E.J., Kim, A., Golovko, D., Horvai, A., Ackerman, L., Sennino, B., McDonald, D., Lotz, J., and Daldrop-Link, H.E. The influence of ferucarbotran on the chondrogenesis of human mesenchymal stem cells. *Contrast Media Mol Imaging* **4**, 165, 2009.
 28. Penick, K.J., Solchaga, L.A., and Welter, J.F. High-throughput aggregate culture system to assess the chondrogenic potential of mesenchymal stem cells. *Biotechniques* **39**, 687, 2005.
 29. Bai, X., Xiao, Z., Pan, Y., Hu, J., Pohl, J., Wen, J., and Li, L. Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. *Biochem Biophys Res Commun* **325**, 453, 2004.
 30. Ramani, A., Dalton, C., Miller, D.H., Tofts, P.S., and Barker, G.J. Precise estimate of fundamental *in-vivo* MT parameters in human brain in clinically feasible times. *Magn Reson Imaging* **20**, 721, 2002.
 31. Potter, K., Butler, J.J., Adams, C., Fishbein, K.W., McFarland, E.W., Horton, W.E., and Spencer, R.G. Cartilage formation in a hollow fiber bioreactor studied by proton magnetic resonance microscopy. *Matrix Biol* **17**, 513, 1998.
 32. Miyata, S., Numano, T., Homma, K., Tateishi, T., and Ushida, T. Feasibility of noninvasive evaluation of biophysical properties of tissue-engineered cartilage by using quantitative MRI. *J Biomech* **40**, 2990, 2007.
 33. Miyata, S., Homma, K., Numano, T., Furukawa, K., Tateishi, T., and Ushida, T. Assessment of fixed charge density in regenerated cartilage by Gd-DTPA-enhanced MRI. *Magn Reson Med Sci* **5**, 73, 2006.
 34. Ramaswamy, S., Greco, J.B., Uluer, M.C., Zhang, Z., Fishbein, K.W., and Spencer, R.G. Magnetic resonance imaging of chondrocytes labeled with superparamagnetic iron oxide nanoparticles in tissue-engineered cartilage. *Tissue Eng Part A* **15**, 3899, 2009.
 35. Kelly, D.J., Crawford, A., Dickinson, S.C., Sims, T.J., Mundy, J., Hollander, A.P., Prendergast, P.J., and Hatton, P.V. Biochemical markers of the mechanical quality of engineered hyaline cartilage. *J Mater Sci Mater Med* **18**, 273, 2007.
 36. Pfeiffer, E., Vickers, S.M., Frank, E., Grodzinsky, A.J., and Spector, M. The effects of glycosaminoglycan content

- on the compressive modulus of cartilage engineered in type II collagen scaffolds. *Osteoarthritis Cartilage* **16**, 1237, 2008.
37. Gray, M.L., Burstein, D., Lesperance, L.M., and Gehrke, L. Magnetization transfer in cartilage and its constituent macromolecules. *Magn Reson Med* **34**, 319, 1995.
38. Wachsmuth, L., Juretschke, H.P., and Raiss, R.X. Can magnetization transfer magnetic resonance imaging follow proteoglycan depletion in articular cartilage? *MAGMA* **5**, 71, 1997.
39. Stanton, S., Salih, V., Bentley, G., and Downes, S. The growth of chondrocytes using Gelfoam® as a biodegradable scaffold. *J Mater Sci Mater Med* **6**, 739, 1995.
40. Ponticello, M.S., Schinagl, R.M., Kadiyala, S., and Barry, F.P. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. *J Biomed Mater Res* **52**, 246, 2000.
41. Awad, H.A., Wickham, M.Q., Leddy, H.A., Gimble, J.M., and Guilak, F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials* **25**, 3211, 2004.
42. Portnoy, S., and Stanis, G.J. Modeling pulsed magnetization transfer. *Magn Reson Med* **58**, 144, 2007.
43. Ropele, S., Seifert, T., Enzinger, C., and Fazekas, F. Method for quantitative imaging of the macromolecular 1H fraction in tissues. *Magn Reson Med* **49**, 864, 2003.
44. O'Driscoll, S.W., and Fitzsimmons, J.S. The role of periosteum in cartilage repair. *Clin Orthop Relat Res* **391**, S190, 2001.
45. Stanis, G.J., Odobina, E.E., Pun, J., Escaravage, M., Graham, S.J., Bronskill, M.J., and Henkelman, R.M. T1, T2 relaxation and magnetization transfer in tissue at 3T. *Magn Reson Med* **54**, 507, 2005.

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