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Determination of collagen and proteoglycan concentration in osteoarthritic and healthy articular cartilage by Fourier transform infrared imaging and partial least square

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ABSTRACT

Fourier transform infrared imaging (FTIRI) combined with chemometrics has potential to determine the molecular and chemical properties of biological tissues. In this study, FTIRI with partial least square (PLS) algorithm was used to quantitatively study the concentration distributions of two principal components (collagen and proteoglycan) in healthy and osteoarthritis (OA) articular cartilage. 10 μm-thick sections of canine tibial cartilage were imaged at 6.25 μm/pixel. The spectra extracted from the infrared images were imported into the PLS model to predict the concentrations of collagen and proteoglycan in the corresponding cartilage sections. Spectral pre-processes in this model included multiplicative scatter correction, normalization, and baseline correction, in order to reduce negative factors in the spectra. Leave-one-out cross validation was also performed in this model. The obtained coefficients of Pearson's *r* (0.964) and root mean square error of calibration sample (RMSEC, 5.4%) suggest the excellent representativeness of the PLS model. The prediction results indicate that proteoglycan concentration is lower than that of collagen in both healthy and OA cartilage, and decreases in the OA cartilage, especially at superficial zone. The prediction with spatial resolution can help to understand the osteoarthritic development and demonstrate that FTIRI combined with PLS is a powerful analytical approach in biomedical research.

1. Introduction

Collagen and proteoglycan (PG), two macromolecules in soft connective tissues, are the major building blocks in the extracellular matrix (ECM) in articular cartilage and play a significant role in maintaining physical and mechanical properties of cartilage. Structurally, PG is embedded in a three-dimensional collagen fibril network, which provides cartilage with the ability to resist external pressures and maintain its resiliency [1]. Furthermore, the properties of articular cartilage across its thin thickness are depth-dependent and anisotropic [2], which is often conceptually sub-divided into three successive layers: superficial zone (SZ), transitional zone (TZ), and radial zone (RZ) from surface to subchondral bone [3,4]. In addition to collagen and PG, a small amount of chondrocytes is interspersed distributed in articular cartilage [5], which is involved in the macromolecular synthesis and metabolism.

Osteoarthritis (OA) is a chronic disease of the joint, commonly accompanied by the reduction of PG concentration, the death of the chondrocytes, the damage of fiber network, joint pain and loss of mobility for the patients [6]. At the present time, there is no clinical procedure that can diagnose and monitor the onset and progression of the disease. Quantitative research on the principal component concentrations in healthy and OA articular cartilage is, therefore, very important since any small changes in the macromolecular concentrations might indicate the disease manifesting.

As a newly developed analytical tool, Fourier transform infrared imaging (FTIRI) can be used to detect the changes in molecular compositions and structures of biologic tissues with high spatial and spectral resolutions. When FTIRI was used to study the PG and collagen in articular cartilage, for example, one could resolve some characteristic bands specifically involving in collagen and PG, including amide I (1700–1600 cm^{-1}), amide II (1600–1500 cm^{-1}), amide III (1300–1200 cm^{-1}) and sugar band (1125–1000 cm^{-1}) [7,8]. Since the noticeable overlaps between the IR characteristic bands of collagen and PG, it is difficult to develop simple imaging relationships between the molecular

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concentrations and infrared absorbance. Therefore, the characteristic bands assigned to collagen (1338 cm^{-1}) and PG (1072 cm^{-1}) can only be used qualitatively in the analysis of the macromolecular distributions in articular cartilages [2,9,10]. The use of chemometrics method is needed in FTIRI post analysis to quantitatively determine the concentrations of bio-specific macromolecules in cartilage [11,12].

Partial least square (PLS) algorithm, a quantitatively spectral decomposition technique, has been used to extract chemical information from complex spectra [13]. The decomposition procedure of the PLS model provides more robust calculation since it extracts information from both spectral and concentration matrices [14,15]. PLS also combines the features of principal component analysis (PCA) and multiple regression approach [16], which utilizes the entire range of the spectrum rather than some individual absorption bands. These merits of PLS algorithm would increase the accuracy and reliability of the regression modeling of complex spectra.

Based on our previous work in which FTIRI combined with PLS was used to predict successfully the percentage concentrations of collagen and PG in bovine nasal cartilage [17], the FTIRI-PLS approach is applied in this study to determine and differentiate the concentration distributions of collagen and PG in healthy and OA articular cartilages. The results are discussed in the contest of better understanding the osteoarthritic development and diagnostics.

2. Materials and methods

2.1. Sample preparation

The specimens of articular cartilage were harvested from the knee joints of 8 mature dogs (7 healthy and 1 osteoarthritis) after they were sacrificed for the biomedical project, which was approved by the institutional review committees. The OA cartilage had been cultivated for 2 years after an anterior cruciate ligament (ACL) transection in one knee joint. The canine model of the ACL transection has been a gold standard in animal studies of OA, can result in the distortion of the joints movement and the damage of cartilage [18,19], which resemble the tissue changes in human OA.

The cartilage samples still attached to the subchondral bones were cut from the tibial locations that were covered with meniscus in the knee joints, by using a table saw with a diamond blade. The size of the cartilage-bone blocks was about 2 mm × 2 mm × 2 mm. After being washed in the saline and quickly frozen by liquid nitrogen, these specimen blocks were sectioned into 10-μm thick sections by using a cryostat (Leica CM 1950, Germany) at −20 °C. The cutting direction was vertical to the cartilage surface, which is from SZ, TZ to RZ. The cartilage sections were mounted on MirrIR slides (Kevley Technologies, Chesterland, OH), which were dried in the air for 2 h before FTIRI experiments.

2.2. FTIR imaging

FTIR spectroscopy and imaging experiments were carried out on a PerkinElmer Spotlight-300 infrared imaging system, which includes a FTIR spectrometer (Spectrum One) and an infrared microscope. The MirrIR slides with cartilage sections were mounted on the scanning stage of the FTIRI system. Infrared imaging data of cartilage were collected by a MCT array detector at 6.25 μm/pixel and 8 cm^{-1} wavelength spacing (namely 16 cm^{-1} resolution) over a mid-infrared range of 4000–744 cm^{-1}. The background data of the MirrIR slide were also collected in the same spectral range for the baseline correction of the spectral data. Two scans per pixel were performed for the background and the samples.

The field-of-view in FTIRI experiments was approximate 650–700 μm, which contained the whole thickness of cartilage from the surface to subchondral bone. Co-add IR spectra, namely averaged spectra of the selected areas, were extracted with 20 μm spacing from the FTIR images that were divided into the rectangular regions (20 μm (length) × 100 μm (width) for the typically healthy cartilages and 20 μm × 160 μm for the typically OA cartilage). Therefore, 35 and 32 extracted spectra of the healthy and OA cartilage would be predicted, respectively, in the PLS model to obtain the concentration profiles of collagen and PG.

To construct the calibration spectral matrix of the PLS model, the standard infrared spectral library of pure chemical components was measured by mixing collagen and chondroitin 6-sulfate (CS6) in different mass ratios. The commercial product of CS6 was purchased from Sigma–Aldrich (MO). Fibrillous type II collagen (Elastin Products Company, MO) was processed into powder by resolving, grinding, and drying in an oven. KBr powder was also dried and grinded thoroughly. Collagen and CS6 were dry-mixed with 90 mg KBr powder in different mass ratios (collagen/PG = 0.59, 1.09, 1.56, 0.4, 0.85, 1.34, 0.98, 1.36, 2.54) and then pressed into 10 mm-diameter pellets. IR spectra of ten pellets were collected by using the Spectrum One in the range of 4000–744 cm^{-1} with 8 cm^{-1} wavenumber spacing. Finally, the ten corresponding concentration ratios of each group were acted as the "concentration matrix" of the calibration dataset.

2.3. PLS model

PLS regression model was built by Unscrambler X software (CAMO Software, Inc., Woodbridge, NJ), which is a multivariate data analysis software that can build the complicated mapping relationships between the absorption intensity and the collagen and PG contents in cartilage. With the importing of a set of co-added spectra of cartilage specimens into the software as a spectral matrix, the regression model can determine the concentration information of the cartilage.

A series of spectral pre-processes were performed to improve the stability of the PLS model, which included multiplicative scatter correction (MSC), normalization and baseline correction. MSC was used to reduce the scattering effects in the spectra. Leave-one-out cross-validation was also carried out in this PLS model, that is, each spectrum of the calibration matrix will be predicted by the residual via this PLS model. Therefore the predictive ability of this model can be tested out through the fitting

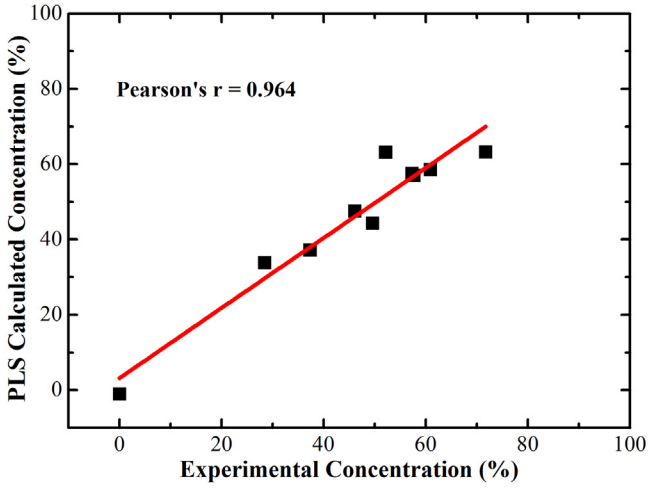

Fig. 1. Correlation between the experimental and PLS-predicted concentrations of collagen in the calibration matrix (factor = 2).