Published in final edited form as:

Spectrochim Acta A Mol Biomol Spectrosc. 2012 March ; 88: 90–96. doi:10.1016/j.saa.2011.12.002.

Concentration Profiles of Collagen and Proteoglycan in Articular Cartilage by Fourier Transform Infrared Imaging and Principal Component Regression

Jianhua Yin, PhD, Yang Xia, PhD*, and Mei Lu, PhD#

Department of Physics and Center for Biomedical Research, Oakland University, Rochester, MI 48309, USA

#Department of Biostatistics and Epidemiology, Henry Ford Health System, Detroit, MI 48202, USA

Abstract

Fourier-transform infrared imaging (FT-IRI) technique with the principal component regression (PCR) method was used to quantitatively determine the 2D images and the depth-dependent concentration profiles of two principal macromolecular components (collagen and proteoglycan) in articular cartilage. Ten 6 μm thick sections of canine humeral cartilage were imaged at a pixel size of 6.25 μm in FT-IRI. The infrared spectra extracted from FT-IRI experiments were imported into a PCR program to calculate the quantitative distributions of both collagen and proteoglycan in dry cartilage, which were subsequently converted into the wet-weight based concentration profiles. The proteoglycan profiles by FT-IRI and PCR significantly correlated in linear regression with the proteoglycan profiles by the non-destructive μMRI (the goodness-of-fit 0.96 and the Pearson coefficient 0.98). Based on these concentration relationships, the concentration images of collagen and proteoglycan in both healthy and lesioned articular cartilage were successfully constructed two dimensionally. The simultaneous construction of both collagen and proteoglycan concentration images demonstrates that this combined imaging and chemometrics approach could be used as a sensitive tool to accurately resolve and visualize the concentration distributions of macromolecules in biological tissues.

Keywords

FT-IRI; PCR; articular cartilage; concentration; collagen; proteoglycan

1. Introduction

Articular cartilage, a thin layer of soft connective tissue that covers the articulating surfaces of bones in synovial joints to maintain normal joint motion and to withstand external loading, has two principal macromolecular components (type II collagen and proteoglycan (PG)) and is saturated with water molecules and mobile ions^{1, 2}. A PG aggrecan consists of

© 2011 Elsevier B.V. All rights reserved.

*Corresponding Author and Address: Yang Xia, PhD, Department of Physics, Oakland University, Rochester, Michigan 48309, USA, Phone: (248) 370-3420, Fax: (248) 370-3408, xia@oakland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

glycosaminoglycans (GAG) attached to a protein core. The collagen fibers are woven into a three-dimensional fibrous network, which enmeshes PG molecules in the tissue. Since the collagen in the fibrous network has a depth-dependent orientation, the uncalcified cartilage is conceptually subdivided into three structural sub-zones from the surface to subchondral bone, superficial zone (SZ), transitional zone (TZ), and radial zone (RZ) ³⁻⁵. The zonal difference in the fibril orientation, shape, and arrangement of chondrocytes distinguishes articular cartilage from the more homogenous nasal cartilage and provides articular cartilage with the functional properties of depth-dependent tensile strength and resiliency ^{3, 6, 7}.

Either the disruption of the collagen network or a loss of PG in cartilage would signal the onset of functional degeneration of the tissue, which will eventually lead to degenerative joint diseases such as osteoarthritis or other forms of arthritis. The ability to determine the concentration profiles of collagen and PG along the tissue depth, therefore, is highly useful in the monitoring of tissue degeneration and therapeutic progress. Several approaches have been used in recent years to investigate the depth-dependent properties of articular cartilage, such as biochemical analysis ⁸⁻¹³, micro-magnetic resonance imaging (μ MRI) ^{12, 13}, biomechanical measurement ^{6, 7}, and various forms of microscopy techniques ^{5, 6, 14-18}. The biochemical and biomechanical methods can determine the contents of molecular components in either bulk tissue or thick parallel slice, not with microscopic resolution in imaging. The dGEMRIC protocol in μ MRI can determine only the PG concentration in cartilage; there is not yet an effective μ MRI protocol to determine the collagen concentration or its distribution in cartilage. Most microscopy techniques (e.g., optical microscopy, scanning and transmission electron microscopies) are commonly used to study tissue morphology and ultrastructure, not molecular concentrations.

Fourier transform infrared imaging (FT-IRI) technique is a newly developed analytical tool with an imaging capability and has been used to study the variations in compositions and structures of biological tissues (chemical compositions and amide anisotropies) with fine spatial and spectral resolutions ^{16, 19-29}. Most FT-IRI studies of articular cartilage have used the peak intensities/areas in the infrared absorption images to represent the molecular contents ^{19, 20, 22, 23, 30}. The considerable overlaps of the characteristic bands of multiple molecular components in cartilage ²¹, however, can introduce large errors in any attempt of a direct intensity-based calculation of concentration ²⁹.

A more accurate approach in FT-IRI is to combine FT-IRI experiments with the chemometrics method, which is possible to determine the concentration distributions of collagen and PG in articular cartilage with microscopic resolution. As the simplest chemometrics method, the principal component regression (PCR) approach can fully utilize all spectral data (multivariate calibration) and iteratively perform factor analysis. As such, it is possible for PCR to identify the number of components in a mixed sample and to predict the component concentrations in unknown samples with higher precision than univariate calibration ^{31, 32}. In our previous work ³², FT-IRI combining PCR (FT-IRI-PCR) was successfully applied in calculating the principal component concentrations in bovine nasal cartilage to obtain reliable results by comparing the infrared results with the biochemical measurement. This project uses the FT-IRI-PCR approach to determine the depth-dependent profiles of collagen and PG concentrations in articular cartilage, quantitatively and simultaneously. In addition, the PCR results were compared with two signature bands in the infrared spectra of articular cartilage, the band of collagen II (around 1338 cm^{-1} , the CH₂ wagging vibration of proline side chains ^{20, 23}), and the band of PG for sugar groups (around 1072 cm^{-1}) ^{19, 20}.

2. Materials and Methods

2.1 Cartilage sample

Three intact humeral heads were harvested immediately from three 1- to 2-year-old healthy dogs shortly after they were sacrificed for an unrelated biomedical study. Articular cartilage still attached to the underlining bones was cut from the joints by using a table saw and a diamond blade. The final size of the cartilage-bone specimen was approximately 2 mm × 2 mm × 2 mm. After the tissue blocks were washed in saline for one minute, they were frozen in water by liquid nitrogen. A cryostat (Reichert HistoStat Cryotome) was used to cut a number of 6-μm thick sections from each frozen block. These sections were rapidly picked up by the MirrIR slides (Kevley Technologies, Chesterland, OH) and left to air dry for 2 hours before the FT-IR experiments were conducted. A total of ten sections from three cartilage-bone blocks were used in this FT-IR study. In addition to the healthy cartilage blocks, as an illustration of the potential application of the method, one tissue block from an ongoing project in our lab that studies the early changes in animals that underwent anterior (cranial) cruciate ligament (ACL) transection surgery was used, which was prepared using the identical procedures as the healthy cartilage.

2.2 FT-IR spectroscopy and imaging

FT-IR experiments were performed on a PerkinElmer Spotlight-300 FT-IR imaging system (Wellesley, MA), which has an FT-IR spectrometer, an infrared microscope, and a liquid N₂ cooled 16-element MCT (mercuric cadmium telluride) focal plane array detector and a single point MCT detector. In the imaging mode, the infrared light focuses on the specimen that is mounted on a movable mechanical stage. The data are collected by the focal plane array detector at 6.25-μm pixel resolution and 16 cm⁻¹ spectral resolution over a range of 4000-744 cm⁻¹. An internal coaxial LED illumination with variable intensity is available in the instrument to produce visible images, which enables the identification of the tissue region for IR imaging.

During the image analysis, a rectangular region-of-interest (ROI: having a width of 20 μm, which is along the direction of the tissue depth, and a fixed height) was selected and the spectra in the region were averaged to obtain one target spectrum by using the function of “Co-add Spectra” in the PerkinElmer software. These target spectra were introduced into a chemometrics software, Spectrum Quant+ from PerkinElmer with the PCR algorithm, to calculate the concentrations of collagen and PG in the specimens. The first and second eigenvalues were comparable with approximate 100% of total variance, suggesting only a little amount of total variance would be missing. Thereby it is doable for the PCR concentration prediction based on two principal components of collagen and PG. No further spectral pre-processing was performed on the raw data ³¹.

A library of the infrared spectra of the pure chemical components of type II collagen (Elastin Products Company, MO) and chondroitin-6-sulfate (CS6) (Sigma-Aldrich, MO) were established in the previous work in our lab ³². Briefly, the pure chemicals were dry-mixed in 11 different ratios (Collagen/CS6 = 1.34, 2.54, 1.56, 1.36, 1.24, 1.09, 0.98, 0.86, 0.60, 0.40, 0 mg/mg) and pressed into 10 mm pellets of potassium bromide (KBr). The KBr pellets were loaded into the sample chamber of the same infrared spectrometer to obtain the reference infrared spectra ranging from 4000 to 744 cm⁻¹ with a spectral resolution of 16 cm⁻¹ and 2 scans per pixel. These spectra from the pure chemicals were used to construct a standard library in the Spectrum Quant+ software. (Note that the PG population in cartilage also contains some other minor sugar components. Since CS6 is the dominant component, it has been used in other published works to represent PG ^{19, 21}.) Cross-validation was performed in our previous work ³², which describes the excellent correlation between the

actual concentration and the PCR predicted concentration about collagen. At this time the optimal number of factor of 2 was verified.

2.3 Statistical Analysis

Pearson correlation coefficient was calculated between μ MRI (as variable Y) and FT-IRI (as variable X) at each depth. The correlation coefficient between variables Y and X, r , has a range of -1 to 1, where $|r|=1$ means a perfect correlation and 0 means no correlation. A positive correlation coefficient indicates that an increased Y is correlated with an increased X; a negative correlation coefficient, in contrary, indicates an increased Y correlated with a decreased X. A linear regression model was used to study the transformation of μ MRI to FT-IRI on the measurement of tissue depths with the estimation of R^2 for modeling goodness-of-fit, where $r = \sqrt{R^2}$.

3. Results

Fig 1a shows a target spectrum obtained from the Spotlight software by PerkinElmer. All target spectra were introduced into the Spectrum Quant+ software with the PCR algorithm to calculate the concentrations of collagen and PG in the specimens. Fig 1b shows two spectra from the pure chemicals of collagen (type II) and PG (CS6) respectively. It is clear that the collagen spectrum has a unique peak at 1338 cm^{-1} , which the PG spectrum lacks; in the meantime, the PG spectrum has a dominant peak at 1072 cm^{-1} . It should also be noted that there is significant overlapping of these two spectra in the wavenumber region-of-interest; consequently, any direct assignment of an IR peak intensity/area as the molecular concentrations will contain significant error.

A set of the FT-IR images from a typical imaging experiment is shown in Fig 2, including the visible image (Fig 2a), the total absorption image (Fig 2b), the amide I image (Fig 2c, in the wavenumber range of $1700 \sim 1600\text{ cm}^{-1}$), the amide II image (Fig 2d, in the wavenumber range of $1600 \sim 1500\text{ cm}^{-1}$), the amide III image (Fig 2e, in the wavenumber range of $1355 \sim 1315\text{ cm}^{-1}$), the 1338 cm^{-1} band image (Fig 2f, in the wavenumber range of $1355 \sim 1315\text{ cm}^{-1}$), and the 1072 cm^{-1} band image (Fig 2g, in the wavenumber range of $1125 \sim 960\text{ cm}^{-1}$). The two spectroscopic band images in Figs 2f and 2g represent *qualitatively* the inhomogeneous distributions of collagen and PG across the uncalcified tissue (SZ, TZ and RZ). The minuscule numbers surrounding each image were the micrometer readings of the moving stage, on which the specimen was scanned. The actual values of these micrometer readings are not important in the final presentation but useful in the data analysis, since they can be used to track and correlate the locations of specific features on the images. The total thickness or width of the uncalcified cartilage was about $660\text{ }\mu\text{m}$.

Based on the absorption images from the FT-IRI experiments, the PCR program extracted all target IR spectra in each rectangular ROI to quantitatively calculate the concentrations of collagen and PG for that ROI. The depth-dependent profiles of collagen and PG concentrations from the section mentioned above are shown in Fig 3a. Since the tissue section was dry, the sum of the collagen and PG concentrations at any location was assumed to be 100 %. The mean and the standard deviation of the collagen and PG concentrations for the section shown Fig 3a are $58.1 \pm 4.8\%$ and $41.9 \pm 4.8\%$ respectively. As a comparison, the band areas of the 1338 and 1072 cm^{-1} signature peaks in these target spectra were also obtained and plotted in Fig 3b. Although there are some similarities in lineshapes between the profiles shown in Fig 3a and 3b, in particular the 1072 sugar bands, the vertical axes in the peak area profiles (Fig 3b) are not calibrated. Interestingly, the ratios of the two sets of profiles in Fig 3a and Fig 3b resemble each other, as shown in Fig 3c. The critical difference

between the two ratio profiles is again its values: the concentration ratio had its meaning (the concentration ratio of collagen over PG) but the peak ratio was not calibrated to molecular concentrations.

A total of ten AC sections were imaged and processed. Since these tissue sections had different depths, the absolute depth coordinates of all tissues were normalized to 0 (articular surface) and 1 (tidemark). All calculated concentration data from the ten sections are shown in Fig 4a, together with the fits of a 3rd order polynomial regression (the solid lines). It is clear that in the dry tissue sections, the highest content of collagen is in the SZ, which decreases as the function of tissue depth approximately to the middle portion of the tissue. The collagen concentration remains relatively constant for the second half of the tissue (the radial zone). The PG concentration shows a reversed trend as the collagen profile, having the lowest concentration in the SZ and increasing to maximum at the middle tissue. The distribution of collagen and PG in articular cartilage resemble those introduced in the reference of ⁷ The mean concentrations of the collagen and PG in dry articular cartilage for all ten sections are $69.3 \pm 8.2\%$ and $30.7 \pm 8.2\%$ (mean $\pm$ standard-deviation), respectively, which are well consistent with previous studies in literature ^{2, 3, 7, 33, 34}. The cause of the concentration distribution along the vertical scale was attributed to the fact that those ten sections were from three different canines that must have had individual differences.

Since the concentrations of collagen and PG are commonly measured on a wet weight basis ¹², the concentration profile of water in articular cartilage has to be considered ^{3, 8, 10, 34, 35}. Fitting the measurement of the water profiles by Brocklehurst et al ¹⁰, shown in Fig 4b, the dry-weight based concentration profiles of collagen and PG in articular cartilage were converted into the wet-weight based profiles, shown in Fig 4c (from a conversion relationship of $1120 \text{ mg/ml} \times (1 - C_{H_2O}) \times C_{PC}$, where 1120 mg/ml is the mass of cartilage tissue in the unit volume; C_{H_2O} and C_{PC} represent the concentrations of principal components). These profiles qualitatively resemble the macromolecular content profiles reported in the literature ^{8, 11, 36}. A notable difference between the dry-based profiles and the wet-based profiles is the monotonic increments of both collagen and PG in the deep tissue. The mean concentrations of collagen and PG on a wet-weight base are 198.9 ± 34.1 and 89.7 ± 28.5 mg/ml respectively.

As a validation, the wet-weight base PG concentration profile by FT-IRI-PCR was plotted in Fig 5a, together with that by a previous μ MRI data ¹³ that used an identical tissue (the same humeral tissue from the identical locations on the canine joint surfaces, where the joints were from a long-term on-going study). The agreement is excellent (the mean PG by μ MRI was 84.3 ± 27.6 mg/ml.). We would like to point out that the PG concentration in our μ MRI work has been extensively validated in several previous publications that used not only ¹H μ MRI but also several histochemical assays, ²³Na inductively coupled plasma emission spectroscopy and ²³Na NMR spectroscopy ^{12, 13, 37–39}. In this project, the statistical correlation between the μ MRI data ¹³ and the averaged profiles of FT-IRI-PCR concentration (the solid line in Fig 4c) were assessed using the linear regression. Fig 5b shows the statistical assessment between the two sets of data, where the depth profile of PG using μ MRI can be expressed as the function of that of FT-IRI-PCR in the formula as

$$Tissuedepth_{MRI} = -37.736 + 1.382 \times Tissuedepth_{FT-IRI-PCR},$$

which has a goodness-of-fit of 0.96 and the Pearson coefficient r of 0.98.

The 2D images of collagen and PG concentrations in articular cartilage were constructed, on the wet-tissue basis and pixel-by-pixel by importing the spectra at each pixel location to the

PCR program and applying the dry-to-wet conversion on the PCR results. A pair of the quantitative concentration images is shown in Fig 6a from the tissue section in Fig 2, in the unit of mg/ml. Also shown in Fig 6a is a visible image from a Leica microscope, which provides better quality visible images than the FT-IRI system. Although the existence of the cells in tissue disturbed the distribution of the PG and collagen concentrations in the extracellular matrix, one can see some similarities between the PG concentration image (Fig 6a) and the sugar image by the peak-area method (Fig 2g). These similarities indicated that characteristic bands play significant roles in structural changes, principal component discrimination and diversity. There are two major differences between the quantitative concentration images and the peak-area images. First, the regions surrounding the chondrocytes and pericellular areas are very different between the quantitative images and the peak-area images. Second, the peak-area images are not calibrated – the values of image absorption cannot be directly correlated to the chemical concentration, which confirms that the individual peak-area images are not suitable to directly represent the chemical composition.

As an illustration of the method, Fig 6b shows a set of the PCR calculated wet-weight based concentration images from a canine tibial cartilage where the animal was sacrificed 12 weeks after undergoing anterior (cranial) cruciate ligament (ACL) transection in one knee joint. While the collagen map of the lesioned cartilage (Fig 6b) has the same feature as in the healthy cartilage (Fig 6a), a noticeable change in the PG map of the lesioned cartilage was the reduction of the PG concentration for the surface tissue (the initial 150 μm tissue), which is consistent with the understanding that the PG reduction is an early sign of the tissue degradation. A comprehensive project is currently ongoing in our lab to investigate the early changes in tissue at different stages of the osteoarthritic lesions using multidisciplinary imaging techniques.

4. Discussion

Collagen and PG each has a characteristic band in FT-IR spectra, centered at 1338 and 1072 cm^{-1} respectively^{19, 20, 23, 30}. Hence the corresponding FT-IR images and profiles of absorption peak areas qualitatively resemble the distribution of collagen and PG in cartilage. However, a direct interpretation of characteristic band images/intensity profiles as the molecular concentration distributions is often problematic, due to two reasons. There are significant cross contaminations between the characteristic bands of collagen and PG in each other's spectrum, as illustrated in Fig 1b. Consequently, these spectral band intensity profiles^{19, 20, 22, 23, 30} can only be used for qualitative reference of molecular content, not accurately interpreted as the molecular concentration. In contrast, by using a spectral library in this project where each element was measured from a combination of pure chemicals, the PCR algorithm in this project used all spectral bands to extract unique information in the range of 4000-960 cm^{-1} and was able to calculate the collagen and PG concentrations accurately.

4.1 Comparison between FT-IRI and FT-IRI-PCR

By using a spectral library in this project where each element was measured from a combination of pure chemicals, the PCR algorithm extracted unique information in the range of 4000-960 cm^{-1} and was able to calculate the collagen and PG concentrations accurately. On the mean values summed over the entire tissue depth, the accuracy of the FT-IRI-PCR based PG concentration (89.7 ± 28.5 mg/ml) agrees well with the non-destructively obtained PG concentration profiles by μMRI (84.3 ± 27.6 mg/ml)¹³ and with other reports in literatures^{40, 41}; likewise, the mean collagen concentration by the FT-IRI-PCR method is 198.9 ± 34.1 mg/ml, also consistent with the previous reports⁴⁰⁻⁴². The quantitative profiles of the collagen and PG concentrations in this report are in agreement with the predicted

results reported by Wilson et al.⁷. They also confirm the depth-dependent nature of these macromolecules in articular cartilage^{2, 3, 6–8, 10–13, 20, 33, 34, 43–45}, which give rise to distinct biomechanical properties of the tissue. *To our best knowledge, this is the first detailed depth dependent profile investigation of quantitative collagen concentration at microscopic resolution.*

4.2 Experimental Limitations and Errors

Any quantitative procedure in analytical science must have its experimental limitations and errors. On the tissue treatment, some recent work on articular cartilage revealed that the tissue could lose a portion of its PG to the soaking solution after the tissue goes through a freeze-thaw cycle^{12, 13}. In this project, although our specimens were frozen by liquid nitrogen, it had never been soaked-thawed in saline for any long time. We therefore do not expect any significant loss of PG from the tissue sections that were studied by FT-IRI in this project.

There are a number of potential sources of error in the methods employed in this paper. Composition-wise, we have ignored the contributions of minor matrix components (other than collagen and PG) and chondrocytes (ca. 1–5% in volume), which could introduce a small error in the PCR calculation. In fact, the influence of chondrocytes is visible in the collagen concentration image of articular cartilage (Fig 6), which showed an increased collagen concentration at some cells and pericellular areas (the highlight areas). This localized high concentration of collagen is not visible in spectroscopic image of the 1338 cm^{-1} band in Fig 2f. Mathematically, this type of localized high concentration is the source of the high fluctuations in the PCR calculated concentration profiles as evident in Fig 4. Morphologically, this type of localized high concentration reflects the complex structure at/around the chondrocytes, which is different than the extracellular matrix of the tissue¹⁷. Since the pixel resolution of the current FT-IRI is limited to a nominal size of 6.25 μm , this instrument is insufficient to effectively resolve any fine structure of chondrocytes and pericellular areas⁴⁶. In addition, the complex cellular structure could increase any scattering of infrared irradiation at/around the cell areas^{46, 47}, which further increases the inaccuracy of the PCR calculation in these areas^{46, 48}. Further investigation by attenuated total reflection-FTIRI with 1.56 μm pixel resolution confirms the mentioned above and discovers that protein mostly distributes around the cells and weak absorption from other cellular components occurs at cellular centers⁴⁶. Another potential source of error was the use of CS6 as the total GAG to represent PG, thus ignoring the contributions of minor core proteins, link proteins, and keratan sulphate in PG on PCR calculation. Finally, the conversion of the dry-weight measurement to the wet-weight measurement adopted the depth dependent profile of water content from literature¹⁰, which might contain some species-specific errors when it was applied to our specimens. In addition, the water content in the diseased sample would be disease-dependent, larger than the healthy sample, but not yet fully determined. A more accurate determination of the water content for tissue at different stages of the disease would require further investigation.

We should also point out that the initial depth region ($\sim 20 \mu\text{m}$) in the SZ has been excluded from all PCR calculations in this report, for the following reasons. This region typically includes a thin layer of membrane-like tissue that is primarily constituted of type II collagen finely aligned in the direction of shear stress and serves as a gliding surface³³. As mentioned by Crockett et al.⁴⁹, the articular surface layer is further covered by a superficial gel-like layer that mainly contains hyaluranan and phospholipid. These components might influence the calculation results on PCR concentrations of collagen and PG in this narrow region. The other important reason is the specular reflection that can easily happen at the tissue edge, which can cause some spectral artifacts⁴⁸.

In conclusion, the depth-dependent profiles of the two principal macromolecular components in articular cartilage were determined both qualitatively and quantitatively using FT-IRI and PCR with fine resolution. The dry and wet profiles of these concentrations have different shapes, depending upon the concentration of water in the tissue. The excellent correlation between the PG profiles from this project and the previously validated PG profiles^{12, 13} demonstrates the accuracy of the macromolecular components by the FT-IRI-PCR approach. The ability to determine the concentration profiles and the mean percentage concentrations of collagen and PG ($69.3 \pm 8.2\%$, $30.7 \pm 8.2\%$ respectively in dry tissue; 198.9 ± 34.1 and 89.7 ± 28.5 mg/ml respectively in wet tissue) demonstrates that the FT-IRI-PCR method can be effectively used to study the distribution of macromolecules accurately in articular cartilage and other biological tissues. The construction of the quantitative images of macromolecules in both lesioned and healthy articular cartilage in two dimensions provides a visual framework to study the fine structure of the tissue chemically. To the best of our knowledge, this is the first 2D collagen image in articular cartilage by the chemometrics method, quantitatively and with fine spatial resolution. Since these chemical concentrations can predict diseased progression and monitor the tissue injury and repair, this combination of FT-IRI and chemometrics approach has potential to improve our understanding of the complex processes in the variations of molecular composition and morphology in disease cartilage.

Acknowledgments

Yang Xia is grateful to the National Institutes of Health for the R01 grants (AR 045172, AR 052353). The authors are indebted to Dr John Matyas (Veterinary Medicine, University of Calgary, Canada) for providing the lesioned specimens to an ongoing project, Drs. Clifford Les and Hani Sabbah (Henry Ford Hospital, Detroit) for providing the healthy specimens, Dr. Shaokuan Zheng and Mr. Farid Badar (Dept of Physics, Oakland University) for preparing the cartilage specimen blocks, and Ms. Carol Searight (Dept of Physics, Oakland University) for the editorial comments on the manuscript.

References

1. Buckwalter, JA.; Rosenberg, LC.; Hunziker, EB. Articular cartilage and knee joint function. Basic science and arthroscopy, Bristol-Myers/Zimmer Orthopaedic Symposium. Ewing, JW., editor. Raven Press; New York: 1990. p. 19-56.
2. Mow VC, Guo XE. *Ann Rev Biomed Eng.* 2002; 4:175–209. [PubMed: 12117756]
3. Maroudas AI. *Nature.* 1976; 260:808–809. [PubMed: 1264261]
4. Buckwalter JA, Mankin H. *J Bone Joint Surgery.* 1997; 79A:600–611.
5. Xia Y, Moody J, Burton-Wurster N, Lust G. *Osteoarthritis Cartilage.* 2001; 9:393–406. [PubMed: 11467887]
6. Chen SS, Falcovitz YH, Schneiderman R, Maroudas A, Sah RL. *Osteoarthritis Cartilage.* 2001; 9:561–569. [PubMed: 11520170]
7. Wilson W, Huyghe JM, van Donkelaar CC. *Biomech Model Mechanobiol.* 2007; 6:43–53. [PubMed: 16710737]
8. Venn M, Maroudas A. *Ann Rheum Dis.* 1977; 36:121–129. [PubMed: 856064]
9. Bayliss MT, Venn M, Maroudas A, Ali SY. *Biochem J.* 1983; 209:387–400. [PubMed: 6405736]
10. Brocklehurst R, Bayliss MT, Maroudas A, Coysh HL, Freeman MA, Revell PA, Ali SY. *J Bone Joint Surg Am.* 1984; 66:95–106. [PubMed: 6690447]
11. Kiviranta I, Jurvelin J, Tammi M, Saamanen AM, Helminen HJ. *Histochemistry.* 1985; 82:249–255. [PubMed: 2581923]
12. Xia Y, Zheng S, Bidthanapally A. *J Magn Reson Imaging.* 2008; 28:151–157. [PubMed: 18581328]
13. Zheng S, Xia Y, Bidthanapally A, Badar F, Ihsar I, Duvoisin N. *Magn Reson Imaging.* 2009; 27:648–655. [PubMed: 19106023]
14. Redler I, Mow VC, Zimny ML, Mansell J. *Clin Orthop and Related Topics.* 1975; 112:357–367.

15. Xia Y, Elder K. *J Microscopy*. 2001; 204:3–16.
16. Xia Y, Ramakrishnan N, Bidthanapally A. *Osteoarthritis Cartilage*. 2007; 15:780–788. [PubMed: 17317225]
17. Bonifacio A, Beleites C, Vittur F, Marsich E, Semeraro S, Paoletti S, Sergio V. *Analyst*. 2010; 135:3193–3204. [PubMed: 20967391]
18. Lim NS, Hamed Z, Yeow CH, Chan C, Huang Z. *J Biomed Opt*. 2011; 16:017003. [PubMed: 21280924]
19. Potter K, Kidder LH, Levin IW, Lewis EN, Spencer RG. *Arthritis Rheum*. 2001; 44:846–855. [PubMed: 11315924]
20. Camacho NP, West P, Torzilli PA, Mendelsohn R. *Biopolymers*. 2001; 62:1–8. [PubMed: 11135186]
21. David-Vaudey E, Burghardt A, Keshari K, Bouchet A, Ries M, Majumdar S. *Eur Cell Mater*. 2005; 10:51–60. [PubMed: 16307426]
22. Bi X, Li G, Doty SB, Camacho NP. *Osteoarthritis Cartilage*. 2005; 13:1050–1058. [PubMed: 16154778]
23. Bi X, Yang X, Bostrom MP, Camacho NP. *Biochim Biophys Acta*. 2006; 1758:934–941. [PubMed: 16815242]
24. Ramakrishnan N, Xia Y, Bidthanapally A. *Phys Med Biol*. 2007; 52:4601–4614. [PubMed: 17634653]
25. Ramakrishnan N, Xia Y, Bidthanapally A, Lu M. *Appl Spectrosc*. 2007; 61:1404–1409. [PubMed: 18198035]
26. Ramakrishnan N, Xia Y, Bidthanapally A. *J Orthop Surg Res*. 2008; 3:48. [PubMed: 18837979]
27. Xia Y, Alhadlaq H, Ramakrishnan N, Bidthanapally A, Badar F, Lu M. *J Struct Biol*. 2008; 164:88–95. [PubMed: 18634884]
28. Rieppo J, Hyttinen MM, Halmesmaki E, Ruotsalainen H, Vasara A, Kiviranta I, Jurvelin JS, Helminen HJ. *Osteoarthritis Cartilage*. 2009; 17:448–455. [PubMed: 18849174]
29. Rieppo L, Saarakkala S, Narhi T, Holopainen J, Lammi M, Helminen HJ, Jurvelin JS, Rieppo J. *Microsc Res Tech*. 2010; 73:503–512. [PubMed: 19839035]
30. Bi X, Yang X, Bostrom MP, Bartusik D, Ramaswamy S, Fishbein KW, Spencer RG, Camacho NP. *Anal Bioanal Chem*. 2007; 387:1601–1612. [PubMed: 17143596]
31. Smith, BC. *Fundamentals of Fourier Transform Infrared Spectroscopy*. CRC Press; Boca Raton: 1996.
32. Yin JH, Xia Y. *Appl Spectrosc*. 2010; 64:1199–1208. [PubMed: 21073787]
33. Buckwalter JA, Mankin HJ. *J Bone and Joint Surgery Am*. 1997; 79:600–611.
34. Rieppo J, Hyttinen MM, Lappalainen R, Jurvelin JS, Helminen HJ. *Trans ORS*. 2004:1021.
35. Torzilli PA. *J Orthop Res*. 1988; 6:766–769. [PubMed: 3404334]
36. Roberts S, Weightman B, Urban J, Chappell D. *J Bone Joint Surg Br*. 1986; 68:418–422. [PubMed: 3733808]
37. Zheng S, Xia Y. *NMR Biomed*. 2009; 22:158–164. [PubMed: 18720450]
38. Zheng S, Xia Y. *Magn Reson Med*. 2010; 63:25–32. [PubMed: 19918900]
39. Zheng S, Xia Y. *Cartilage*. 2010; 1:55–64. [PubMed: 21379397]
40. Carver SE, Heath CA. *Biotechnol Bioeng*. 1999; 62:166–174. [PubMed: 10099526]
41. Carver SE, Heath CA. *Biotechnol Bioeng*. 1999; 65:274–281. [PubMed: 10486125]
42. Deng Y, Lin XS, Zheng Z, Deng JG, Chen JC, Ma H, Chen GQ. *Biomaterials*. 2003; 24:4273–4281. [PubMed: 12853259]
43. Maroudas A, Bayliss MT, Venn M. *Ann Rheum Dis*. 1980; 39:514–534. [PubMed: 7436585]
44. Mow VC, Kuei SC, Lai WM, Armstrong CG. *J Biomech Eng*. 1980; 102:73–84. [PubMed: 7382457]
45. Mow, VC.; Gu, WY.; Chen, FH. *Basic Orthopaedic Biomechanics and Mechano-biology*. Van, RH.; Mow, C., editors. Lippincott Williams & Wilkins; Philadelphia: 2005. p. 181–258.
46. Yin JH, Xia Y. *Biomedical Optics Express*. 2011; 2:937–945. [PubMed: 21483615]

47. Lee J, Gazi E, Dwyer J, Brown MD, Clarke NW, Nicholson JM, Gardner P. *Analyst*. 2007; 132:750–755. [PubMed: 17646874]
48. Gulley-Stahl HJ, Bledsoe SB, Evan AP, Sommer AJ. *Appl Spectrosc*. 2010; 64:15–22. [PubMed: 20132593]
49. Crockett R. *Tribol Lett*. 2009; 35:77–84.

Fig 1.

(a) An infrared spectrum from the FT-IR image of articular cartilage. Two arrows point to two signature peaks centered at 1338 and 1072 cm^{-1} , which were characteristic peaks of type II collagen and PG, respectively. (b) The overlapping nature of the pure chemical spectra in FT-IR spectroscopy: type II collagen of 0.14 mg and CS6 (PG) of 0.34 mg (dotted line), pressed individually in KBr pellets.

Fig. 2.

The images of a ROI on a cartilage section: (a) visible image, (b) the total IR absorption image, (c) the amide I image ($1700\text{--}1600\text{ cm}^{-1}$), (d) the amide II image ($1600\text{--}1500\text{ cm}^{-1}$), (e) the amide III image ($1300\text{--}1200\text{ cm}^{-1}$), (f) the 1338 cm^{-1} collagen image ($1355\text{--}1315\text{ cm}^{-1}$), and (g) the 1072 cm^{-1} sugar image ($1125\text{--}960\text{ cm}^{-1}$). The same color scale was used to plot the FT-IR images, with the max absorptions for the (b) to (g) images being 1.5, 0.45, 0.35, 0.04, 0.30, respectively. The tiny numbers surrounding the images are the coordinates in microns, which merely reflect the actual position of the cartilage section on the moveable stage of the FT-IR instrument. The division of the histological zones was based on the previous studies using the same type of specimens: SZ: superficial zone; TZ: transitional zone; RZ: radial zone; TM: tidemark.

Fig. 3.

(a) The depth dependencies of collagen (solid dots) and PG (open circles) concentrations in articular cartilage by FT-IRI and PCR method. (b) The depth dependent profiles of peak areas of 1338 and 1072 cm^{-1} bands in the same tissue section. (c) The depth dependent profiles of the PCR concentration ratio of collagen to PG and the peak area ratio of 1338 to 1072 cm^{-1} .

Fig. 4. The depth-dependent profiles of (a) quantitative PCR concentrations in articular cartilage on the dry-weight base, (b) the water concentration profile (fitted the data from the Reference ¹⁰), and (c) the quantitative PCR concentrations of collagen and PG on the wet-weight base.

Fig. 5.

(a) The correlation between the depth-dependent profiles of the PG concentrations by FT-IRI-PCR and μMRI . (b) The linear regression correlation between the data in (a). The central shaded band marks the 95% confidence limits. Two outlines mark the 95% prediction limits. The Pearson correlation coefficient between the two variables is 0.98.

(a) Healthy Cartilage **(b) OA Cartilage** **Fig. 6.**

(a) The wet-weight based 2D concentration images of collagen and PG in a healthy articular cartilage by the FT-IRI and PCR methods. (b) The wet-weight based 2D concentration images of collagen and PG in a lesioned articular cartilage by the FT-IRI and PCR methods. The visible images of the specimens were also shown. The max and min concentrations for the collagen maps in both healthy and lesioned cartilage were 260 and 130 mg/ml, respectively. The max and min concentrations for the PG maps in both healthy and lesioned cartilage were 170 and 40 mg/ml, respectively.