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Assessment of hyaline cartilage matrix composition using near infrared spectroscopy

Uday P. Palukuru, Cushla M. McGoverin, Nancy Pleshko *

Department of Bioengineering, Temple University, 1947 N. 12th St, Philadelphia, PA, USA

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ABSTRACT

Changes in the composition of the extracellular matrix (ECM) are characteristic of injury or disease in cartilage tissue. Various imaging modalities and biochemical techniques have been used to assess the changes in cartilage tissue but lack adequate sensitivity, or in the case of biochemical techniques, result in destruction of the sample. Fourier transform near infrared (FT-NIR) spectroscopy has shown promise for the study of cartilage composition. In the current study NIR spectroscopy was used to identify the contributions of individual components of cartilage in the NIR spectra by assessment of the major cartilage components, collagen and chondroitin sulfate, in pure component mixtures. The NIR spectra were obtained using homogenous pellets made by dilution with potassium bromide. A partial least squares (PLS) model was calculated to predict composition in bovine cartilage samples. Characteristic absorbance peaks between 4000 and 5000 cm^{-1} could be attributed to components of cartilage, i.e. collagen and chondroitin sulfate. Prediction of the amount of collagen and chondroitin sulfate in tissues was possible within 8% (w/dw) of values obtained by gold standard biochemical assessment. These results support the use of NIR spectroscopy for in vitro and in vivo applications to assess matrix composition of cartilage tissues, especially when tissue destruction should be avoided.

1. Introduction

Articular cartilage is a type of hyaline cartilage found on the articular surfaces of bones in diarthrodial joints and functions to provide a near frictionless and load bearing surface to facilitate smooth joint movement. This tissue is hypocellular, avascular, aneural, alymphatic and contains only one type of differentiated cell, chondrocytes, which maintain the extra cellular matrix (ECM) (Fox et al., 2009). The functionality of articular cartilage is inherently linked to chemical composition. The ECM of articular cartilage is primarily composed of collagen type II fibrils (15–25% wet weight), proteoglycans (PG) (5–10% wet weight) and water (70–80% wet weight) (Cohen et al., 1998). Mature articular cartilage displays a zonal architecture with varied chemical composition, collagen fibril orientation and chondrocyte shape in the superficial, transitional, and deep zones (Buckwalter et al., 1994).

Injury or disease [e.g. osteoarthritis (OA)] in articular cartilage may cause the degradation of ECM structure and inhibit function; subsequently, joint pain and a loss in mobility may occur. Degenerative changes in ECM include the disruption of collagen fibrils which restricts PG water binding capacity and leads to swelling. The changes in collagen and PG content play an important factor in disease progression and need to be evaluated accurately for the development of successful intervention strategies. Several modalities are utilized to evaluate the composition of cartilage including optical arthroscopy, computed tomography (CT) based arthrography and magnetic resonance imaging (MRI) for *in vivo* studies (Hayes and Conway, 1992; Chung et al., 2001; Cockman et al., 2006; Domayer et al., 2008; Piscaer et al., 2008; Roemer et al., 2009; Siebelt et al., 2011b). MRI has been used extensively to evaluate the morphology and composition of osteoarthritic tissue non-invasively but has inadequate specificity and sensitivity at a molecular level, is expensive, and cannot be used intra-operatively (Gelb et al., 1996; Nishii et al., 2005; Lin et al., 2009a,b; Sutter et al., 2014). CT has also been used extensively to evaluate morphology of osteoarthritic tissue non-invasively but suffers from lack of sensitivity, has the added disadvantage of incorporating ionizing radiation, and also is not used intra-operatively (Daenen et al., 1998; Rand et al., 2000; Bansal et al., 2011; Siebelt et al., 2011a).

Infrared (IR) spectroscopy is a technique based on the interactions between infrared radiation and matter that enables molecular characterization of samples (Siebert, 1995). The chemical bonds within molecules of a sample have unique vibrational frequencies and infrared wavelengths incident on a sample that resonant with these frequencies are absorbed. This allows the molecular characterization of a sample as each molecule has a unique absorption profile across the infrared spectral range. Fourier transform infrared (FTIR) spectroscopy has been used to investigate the chemical composition of many biological tissues including cartilage in both the mid infrared (MIR) spectral range (4000–400 cm^{-1}) (Camacho et al., 2001; Potter et al., 2001; Kim et al.,

* Corresponding author at: Department of Bioengineering, Temple University, 1947 N. 12th St, Rm 839, Philadelphia, PA 19122, USA. Tel.: +1 2152044280.
E-mail address: npleshko@temple.edu (N. Pleshko).

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2005; Krafft and Sergo, 2006; Boskey and Pleshko Camacho, 2007; Xia et al., 2007; Movasaghi et al., 2008; Rieppo et al., 2012b) and near infrared (NIR) spectral range (10,000–4000 cm^{-1}) (Brown et al., 2009; Baykal et al., 2010; Afara et al., 2012; Padalkar et al., 2013). Studies have been performed in the MIR region to assess the spectral signatures of the pure components present within the cartilage matrix (Camacho et al., 2001; Potter et al., 2001). An infrared fiber optic probe (IFOP) coupled to an FTIR spectrometer may be used to collect spectra directly from the surface of an intact sample using attenuated total reflectance in the MIR region; this method has shown promise for clinical studies (West et al., 2004; Li et al., 2005; Hanifi et al., 2013). However, the MIR region has limited penetration depth (up to 10 μm maximum); hence, these studies incorporate information from only the first few micrometers of the tissue. For greater penetration depth, NIR spectroscopy is superior. However, the absorbances in NIR spectra are very low, and generally arise from a combination of molecular species, making assignments of specific peaks challenging. Nevertheless, several studies have demonstrated the effectiveness of NIR spectroscopy in evaluating cartilage tissue quality non-destructively (Spahn et al., 2007, 2008; Brown et al., 2009; Baykal et al., 2010; Afara et al., 2012, 2013a, 2013b, 2013c; Padalkar et al., 2013). Studies have also shown that the NIR spectra obtained using an IFOP can be used to monitor disease progression in OA cartilage, and that this modality can perform better than other clinically used procedures to evaluate cartilage degeneration (Hofmann et al., 2010; Brown et al., 2011, 2012; Spahn et al., 2013b). Most of these studies are focused on regions of the NIR spectra that are based on water absorbance and where cartilage tissue component contributions highly overlap. Many of these studies have also focused on using NIR spectra for evaluation of tissue composition without attributing specific absorbances in NIR spectra to individual tissue components, other than water. Knowledge of specific absorbance peak assignments in the NIR spectral region would enable increased understanding of these complicated spectra.

Problems associated with NIR spectroscopy, including signal overlap, can be overcome by using multivariate data analysis techniques [for example principal component analysis (PCA) and partial least square regression (PLS-R) coupled with signal preprocessing techniques e.g. extended multiplicative scatter correction (EMSC) and second derivatives]. The multivariate techniques PCA and PLS-R were used to successfully to evaluate cartilage tissue and pure components of cartilage in the MIR region (Yin and Xia, 2010; Hanifi et al., 2012; Rieppo et al., 2012a). In work by Padalkar et al. (2013) the composition of water in hyaline cartilage was evaluated quantitatively using NIR spectroscopy, but the study did not evaluate cartilage pure matrix components.

There are two primary goals of the current study: 1) identify the contributions of individual matrix components of cartilage to absorbances in the NIR spectral range and 2) demonstrate the feasibility of NIR spectroscopy for quantitative compositional assessment of cartilage. Cartilage pure components, namely collagen and chondroitin sulfate (which is a major component of proteoglycans), were mixed in various ratios and NIR spectra collected. Pure component mixture NIR spectra and multivariate techniques were used to predict the composition of bovine articular and nasal cartilage from their corresponding NIR spectra, and these predictions were compared to gold standard biochemistry measurements. Collectively these studies demonstrate that NIR spectroscopy can be used to quantitatively, and non-destructively, evaluate the composition of cartilage tissue.

2. Results

2.1. NIR spectral analysis of KBr pellets

The NIR spectra of multicomponent samples often contain broad features due to overlap of contributions from individual components. This is clearly evident in the scatter corrected and normalized spectra of the collagen and chondroitin sulfate mixtures [Fig. 1a]. Bands centered at ~4610 and 4890 cm^{-1} are observed to increase in intensity with increasing collagen content in the scatter corrected and normalized spectra. However, no readily discernible peaks that can be attributed to increasing chondroitin sulfate content are observable. Second derivative processing revealed additional features of the data. With increasing collagen content, peaks centered near 4050 and 4260 cm^{-1} appeared, and similarly, peaks centered near ~4020 and 4310 cm^{-1} increased in intensity with increasing chondroitin sulfate content [Fig. 1b, (Note: second derivative peaks are negative)]. The second derivative NIR spectra of biological samples (young and adult articular cartilage, and adult nasal cartilage) supported these absorbance band assignments [Fig. 2]. Collagen peaks were most intense in young and adult articular cartilage, and were least intense in nasal cartilage, as was expected from a literature assessment of collagen content within these tissue types.(Goh and Lowther, 1966; Campo and Tourtellotte, 1967; Eyre and Muir, 1975; Heinegard and Paulsson, 1987).

2.2. Biochemical assessment of powders

The collagen content of young articular cartilage, adult articular cartilage, and young nasal cartilage was assessed by measuring the amount of hydroxyproline present in the samples [Table 1]. Hydroxyproline which is one of the three major amino acids present in the collagen protein chain is frequently used to estimate the total collagen content in samples (Hosseininia et al., 2013; McAlinden et al., 2014). The amount of collagen in the cartilage powders averaged 72.9% weight/dry weight (w/dw) in adult articular cartilage, 64.1% w/dw in young articular cartilage and 41.5% w/dw in nasal cartilage. Among the cartilage powders the amount of sulfated glycosaminoglycans (sGAG) varied from an average of 19.7% w/dw in adult articular cartilage to 52.7% w/dw in nasal cartilage [Table 1].

2.3. Prediction of major cartilage components using NIR spectral data

A partial least squares (PLS) model was calculated from the pure component mixture spectra for collagen and chondroitin sulfate content; the root mean square error of prediction (RMSEP) was 8% w/dw and R^2 was 0.95 [Fig. 3]. The loading weights of the factors used in the PLS model incorporated the unique spectral features attributed to the individual components [Fig. 4]. Unsurprisingly, features characteristic to collagen and chondroitin sulfate were present predominantly in the loading weights of the first factor, which accounted for nearly 90% of variation in the samples. The PLS model predicted the highest percentage of collagen in adult articular cartilage, followed by young articular cartilage, and then nasal cartilage powder [Table 1]. Chondroitin sulfate content as predicted by the PLS model followed the reverse trend [Table 1].

3. Discussion

To more effectively use NIR spectroscopy to assess changes in the ECM of biological tissues such as hyaline cartilage, it is important to understand the NIR spectral signatures of the individual components of the tissue matrix. The use of sample powders diluted with a NIR transmissive salt to extract NIR signatures is a standard practice, and was employed in this work to observe the NIR spectra of pure cartilage ECM components. NIR spectra of collagen and chondroitin sulfate mixtures had differences which were attributed to the individual components, in particular when the second derivative spectra were examined. These spectral differences were also present in cartilage powders of varying biochemical composition [Figs. 1b and 2b]. Although there are no previous studies available on the specific interpretation of the NIR spectra of cartilage, NIR studies of agricultural and meat products with similar components (proteins and sugars) identified absorbance peaks attributable to protein, and cellulose and starch, two biopolymers with high sugar content (Osborne and Douglas, 1981; Weyer, 1985;