OPTICAL NON-DESTRUCTIVE EVALUATION OF ARTICULAR CARTILAGE INTEGRITY: A REVIEW

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Abstract: This paper reviews the current status of the application of optical non-destructive methods, particularly infrared (IR) and near infrared (NIR), in the evaluation of the physiological integrity of articular cartilage. It is concluded that a significant amount of work is still required in order to achieve specificity and clinical applicability of these methods in the assessment and treatment of dysfunctional articular joints.

Key words: Articular cartilage, near infrared (NIR), infrared (IR), spectroscopy.

1 INTRODUCTION

Optical spectroscopy is a proven and effective method of determining the structural composition of materials, including those from biological origin. Prominent among these methods are those based on the mid-infrared (mid-IR) and near infrared (NIR) regions of the electromagnetic (EM) spectrum. Infrared (IR) radiation is electromagnetic energy with wavelength longer than visible light, but shorter than microwaves. Generally, wavelength from 0.8 to 100 micrometers (μm) is often used for IR spectroscopy and are divided into the near infrared (0.78 – 2.5μm), the mid-IR (2.5 – 15μm), and the far-IR (15 – 100μm) regions. The near infrared (NIR) and the mid-IR (referred to as IR in this paper) regions are the most useful for qualitative and quantitative analysis and assessment of structural networks and composition of food (Nielsen, 2010) and biological-materials such as meat (Tøgersen et al., 1999). More recently, these methods are increasingly being applied for the non-destructive evaluation of the functional architecture/structure of articular cartilage, with the objective of clinical application in the treatment of the mammalian articular joint, and the alleviation of pain from osteoarthritis and related conditions.

Articular cartilage is a dense semitranslucent layer of tissue covering the ends of bones in articulating joints in humans and animals. Comprising of 65-85% water, 10-20% w/w collagen fibrils, 2-10% w/w proteoglycan aggregates, and a few chondrocytes cells (3%), this tissue functions to distribute or spread any contact load applied to the joint with minimal effect on the bone, as well as providing joint lubrication. Of importance to structural evaluation is that the collagen and proteoglycans, whose architectural design is such that the collagen 3D meshwork entraps the swollen proteoglycan to create a functional material, comprise hierarchical micro to ultramicroscopic chain networks which according to their physics these optical methods can detect and characterise.

Collagen accounts for about two-thirds of the dry weight of adult human articular cartilage. The tissue‘s material strength depends on the extensive cross-linking of the collagen fibrils and the apparent zonal changes in fibrillar architecture with tissue depth (Eyre, 2002). With respect to collagen, articular cartilage comprises cross-linked hierarchical long chain nano and microscopic polymeric networks (microfibrils and heterofibrils) of collagen II, IX and XI. Proteoglycans, on the other hand, are negatively charged, and immobile constituting the fixed charge density (FCD) of the matrix (Maroudas, 1976), and are instrumental to the development of the intrinsic osmotic pressure of the matrix. Again, this component is an agglomeration of nano and micro polymeric network of several small and large-chain molecules (Roughley, 2006) which, in theory, suits detectability and categorisation by the optical IR and NIR methods.

Cartilage dysfunction is often manifested as disruption of collagen connectivity, including the fibrillation of the superficial layer in the early stages of degeneration (Carney, Billingham, Muir, & Sandy, 1984) and/or proteoglycan loss. Whichever is the case, the effect can be argued to be one that can hypothetically be measured or qualified with optical spectrometry as reviewed herein.

The assessment and evaluation of articular cartilage matrix viability using optical spectroscopic techniques is based on the sensitivity of infrared (IR) and near infrared (NIR) to the vibration of chemical bonds between the polymeric chain monomers of C–H, N–H, O–H and S–H. This paper presents a comparative review of these two methods relative to their capacity to characterise human articular cartilage which is approximately between 1 - 4 mm thick.

2 PERTINENT THEORY OF OPTICAL SPECTROSCOPY

The conceptual basis of optical spectroscopy, also known as vibrational spectroscopy is that at temperatures above absolute zero, all the atoms in molecules are in continuous vibration with respect to each other. When the frequency of a specific vibration is equal to the frequency of the IR or NIR radiation directed on the molecule, the molecule absorbs the radiation (Sherman Hsu, 1997). These atom-to-atom bonds within molecules vibrate with frequencies that may be described by the laws of physics and can therefore be quantified. When vibrating molecules absorb light of a particular frequency (IR and NIR in this case), they are excited to a higher energy level (Ingle Jr. & Crouch, 1988). Using a spectrometer, a scan of the IR or NIR spectral region of the electromagnetic (EM) spectrum showing the energy absorbed by each, or group, of vibrating molecules in a material can be obtained.

The vibration of molecules is described by the harmonic oscillator model, where the energy of the different, equally spaced levels can be calculated from

$$Evib = \left(v + \frac{1}{2}\right)\frac{h}{2\pi}\sqrt{\frac{k}{\mu}}, \quad (1)$$

Where, **v** is the vibrational quantum number, ***h*** the Planck constant, ***k*** the force constant and ***μ*** the reduced mass of the bonding atoms.

Only those transitions between consecutive energy levels ($\Delta\nu = \pm 1$) that cause a change in dipole moment are possible,

$$\Delta E_{vib} = \Delta E_{rad} = h\nu, \quad (2)$$

where **ν** is the fundamental vibrational frequency of the bond in the IR region. However, the harmonic oscillator model cannot explain the behavior of actual molecules, as it does not take account of Coulombic repulsion between atoms or dissociation of bonds. As a result, the behavior of molecules more closely resembles the model of an anharmonic oscillator, where the energy levels are not equally spaced, leading to a decrease in the energy difference with increasing vibrational frequency **ν.** In this case:

$$\Delta E_{vib} = h\nu\,[1 - (2\nu + \Delta\nu + 1)y], \quad (3)$$

where ***y*** is the anharmonicity factor. The anharmonicity can result in transitions between vibrational energy states where $\Delta\nu = \pm 2, \pm 3....$ As a result of this anharmonicity, additional absorption bands are generated by the appearance of overtones (approximately integral multiples of the fundamental absorption frequencies), combinations of fundamental frequencies, differences of fundamental frequencies, coupling interactions of two fundamental absorption frequencies, and coupling interactions between fundamental vibrations and overtones or combination bands (Fermi resonance) (Sherman Hsu, 1997). These intensities are particular to NIR and are much less likely than the fundamental transitions, so the bands are much weaker. The band for the first overtone is 10 – 100 times weaker than that for the fundamental frequency, depending on the particular bond. They appear between 0.78 – 2µm, depending on the overtone order and the bond nature and strength (Blanco & Villarroya, 2002). The combination bands appear between 1.9 – 2.5µm. The influence and blending of all these factors thus create a unique IR spectrum for each compound (Sherman Hsu, 1997).

2.1 **Infrared (IR) and near infrared spectroscopy (NIR) spectroscopy – Capacity and limitations**

Less popular as an analytical technique compared to NIR, IR absorptions are primarily due to molecular vibrations resulting from bending, stretching and rotational motions of atoms in the molecule. These absorptions are characteristic of both organic, such as biological materials, and inorganic constituents, such as metal and heavy metals. Examples of successful analysis include predictions of contents of organic carbon and nitrogen contents of sand, silt, and clay, contents of Al_2O_3, CaO, Fe_2O_3, K_2O, MgO, P_2O_5, SiO_2, and TiO, lime requirements of agricultural soils, and amounts of several extractable or exchangeable elements (Ludwig et al., 2008). Fundamental absorption frequencies which appear in the NIR region as overtones and combination usually originate from the IR region.

On the other hand absorption bands observed in the NIR regions are primarily overtones and combinations (due to anharmonicity), and they tend to be weak. However, this does not pose a disadvantage since absorption bands that have sufficient intensity to be observed in the NIR region arise primarily from functional groups such as C–H, N–H, O–H and S–H, which are common groups in major constituents of food and biological materials, namely water, protein, lipids and carbohydrates (Nielsen, 2010). Interactions between atoms in different molecules (for example hydrogen bonding and dipole interactions) alter vibrational energy states, thereby shifting existing absorption bands and giving rise to new ones through differences in crystal structure. This allows crystal forms to be distinguished and physical properties (such as density, viscosity, and particle size in pulverulent solids) determined. In other words, the NIR spectrum contains not only chemical information of use to determine compositions, but also physical information that can be employed to determine physical properties of samples (Blanco & Villarroya, 2002).

Whilst these two spectroscopic methods are capable of characterising a networked tissue like articular cartilage, the IR method suffers from an extremely limited depth of penetration where it can only travel a few micrometers into the tissue. This limitation practically confines its application to the characterisation of thin cartilages sections (Bi, Li, Doty, & Camacho, 2005). Near infrared is able to travel further into a given tissue (up to 8.5 mm in neonatal head) (Faris et al., 1991) thereby providing a distinct advantage for probing right through cartilage which as stated earlier is approximately 1-4 mm in the human.

2.2 **General Spectral analysis and Chemometrics**

In order to improve the specificity of both IR and NIR measurements, statistical techniques involving *curve fitting* are usually employed. These include advanced multivariate analytical tools such as *multiple linear regression (MLR), principal component regression* (PCR) and *partial least squares regression* (PLSR), which have dramatically improved the interpretation of the results from the raw data leading to the approximation of the absolute amounts of (single or multiple) constituents in a sample from a single FT-IRIS spectrum (Martens & Martens, 2001).

Similarly, the analytical information contained in the NIR spectra of samples can be extracted by using various multivariate analysis techniques, such as those listed above, that relate several analytical variables (as in a NIR spectrum) to properties (such as concentration) of the analyte(s). The multivariate techniques most frequently used allow samples with similar characteristics to be grouped, in order to establish classification methods for unknown samples (qualitative analysis) or to perform methods determining some property of unknown samples (quantitative analysis) (Blanco & Villarroya, 2002).

In addition, the IR and NIR spectra of solid samples are influenced by the physical properties of the solid samples. This may pose some problems in evaluating aspects of samples for which physical appearance is not important (such as identification of raw materials and determination of composition). In these situations, spectral pretreatment is used to minimize such contributions incorporating irrelevant information into spectra. While NIR analysis of some samples have been shown to achieve good resolution with raw spectra and no pretreatment, IR analysis of the same samples requires pretreatment to improve its resolution (Calderón et al., 2009). Some of the more frequent pretreatments for NIR spectra include: normalization, derivatives (usually first or second), multiplicative scatter correction (MSC), the standard normal variate (SNV) and de-trending (DT); or, a combination thereof.

3 SPECTROSCOPIC ASSESSMENT OF ARTICULAR CARTILAGE

The application of optical spectroscopy in articular cartilage research mainly adopts Fourier transform instrumentation. Fourier transforms infrared imaging spectroscopy (FT-IRIS) has been used to identify specific molecular components of the tissue‘s collagenous network, proteoglycans, and chondrocytes that contribute to its IR spectrum (Boskey & Camacho, 2007). FT-IRIS spectral data are typically obtained from single, 7–10µm thick sections of tissue which are generally dehydrated and may not be representative of the entire tissue‘s structure. In contrast, using FT-NIR, NIR‘s deep penetration into the tissue allows for acquisition of spectral data from full thickness cartilage samples without sample preparation. This permits testing of the sample in its physiological state, giving a more representative view of the matrix

structure.

FT-IRIS has been used to characterize the spatial distribution of collagen and proteoglycans (PG) and to determine the orientation of collagen fibrils, as well as to assess specific molecular vibration that arises directly from the PG, the sugar ring vibration (West, Bostrom, Torzilli, & Camacho, 2004). In addition, the orientation and organization of the collagen network determined using this method has been suggested to correlate with subtle changes in cartilage as a result of degradation (Rieppo et al., 2008; West et al., 2004), with Amide bond vibrations at 1250, 1550 and 1655 cm^{-1} proposed to be characteristic of collagen, and those at 1545 and 1640 cm^{-1} characteristic of proteoglycans (Camacho, West, Torzilli, & Mendelsohn, 2001). On the contrary, due to the typically broad and extensively overlapped bands of NIR spectra (Blanco & Villarroya, 2002), it is generally challenging to clearly define and associate specific peaks of the spectra to cartilage collagen and proteoglycan. However, this problem is overcome with the use of chemometric and statistical techniques to extract the necessary information from the spectra.

Critical evaluation of FT-IRIS for the assessment and characterization of cartilage composition demonstrates that existing FT-IRIS methods for PG quantification are not fully consistent and lack the specificity for quantitative assessment, and the proposed collagen-to-PG ratio parameters are inaccurate (Rieppo et al., 2008). In addition, degraded macromolecules of collagen and PG are not easily detected by FT-IRIS spectroscopy, making this method ineffective for resolving cartilage degeneration in the initial stages of disease. However, existing IR technology has been reported to recognize advanced stages of tissue degeneration such as lacerations, ruptures and chondral fractures (Buckwalter & Mow, 1994). On the other hand, employment of NIR in such applications as non-invasive *in vivo* measurement of neonatal head, based on the penetrating capability of NIR light (Faris et al., 1991), and prediction of joint condition based on NIR analysis of the synovial fluid (Shaw, Kotowich, Eysel, & Jackson, 1995) demonstrates the potential of this techniques for articular cartilage assessment. Recent reports have show that NIR is capable of distinguishing normal from enzymatically digested cartilage (Brown et al., 2009), and in a pilot study, Spahn et al. (2007) demonstrate the potential of NIR to evaluate low grade degenerated cartilage lesion in the human medial knee compartment. Furthermore, in recent, yet to be published results, the authors have been able to correlate the NIR spectra of cartilage to both its physical and mechanical parameters, such as thickness, effective stress, and physicochemical properties such as osmotic reswelling of the tissue after mechanical compression.

4 CONCLUSIONS

Despite these advances in the use of IR for identification and characterisation of cartilage matrix components, clinical applications based on this method are extremely limited due to its poor penetration depth into the tissue, and the need for sample preparation. Although cartilage surface layer characterisation may be achievable using IR, non-destructive full thickness, whole matrix evaluation is practically impossible using this technique. NIR, on the other hand, penetrates deep into the tissue sample, providing insight into its network structure and architecture, and needs no sample preparation. These properties make NIR a viable option for clinical characterisation of articular cartilage integrity. However, more work is needed in order to bring NIR application to the advanced state of IR characterisation of articular cartilage.

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