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Future spectroscopic diagnostics in osteoarthritis

Michael D Morris[†]
& Blake J Roessler

[†]*Author for correspondence*
University of Michigan, Department of Chemistry, Ann Arbor, MI 48109, USA
Tel.: +1 734 764 7360;
Fax: +1 734 647 1174;
mdmorris@umich.edu

'The vibrational spectrum represents an optical fingerprint for a given complex organic molecule.'

Imaging of musculoskeletal tissues is an important aspect of diagnostic and therapeutic rheumatology. The past 20 years have witnessed the development and refinement of multiple technical platforms for imaging of musculoskeletal tissues. In addition to conventional radiographs, these include computed tomography, magnetic resonance imaging, and ultrasound [1]. Each of these platforms provide important and often complementary diagnostic and disease progression information to the clinical rheumatologist. However, every one of these platforms fails to provide critical information about the structural composition of extracellular matrix (ECM) components at the molecular level. There is increasing experimental evidence that molecular changes in ECM components may determine macroscopic changes in bone, tendon, synovium and cartilage, which are recognized by available imaging techniques and associated with clinical disease. Therefore, an unmet medical need remains for imaging technologies that can identify early molecular changes in ECM components that may predict disease risk or progression.

Over the same period of time, there has been extensive development of visible and infrared light-based vibrational spectroscopy techniques for the study of complex organic polymers, including ECM of musculoskeletal tissues. These studies owe much to extensive research on the spectroscopy of synthetic polymers of industrial interest, including the ultrahigh-molecular-weight polyethylene used in artificial joint implants. Much of the effort has been directed at the analysis of bone ECM, but in the last few years, several groups have also begun to investigate the spectroscopic properties of articular cartilage ECM. The term 'vibrational spectroscopy' includes several very different techniques that share the ability to detect and analyze molecular vibrations: the bending and stretching motions of the chemical bonds that hold organic molecules together. The strength of these methods is that molecular vibrations occur as specific and well-known optical frequencies. The vibrational spectrum represents an optical fingerprint for a given complex organic molecule. For example, collagen proteins are a major ECM component in bone, tendon and articular cartilage, and, at a molecular level, are derivatized organic polymers composed of repetitive amide linkages. Since structural collagens are characterized by long half lives, aging or disease can cause alterations in the secondary structure of the collagen fibrils that will be characterized by small changes in the vibrational frequencies and intensities of the amide backbone spectra.

Because vibrational spectroscopy is unfamiliar to most rheumatologists, we will briefly review the basis for several available methods, discuss the current research using osteoarthritis (OA) as a disease model, and assess prospects for clinical application in OA. Introductory texts on both infrared [2] and Raman [3] spectroscopy are available, and although these are aimed at advanced undergradutate chemistry students, they should be easily understood by anyone who has completed approximately 2 years of college chemistry.

Fourier-transform infrared spectroscopy

Molecules absorb light at specific frequencies in the infrared. Absorption can occur when the optical frequency of the light corresponds to the frequency of a bending or stretching motion of the molecule. The frequencies are usually expressed in wave numbers (the inverse of the wavelength of light), with cm^{-1} as unit convention. Modern laboratory instruments acquire infrared spectra in a format that is not easily interpreted, and a mathematical operation termed a Fourier transform is required to make spectra usable. Therefore, infrared spectroscopy is usually called Fourier-transform infrared spectroscopy (FTIR). Experimentally, FTIR has the technical problem that water is a strong absorber of infrared radiation. Very thin (~20 µm thick) or dehydrated specimens must be used, or the water absorption renders measurement impossible. Similarly, silica is a strong absorber of infrared light, meaning that conventional fiber

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optics (including arthroscopes) cannot be used in FTIR. Special infrared-transmitting fibers are available, but these do not transmit visible light. While FTIR instruments generally use electrically heated bars of silicon carbide or other ceramics to generate infrared illumination, lasers can be used for special purposes. Recently, reliable and inexpensive quantum cascade lasers have become available. These operate on single wavelengths, but two or more can be used to make absorption and background correction measurements. Current generation quantum cascade lasers are only a little larger than a typical laser pointer, and are under investigation for use in the clinical market.

Raman spectroscopy

When a specimen is irradiated by a laser, some of the light is scattered. Most of the scattering occurs with no change of wavelength, but a small amount is scattered at slightly longer wavelengths. The wavelength shifts correspond to vibrational frequencies of the molecules comprising the specimen. This scattered light, called Raman scattering, is usually generated with a laser operating in the deep red (~785 nm) or near-infrared (typically 830 or 1064 nm) wavelengths. These wavelengths are used because they can be generated with readily available solid state lasers. In turn, standard spectroscopic instrumentation can be used to detect and record Raman spectra. Water does not generate significant Raman scatter, so the technique can be used with fresh tissue or aqueous solutions. In addition, conventional silica optical fibers transmit both the laser and Raman-shifted light well. Although prototype Raman endoscopes for use in the gastrointestinal or genito-urinary systems have been described in the biomedical optics literature, no reports of a Raman arthroscope have appeared to date. With few exceptions, Raman instruments are currently more expensive than FTIR instruments, and the time to acquire spectra is longer. New detector types are decreasing measurement times by factors of 10–50 [4], and instrument prices are dropping with wider application of Raman spectroscopy.

Surface-enhanced Raman spectroscopy

It has been known for many years that Raman spectra of molecules absorbed on patterned gold or silver surfaces are thousands or even millions of times more intense than if the same molecules are observed on smooth metal or surfaces such as glass. The origin of surface-enhanced Raman spectroscopy (SERS) lies in the peculiar localized electromagnetic fields on these metals. In general, SERS has been a specialist technique; however, the recent commercial development of reproducible patterned surfaces has made it possible for any chemist to use the method.

Spectroscopy of articular cartilage

FTIR spectroscopy of articular cartilage has been more extensively investigated than Raman spectroscopy. Systematic exploration using FTIR began early in this decade with demonstrations by Camacho and colleagues at the Hospital for Special Surgery (NY, USA) [5], and shortly thereafter by Potter and colleagues at National Institutes of Health (MD, USA) [6], that information in infrared spectra could be used to map collagen and proteoglycan content in articular cartilage.

The Camacho group has suggested that inherent FTIR spectral information could be used as early indicators of ECM damage and, in turn, early osteoarthritis. While they initially suggested that polarization (i.e., intensity under polarized infrared light of certain collagen amide bands) would decrease if the articular cartilage was damaged by any process that would alter/degrade the normal organization of the ECM into parallel collagen fibrils. However, from a practical standpoint, this remains problematic since polarized light measurements cannot be made through most commercially available optical fibers.

In more recent work, the same investigators have used infrared-transmitting optical fibers as analytical tools. In these experiments, the test specimens were osteoarthritic human tibeal plateaus obtained during knee replacement surgeries. Initially, ratios of intensities of two amide bands were used as a measure of articular cartilage damage [7]. This metric is based on the fact that the intensities of some bands are sensitive to the order in the collagen triple helix and will decrease when the collagen molecules are damaged. Using the Collins scale as a clinical measure of articular cartilage damage, the FTIR band areas tracked Collins scale measures accurately. More sophisticated data treatments can be applied [8]. Using partial least squares (PLS) a multivariate (also termed chemometric) calibration method enables inclusion of information from much of infrared spectrum, resulting in a correlation coefficient (n = 61) against the Collins score of approximately 0.82.

It should be cautioned that using PLS calibration curves to measure damage to articular cartilage (or any other ECM component) is inherently difficult. Measurements can be in error from small changes in temperature, instrument instability and other changes in ambient experimental conditions. How susceptible musculoskeletal ECM components are to these potential infrared errors are not known. Encouragingly, the fiber-optic measurements performed in these experiments required less than 1 min and are compatible with conventional arthroscopy, although not with fused silica optical probes. However, it remains to be firmly established whether infrared spectroscopy can detect disease-inducing damage to articular cartilage before it becomes visible using current clinical imaging methods.

Our own group is taking a somewhat different translational approach. Preliminary studies have been exploring the direct analysis of ECM components using Raman spectroscopy. These experiments have focussed on the Raman spectroscopy analysis of collagen in the sclera, articular cartilage and subchondral bone of wild-type and transgenic mice harboring structural truncations in the introduced Type II collagen transgene [9]. In addition, we have also been developing SERS methods for measurement of proteoglycans in synovial fluid as potential biomarkers of articular disease. Although our initial studies have focussed on the analysis of synovial fluid, our working hypothesis is that SERS may be applicable to the analysis of specific proteoglycans present in serum or urine. While the infrared spectra of most articular proteoglycans are strong [10], the Raman spectra are weak. For example, hyaluronic acid, a major proteoglycan component of synovial fluid, is undetectable by Raman spectroscopy at concentrations normally found in synovial fluid and serum. However, by SERS, microliter quantities of synovial fluid, which are spotted onto a patterned gold substrate and dried, yield intense hyaluronic acid spectra with bands that readily identify the molecule [11]. We have found that, at high concentrations, the width of the band rather than its height is the most useful measure of hyaluronic acid concentration. Proteins have Raman spectral bands that partially obscure some hyaluronic acid bands, but proteins are readily removed by established extraction methods, such as treatment with trichloroacetic acid. A further advantage of the SERS methodology is that, because signals are strong, it is likely that SERS can be performed with small instruments intended for routine applications rather than with the expensive instruments designed for laboratory use.

Summary

Spectroscopic analysis has been widely used to characterize the polymer components in artificial joint implants, but has only recently been applied to the components of the joints themselves. Both FTIR and Raman spectroscopy have potential applications to study and diagnose mulsculoskeletal diseases and may find use as methods for direct assessment of cartilage, bone tendon and synovium, or as methods for the identification of relevant biomarkers derived from these tissues. Preliminary FTIR and Raman data *in situ* and *in vivo* have yielded promising results. Further study and technological refinement will be needed in order to establish a role for these technologies in clinical rheumatology.

Bibliography

1. Sharma L, Kapoor D, Issa S: Epidemiology of osteoarthritis: an update. *Curr. Opin. Rheumatol.* 18, 157–162 (2006).
2. Stuart BH: *Infrared Spectroscopy: Fundamentals and Applications.* John Wiley and Sons, Chichester, UK, 242 (2004).
3. Smith E, Dent G: *Dent: Modern Raman Spectroscopy: A Practical Approach*. John Wiley and Sons, Chichester, UK 222 (2005).
4. Coates CG, Denvir DJ, McHale NG, Thornbury KD, Hollywood MA: Optimizing low-light microscopy with back-illuminated electron multiplying charge-coupled device: enhanced sensitivity, speed and resolution. *J. Biomed. Optics* 9, 1244–1252 (2004).
5. Camacho NP, West P, Torzilli PA, Mendelsohn R: FTIR microscopic imaging of collagen and proteoglycan in bovine cartilage. *Biopolymers (Biospectroscopy)* 62, 1–8 (2001).
6. 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 Rheum.* 44, 846–855 (2001).
7. West PA, Bostrom MPG, Torzilli PA, Camacho NP: Fourier transform infrared spectral analysis of degenerative cartilage: an infrared fiber optic probe and imaging study. *Appl. Spectrosc.* 58, 376–381 (2004).
8. 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.* 59, 1527–1533 (2005).
9. Dehring KA, Smukler AR, Roessler BA, Morris MD: Correlating changes in collagen secondary structure with aging and defective Type II collagen by Raman spectroscopy. *Appl. Spectrosc.* 60, 366–372 (2006).
10. Servaty R, Schiller J, Binder H, Arnold K: Hydration of polymeric components of cartilage – an infrared spectroscopic study on hyaluronic acid and chondroitin sulfate. *Internat. J. Bio. Macromol.* 28, 121 (2001).
11. Mandair GS, Dehring KA, Roessler BJ, Morris MD: Detection of potential osteoarthritis biomarkers using surface-enhanced Raman spectroscopy in the near-infrared. *Proc. SPIE* 6093, H60930 (2006).

Affiliations

- *Michael D Morris, PhD*
  University of Michigan, Department of Chemistry, Ann Arbor, MI 48109, USA
  Tel.: +1 734 764 7360;
  Fax: +1 734 647 1174;
  mdmorris@umich.edu
- *Blake J Roessler, MD*
  University of Michigan, Division of Rheumatology, Department of Internal Medicine, Medical School, Ann Arbor, MI 48109, USA
  Tel.: +1 734 647 3413;
  Fax: +1 734 764 3596;
  roessler@umich.edu