Published in final edited form as:

Anal Bioanal Chem. 2007 March ; 387(5): 1601–1612. doi:10.1007/s00216-006-0910-7.

Fourier Transform Infrared Imaging and MR Microscopy Studies Detect Compositional and Structural Changes in Cartilage in a Rabbit Model of Osteoarthritis

Xiaohong Bi¹, Xu Yang¹, Mathias P.G. Bostrom¹, Dorota Bartusik², Sharan Ramaswamy², Kenneth W. Fishbein², Richard G. Spencer², and Nancy Pleshko Camacho¹

¹ Research Division, Hospital for Special Surgery, 535 E 70th Street, New York, NY 10021

² National Institute on Aging, Intramural Research Program, GRC 4D-08, 5600 Nathan Shock Drive, Baltimore, MD 21224

Abstract

Assessment of subtle changes in the primary macromolecular components of cartilage, proteoglycan (PG) and collagen, is critical for the diagnosis of early stages of osteoarthritis (OA), but to date has remained a challenge. In the current study, we induced osteoarthritic cartilage changes in a rabbit model via ligament transection and medial meniscectomy and monitored disease progression with the techniques of infrared fiber optic probe (IFOP) spectroscopy, Fourier transform infrared imaging spectroscopy (FT-IRIS), and magnetic resonance imaging (MRI) microscopy. IFOP studies combined with chemometric partial least squares analysis enabled us to monitor progressive cartilage surface changes from two to twelve weeks post-surgery. FT-IRIS studies of histological sections of femoral condyle cartilage demonstrated that compared to control cartilage, the OA cartilage had significantly reduced PG content 2 and 4 weeks post-surgery, collagen fibril orientation changes 2 and 4 weeks post-surgery, and changes in collagen integrity 2 and 10 weeks post-surgery, but no significant changes in collagen content at any time point. MR microscopy studies found reduced fixed charge density (FCD), reflective of reduced PG content, in the OA cartilage compared to controls 4 weeks post-surgery. In addition, a non-significant trend towards higher apparent MT exchange rate k_m was found in the OA cartilage at this time point, suggesting changes in collagen structural features. These two MR findings, for FCD and k_m , parallel the FT-IRIS findings of reduced PG content, and altered collagen integrity, respectively. Further, MR microscopy studies at the 12 week time point cartilage found a trend towards longer T_2 values and decreased anisotropy in the deep zone of the OA cartilage, consistent with increased hydration and less ordered collagen. These studies demonstrate that together, FT-IRIS and MR microscopy provide complementary data on compositional changes in articular cartilage at early stages of osteoarthritic degradation.

Keywords

Infrared spectroscopy; magnetic resonance imaging; osteoarthritis; cartilage; collagen; proteoglycan

Introduction

Osteoarthritis (OA) is an age-related disease of the joints that results in pain, frequent disability and considerable economic burden. In the United States, an average of 16 to 21 million

individuals are diagnosed annually with OA, and the prevalence of the disease is further increasing because of an aging population [1–3]. The treatment of OA is hindered by the inability to identify early-stage cartilage degeneration and the lack of effective pharmaceutical interventions [4]. Development of novel non-invasive or non-destructive detection techniques for OA, along with knowledge of specific ultrastructural changes at early stages of degeneration, would significantly enhance capabilities for early diagnosis and treatment, and facilitate the development of tissue engineering and other therapeutic interventions.

The development of OA involves progressive degeneration of articular cartilage, changes in subchondral bone and limited synovitis [5]. Articular cartilage is comprised of a collagenous network, highly negatively charged proteoglycans (PGs), water, non-collagenous proteins and chondrocytes [6]. Three structural cartilage zones have been defined based on morphological differences in chondrocytes and their extracellular matrix. In the superficial zone of cartilage, collagen fibrils are densely packed and oriented parallel to the articular surface [6]. The middle, or transitional zone of cartilage contains less organized collagen fibrils and an increased concentration of PGs. In the deep zone, collagen fibrils are oriented perpendicular to the articular surface. The highest concentration of PGs and lowest water content are present in this zone. Osteoarthritic degeneration of cartilage is associated with progressive deterioration in the collagen framework, increased swelling of the tissue and loss of proteoglycan [7;8].

Animal models of OA have been developed to better understand the disease and to evaluate therapeutic interventions. Anterior cruciate ligament transection (ACLT) in the rabbit is a frequently-used and well-established technique to surgically induce joint degeneration resembling post-traumatic human OA [9–13], which occurs as a consequence of altered load distribution and joint destabilization. In these studies, osteoarthritic degeneration was evidenced by progressive changes in morphology, histopathology and physical properties of articular cartilage in the operated knees. In one study, early-stage cartilage degradation was observed as early as three weeks post-surgery [11].

Magnetic resonance imaging (MRI) is the most widely-used modality for non-invasive detection of pathologic changes in cartilage. Specific MRI parameters have been correlated with the tissue changes associated with OA, such as tissue swelling, PG loss and collagen network destruction [14–19]. However, only with a high field strength magnet, such as that used for *in vitro* studies [20–22], can detailed information on compositional cartilage changes be identified in each cartilage zone individually. The MRI-measured parameters T_1 and T_2 have been shown to respond to PG depletion [22] and changes in hydration [16], respectively. Other MRI microscopy studies have demonstrated orientational dependence of T_2 relaxation in articular cartilage explants [23]. The T_2 anisotropy phenomenon in cartilage MRI has been shown to arise from long-range order in collagen fibril packing [24–26], and thus might be a useful tool for the detection of early changes in collagen fiber architecture associated with the development of OA [27]. Finally, magnetization transfer (MT) imaging provides a non-invasive means of evaluating collagen content and structure in cartilage [28–30].

Fourier transform infrared (FT-IR) spectroscopy has been used extensively to study the structure and orientation of biomolecules isolated from normal and pathologic tissues [31–35]. The advantage of this technique is its ability to provide simple and often non-destructive measurements based on the unique molecular spectral signatures of tissue components. Advances in FT-IR imaging spectroscopy (FT-IRIS), a technique that couples an FT-IR spectrometer with an array detector and an optical microscope to produce an FTIR spectrum at each pixel in a defined field of view, permits mapping of spectral features and hence tissue components with a pixel resolution of 6.25 microns. In cartilage research, FT-IRIS has been applied to characterize the spatial distribution of collagen and PG, [36–40], and, with a polarizer [41;42], to determine the orientation of collagen fibrils. We have also successfully correlated

changes in IR parameters with subtle cartilage degradation [39;42]. Together, these advances have made FT-IRIS a powerful tool with which to monitor cartilage degradation and to evaluate cartilage repair techniques and the composition of engineered tissues. Similarly, an infrared fiber optic probe (IFOP) has been applied in our laboratory to map the cartilage surface *in vitro* and in animal models [39;43], and we envision clinical application of this methodology following further development.

In the present study, two infrared spectroscopic techniques, IFOP and FT-IRIS analysis, were utilized to monitor disease progression in an ACLT-induced OA rabbit model. MRI microscopy was also performed on selected tissues to examine the correspondence of outcome measures obtained from these state-of-the-art technologies.

Materials and Methods

Osteoarthritis Model

Surgery was performed under an IACUC-approved protocol on the right knee of 6 month old male New Zealand white rabbits to create osteoarthritic changes similar to those described in the Hulth-Tehlag Model [44]. The surgery involved transection of the anterior and posterior cruciate ligaments and excision of the medial meniscus. A sham procedure was performed on the left hind leg to serve as a surgical control within the same animal. The rabbits were sacrificed at 0, 2, 4, 6, 8, 10 and 12 weeks post-surgery (N = 5 for each group). Animals from a group of corresponding age-matched unoperated rabbits (N = 3, except at 8 weeks, where N = 1) were sacrificed at each of these timepoints to serve as non-surgical controls.

Tissue Collection and Preparation

The femoral condyles from the ACLT-operated, sham-operated and unoperated legs were excised, followed immediately by IFOP data collection. Tissues were then fixed with 80% ethanol and 1% cetylpyridinium chloride (CPC), decalcified with 10% EDTA in Tris buffer, and embedded in paraffin. Histological sections were cut at 7 μ m thickness perpendicular to the articular surface and mounted onto BaF₂ IR windows and glass slides for FT-IRIS and histologic analysis, respectively.

IFOP Data Acquisition

The design of the IFOP system has been described previously [43]. Briefly, a flat-tipped, 1 mm diameter ZnS attenuated total reflectance (ATR) crystal was attached to the end of a fiber-optic bundle which was coupled to a Bruker (Billerica, MA) IR spectrometer equipped with a mercury cadmium telluride (MCT) detector. Data collection was initiated after ca. 60 seconds contact between the femoral condyle cartilage and crystal. Spectra were acquired, with 256 scans each from the weight-bearing site on each medial femoral condyle as input into the chemometric model. Spectra were collected in triplicate in order to establish a measure of spectral variability for incorporation into the model. A typical IFOP spectrum of cartilage is shown in Figure 1 with peak assignments based on previous studies of model compounds [37;40].

Chemometric Partial Least Squares (PLS) Model

Chemometric methods were utilized to analyze the IFOP data. The details of the PLS method, as applied to data from human cartilage, have been previously described [43]. Briefly, a model was developed for the current study utilizing post-surgical time as a surrogate for disease severity and based upon the PLS regression algorithm of QuantII OPUS NT software (Bruker). The infrared spectra were pre-processed for PLS analysis by performing straight line subtraction in the spectral range of 1586–999 cm^{-1} . One hundred and thirty seven spectra were

used for calibration of the PLS model, and 59 independent spectra were utilized as test input to compare the actual time post-surgery to the predictions of the model.

FT-IRIS Data Acquisition

Transmission FT-IRIS data were acquired from histological sections of femoral condyle cartilage at 8 cm^{-1} spectral resolution using a Spectrum Spotlight FT-IR Imaging system (Perkin-Elmer, Bucks, UK). This system is comprised of an FTIR spectrometer coupled with a light microscope and an 8×2 staggered linear array detector, and allows data collection over a user-defined rectangular region at $6.25\text{ }\mu\text{m}$ pixel resolution. FT-IRIS data were also collected with a polarizer inserted into the light path to obtain information on collagen fibril orientation.

FT-IRIS Image Processing

FT-IRIS images were created based on the specific vibrational absorbances for each component of interest using ISys software v3.1 (Spectral Dimensions, Olney, MD). The absorbance bands were baseline-corrected and then integrated. Previous studies have correlated collagen and PG content with the area under the protein amide I band ($1598\text{--}1710\text{ cm}^{-1}$) and the integrated area of the absorbance in the range of $950\text{--}1150\text{ cm}^{-1}$, respectively [37;40]. The area under the infrared absorbance centered at 1338 cm^{-1} ($1300\text{--}1356\text{ cm}^{-1}$), a feature attributed to side-chain CH_2 vibrations, has previously been shown to decrease in intensity with collagen denaturation [39]. This area was divided by that of the amide II band ($1492\text{--}1598\text{ cm}^{-1}$) to yield a measurement of collagen integrity [39;42]. Images were calculated from data acquired with the polarizer by dividing the areas of the amide I and amide II collagen peaks for each pixel (amide I/amide II). We have previously demonstrated that this ratio provides an index of collagen fibril orientation as follows: ≥ 2.7 : fibrils parallel to the articular surface; ≤ 1.7 : fibrils perpendicular to the articular surface; $2.7\text{--}1.7$: random or mixed fibril orientation [41]. For quantitative analysis, all the pixels in the polarized FT-IRIS image were divided into three orientation categories as above, and the percentage of pixels falling into each category was calculated.

Histology

Histological slides were stained with Alcian blue and picosirius red to demonstrate the distribution of proteoglycan and collagen, respectively. Picosirius red, which stains collagen type I, II and III, can be used to indicate the quantity of collagen [45]. On the Alcian blue-stained sections, dark blue corresponds to PG, red/pink to nuclei, and pale pink to cytoplasm [46].

MRI Methods

T₂ Anisotropy in Intact Femoral Condyles

MRI studies were performed on sham-operated (control; left) and operated (OA; right) femurs of three rabbits sacrificed 12 months after surgery. Experiments were performed at $4.0 \pm 0.1\text{ }^{\circ}\text{C}$ using a 400 MHz Bruker Avance DMX NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a Magnex 9.4 T/105 mm vertical bore magnet (Magnex scientific, Abingdon, UK) and a Bruker three-axis shielded Micro2.5 microimaging gradient set and 25 mm proton birdcage resonator probe.

Proton T₂ was measured in a sagittal slice (thickness 0.5 mm) through the center of the medial condyle using a spin-echo sequence with a minimum TE = 9.2 ms and 16 echoes, TR=6 s, number of averages (NEX) = 8, field of view (FOV) = $0.7 \times 2.4\text{ cm}$, matrix size (MTX) = 256×256 , and in-plane resolution of $27 \times 94\text{ }\mu\text{m}$. The read gradient direction was parallel to the main magnetic field, B₀. Analysis was restricted to the radial cartilage zone [24]. Experiments

were repeated with the sample rotated by approximately 55° , with the FOV increased to 1.2×2.4 cm, resulting in in-plane resolution of $47 \times 94 \mu\text{m}$, to enable visualization of the entire articular surface in the tilted configuration. T_2 analysis was carried out in a region of interest corresponding to that analyzed in the non-tilted configuration. An anisotropy index was calculated according to:

$$\eta = [R_2(55^\circ) - R_2(0^\circ)] / R_2(55^\circ) \quad [1]$$

where $R_2 = 1/T_2$. With this definition, a sample containing collagen fibrils randomly oriented relative to B_0 would yield $\eta = 0$, while larger values would indicate more regular orientation.

Fixed Charge Density in Osteochondral Plugs

In a separate group of six rabbits sacrificed 4 weeks post-surgery, an osteochondral plug was harvested from the weight-bearing region of the medial femoral condyle of unoperated (control; N=3) or operated (OA; N=3) legs of each animal (Figure 2). MRI microscopy was performed at 4°C with the plugs embedded in agarose gel using the hardware described above but with a 15 mm proton birdcage resonator. A 1.5 mm-thick slice was defined through the center of each plug along the anterior-posterior direction and imaged with a spin-echo sequence with FOV = 0.5×1.4 cm and MTX = 256×256 , resulting in an in-plane resolution of $20 \times 55 \mu\text{m}$. The read direction was parallel to B_0 and approximately perpendicular to the cartilage articular surface. Magnetization transfer (MT) experiments were performed with a presaturation pulse with offset = + 6 kHz relative to water, amplitude $B_1 = 12 \mu\text{T}$, and duration t_p ranging between 0.1 and 4.6 s. Values of TE = 12.8 ms, TR = 5 s and NEX = 2 were used. The MT exchange rate, k_m , was calculated from a fit of signal intensity as a function of saturation time t_p [47]. Matrix fixed charge density was estimated with the dGEMRIC technique [48], using a concentration of Gd-DTPA^{2-} of 2 mM.

Statistics

For each FT-IRIS parameter examined, a two-way ANOVA test was performed to test for the simultaneous effects of time after surgery and disease state (OA or control) on the outcome variables. Bonferroni post-hoc tests were performed for comparison of OA and control groups. Statistical significance was defined as $p < 0.05$. All MRI data are reported as mean $\pm$ SD. For MRI data obtained from intact condyles, paired t-tests were used to compare mean T_2 values and anisotropy indices between operated and contralateral legs. MT, T_1 and FCD values obtained on osteochondral plugs taken from operated legs and unoperated controls animals were compared using unpaired t-tests.

Results

IFOP Analysis

The PLS correlation between the calibrated values and the actual weeks post-surgery was $R^2 = 94.8$ using the suggested rank of 15 (Figure 3i). A prediction validation was performed against this PLS model with 59 test spectra and resulted in $R^2 = 93.6$ with a standard error of prediction of 1.02 (Figure 3ii). This prediction gave an accuracy of ± 2 weeks for 95% percent of the test spectra.

FT-IRIS and MRI Analyses

Figure 4 shows typical FT-IRIS images and histology from medial femoral condyles obtained two weeks post-surgery from the articular surface down to the tidemark in an OA and age-matched non-surgical control animal. Qualitative differences in the spatial distribution of collagen (i: amide I, iii: picrosirius red) and proteoglycan (ii: PG/amide I, iv: Alcian Blue)

between the control and OA tissues are evident; there appears to be more collagen and less PG in the OA tissue at this timepoint. Quantitative FT-IRIS data indicative of collagen content, PG content and collagen integrity averaged over the full thickness of cartilage are shown in Figure 5 at each post-surgical timepoint.

Collagen Content from FT-IRIS—There were no significant differences in collagen content in OA versus non-surgical control at any timepoint, and no differences within each group over time (Figure 5i).

PG Content—Reduced PG content, as reflected in the PG-to-collagen ratio, was found in the OA compared to the control groups at 2 and 4 weeks post-surgery by FT-IRIS, but no differences were detected within each group over time (Figure 5ii). MRI T_1 and FCD values for radial zone cartilage in the osteochondral plug samples are shown in Table 1. There were no significant baseline differences in T_1 between samples cut from control versus OA legs. After equilibration with $Gd-DTPA^{2-}$, cartilage T_1 was significantly longer in the control samples, indicating a greater matrix fixed charge density and hence greater PG content. Thus, both the MRI-derived FCD data and the FT-IRIS data show a significant reduction in PG content in the OA group at 4 weeks post-surgery.

Collagen Structure—The FT-IRIS-derived collagen integrity parameter was significantly greater in the OA groups at 2 and 10 weeks post-surgery compared to age-matched non-surgical controls (Figure 5iii), possibly indicative of a hypertrophic response consisting in part of new collagen formation or repair. There were no significant differences within each group over time. Figure 6 shows a typical T_2 -weighted MRI image of a plug sample from a control knee. Images from OA knees were similar in appearance. MRI magnetization transfer data for radial zone cartilage in these plugs at 4 weeks post-surgery exhibited a non-significant trend towards higher apparent MT exchange rate k_m in the OA group: $0.44 \pm 0.17 \text{ s}^{-1}$ (OA) versus $0.23 \pm 0.05 \text{ s}^{-1}$ (control); $p = 0.16$. This trend could reflect changes in collagen structural features, especially in the absence of large changes in collagen content.

Collagen Orientation—The polarized FT-IRIS images indicate qualitative collagen fibril orientation changes at 2, 6 and 12 weeks post-surgery in the OA group, and are compared to polarized light microscopy (PLM) images from the same tissues (Figure 7). Collagen fibrils in the cartilage of the 2-week OA group tended to adopt a disorganized orientation, with fewer fibrils perpendicular to the articular surface as compared to the non-surgical control (Figure 7i). This phenomenon was also apparent in the PLM image. At 6 weeks post-surgery, this effect was also noted to a lesser extent (Figure 7ii), where two of five specimens showed a more random collagen fibril orientation and decreased fraction of fibrils with perpendicular orientation than controls. At 8 weeks post surgery, none of the five rabbits exhibited such effects. By 12 weeks post-surgery, severe cartilage damage, including pronounced surface fibrillation, was observed (Figure 7iii). The percentage of collagen fibrils exhibiting each of the three orientations was extracted from the polarized FT-IRIS images (Figure 8). A significantly lower fraction of pixels exhibiting perpendicular fibril orientation was observed for the 2-week and 4-week OA groups compared to controls (Figure 8iii).

MR microscopy T_2 values for radial zone cartilage of the rabbit medial femoral condyles 12 weeks post-surgery are summarized in Table 2. We found a pronounced though non-significant increase in T_2 with osteoarthritic degradation. This trend was more pronounced in the 0° sample orientation, for which radial zone collagen fibrils were expected to be oriented primarily parallel to the B_0 field, as compared to the 55° data. Values for the anisotropy index η demonstrated a trend toward decreased anisotropy in the osteoarthritic cartilage, although again the small sample size prevented this difference from being statistically significant.

Discussion

Collagen content, integrity and orientation, as well as proteoglycan content, are important determinants of cartilage function. In the current study, utilizing FT-IRIS and MRI microscopy, we investigated changes in these parameters in a rabbit model of osteoarthritis. Although similar changes have previously been found by FT-IRIS in a small number of human cartilage samples [49], our use of an animal model here permitted a controlled study at defined points in the progression of the disease. Further, it was possible to evaluate tissue at early stages of the disease; both FT-IRIS and MR microscopy uncovered composition changes which occurred prior to onset of gross pathologic changes.

Another recent study of experimental OA in a rabbit model found changes in type II collagen synthesis at 1, 2 and 4 weeks post-surgery [50]. The expression of type II collagen C-propeptide, reflective of type II collagen synthesis, was found to increase over time at some sites in the OA cartilage, and to decrease at others. However, expression was always greater than in control regions. Analogous results reported in the present work are an increased FT-IRIS collagen integrity parameter in the OA tissues 2 and 10 weeks post-surgery, and a trend towards higher MT exchange rate k_m in the OA group at 4 weeks post-surgery as detected by MRI. All of these findings are consistent with increased type II collagen synthesis early in the development of OA, potentially indicating an active repair response. The qualitatively increased collagen content of the OA tissues compared to controls at earlier timepoints may also be attributable to this phenomenon, but did not reach statistical significance due to small sample size. Thus, it appears that the collagen integrity parameter may be a more sensitive indicator of active repair than collagen content in cartilage.

We used k_m rather than MTR as a probe of collagen alterations in OA, since MTR depends upon T_{1sat} , which may itself be affected by the induction of OA. Previous studies of magnetization transfer *in vitro* have demonstrated that both MT ratio and k_m increase with increasing collagen content [28;30]. The observed trend towards increased k_m with OA may be due to factors other than increased collagen content. We note that k_m may be sensitive to a variety of collagen structural factors such as crosslinking [51], denaturation, fibril assembly and packing in the extracellular matrix [28;52]. In agreement with our findings Laurent et al. [53] utilized a 3T magnet to assess changes *in vivo* in a goat model of experimental OA and found that k_m was increased 2 weeks post surgery.

In our studies, the consistency in baseline T_1 values between control and OA samples is in agreement with published data showing only a minor increase in cartilage T_1 upon enzymatic 2-depletion of PG species [54]. Measurement of T_1 values after equilibration with Gd-DTPA indicated a higher proteoglycan content in the controls than in the OA samples, consistent with the substantial GAG loss from cartilage following surgical induction of experimental OA described previously [53], and with the FT-IRIS data in the current study.

Although MRI and FT-IRIS both provide insight into macromolecular content and structure, they are in many respects complementary. FT-IRIS is performed on dehydrated tissue sections, while MRI studies are performed on hydrated, intact tissues, including *in vivo* tissues in many instances. To assess PG, MRI studies indirectly measure fixed charge density through known effects of Gd-DTPA²⁻ on tissue water relaxation, while FT-IRIS assesses a specific molecular vibration that arises directly from the PG, the sugar ring vibration [37,40]. Both approaches yield semi-quantitative information on local PG content. The differences in the manner in which PG content is determined between the two modalities could account for the discrepancy found in the percent loss of PG, which, based on FT-IRIS, was approximately 36% at the 4 week time point, while the MRI-based FCD data indicated a PG loss of 68%, nearly twice as great. Alternatively, this discrepancy could be attributable to the fact that the MRI data were

obtained on an entire intact osteochondral plug, while the FT-IRIS data were obtained from a single seven-micron-thick histological section, which may not have been representative of the entire cartilage sample. Notwithstanding these differences, to our knowledge, this is the first study to demonstrate specific correspondence between parameters obtained by FT-IRIS and MRI.

In contrast to FT-IRIS, IFOP assessment is performed on hydrated tissues in a nondestructive manner. Indeed, we have previously described changes in water content in human osteoarthritic tissues [43]. In the current study, using PLS, we were able to predict the time after surgery, a surrogate for disease severity, to within an accuracy of two weeks for the data set tested. Although this specific animal-based chemometric model cannot be directly applied to human cartilage, these results support the potential use of IFOP-based methodology for OA staging in humans, which can be performed in a minimally-invasive fashion in conjunction with arthroscopy. From a clinical perspective, the use of such a minimally-invasive technique for evaluation of cartilage quality could facilitate early intervention and assessment of repair cartilage. The ability of the chemometric analysis of IFOP results to stage early cartilage degeneration is attributable to its ability to detect subtle molecular changes in cartilage structure.

In the current study, FT-IRIS results indicated significant changes in collagen fibril orientation in the earliest stages of disease progression, but no significant changes at the later stages. Using MRI, the dependence of cartilage T_2 on sample orientation was expressed as an anisotropy index η . A trend towards a smaller value for this index in the OA samples obtained 12 weeks after surgery was seen, and is somewhat in contrast to the FT-IRIS assessment of fibril orientation at this time point. However, the larger value of T_2 in the OA samples is consistent with observations both in animal models [55;56] and in *in vitro* samples which have undergone pathomimetic enzymatic degradation [57;58]. The fact that this increase is more pronounced with fibrils oriented nominally parallel to B_0 than at the magic angle suggests an alteration in fibril alignment with degradation. In addition, in each sample, radial zone T_2 was greater at 55° than at 0° (data not shown). This is consistent with radial zone collagen fibril orientation being approximately perpendicular to the articular surface in both the control and OA legs. The trend towards a decrease in η with osteoarthritic degradation is consistent with a randomization of collagen fibril orientation in response to osteoarthritic degradation, a result which, to our knowledge, has not heretofore been reported in the MRI literature.

In summary, FT-IRIS and MRI microscopy together provide valuable compositional and structural information related to changes in articular cartilage at early stages of osteoarthritic degradation. These techniques together provide a powerful complementary approach that results in a comprehensive picture of molecular and biophysical changes in diseased cartilage.

Acknowledgments

This study was supported by NIH EB000744 (NPC) and the Intramural Research Program of the NIH National Institute on Aging, and utilized the facilities of the HSS Core Center for Musculoskeletal Integrity, supported by NIH AR46121. The authors wish to thank Dr. Holly Canuto (NIA) for useful suggestions.

References

1. Hogue JH, Mersfelder TL. Pathophysiology and first-line treatment of osteoarthritis. *Ann Pharmacother* 2002 Apr;36(4):679–86. 2002. 36:679-86. [PubMed: 11918520]
2. Lawrence RC, Hochberg MC, Kelsey JL, et al. Estimates of the prevalence of selected arthritic and musculoskeletal diseases in the United States. *J Rheumatol* 1989;16:427–41. [PubMed: 2746583]
3. Leveille SG. Musculoskeletal aging. *Curr Opin Rheumatol* 2004 Mar;16(2):114–8. 2004;16:114-8. [PubMed: 14770095]

4. Burstein D, Bashir A, Gray ML. MRI techniques in early stages of cartilage disease. *Investigative Radiology* 2000;35:622–38. [PubMed: 11041156]
5. Poole AR. An introduction to the pathophysiology of osteoarthritis. *Frontiers in Bioscience* 1999;4:662–70.
6. Huber M, Trattning S, Lintner F. Anatomy, biochemistry, and physiology of articular cartilage. *Investigative Radiology* 2000;35:573–80. [PubMed: 11041151]
7. Freeman MA. Is collagen fatigue failure a cause of osteoarthritis and prosthetic component migration? A hypothesis. *J Orthop Res* 1999;17:3–8. [PubMed: 10073641]
8. Bank RA, Soudry M, Maroudas A, Mizrahi J, TeKoppele JM. The increased swelling and instantaneous deformation of osteoarthritic cartilage is highly correlated with collagen degradation. *Arthritis Rheum* 2000 Oct;43(10):2202–10. 2000;43:2202-10. [PubMed: 11037879]
9. Yoshioka M, Coutts RD, Amiel D, Hacker SA. Characterization of a model of osteoarthritis in the rabbit knee. *Osteoarthritis Cartilage* 1996;4:87–98. [PubMed: 8806111]
10. Sah RL, Yang AS, Chen AC, et al. Physical properties of rabbit articular cartilage after transection of the anterior cruciate ligament. *J Orthop Res* 1997;15:197–203. [PubMed: 9167621]
11. Rogart JN, Barrach HJ, Chichester CO. Articular collagen degradation in the Hulth- Telhag model of osteoarthritis. *Osteoarthritis Cartilage* 1999;7:539–47. [PubMed: 10558852]
12. Batiste DL, Kirkley A, Laverty S, Thain LM, Spouge AR, Holdsworth DW. Ex vivo characterization of articular cartilage and bone lesions in a rabbit ACL transection model of osteoarthritis using MRI and micro-CT. *Osteoarthritis Cartilage* 2004 Dec;12(12):986–96. 2004;12:986-96. [PubMed: 15564066]
13. Batiste DL, Kirkley A, Laverty S, et al. High-resolution MRI and micro-CT in an ex vivo rabbit anterior cruciate ligament transection model of osteoarthritis. *Osteoarthritis Cartilage* 2004 Aug;12(8):614–26. 2004;12:614-26. [PubMed: 15262241]
14. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. *Magnetic Resonance in Medicine* 1999;41:857–65. [PubMed: 10332865]
15. Akella SV, Regatte RR, Gougoutas AJ, et al. Proteoglycan-induced changes in T1rho-relaxation of articular cartilage at 4T. *Magn Reson Med* 2001 Sep;46(3):419–23. 2001;46:419-23. [PubMed: 11550230]
16. Gray ML, Burstein D, Xia Y. Biochemical (and functional) imaging of articular cartilage. *Semin Musculoskelet Radiol* 2001 Dec;5(4):329–43. 2001;5:329-43. [PubMed: 11745049]
17. Toffanin R, Mlynarik V, Russo S, Szomolanyi P, Piras A, Vittur F. Proteoglycan depletion and magnetic resonance parameters of articular cartilage. *Archives of Biochemistry and Biophysics* 2001;390:235–42. [PubMed: 11396926]
18. Wayne JS, Kraft KA, Shields KJ, Yin C, Owen JR, Disler DG. MR imaging of normal and matrix-depleted cartilage: correlation with biomechanical function and biochemical composition. *Radiology* 2003;228:493–9. [PubMed: 12893905]
19. Burstein D, Gray M. New MRI techniques for imaging cartilage. *J Bone Joint Surg Am* 2003;85-A (Suppl 2):70–7. [PubMed: 12721347]
20. Petersen EF, Fishbein KW, Laouar L, Spencer RG, Wenz JF. Ex vivo magnetic resonance microscopy of an osteochondral transfer. *J Magn Reson Imaging* 2003 May;17(5):603–8. 2003;17:603-8. [PubMed: 12720271]
21. Chen C-T, Fishbein KW, Torzilli PA, Hilger A, Spencer RG, Horton WE Jr. Matrix fixed-charge density as determined by magnetic resonance microscopy of bioractor-derived hyaline cartilage correlates with biochemical and biomechanical properties. *Arthritis and Rheumatism* 2003;48:1047–56. [PubMed: 12687548]
22. Potter K, Butler JJ, Horton WE, Spencer RG. Response of engineered cartilage tissue to biochemical agents as studied by proton magnetic resonance microscopy. *Arthritis Rheum* 2000 Jul;43(7):1580–90. 2000;43:1580-90. [PubMed: 10902763]
23. Xia Y, Moody JB, Alhadlaq H. Orientational dependence of T-2 relaxation in articular cartilage: A microscopic MRI (mu MRI) study. *Magnetic Resonance in Medicine* 2002;48:460–9. [PubMed: 12210910]

24. Xia Y. Relaxation anisotropy in cartilage by NMR microscopy (mu MRI) at 14-mu m resolution. *Magnetic Resonance in Medicine* 1998;39:941–9. [PubMed: 9621918]
25. Xia, Y. Relaxation anisotropy as a possible marker for macromolecular orientations in articular cartilage. In: Blumler, P.; Blumich, B.; Botto, R.; Fukushima, E., editors. *Spatially Resolved Magnetic Resonance: Methods, Materials, Medicine, Biology, Rheology, Geology, Ecology, Hardware*. Weinheim, Germany: Wiley-VCH; 1998. p. 351–62.
26. Nieminen MT, Rieppi J, Toyra J, et al. T2 relaxation reveals spatial collagen architecture in articular cartilage: a comparative quantitative MRI and polarized light microscopic study. *Magnetic Resonance in Medicine* 2001;46:487–93. [PubMed: 11550240]
27. Alhadlaq HA, Xia Y, Moody JB, Matyas JR. Detecting structural changes in early experimental osteoarthritis of tibial cartilage by microscopic magnetic resonance imaging and polarised light microscopy. *Ann Rheum Dis* 2004 Jun;63(6):709–17. 2004;63:709–17. [PubMed: 15140779]
28. Laurent D, Wasvary J, Yin J, Rudin M, Pellas TC, O'Byrne E. Quantitative and qualitative assessment of articular cartilage in the goat knee with magnetization transfer imaging. *Magn Reson Imaging JID* - 8214883 2001;19:1279–86.
29. Potter K, Butler JJ, Adams C, et al. Cartilage formation in a hollow fiber bioreactor studied by proton magnetic resonance microscopy. *Matrix Biol* 1998;17:513–23. [PubMed: 9881603]
30. Seo GS, Aoki J, Moriya H, et al. Hyaline cartilage: in vivo and in vitro assessment with magnetization transfer imaging. *Radiology* 1996;201:525–30. [PubMed: 8888253]
31. Pastrana B, Mautone AJ, Mendelsohn R. Fourier transform infrared studies of secondary structure and orientation of pulmonary surfactant SP-C and its effect on the dynamic surface properties of phospholipids. *Biochemistry* 1991;30:10058–64. [PubMed: 1911771]
32. Moore DJ, Wyrwa M, Reboulleau CP, Mendelsohn R. Quantitative IR studies of acyl chain conformational order in fatty acid homogeneous membranes of live cells of *Acholeplasma laidlawii* B. *Biochemistry* 1993;32:6281–7. [PubMed: 8512939]
33. Moore DJ, Gioioso S, Sills RH, Mendelsohn R. Some relationships between membrane phospholipid domains, conformational order, and cell shape in intact human erythrocytes. *Biochim Biophys Acta* 1999;1415:342–8. [PubMed: 9889394]
34. Haris PI, Chapman D. The conformational analysis of peptides using Fourier transform IR spectroscopy. *Biopolymers* 1995;37:251–63. [PubMed: 7540054]
35. Pevsner A, Diem M. IR spectroscopic studies of major cellular components. III. Hydration of protein, nucleic acid, and phospholipid films. *Biopolymers* 2003;72(4):282–9. 2003;72:282–9. [PubMed: 12833483]
36. Rieppo J, Hyttinen MM, Jurvelin JS, Helminen HJ. Reference sample method reduces the error caused by variable cryosection thickness in Fourier transform infrared imaging. *Applied Spectroscopy* 2004;58:137–40. [PubMed: 14727731]
37. Camacho NP, West P, Torzilli PA, Mendelsohn R. FTIR microscopic imaging of collagen and proteoglycan in bovine cartilage. *Biopolymers* 2001;62:1–8. [PubMed: 11135186]
38. Potter K, Kidder LH, Levin IW, Lewis EN, Spencer RGS. Imaging of collagen and proteoglycan in cartilage sections using fourier transform infrared spectral imaging. *Arthritis and Rheumatism* 2001;44:846–55. [PubMed: 11315924]
39. West PA, Bostrom MP, Torzilli PA, Camacho NP. Fourier transform infrared spectral analysis of degenerative cartilage: an infrared fiber optic probe and imaging study. *Appl Spectrosc* 2004;58:376–81. [PubMed: 15104805]
40. Kim M, Bi X, Horton WE, Spencer RG, Camacho NP. Fourier transform infrared imaging spectroscopic analysis of tissue engineered cartilage: histologic and biochemical correlations. *J Biomed Opt* 2005 May-Jun;10(3):031105. 2005;10:031105. [PubMed: 16229630]
41. Bi X, Li G, Doty SB, Camacho NP. A novel method for determination of collagen orientation in cartilage by Fourier transform infrared imaging spectroscopy (FT-IRIS). *Osteoarthritis Cartilage* 2005;13:1050–8. [PubMed: 16154778]
42. West PA, Torzilli PA, Chen C, Lin P, Camacho NP. Fourier transform infrared imaging spectroscopy analysis of collagenase-induced cartilage degradation. *J Biomed Opt* 2005;10:14015. [PubMed: 15847596]

43. Li G, Thomson M, Dicarlo E, et al. A chemometric analysis for evaluation of early-stage cartilage degradation by infrared fiber-optic probe spectroscopy. *Appl Spectrosc* 2005;59:1527–33. [PubMed: 16390593]
44. Hulth A, Lindberg L, Telhag H. Experimental osteoarthritis in rabbits. Preliminary report. *Acta Orthop Scand* 1970;41:522–30. [PubMed: 5507896]
45. Junqueira LC, Bignolas G, Brentani R. Picrosirius staining plus polarization microscopy, a specific method for collagen detection. *Histochem J* 1979;11:447–55. [PubMed: 91593]
46. Prophet, EB.; Mills, B.; Arrington, JB.; Sobin, LH. *Laboratory Methods in Histotechnology*. Washington, D.C: American Registry of Pathology; 1992.
47. Hajnal J, Baudouin CJ, Oatridge A, Young I, Bydder G. Design and implementation of magnetization transfer pulse sequences for clinical use. *J Comput Assist Tomogr* 1992;16:7–18. [PubMed: 1729310]
48. Bashir A, Gray ML, Burstein D. Gd-DTPA2- as a measure of cartilage degradation. *Magn Reson Med* 1996;36:665–73. [PubMed: 8916016]
49. Bi X, Yang X, Bostrom MP, Camacho NP. Fourier transform infrared imaging spectroscopy investigations in the pathogenesis and repair of cartilage. *Biochim Biophys Acta*. 2006 May 23; 2006.
50. Hotta H, Yamada H, Takaishi H, et al. Type II collagen synthesis in the articular cartilage of a rabbit model of osteoarthritis: expression of type II collagen C-propeptide and mRNA especially during early-stage osteoarthritis. *J Orthop Sci* 2005;10:595–607. [PubMed: 16307185]
51. Fishbein KW, Gluzband Y, Kaku M, Togore H, Shapses S, Yamauchi M, Spencer RG. The effects of formalin fixation and crosslinking on MRI contrast in bovine nasal cartilage explants. *Proc Intl Soc Magn Reson Med* 2006;14:1254.
52. Gray ML, Burstein D, Lesperance L, Gehrke L. Magnetization transfer in cartilage and its constituent macromolecules. *Magn Reson Med* 1995;34:319–25. [PubMed: 7500869]
53. Laurent D, O'Byrne E, Wasvary J, Pellas TC. In vivo MRI of cartilage pathogenesis in surgical models of osteoarthritis. *Skeletal Radiol* 2006;35:555–64. [PubMed: 16639625]
54. Gillis A, Gray M, Burstein D. Relaxivity and diffusion of gadolinium agents in cartilage. *Magn Reson Med* 2002;48:1068–71. [PubMed: 12465119]
55. Gahunia HK, Lemaire C, Babyn PS, Cross AR, Kessler MJ, Pritzker KP. Osteoarthritis in rhesus macaque knee joint: quantitative magnetic resonance imaging tissue characterization of articular cartilage. *J Rheumatol* 1995;22:1747–56. [PubMed: 8523356]
56. Watson PJ, Carpenter TA, Hall LD, Tyler JA. Cartilage swelling and loss in a spontaneous model of osteoarthritis visualized by magnetic resonance imaging. *Osteoarthritis Cartilage* 1996;4:197–207. [PubMed: 8895221]
57. Nieminen MT, Toyras J, Rieppo J, et al. Quantitative MR microscopy of enzymatically degraded articular cartilage. *Magnetic Resonance in Medicine* 2000;43:676–81. [PubMed: 10800032]
58. Jelicks LA, Paul PK, O'Byrne E, Gupta RK. Hydrogen-1, sodium-23, and carbon-13 MR spectroscopy of cartilage degradation in vitro. *J Magn Reson Imaging* 1993;3:565–8. [PubMed: 8347947]

Figure 1.
A representative infrared fiber optic probe spectrum obtained from medial femoral condyle cartilage of a control rabbit with the absorbances of interest labeled.

Figure 2.

Distal femur of a rabbit sacrificed 4 weeks post-surgery illustrating the site of plug harvest from the weight-bearing region of the medial femoral condyle. MR imaging slices were defined through the center of each plug along the anterior-posterior axis and histological sections for FT-IRIS analysis were obtained from similar regions as those of the plug harvest.

Figure 3. Cross validation (i) and test validation (ii) results for a 15-factor PLS model using the spectral range of 1586–999 cm^{-1} : $R^2 = 94.8$ and 93.6 , respectively. The red points are outliers as determined by the Quant II OPUS software.

Figure 4.

FT-IRIS-derived images of collagen content (Amide I) (i), and proteoglycan content (PG/collagen) (ii) from the articular surface down to the tidemark, acquired from the medial femoral condyle of an OA (right panels) and non-surgical control (left panels) animal at the two week timepoint. The color scale indicates the pixel values for collagen content and PG/collagen, where red and dark blue represent highest and lowest values, respectively. The histological images were acquired from the same tissue stained by picrosirius red for collagen content (iii) and Alcian blue for proteoglycan content (iv).

Figure 5.

FT-IRIS measurements (mean $\pm$ standard deviation) for collagen content (i), PG/collagen ratio (ii) and collagen integrity (iii) averaged over the entire depth of cartilage at 2 through 12 weeks post-surgery. Pink and blue symbols represent the values at each timepoint for the OA and the non-surgical control groups, respectively. Statistically significant differences between OA and control at specific time points are noted with an asterisk (* $p < 0.05$).

Figure 6.

T₂-weighted MRI image of an osteochondral plug cut from the medial femoral condyle of a non-surgical control rabbit age-matched to the 4 week post-surgical time point. Compact subchondral bone appears as a dark band lying between the articular cartilage and the trabecular bone. Radial zone cartilage is indicated by the defined region of interest (ROI). Pixel intensities within such ROI's were used to determine the MRI parameters k_m and T_1 , and to calculate matrix fixed charge density.

Figure 7.

Polarized FT-IRIS images at 2, 6 and 12 weeks post-surgery, and the corresponding polarized light microscopy (PLM) images from picrosirius red stained slides. The boundaries on the color bar indicate the three collagen fibril orientation categories with respect to the articular surface, corresponding to amide I/amide II peak area ratios ≥ 2.7 (parallel), between 2.7 and 1.7 (random), and ≤ 1.7 (perpendicular), respectively. In the PLM images, highly ordered collagen appears bright, while collagen that is non-birefringent appears dark.

Figure 8.

Percentage of pixels from the entire IR image showing parallel (i), random (ii) or perpendicular (radial) (iii) collagen fibril orientations at different timepoints. Pink and blue symbols represent the mean values $\pm$ standard error of the mean at each timepoint for the OA and non-surgical control groups, respectively. Statistically significant differences between OA and control at specific time points are noted with an asterisk (* $p < 0.05$).

Table 1

Proton T_1 relaxation data for radial zone cartilage in plugs cut from rabbit medial femoral condyles 4 weeks post-surgery. N=3 animals in each group.

	Non-Surgical Control Osteochondral Plugs	Operated (OA) Osteochondral Plugs	p value
Pre-Gd T_1 , ms	1209 $\pm$ 93	1278 $\pm$ 52	0.34
Post-Gd T_1 , ms	216.4 $\pm$ 9.0	126.6 $\pm$ 7.6	< 0.01
FCD, mM	-132.7 $\pm$ 12.4	-41.6 $\pm$ 11.0	< 0.01

Table 2

Proton T_2 relaxation data for radial zone cartilage of the rabbit medial femoral condyle at 12 weeks post-surgery. N=3 animals in each group.

	Sham-Operated (Control) Femoral Condyles	Operated (OA) Femoral Condyles	p value
T_2 , ms, at $\theta \approx 0^\circ$	27.1 ± 4.1	39.0 ± 1.3	0.06
T_2 , ms, at $\theta \approx 55^\circ$	41.4 ± 4.0	51.9 ± 10.5	0.14
Anisotropy index η	-0.565 ± 0.37	-0.326 ± 0.23	0.22