INTRODUCTION:

Fourier-transform infrared imaging (FTIRI) can spatially resolve chemical signatures of a biological specimen with fine spatial and frequency resolutions, and is used in this work to study the anisotropic characteristics of infrared absorbance in individual histological zones of articular cartilage at 6.25μm in-depth resolution. We aim to explore novel usages of FTIRI in cartilage research because many changes in the tissue's fine histological structure and delicate chemical / molecular composition proceed significantly the development of osteoarthritis as a clinical disease, a sensitive technique for detecting the early changes in cartilage leading to OA therefore can be valuable in monitoring the disease progression and evaluating the efficacy of treatment.

METHODS:

A canine humeral cartilage-bone block was paraffin-embedded and microtomed into 6μm sections. (The interface between the soft tissue and the bone was preserved in the thin sections.) The infrared imaging was done on a PerkinElmer Spotlight 300 FTIRI system, with a square pixel of 6.25μm and a frequency resolution of 8 cm⁻¹. Each of the five sections was infrared-imaged twenty-six times with the identical acquisition parameters, each time with a 5°-10° increment of the polarization in the 0°-180° angular space. Following the infrared imaging experiments, the same tissue sections were also imaged in a polarized light microscope (PLM) with a square pixel of 2.72μm at 5x objective. For FTIRI experiments, the images of four major peaks of interest were studied in detail (amide I at 1700–1600 cm⁻¹, amide II at 1600–1500 cm⁻¹, sulfate at 1300–1200 cm⁻¹, and sugar 1125–1000 cm⁻¹). For PLM experiments, the images of fibril angle and retardation were analyzed.

RESULTS:

Fig 1 shows the visible and two infrared images of the specimen from the experiments. At each polarization, the selected region of interest in the tissue contains 6429 individual infrared spectra. The four major molecular vibrational peaks of the infrared spectra (amide I, amide II, sulfate, sugar) exhibit distinctly different characteristics in different histological zones upon polarization, which demonstrates the anisotropic orientation of various chemical compositions in different parts of the tissue. Fig 1 also shows the depth profiles of the absorbance bands of amide I and amide II in the first quadrant of the polarizer rotation.

A novel type of 2D infrared image, “the absorbance anisotropy image”, was constructed for each of the major components (Fig 2a-b), where each row is one absorbance profile as a function of the tissue depth at a fixed angle (Fig 1), and each column is a plot of absorbance versus the polarization angle of the sample (Fig 2c-d). The cross-sections of these 2D images offer several distinct features regarding the depth dependent anisotropy of each infrared component (Fig 2c-d). (1) For both amide I and amide II absorbance bands, the tissues in the superficial zone and in the radial zone have the opposite anisotropic characteristics, indicating the perpendicular orientation of the fibrils in these two zones. (2) The anisotropic periodicities of the amide I and amide II absorbance bands have the reversed characteristics, which indicate the 90° angle difference between the amide I and amide II vibrations.

Fig 3 shows the angle and retardation profiles from a 2D PLM experiment, which has the consistent features as in some of the previous MRI/PLM correlation studies, namely the angle profile of the tissue has a hyperbolic tangent transition in the transitional zone where the retardation is the minimum. Two vertical lines indicate the approximate division of histological zones in cartilage.

CONCLUSIONS:

This type of infrared anisotropic study has the potential to monitor the individual chemical concentrations in articular cartilage at high resolution. The ability of examining the same tissue section using both FTIRI and PLM offers the possibility of correlating between the morphology of and chemical distribution in the tissue.

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