Assessment of early osteoarthritis by Raman spectroscopy

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Introduction: Measures of outcomes in osteoarthritis (OA) that can predict disease progression or amelioration are essential for the assessment of pharmaceutical and surgical interventions, particularly at early disease stage when it is treatable. The earliest observed molecular change with onset of OA is the loss of sulphated GAGs and type II collagen cleavage, later leading to collagen degradation, increased tissue hydration, and eventual degeneration of the extracellular matrix. We have taken advantage of the changes in sulphated GAGs, collagen and hydration for the examination of cartilage by Raman spectroscopy (RS) to compare between healthy tissue and tissue at early and late disease stage. RS is a sensitive light scattering technique used to characterise vibrational energies of molecular bonds. It has been applied to bone tissue to observe both the organic (collagen) and inorganic (hydroxypatite) components and spectra can be recorded in vivo using minimally invasive fibre-optic probes or through skin. Such developments in RS show it to have great potential that is poised to become a routine diagnostic tool in medicine. The aim of this study was to apply RS to hyaline cartilage to map molecular changes associated with early onset OA and apply an assessment strategy to correlate with histological grading.

Materials and Methods: Full-depth cartilage was obtained under Institutional guidelines from an age range of normal specimens (5-80yrs, n=14) and stored at -80°C without further processing. OA cartilage from patients (54-76 yrs n=4) undergoing total knee arthroplasty, to analyse residual cartilage. Cartilage was also prepared from macroscopically normal specimens (46-73yrs, n=4) but for the presence of a single focal lesion (~0.5-1 cm²). These are considered early OA lesions as they are small and non-symptomatic. The lesion site, adjacent and distal regions were compared. Raman spectra were obtained at room temperature of thawed cartilage immersed in water using a Raman labInVia spectrometer (Renishaw plc) with a 150mwatt laser diode in-line with a microscope and x50 objective. Multiple sites per specimen were scanned, with each spectrum recorded as the sum of four exposures of 300 seconds each. In further work, normal cartilage (n=4 specimens) was cultured in the presence of 10 ng/ml IL-1ß at time points up to 12 days prior to analysis. Cartilage was graded by safranin O histology from 0-3, to give global scores where 0 = normal; 1 = smooth, unfractured synovial surface, some loss of GAG staining; II = surface roughening, obvious GAG loss from superficial layer; III = thinning, eroded cartilage, poor GAG staining, loss of cells. Because of the complexity of Raman spectrum of hyaline cartilage, we focussed on the well documented biochemical changes that occur in early OA. Thus, the analysis concentrated on spectral features related to sulphate (SO₄²⁻), water and collagen components. A Lorentzian curve fit was applied to measure band area, position, and width.

Results: Raman spectral features common to all specimens included a high-frequency region (2500-3700 cm⁻¹) denoting O-H (H₂O) stretching vibrations and a region associated with characteristic modes of the organic components of the extracellular matrix which included vibrations of SO₄²⁻ (Figure 1). These spectra of cartilage immersed in water were indistinguishable from spectra recorded under synovial fluid (data not shown). The H₂O associated bands remained constant in intensity with degeneration grade; however there was a distinct reduction in all other bands with increasing grade of degeneration indicating a relative increase in hydration. To quantify spectral output with disease grade, we established a reproducible measure of the integrated intensity ratio between characteristic peak areas. Thus, the SO₄²⁻ peak significantly loses mean relative intensity with degeneration, consistent with the known loss of aggrecan both in early and later stages of OA (Figure 2A). Grades II & III disease could be unambiguously identified with this quantification. Similarly, the analysis demonstrated a significant increase in hydration of the tissue with disease (Figure 2B) and the ratio of proline:hydroxyproline was significantly increased with disease grade (Figure 2C). No age-related differences in spectra of healthy cartilage were observed; indicative that the above changes were associated with disease stage and not due to normal age-related changes. Grade II-III treatment showed advanced disease grade with early loss of proteoglycan (grade I and II) and later by increased hydration and proline:hydroxyproline ratios (grade II & III) with culture time (not shown).

Discussion: Reproducible Raman spectra were obtained from healthy and diseased articular cartilage, allowing an objective assessment of the degeneration grade, indicated as 0-III. A, Full spectra; B, Detailed spectra of arrows a-c in A, between 800-1200cm⁻¹. Numbers indicate the band centres with those in bold used in assessment analysis - proline ~850cm⁻¹, hydroxyproline ~877cm⁻¹, phenylalanine ring-breathing band ~1003cm⁻¹ and sulphate v1 band ~1063cm⁻¹; C, Detailed spectra of arrows d-e, between 2750 and 3700cm⁻¹ with CH2 / CH3 stretch bands at ~2940 and O-H (water) stretch doublet at ~3210 and 3380cm⁻¹.

References: