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Prediction of compressive stiffness of articular cartilage using Fourier transform infrared spectroscopy

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ABSTRACT

Unique biomechanical behavior of articular cartilage is a result of its structure and composition. Interrelationships of tissue constituents (collagen, proteoglycans (PGs) and water) and tissue biomechanical parameters have been studied, but it is evident that no constituent alone explains the tissue mechanics. Fourier transform infrared (FT-IR) spectra can provide detailed information about the biochemical composition of articular cartilage. In this study, a chemometric approach to predict the biomechanical behavior of articular cartilage directly from the FT-IR spectra, i.e., without converting the data into collagen and PG information, was investigated. Partial least squares regression (PLSR) was used to predict equilibrium modulus ($n=32$) and dynamic modulus ($n=24$) of bovine cartilage samples from their average FT-IR spectra. The linear correlation coefficients between the reference and predicted values of Young's modulus and dynamic modulus were $r=0.866$ ($p<0.001$) and $r=0.898$ ($p<0.001$), respectively. When the compressive biomechanical behavior of AC is predicted, the present study indicates that similar or improved results can be obtained with FT-IR spectroscopy as compared to those of traditional biochemical methods.

1. Introduction

Articular cartilage (AC) is a functional tissue that covers the bone-ends of diarthroidal joints. Its unique biomechanical features are required to withstand the everyday stresses generated during typical joint movements (Mow et al., 1992). From the biomechanical point of view, it provides nearly frictionless gliding surfaces within a joint and redistributes the stresses generated in normal locomotion to contact surfaces with larger areas. AC is compressible or nearly incompressible depending on the loading conditions. This biomechanical behavior of AC is a result of its composition and structure. Majority of AC is composed by water, fibrillous collagen network, proteoglycans (PGs) and chondrocytes (Mow et al., 1992). The exact mechanisms how the tissue macromolecular composition and architecture relate to its biomechanical properties have been under active research, and understanding of the structure–function relationships of AC is essential, e.g., if artificial cartilage tissue is developed. It is evident that the inhomogeneous distribution of all macromolecular components in AC and complex 3-dimensional collagen network provide the arrangement required for AC to function optimally.

Research efforts toward understanding the biomechanical properties of AC cartilage have evolved from simple linear correlation analyses between the tissue constituents and biomechanical parameters to complex mathematical models of AC biomechanics (Kempson et al., 1970; Mow et al., 1992; Wilson et al., 2004; Korhonen et al., 2008). Overall goal of the past studies has been to interrelate to the biomechanical behavior of the tissue with its composition. However, different biochemical constitutes interact with each other and consequently the link between the biomechanical behavior and specific macromolecular composition is not trivial. Unfortunately, the currently applied biochemical methods are not ideal for taking into account of the biochemical composition as a whole. Therefore, different compositional parameters usually are individually correlated with biomechanical parameters. Further, specific interactions between the macromolecules are not clear, and therefore they cannot be included in the mathematical models.

Fourier transform infrared (FT-IR) spectroscopy offers a possibility to collect detailed information about the tissue composition. The method is based on the absorption of

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infrared energy in the material under interest. Energy is absorbed by the intramolecular bonds at characteristic resonance frequencies. Collected absorption spectra carry a vast amount of information about the tissue biochemical composition. However, as absorption peaks overlap with each other, specific macromolecular information is difficult to retrieve from the data. Several methods, such as integrated absorbances, second derivative spectroscopy, curve fitting and multivariate models, have been used to obtain collagen and PG contents in AC (Yin et al., 2012; Camacho et al., 2001; Rieppo et al., 2010; Yin and Xia, 2010; Rieppo et al., 2012). The estimated contents could be then correlated with the biomechanical parameters (Mahmoodian et al., 2011), but such approaches would offer little improvement compared to the earlier biochemical analyses methods.

Multivariate analysis makes possible to consider the collected spectral data as an overall description of material biochemical properties. Multivariate regression models, such as Partial least squares regression (PLSR), are calibrated against a quantitative or qualitative feature of the specimen provided by a reference method. Application requires no *a priori* information about how or which part of the data is related to the phenomenon of interest. This resolves the problems associated with the application of potentially interdependent variables (collagen, cross-links, proteoglycans etc.). Indeed, structure–function relationships are not studied by correlating the measured variables one by one with the functional properties, but instead, the multivariate regression methods build new uncorrelated variables that are used in the regression to explain the studied phenomenon. Obviously, multivariate regression analysis provides no direct information about the structure–function relationships but it allows evaluating how well the average biochemical composition can predict the biomechanical parameters.

This study was designed to evaluate whether a direct relation of FT-IR data with the biomechanical properties of AC can be established by using multivariate regression techniques. As far as we know, this study is the first attempt to directly, i.e., without converting the spectral data to estimate specific macromolecules, link spectral characteristics to biomechanical properties of AC. We hypothesize that multivariate analysis can improve the prediction of biomechanical properties over that obtained previously with the traditional correlation analysis. This study also reveals new information about which spectral regions hold the most valuable information regarding specific functional properties.

2. Materials and methods

2.1. Sample preparation

The samples used in this study were originally collected in other studies (Saarakkala et al., 2003; Töyräs et al., 2003). Briefly, bovine patellae ($n=32$) with variable visual signs of spontaneous degeneration were obtained. A cylindrical osteochondral sample ($d=19$ mm) was drilled from each patella. The samples were stored in a freezer (-20 °C) for 2 weeks. Subsequently, the sample was split into two halves. From the first block, a cylindrical ($d=3.7$ mm) full-thickness cartilage sample was taken for biomechanical reference measurements while the remaining cartilage was used for biochemical reference measurements. The second block was fixed with 10% formalin, decalcified with EDTA, dehydrated and embedded in paraffin.

2.2. Biomechanical testing

Biomechanical testing was originally conducted in an earlier study (Saarakkala et al., 2003; Töyräs et al., 2003). Briefly, the biomechanical measurements were conducted immediately after preparing the smaller cylindrical sample ($d=3.7$ mm) from the larger osteochondral block. A custom-made material testing instrument (with resolutions of 5 mN and 0.1 μm for the force and position, respectively) was used for biomechanical reference measurements (stress–relaxation in unconfined compression geometry, 10% pre-strain followed by 10% strain with 2 mm/s ramp speed and relaxation time of 2400 s). Young's modulus (or equilibrium modulus) and dynamic (or instantaneous modulus) modulus were calculated as a stress–strain ratio after the relaxation and instantaneously after a 10% step, respectively (Saarakkala et al., 2003; Töyräs et al., 2003).

2.3. FT-IR spectroscopic imaging

Three 5-μm-thick sections were cut from each paraffin-embedded sample with a microtome and transferred onto standard microscope slides. Paraffin was dissolved with xylene prior to transferring the sections onto 2-mm-thick ZnSe windows for the FT-IR spectroscopic imaging. Measurements were conducted with the Perkin Elmer Spotlight 300 FT-IR imaging system (Perkin Elmer, Shelton, CO, USA) in transmission mode using spectral resolution and pixel resolution of 4 cm^{-1} and 25 μm, respectively. Eight repetitive scans per pixel were averaged. The imaging system and the sample box were purged with CO_2-free dried air during the measurements to standardize the measurement conditions (Parker Balston, Haverhill, MA, USA). A 400-μm-wide area was imaged from cartilage surface to cartilage-bone junction within each section.

2.4. Data pre-processing

Since only bulk reference biomechanical values for each sample were available, spectra of each section were also averaged to obtain one mean spectrum. Further, the mean spectra of parallel sections of the samples were averaged. Thereafter, Extended Multiplicative Signal Correction (EMSC) was used to remove scattering-related baseline variations from the spectra (Kohler et al., 2007).

2.5. Univariate analysis

Univariate parameters for estimation of the collagen content (amide I region, 1584–1720 cm^{-1}) and PG content (carbohydrate region, 984–1140 cm^{-1}) were calculated from the FT-IR spectra (Camacho et al., 2001). The univariate parameters were correlated with Young's modulus and dynamic modulus of the same samples.

2.6. Multivariate analysis models

Spectral region of 900–1800 cm^{-1} was used for both Young's modulus and dynamic modulus. Optimal number of components for the models was chosen by performing a leave-one-out cross-validation and calculating the root-mean-square error of cross validation (RMSECV) for all models. Minimum value of RMSECV indicated the best model. The best models were evaluated by calculating RMSECV and by Pearson's correlation coefficient between the predicted values and the reference values.

2.7. Genetic algorithm for wavenumber selection

Genetic algorithm was used to select the spectral variables in order to see if the models built using the full spectral range could be improved. Genetic algorithms are optimization methods based on the principles of natural evolution (Leardi et al., 2002). Variables are called genes, and the solution vector that contains the selected variables is called a chromosome. In variable selection, chromosomes are binary vectors with 1's indicating the variables to be selected. A population contains multiple chromosomes. In the beginning, a population with a pre-defined population size consisting of random chromosomes is made. A PLSR model is built for each chromosome, and its performance is evaluated by evaluating their RMSECV. The smallest RMSECV value indicates the best chromosome of the population. Typically, the best solution of the population is copied to the next population. The next population is formed by recombining the initial chromosomes by using cross-over and mutation. In cross-over, two chromosomes are combined by choosing a random splitting point and then combining the parts. A mutation is a change in a single gene (variable), and it has a low probability. The algorithm usually runs for a pre-defined number of generations, and the best chromosome of the last generation is considered as the optimal solution for the problem.

The parameters used in the genetic algorithm were as follows— the population size: 100, gene initialization probability: 5%, cross-over method: one-point, cross-over probability: 80%, mutation probability: 1%, number of generations: 100, response (to be minimized): RMSECV of the prediction. The number of PLS components for Young's modulus was chosen based on the full spectrum model. For dynamic modulus, the full spectrum model used a relatively high number of PLS components. A simpler model was preferred when genetic algorithm was