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Original Article

Hylonome

MR imaging and early cartilage degeneration and strategies for monitoring regeneration

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Introduction

Interactions between all the major joint tissues, including the articular cartilage, synovium, bone marrow, subchondral bone, trabecular bone and muscle, have been implicated in osteoarthritis (OA)[1]. Magnetic resonance (MR) images have been used to quantify the cartilage morphology, volume and thickness[2] and focal defects[3], and may reflect changes in biochemical composition of articular cartilage[4-6]. Cartilage loss in OA is preceded by damage to the collagen-proteoglycan matrix and elevation of cartilage water content. These changes are associated with changes in cartilage relaxation times T_2 and $T_{1\varrho}$[7], as well as in the uptake of contrast agents such as Gd-DTPA in the cartilage matrix (dGEMRIC)[4]. Furthermore, injury and OA-related changes in bone marrow manifested by an increase in the signal intensity in bone marrow on fat-saturated T_2-weighted images (bone marrow edema, BME) have been associated with severity and progression of OA[8]. Such marrow changes are also associated with acute injuries such as anterior cruciate ligament injuries[9] that sometimes progress to OA and joint degeneration. The purpose of this paper is to review the interrelationships of T_2, $T_{1\varrho}$, cartilage volume and BME in patients with OA versus those with acute knee injury, and markers of early cartilage degeneration. Stem cell based regeneration strategies are being proposed for cartilage repair. The ability to monitor stem cell therapies *in vivo* is rapidly becoming a significant consideration. Recently, it has been shown that stem cells can be labeled with super paramagnetic iron oxide (SPIO) particles and detected using MRI[10]. The SPIO contrast agent is metabolized by the cell, increasing the cell's magnetic susceptibility. As negative contrast agents, labeled cell populations appear as hypo-intense regions, making them distinguishable on MR images. Using these labeled cells for tracking cartilage regeneration would be a potential tool for understanding the regenerative mechanisms.

Methods

In controls and subjects with OA and ACL injury studies relating cartilage volume, thickness or degeneration to relaxation time T_2, $T_{1\varrho}$ and bone marrow edema changes were conducted. High-resolution, fat-suppressed, sagittal images were acquired for assessing cartilage structure, using a 3-D spoiled gradient echo (SPGR) sequence. The cartilage was segmented using a spline-based, semi-automatic technique and was defined in four distinct regions: medial and lateral tibia, and medial and lateral femur. Total cartilage volume and average thickness were calculated for each region. Sagittal images were acquired for measuring T_2 relaxation time, using a dual echo spin echo sequence. A map of T_2 values was calculated. Using a $T_{1\varrho}$ relaxation time mapping technique $T_{1\varrho}$ maps were reconstructed. In patients with OA and with ACL (anterior cruciate ligament) tears, who showed BME $T_{1\varrho}$-weighted and fat-saturated T_2-weighted fast spin-echo images were acquired. Point RESovled Spectroscopy (PRESS) volume selection was used to acquire spectroscopic fat/water quantitative data in the edema. BME was semi-automatically segmented using a threshold method based on T_2-weighted images and volume of BME was calculated.

Human mesenchymal stem cells (hMSCs) obtained from the VA Medical Center (San Francisco, CA) were labeled using Feridex IV (Fe) (Berlex Laboratories, Wayne, NJ). Protamine Sulfate (Pro) (APP, Schaumberg, Illinois) was used as a transfection agent. Cells were labeled using the Fe-Pro complex as described in Arbab et al.[11]. Following labeling, a Trypan Blue Assay was performed to assess of cell viability. For *in vitro* imaging, cells were placed in tubes (10^3-10^6 cells/mL) containing Ficoll (Amersham Biosciences,

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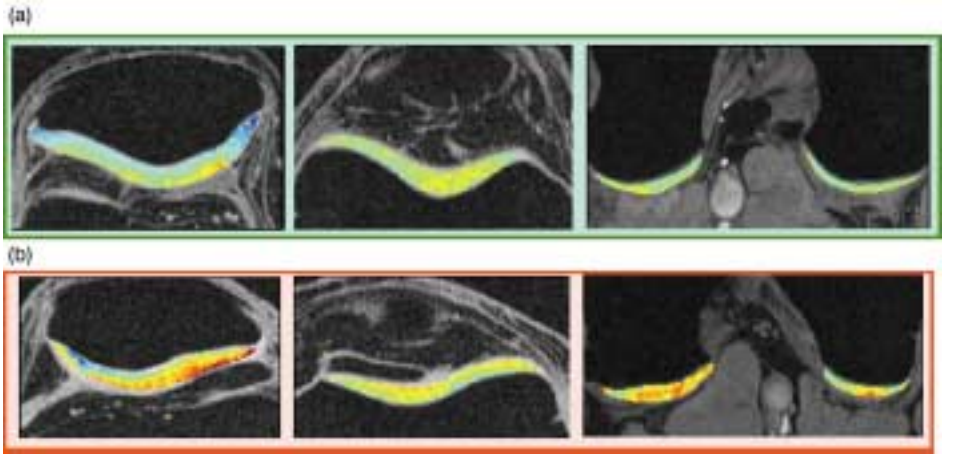

Figure 1. Color-coded $T_{1\varrho}$ map overlays on SPGR images. Left: patellar cartilage; middle: anterior femoral (trochlea) cartilage; right: posterior femoral cartilage. (a) a healthy volunteer, male, 30; (b) a patient with early OA, female, 27. The $T_{1\varrho}$ values were 40.05±11.43 ms in the volunteer and 50.56±19.26 ms in the patient, respectively.

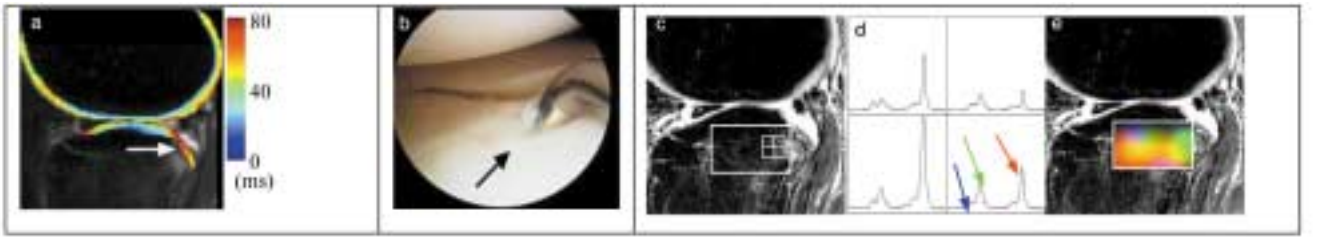

Figure 2. A patient with ACL tear showed significantly elevated $T_{1\varrho}$ values in bone marrow edema overlying cartilage (a) and the cartilage damage was confirmed with arthroscopy (b). The 3-D spectral data in the BME region (c) showed elevated water and unsaturated lipids (d). The water elevated and saturated lipids decreased in the edema while unsaturated lipids elevated in the peripheral region of edema (e). Green: Water; red: SatLip; blue: UnsatLip.

Piscataway, NJ), to maintain osmolarity. Tubes were placed in a water-containing plastic container and imaged at room temperature (20°C). Imaging was done on a 3T MR scanner (GE Medical Systems, Waukesha, Wisconsin). Spin-echo sequence was used to determine T_1(T=12ms, TR increasing from 60-1000ms) and T_2(TR=4000ms, TE increasing from 12-48ms) relaxation times. A gradient-echo (GRE) sequence was used to measure T_2* relaxation times using $\alpha=90^o$ flip angle, TR = 34ms, and TE increasing from 4-20ms. Dedicated fitting software was used to quantify relaxation times. For *ex vivo* imaging, two osteochondral defects (4mm diameter, 4mm height) were created in the trochlea of the rabbit distal femur. Gelfoam Size 100 (Pharmacia and Upjohn, Kalamazoo, Michigan) cut to the size of the defect was used as a scaffold to load the labeled hMSCs. The two defects were used as follows: (1) labeled cells on Gelfoam ($3x10^6$ cells/mL); (2) empty Gelfoam. The excised knee was imaged using a T_2*-weighted 3-D GRE sequence.

Results

The studies revealed that higher medial T_2 results in greater loss of medial cartilage volume at twelve months. Specifically, the correlation between baseline medial femoral T_2 and change in medial femoral cartilage volume was r=-0.38 ($p<0.05$).

Figure 1 shows a map of $T_{1\varrho}$. A significant correlation was found between average $T_{1\varrho}$ and T_2 values within the cartilage, with a correlation co-efficient $R^2=0.69$ and $p=0.017$. The increase of average $T_{1\varrho}$ in cartilage from the controls to patients was 19.1% (43.90 ms for controls and 52.28 ms for patients), while the increase was 9.6% for the average T_2 (34.94 ms for controls and 38.31 ms for patients). The difference in average $T_{1\varrho}$ in cartilage between controls and patients was significant ($p=0.003$) while it was not significant for average T_2 ($p=0.202$). Patients with similar average T_2 may have different $T_{1\varrho}$, or vice versa.

The average $T_{1\varrho}$ values in BME-overlying cartilage were significantly higher than that in surrounding cartilage (51.8±10.8

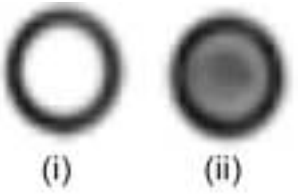

Figure 3. Gradient echo image TE=4 ms: (i) unlabeled MSCs; 10^6cells/mL (ii) MSCs labeled with Fe-Pro; 10^6 cells/mL.

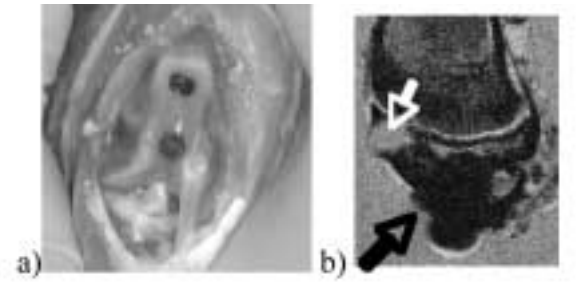

Figure 4. a: Osteochondral defects simulated in trochlea: Top: empty defect, bottom is filled with gelfoam. **b:** gradient echo image, light arrow showing the empty defect filled with water, black arrow showing the defect with gelfoam + labeled cells as a loss of signal.

ms vs. 43.0±8.3 ms, P=0.032) in the ten patients, as one example shown in Figure 2(a). Patients with ACL tears tended to have a higher increase percentage than patients with OA, but it was not significant (6.31%±11.40% vs. 29.15%±20.75%, P=0.06). Volume of BME correlated significantly with volume of elevated water based on 3-D-MRSI (R=84.4%, P=0.004) but not with volume of elevated unsaturated lipids. Spatially elevated water correlated with BME while elevated unsaturated lipids generally were most significant in the peripheral regions of the BME, Figure 2(e). None of the BME parameters (volume, MRSI-based water and unsatlip volume) were correlated with increase percentage of $T_{1\rho}$ values.

The results of the *in vitro* experiments indicate efficient cellular uptake of the Fe-Pro complex, and resulting MR signal intensity loss (Figure 3). In addition, labeling did not affect cell viability. While 10^6 cells/mL results in a strong signal loss, particularly in T_2* measurements, loss of SI of 10^5 cells/mL can also be distinguished relative to unlabeled cells (not shown). In the rabbit model, cells labeled with Fe-Pro appear as a signal void on the MR image (Figure 3). In contrast, the defect loaded without cells (empty Gelfoam) appears brighter with a signal resulting from the scaffold.

Discussion and conclusions

Quantitative imaging appears promising and may potentially provide information beyond morphological changes in articular cartilage, with regards to early cartilage degeneration and biochemistry and further studies are clearly warranted. Stem cell labeling with Fe-Pro results in a significant loss of SI in MRI. The *in vitro* and *ex vivo* results suggest that this method of cell tracking could be applied to *in vivo* detection of stem cell therapies. Following initial detection of transplanted cell populations, this minimally invasive technique could allow for *in vivo* longitudinal tracking of therapy.

References

1. Mankin HJ, Brandt KD. Pathogenesis of arthritis. Textbook of Rheumatology. WB Saunders, Philadelphia; 1993.
2. Eckstein F, Adam C, Sittek H, Becker C, Milz S, Schulte E, Reiser M, Putz R. Non-invasive determination of cartilage thickness throughout joint surfaces using magnetic resonance imaging. J Biomech 1997; 30:285-289.
3. Link TM, Lindner N, Haussler M, Reimer P, Allkemper T, Jerosch J, Peterfy C, Majumdar S, Peters PE. Artifically produced cartilage lesions in small joints: detection with optimized MRI-sequences. Magn Reson Imaging 1997; 14:949-956.
4. Bashir A, Gray M, Burstein D. Gd-DTPA2- as a measure of cartilage degradation. Magn Reson Med 1996; 36:665-673.
5. Dardzinski BJ, Mosher TJ, Li S, Van Slyke MA, Smith MB. Spatial variation of T2 in human articular cartilage. Radiology 1997; 205:546-550.
6. Gray ML, Burstein D, Lesperance LM, Gehrke L. Magnetization transfer in cartilage and its constituent macromolecules. Magn Reson Med 1995; 34:319-325.
7. Akella SV, Regatte RR, Gougoutas AJ, Borthakur A, Shapiro EM, Kneeland JB, Leigh JS, Reddy R. Proteoglycan-induced changes in T1rho-relaxation of articular cartilage at 4T. Magn Reson Med 2001; 46:419-423.
8. Felson DT, Chaisson CE, Hill CL, Totterman SM, Gale ME, Skinner KM, Kazis L, Gale DR. The association of bone marrow lesions with pain in knee osteoarthritis. Ann Intern Med 2001; 134:541-549.
9. Costa-Paz M, Muscolo DL, Ayerza M, Makino A, Aponte-Tinao L. Magnetic resonance imaging follow-up study of bone bruises associated with anterior cruciate ligament ruptures. Arthroscopy 2001; 17:445-449.
10. Bulte J, Duncan I, Ziegler JF. *In vivo* magnetic resonance tracking of magnetically labeled cells after transplantation. J Cereb Blood Flow Metab 2002; 22:899-907.
11. Arbab A, Yocum G, Kalish H, Jordan EK, Anderson SA, Khakoo AY, Read EJ, Frank JA. Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI. Blood 2004; 104.4:1217-1223.