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Fourier transform infrared spectroscopic imaging application for multi-stage discrimination in cartilage degeneration

Xiao Wang, Ming-Yang Zhai[1], Zhi-Hua Mao[1], Yan-Fei Lu, Jian-Hua Yin*

Department of Biomedical Engineering, Nanjing University of Aeronautics and Astronautics, Nanjing 210016, China

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ABSTRACT

Fourier transform infrared spectroscopic imaging (FTIRI) technique can be used to obtain rich information about the chemical components in biological tissue such as articular cartilage, by employing quantitative chemometrics methods. In this study, the imaging technique was used for spectroscopic imaging on cartilage sections. The support vector machine discriminant analysis (SVM-DA) was then employed in imaging data analysis to distinguish among healthy and osteoarthritic (OA) articular cartilages at different degeneration stages for the first time. Briefly, the infrared spectra were extracted from the FTIR images and imported into Unscrambler software for the SVM-DA model construction and prediction. When the 3rd-polynomial kernel function was chosen as the kernel function and the parameters C in each range of 2782.5–10000, 2.3714–10 and 0.0032–0.01, respectively, and the corresponding optimal G was equal to 0.1, 1 and 10, the healthy, OA-8W (8 week after surgery) and OA-2Y (2 years after surgery) cartilage samples were effectively differentiated by the SVM-DA method with high accuracy of 100% for the training set and 86.67% for the prediction group. FTIRI with the use of chemometrics (SVM-DA) may become an effective method to distinguish multi-stage tissue degradation in the future.

1. Introduction

Articular cartilage (AC), which covers the end of articulating bone to reduce friction and distribute pressure, plays an important role during joint motion and loading [1]. The major macromolecular components of AC are type II collagen and proteoglycan (PG) [2]. The main function of collagen is to form the fibril network of cartilage [3], which provides the structural integrity of AC and enmeshes the PG. Since PG possesses a high negative charge density, the close enmeshment of PG provides the resiliency and compressive strength of AC [4]. Histologically, the uncalcified cartilage can be conceptually subdivided, from the articular surface to subchondral bone, into three zones including superficial zone (SZ), transitional zone (TZ), and radial zone (RZ) [5].

The reduction of PG concentration and the disruption of collagen network are the signs of cartilage degeneration, which would eventually lead to osteoarthritis (OA) [6,7]. In the early stage of OA, cartilage would only have the reduction of the molecular concentrations and microscopic damage but not macroscopic injury [8]. Since the joint diseases are the number one cause of disability in the developed countries, OA has been studied in recent years by both in vivo and in vitro technologies, including magnetic resonance imaging, biomechanical measurement, biochemical analysis, and various forms of microscopy techniques [9–11]. It is however still challenging to measure PG and collagen contents in degraded cartilage with sufficient spatial resolution, quantitatively and simultaneously.

As a spectroscopic imaging tool, Fourier transform infrared spectroscopic imaging (FTIRI) is able to provide morphology and spectroscopy information of samples simultaneously with fine spatial and spectral resolutions [12,13]. By extracting FTIR spectra from FTIR image and employing various chemometrics methods in the data analysis of FTIRI, the technology is capable of quantifying the spatial distributions of the macromolecular concentrations in cartilage [8,12,14–16]. In our previous work, the use of partial least square-discriminant analysis (PLS-DA) and principal component analysis-Fisher discriminant analysis (PCA-FDA) in FTIRI enabled the differentiation between healthy and OA samples with high accuracy [14,17]. But these methods had limitations in the multiple discriminant analysis.

Support vector machine discriminant analysis (SVM-DA) is a machine learning method on the basis of statistic learning theory [18]. It constructs a classification model by using the kernel function, which has advantages in non-linear identification [19]. By testing different kernel functions, the classification model can adapt to different types of

* Corresponding author.
E-mail address: yin@nuaa.edu.cn (J.-H. Yin).
[1] These authors contributed equally to this study.

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spectroscopic distribution. FTIR spectroscopy technique combining with SVM-DA has been used to realize the early diagnosis of colon cancer and classification of normal, dysplasia, early carcinoma, and advanced carcinoma [20], as well as working for rapid and non-destructive discrimination of different types of polyacrylamide [21], which suggests that FTIRI with SVM-DA has shown potential in biomedical research. In this project, SVM-DA was combined with FTIRI to differentiate three different kinds of cartilage tissues: healthy cartilage, mildly OA cartilage (8 weeks after the joint surgery) and severe OA cartilage (2 years after the joint surgery). These OA cartilage models were built by anterior cruciate ligament (ACL) transection surgery in one of knee joints.

2. Materials and methods

2.1. Sample preparation

The cartilage samples were harvested from mature dogs after they were sacrificed for an unrelated project [22]. All of the procedures complied with Canadian Council of Animal Care guideline and were approved by Animal Ethics Committee. To induce cartilage degradation, the transectional surgeries of the ACL in one knee (hind limb) were performed 8 weeks or 2 years before the sacrifice, to generate the OA-8W group and OA-2Y group, respectively. The corresponding OA features were experimentally proven before [22], for example, the increase in size and reduce in quantity of chondrocyte at different OA stage [17,22], as well as the different PG loss (PG concentration) [22]. Three dogs/joints constituted each group, as well as the healthy group that had similar individual features both in weight and age. Vertically to cartilage surface, tibia end covered with cartilage were cut into some blocks of 2 mm × 2 mm × 2 mm size by a table saw with a diamond blade when the OA model was harvested. The individual block specimens had the full-thickness cartilage that still attached to the bones in rectangular shape. The specimen blocks were rinsed in saline for 1 min, snap frozen by using liquid N_2 and stored frozen at −20 °C. Subsequently, 10-μm-thick sections were sectioned along the perpendicular direction to cartilage surface by using a cryostat (Leica CM 1950, Germany). Two sections were selected from each dog for imaging; meanwhile, the depth of sections was standardized by calculating from cartilage surface to cartilage/subchondral bone interface. The sections were mounted on the MirrIR slides (Kevley Technologies, Chesterland, OH), which were air-dried for 2 h before FTIRI experiments.

2.2. FTIRI experiment

FTIRI experiments used a PerkinElmer Spotlight-300 infrared imaging system [8], to scan the tissue slides that were mounted on a movable mechanical stage. The sample was in left-right orientation and top-down view when FTIR imaging. The imaging data of cartilage sections were collected at 6.25 μm pixel size and 8 cm^{-1} wavelength spacing over the IR spectra ranging from 4000 to 744 cm^{-1}. Background spectra (MirrIR slide) were also collected in the same range for the baseline correction. The FTIR spectra were extracted from the FTIR images of 18 sections (6 healthy, 6 OA-8W and 6 OA-2Y), which were numbered in the order of AC-1 to AC-6, OA-8W-1 to OA-8W-6 and OA-2Y-1 to OA-2Y-6, respectively. Because of the scattering and diffuse reflection, the IR spectrum from the edge of the tissue sections could become distorted [8]; so the first one or two spectra from the SZ edge were excluded in the analysis, whose boundary could be estimated with MRI [3,22,23].

Since the very obvious change at the early stage of OA was the reduction of PG concentration in SZ and TZ [22,24], the SZ spectra were considered the most representative of OA degradation, and used for the training set. For the training group, 60 spectra (10 from each section) were randomly extracted from SZ of AC-1, AC-3, OA-8W-4, OA-8W-6, OA-2Y-1 and OA-2Y-2 sections with a 10 μm interval. They came from 3 dogs (1 healthy, 1 OA-8W and 1 OA-2Y). The other 20 × 12 spectra extracted from the other 2 × 6 = 12 sections (AC-2, AC-4, AC-5 and AC-6; OA-8W-1, OA-8W-2, OA-8W-3 and OA-8W-5; OA-2Y-3, OA-2Y-4, OA-2Y-5 and OA-2Y-6) belonged to the other 6 dogs (2 healthy, 2 OA-8W and 2 OA-2Y). The 240 spectra (20 from each section) were composed of prediction group. The prediction group also included the TZ and RZ spectra.

2.3. Support vector machine discriminant analysis

SVM-DA was carried out in Unscrambler software (version 10). The spectral range of 1800–1000 cm^{-1} was chosen for SVM-DA model construction. First, an appropriate kernel function with the optimal parameters was selected by trying different combinations of kernel functions and corresponding parameters. The detailed description about SVM including the kernel functions can be found in the related literatures [25]. The kernel functions used in this project included the linear kernel function (LF), radial basis kernel function (RBF) and polynomial kernel function (PF). By comparing all the combinations of kernel functions and corresponding parameters, the optimal kernel function and corresponding parameters (C: regularization parameter; G: kernel parameter) were determined and used to construct the SVM-DA model. Parameter C determines the trade-off between maximum margin and minimum classification error. G was a built-in parameter in radial basis kernel function and polynomial kernel function, which determined the distribution of the data that were mapped to new feature space. Trial-and-error method was applied in the SVM-DA model for all samples to get satisfied results. Subsequently, the model was applied to identify all healthy, OA-8W and OA-2Y samples. The prediction results of all specimens with SVM-DA were analyzed statistically by using the software of IBM SPSS Statistics 20.

3. Results

3.1. Infrared spectrum and image analysis

Fig. 1 shows three infrared absorption spectra, which were extracted from single section of each category of healthy, OA-8W and OA-2Y, respectively. The main characteristic bands of collagen and PG were amide I (1700–1600 cm^{-1}), amide II (1600–1500 cm^{-1}), amide III (1300–1200 cm^{-1}) and sugar bands (1125–960 cm^{-1}) [8,12,22]. Based on the spectral analysis, it was clear that the absorbance of amide I, amide II, sugar and 1338 cm^{-1} bands decreased in the sequence of healthy, OA-8W and OA-2Y, which was ascribed that the FTIR spectra of cartilage were contributed from both collagen and PG [8]. Either

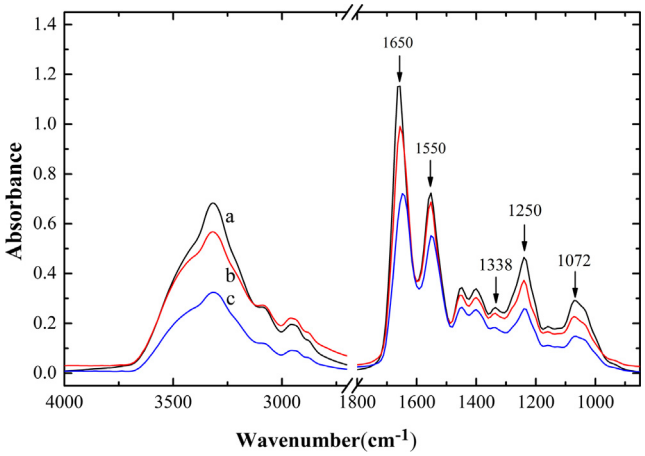

Fig. 1. The infrared absorption spectra extracted from healthy (curve a), OA-8W (curve b) and OA-2Y (curve c) cartilage sections. OA: osteoarthritis; W: week; Y: year.