Spectroscopic Diagnostics for Diseases of Bone and Cartilage

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Introduction

There is now a substantial body of literature on the infrared and Raman spectroscopy of bone and cartilage. The several hundred papers that appear have been devoted to understanding the spectra, to correlating features in spectra with age-related or disease-related changes in animal models and, in a few cases, to human tissue, and to studying the osteointegration or function of prosthetic devices. It has only been within the last few years that researchers have been actively considering how vibrational spectroscopy might actually be used in the diagnosis of damage to and disease of musculoskeletal tissue.

Conventional histology plays a relatively minor role in the diagnosis of bone or cartilage diseases. The iliac crest is the only really feasible site for bone biopsy. However, the procedure is time-consuming, painful and expensive and is not used routinely. Rather, the health of bone is measured by non-invasive techniques that probe tissue architecture at various scales and with varying detail. Similarly, cartilage health is assessed by in vivo examination, rather than by histology of excised specimens. Vibrational spectroscopy is potentially complementary to these methods, because it reports on composition and physical/chemical environment of important tissue constituents.

We discuss next osteoporosis, a metabolic bone disease, and osteogenesis imperfecta, a family of genetic defects that affect the skeleton. While there are many other bone disorders, these are two of the most important. Almost all bone disorders will result in composition abnormalities. In principle, vibrational spectroscopy may have a place as either diagnostic tool or a method for monitoring the progress of therapy.

1. Osteoporosis: By far the most common bone disorder is osteoporosis. The most common sites for osteoporotic fragility fractures are the proximal femur - frequently at the femoral neck -the distal radius and the vertebrae. The standard diagnostic method is dual wavelength X-ray absorption (DXA, DEXA), which measures average calcium content of bone at selected sites. The radiation dose is low and is generally considered to pose minimal risk to the patient. Emerging methods include magnetic resonance imaging, which reports tissue architecture with 0.1 mm resolution, and ultrasound, which provides lower resolution images, but which can be implemented inexpensively. X-ray computed tomography provides high resolution images, but with high doses of radiation.

The infrared spectroscopy[1, 2] and Raman spectroscopy[3, 4] of bone have been reviewed. Both infrared and Raman spectra contain signatures for osteoporotic damage. Osteoporotic cancellous bone tissue has higher crystallinity and a narrower range of crystallinities than normal tissue.[5, 6] The changes are reflected in the ratio of the 1030 cm^{-1}/1020 cm^{-1} band intensities (infrared) and phosphate P-O symmetric stretch (ν_1, ca. 957-959 cm^{-1}) band width.[7] The carbonate

ν_1/phosphate ν_1 band area and height ratios are higher in osteoporotic tissue removed from hip transplant patients compared to matched tissue from people who died causes unrelated to bone tissue properties.[8] Together these findings suggest that either infrared or Raman spectroscopy may be useful in diagnosing susceptibility to osteoporotic fragility fractures.

2. Osteogenesis imperfecta (OI): OI is a family of genetic defects arising from mutations in one of the genes (COL1A1 and COL1A2) that code for the α chains of type I collagen. The mutation usually results in the substitution of a larger amino acid for glycine. Replacement of glycine by any other amino acid causes disruption of the collagen triple helix, resulting in bone fragility (from which the vernacular "brittle bone disease" arises) and low bone mass, often with abnormalities in other tissues (teeth, eyes, skin, bones of the ear) containing collagen I.[9] Some defects result in mild reduction of properties, while others are lethal. While infrared [10-13] and Raman [14] spectroscopies have shown able to characterize the tissue abnormalities in mouse models, there has been little done yet in humans. Indeed, the defect is identified early from genetic tests and visibly abnormal tissue. Bisphosphonates are currently used to treat OI and there may a role for spectroscopy in monitoring the progress of treatment.[10]

3. Bone sampling methods: Where iliac crest biopsy specimens are available, infrared or Raman spectroscopy can be used to evaluate them. Infrared imaging may prove useful.[15] However, because non-invasive measurements are strongly preferred Raman spectroscopy is likely to have an important role. While transcutaneous Kerr-gated time-resolved Raman spectroscopy of bone has been demonstrated [16], more recently developed fiber-optic probe methodsthat use conventional Raman spectroscopy systems are far less expensive and offer much shorter measurement time. These methods use spatial separation of the point(s) at which laser light enters the skin and the point(s) at which it is collected. [17, 18] The use of spatially separated excitation and collection sites is well-known in tissue fluorescence and infrared spectroscopies and has been developed to take advantage of or overcome the effects of multiple scattering. Multiple scattering results in an initially focused beam of light becoming more spread out as it propagates through the tissue. Scattering is usually modeled as a random walk and multiple scattering is called photon diffusion in the biomedical optics literature. The combined process of scattering and absorption is called photon migration.

In the deep red to near-infrared scattering is a more important source of attenuation of a light beam than is absorption. Absorption is at a minimum in the 750-950 nm region, which is often called the therapeutic window. The scattering coefficient declines monotonically with increasing wavelength, and is typically between 1-10/cm in the therapeutic window, depending on the tissue type. Light penetration is measured in centimeters.[19] The vast majority of non-invasive tissue spectroscopy, whether fluorescence, absorption or Raman scattering is performed in this window.

4. Bone Raman Spectroscopy in the Therapeutic Window: Our own group has reported canine tibia Raman scattering from depths of 5 mm below the skin [20] using a ring/disk geometry independently developed by our group [21] and Matousek.[22] We have demonstrated that carbonate/phosphate ratio, an indicator of mineral crystallinity and a potential marker for susceptibility to osteoporotic damage can be recovered with 3%-5% accuracy. We have previously reported bone spectra from human cadaveric tissue using a global illumination fiber optic probe.[23] This probe uses a defocused laser beam, with circular array of collection fibers whose field of view is coincident with the illuminated area. However, we were unable to obtain reasonable results at depths exceeding about 3mm.

5. Osteoarthritis: Osteoarthritis (OA), the degradation of articular cartilage and nearby bone tissue and ligaments, is mostly caused by excessive wear on the joints, although it can also have biological origins or contributions.[24, 25] Because cartilage is avascular it repairs very slowly, if at all. The most important site is the knee joint, which is heavily loaded even during normal activities such as walking, but especially so in certain athletic activities, including marathon running or downhill skiing. In the OA victim, the cartilage becomes eventually sufficiently thin that the femur and tibia rub against each other, resulting in intense pain. Direct measurements of the state of articular cartilage and subchondral bone are possible spectroscopic diagnostics for OA. In addition, the synovial fluid is degraded, suggesting that measurements of the state of hyaluronic acid (hyaluronan), is another possible diagnostic.

6. Spectroscopy of articular cartilage, subchondral bone and synovial fluid: FTIR spectroscopy of articular cartilage has been more extensively investigated than Raman spectroscopy. Systematic began early in this decade with demonstrations by Camacho et al.)[26] and shortly thereafter by Potter et al.[27] that information in infrared spectra could be used to map collagen and proteoglycan content in articular cartilage.

The Camacho group has suggested that FTIR spectral changes could be used as early indicators of ECM damage, and therefore early OA. They initially suggested that polarization of certain collagen amide bands would decrease if articular cartilage was damaged by any process that would alter or degrade the normal organization of the ECM as parallel collagen fibrils. However, from a practical standpoint this remains problematic for in vivo work because polarized light measurements can not be made through commercially available IR-transmitting optical fibers.

More recently Li et al. have used IR-transmitting optical fibers to obtain cartilage spectra from 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 [28]. 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 did track Collins scale measures accurately. More sophisticated data treatments, including partial least squares can be applied [29]. With PLS a correlation coefficient (N = 61) against the Collins score of about 0.82.
[30]
Encouragingly, the fiber optic measurements performed in these experiments required less than a minute and are compatible with conventional arthroscopy, although not with fused silica optical probes. However, it remains to be firmly established that infrared spectroscopy can detect disease inducing damage to articular cartilage before it becomes visible using current clinical imaging methods.
Our group has shown that abnormally now mineral/matrix ratio (i.e. phosphate ν_1/amide I band area) ratio is lower in Del1 mice than in the matched wild-type mice up until about 14-15

months.[31] Del1 is a model for early onset osteoarthritis, so these results suggest that similar measures might be useful in humans. No human tissue studies have yet been reported.

Degradation fluid does give rise to measureable changes in the infrared spectrum of synovial fluid.[32] Surface-enhancement is necessary to obtain Raman spectra in a reasonable time.[30] There is still insufficient evidence to know if Raman spectroscopy of synovial fluid can become an important diagnostic tool.

7. Standardization of measurement protocols: It is clear that non-invasive (bone) or minimally invasive (bone and cartilage methods) are desired by clinicians. For these reasons it is likely that all bone and cartilage spectroscopic diagnostics will be made with fiber optic probes. Measurement of Raman spectra deep (several millimeters) below the skin is a field in its infancy. There is no consensus about what an optimum probe design might be. We offer some suggestions.

In almost every case Raman measurements will be made in a scattering medium. Probe designs will need to have probably have to be somewhat different for different anatomical sites, which all have different morphologies. Cartilage and subchondral bone measurements will have to be compatible with standard arthroscopes.

The role of infrared spectroscopy in arthroscopy is likely to be greater than in bone measurement. FTIR imaging is a superb tool for examination of iliac crest biopsy specimens, but this procedure is performed on a small fraction of the people who are at risk for osteoporosis. On the other hand, a diamond-tipped or other ATR probe can be probably be developed as an accessory for standard arthroscopes and could become highly useful there. Synovial fluid measurement by either Raman or FTIR is feasible with conventional methodologies.

There is not yet a consensus on how data collection should proceed, except that in vivo measurements should be as rapid as possible and ideally require no more than 1 minute. Single point (or a few points) measurement, mapping (20-200 points) and imaging (typically 4K points or more) have all been proposed.

Because all measurements except iliac crest biopsy and synovial fluid measurements will be made in vivo, there are few issues of specimen preparation and fixation.

8. Data reduction methods: There is no consensus on data reduction methods. Infrared or Raman images can be analyzed by univariate or multivariate procedures. Because bone is heterogeneous it can be advantageous to take a pixel-by-pixel approach to measurement of important metrics, typically band area ratios or band widths. In many cases these will differ between normal and diseased tissue. At present, the only applicable specimens are iliac crest biopsies. Non-invasive measurements of bone tissue have yielded only averaged spectra obtained through self-modeling curve resolution (SMCR) on the ensemble of spectra collected with fiber optic Raman probes. Partial least squares (PLS) or other modeling procedures have not been attempted yet, but provide an interesting alternative. There is no reason why PLS could not yield usable results on, for example, bone mineral carbonate/phosphate ratios. The usual cautions about modeling certainly would apply. Similarly, reconstruction of spectra through basis sets is also a promising, but untested, alternative to SMCR.

9. Instrument standardization: There has been no attempt to design instruments specifically for the musculoskeletal tissue diagnostics market. It is unlikely that such instruments would resemble general-purpose laboratory spectrometers or imagers. It is too early to discuss what such instruments would look like.

10. Validation: It will be necessary both to compare the results of non-invasive measurements of bone or cartilage spectra with those made directly on the exposed tissue. The only practical methods for human subjects are to make the exposed tissue measurements during the course of surgery that exposes the tissue or on tissue that is removed as part of a surgery. Our laboratory has funding to make exposed tissue measurements in the course of development of non-invasive spectroscopic diagnostics for susceptibility to fragility fracture of osteoporotic tissue. In addition to the usual concerns for testing instruments in the operating room (sterility, measurement time, etc.), we face the issue that there are no standards for safe laser irradiation of exposed bone tissue. It will be necessary to prove that the required time and laser power do no harm.

It will also be necessary to compare IR and Raman methods to other diagnostic methods for bone and cartilage diseases. In most cases there is no real gold standard, because spectroscopy provides information that is simply unavailable otherwise. It is the author's opinion that spectroscopy will complement rather than displace existing or emerging diagnostics because these provide architectural information with little or no composition information. Prospective studies are especially important. It will take several years and large data sets to know if spectroscopy has provided uniquely helpful information or has simply confirmed the state of the tissue measured directly or indirectly by other methods.

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