Raman spectroscopy can non-invasively distinguish between ochronotic and non-ochronotic cartilage

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**Purpose** Alkaptonuria is a rare genetically inherited form of OA which is characterised by urine which darkens on standing, pigmentation of articular cartilages and early-onset, rapidly progressing osteoarthropathy of multiple weight bearing joints. The pigmentation is caused by presence of a tyrosine metabolite; homogentisic acid (HGA), which polymerises over time to for a pigment in the articular cartilages. The presence of pigment changes the biochemical and biomechanical properties of the cartilage causing anatomical and physiological dysfunction. To date there is no evidence to suggest the exact time when ochronosis and therefore pathological change in tissues commences. Our study will examine ochronotic and non-ochronotic cartilage to determine if Raman spectroscopy can detect pigmentation in macroscopically non-ochronotic cartilage. It will also compare the spectra of ochronotic and non-ochronotic cartilage to normal cartilage spectra in the current literature.

**Methods** Cartilage samples were obtained as surgical waste with informed patient consent following ethical approval. The samples were dissected immediately following surgery into ochronotic and non-ochronotic pairs and stored unfixed at -80 ͦC. Individual cartilage samples were placed on to calcium fluoride discs for Raman spectral analysis.

Spectra were acquired using an InVia Raman microspectrometer (Renishaw plc, UK) with an 785 nm laser. Spectra were collected using 20 s and 3 accumulations, at 100 % power (~10mW at sample) from the non-ochronotic tissue. However, applying the same settings to the ochronotic tissue resulted in detector saturation. Therefore spectra were collected using 1 s and 10 accumulations, at 100 % power. Data were baseline corrected, using polynomial subtraction, to remove fluorescence and normalised.

**Results** Macroscopically non-ochronotic cartilage displayed spectra typical of normal articular cartilage with peaks corresponding to all major functional groups and amino acids: proline, hydroxyproline, phenylalanine, amide I (carbonyl group) and amide III (C-N and N-H). Cartilage which showed observable macroscopic ochronotic pigmentation and those which were completely ochronotic displayed an absence of a recognisable spectra with no discernible peaks corresponding to functional groups seen in the non-ochronotic sample, but were highly fluorescent. The macroscopically pigmented sample showed two peaks, previously described in the literature, corresponding to aromatic C-C twisting and phosphatidylinositol (lipid).

**Conclusions** Our data demonstrates that the semi-pigmented and pigmented samples of cartilage were in stark contrast to the non-pigmented samples. While it was relatively straightforward to collect spectra from the non-ochronotic cartilage, which produced ‘typical’ cartilage spectra, the ochronotic cartilage was very fluorescent, which masked the Raman signal. Therefore, there were very few identifiable peaks; two were identified at 626 and 775 cm-1, but were not present in the non-pigmented spectra. The presence of these peaks may be due to the HGA polymer or an alteration to the cartilage. The lack of prominent peaks in the samples is most likely due to a high level of fluorescence that appears to occur when the HGA polymer becomes incorporated within the cartilage matrix. As a result it was not possible to discern how specifically the chemical structure had changed, just that there had indeed been an alteration in structure. This data shows the significant biochemical and structural changes that occur focally in AKU cartilage and contribute to its rapid and devastating progression. Our results suggest Raman spectroscopy may have application in the development of a non-invasive test for AKU severity.