POSTER 2124

FTIR-I Chemical Mapping of Articular Cartilage as a Function of Tissue Degeneration - A Preliminary Study

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INTRODUCTION

- From early to late osteoarthritis (OA), the tissue exhibits a loss of proteoglycan (PG) content, while also presenting with alterations of tissue structure and cellular features[1].
- Over the past decade, there have been increasing interests in Fourier Transform Infrared Spectroscopy (FTIR) to analyze biological specimens, including articular cartilage[2-4].
- FTIR is capable of *simultaneously* measuring multiple constituents and structural features, including proteoglycans, collagens, collagen integrity, and collagen orientation, and can be combined with imaging (FTIR-I) to measure spatial variations in biochemical composition.
- The purpose of this preliminary work is to:
  1) Validate that FTIR-I is capable of replicating PG features that are identifiable using Safranin O
  2) Assess additional chemical features that Safranin O staining cannot determine.

Research Aim: Evaluate biochemical composition and structure of human donor ankle articular cartilage at varying states of degeneration using histology combined with FTIR-I.

METHODS

Sample Preparation

- 14 articular cartilage explants from 12 human tali.
  - Donors provided by the Gift of Hope Organ & Tissue Donor Network.
  - Collins Grade- [G0, n=4; G1, n=2; G2, n=5; G3, n=1; G4, n=2].
- Cartilage explants were fixed in 10% formalin solution, embedded in paraffin, cut into 6 µm.
- Histology: Samples were stained using Safranin-O/Fast Green & scored using a Modified Mankin Score[5].
- FTIR-I: Performed in transmission mode, with a 4 cm^{-1} spectral resolution, at a spectral range of 3750-950 cm^{-1} (Agilent Cary 670/620 system, 128-by-128 MCT focal array detector).

Data Analysis

- Univariate (TAB. 1, FIG. 1) analyses were applied to the spectra data to generate chemical maps (FIG. 2A)
- K-means clustering (KMC) was applied to reconstruct the image domain into a pseudo-color-coded cluster images (FIG. 2B) to reveal areas with differences in chemical structures within each image

RESULTS

- Carbohydrate content (representing proteoglycan) measured using FTIR-I was found to correlate with Safranin-O staining. Both staining and FTIR-I data demonstrated a loss of the hierarchal layered structure with increasing severity of cartilage degeneration.
- Type II collagen, represented by the areas under the amide I and amide II peaks, was found to be highly abundant across all samples.
  - FTIR-I revealed that samples with little to no degeneration (low Mankin score) exhibited relatively uniform collagen content through the depth of the tissue.
  - With increasing degeneration, the chemical map for type II collagen was less structured.
- For samples with low Mankin Scores, KMC analysis revealed three distinct areas with different chemical structure, correlating to areas of high proteoglycan, collagen, and cellular content.

DISCUSSION

- FTIR-I is sensitive enough to detect subtle variations in biochemical constituents that may not be as identifiable with Safranin-O staining.
- FTIR-I adds assessment opportunities of collagen and extracellular matrix components other than PGs.
- KMC analysis was useful for identification of unique structures based on biochemical makeup, which seem to correlate with various features that are visible in the univariate assessments for PG and collagen.
- **Limitations:** More work is necessary to determine meaningful patterns within clusters generated by KMC, especially in the case of degenerative tissues. Furthermore, some specimens may have resulted in clusters based on only nuanced differences.
- **Future Work:** 1) Implementation of additional multivariate analysis that utilize dimensionality reduction to better understand changes occurring with cartilage degeneration. 2) This work will be expanded to determine predictive spectral biomarkers of early cartilage degeneration.

It is well known that OA progression involves biochemical and structural changes to the tissue. This work highlights the use of an advanced high throughput characterization method to measure biochemical changes in articular cartilage due to degeneration.

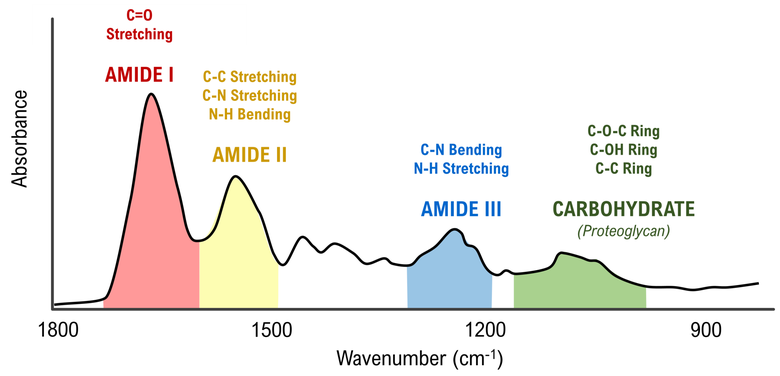

FIGURE 1: Example of spectra obtained for articular cartilage. Areas under the peak represent the content of specific biochemical constituents.

TABLE 1: FTIR univariate assessments

Parameter	Assignment	Wavenumber
Carbohydrate Peak	Proteoglycan Content	975-1140 cm^{-1}
Amide I Peak	Collagen Content	1580-1720 cm^{-1}
Amide II Peak	Collagen Content	1490-1580 cm^{-1}
$\frac{\text{Amide I}}{\text{Amide II}}$	Collagen Orientation	$\frac{1580-1720}{1490-1580}$ cm^{-1}
$\frac{\text{CH2}}{\text{Amide II}}$	Collagen Integrity	$\frac{1338}{1490-1580}$ cm^{-1}

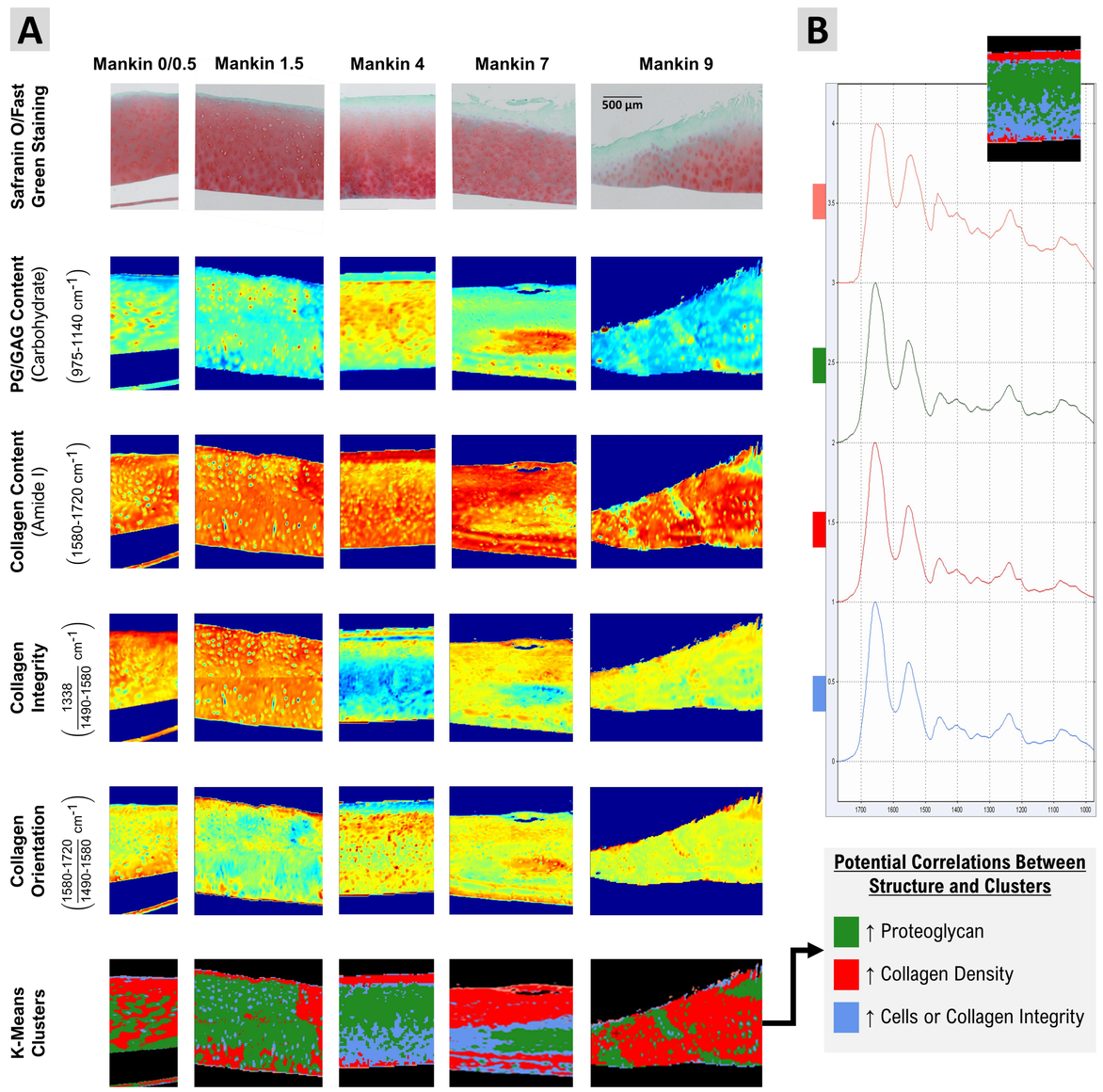

FIGURE 2: A) Histological and FTIR-I results from representative samples at different levels of degeneration, including Safranin O, Univariate Heat Maps, and the resulting KMC Map. Note: Samples are not normalized- therefore, scales between samples are not consistent and should not be used for comparison. **B)** Representative average spectra taken from KMC analysis.

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