Revealing the Hidden Depths of Articular Cartilage Damage with Spatially Offset Raman Spectroscopy

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Introduction

Current imaging modalities for detecting osteoarthritis (OA) such as X-ray and MRI lack molecular specificity and primarily detect late-stage OA. Raman spectroscopy is a non-invasive, label-free spectroscopic technique that can address the need for molecular specificity in cartilage assessment and OA diagnostics. Recently, the application of Raman spectroscopy in the investigation of OA has gained significant attention[1,2] and been used to analyze various joint tissues such as articular cartilage, synovium, and bone[3]. However, current methods for Raman spectroscopy in cartilage assessment are limited in two critical aspects: lower penetration depth and the lack of direct correlation between spectroscopic values and damage gradation. Given that GAG depletion during osteoarthritis (OA) can occur in a depth dependent manner, a depth-based assessment of GAG is crucial to comprehensively evaluate cartilage health. By offsetting the illumination and collection points spatially, SORS has shown the ability to selectively probe biochemical information from different depths within bones and soft tissues[4]. In this study, we demonstrate the first utilization of spatially offset Raman spectroscopy (SORS) for detecting articular cartilage degeneration.

Methods

Bovine femoral condylar cartilage was obtained and then subjected to trypsin treatment for one of four durations: 0 hours, 3 hours, 6 hours, or 9 hours. The treatment was applied to the top surface of the cartilage to ensure depth dependent degradation. To assess the depth-dependent changes, SORS measurements were performed. This approach requires the use of two probes set at specific spatial offsets of 0 mm, 4 mm, 8 mm, and 12 mm (Fig 1a). Each spatial offset corresponds to a different penetration depth of the measurement (Fig 1b). To quantify GAG content within each depth of the cartilage, Raman spectra were analyzed by identifying the 1080 cm^{-1} peak[5] (Fig 1a). After completing the Raman spectral acquisition, the samples underwent unconfined compression testing with a series of five 5% strain steps until 25% compressive strain was reached. Prior to mechanical testing, cylindrical samples were created with a 6 mm diameter biopsy punch and height of approximately 4 mm. After mechanical testing, Safranin-O/Fast Green histology was performed to qualitatively assess GAG content in each sample.

Results

This study shows a direct correspondence between the reduction in GAG signal measured by Raman spectroscopy and the Young's modulus measured through mechanical testing. First, by increasing the distance between the two probes during the SOR measurements, depth dependent changes in GAG content can be identified (Fig 1d). Specifically probe offsets of 0mm, 4mm, 8mm, and 12mm, correspond to a distribution of biochemical information with the most information from depths of 1mm, 2mm, 3 mm and 5 mm respectively (Fig 1b). Histology images confirmed the depth dependent increase in GAG depletion to depths below 2 mm, 4 mm, and 5 mm at 3 hours, 6 hours, and 9 hours of trypsin treatment, respectively. Then, global measures of young's modulus show increasing trypsin treatment exponentially decreases the young's modulus of the tissue (Fig 1c). Finally, a strong correlation between the on-axis Raman measurement and the logarithm of Young's modulus exists, with a correlation coefficient of 0.93 and 0.9 for on-axis and spatial offset 4 mm measurements (Fig 1e).

Discussion

This study demonstrates the potential of the nondestructive SORS in capturing depth dependent molecular changes that occur early in the pathogenesis of osteoarthritis (OA). For spatial offsets of 8 mm and 12 mm, where a substantial portion of the signal originated from deeper layers, the correlation between global mechanics and GAG signal was weaker. Future work could measure depth dependent mechanical properties of the articular cartilage[6] and correlate these properties with depth dependent SORS measurements. This current work builds on existing spectroscopy analysis of articular cartilage[1] by establishing the potential to probe biochemical changes that occur deep in the cartilage. Future work could use this technique to track sequential changes in osteoarthritis progression at the cartilage surface, cartilage middle zone, and the transition region between the cartilage and bone. This sequential tracking in human patients or animal models could further our understanding of patient specific OA progression and treatment options.

Significance/Clinical Relevance

By nondestructively and accurately measuring depth dependent cartilage biochemical changes and providing molecular-specific information, SORS offers new opportunities for advancing understanding, increasing early detection, and improved monitoring of OA progression.

References: [1] Kroupa et al. Journal of Orthopaedic Research (2021). [2] Bergholt et al. ACS Central Science (2016). [3] Hosu et al. Lasers Med. Sci. (2019). [4] Mosca et al. Nat. Rev. Methods Primer (2021). [5] Pezzotti et al. Materials Today Bio (2022). [6] Klien et al. Journal of Biomechanics (2007).

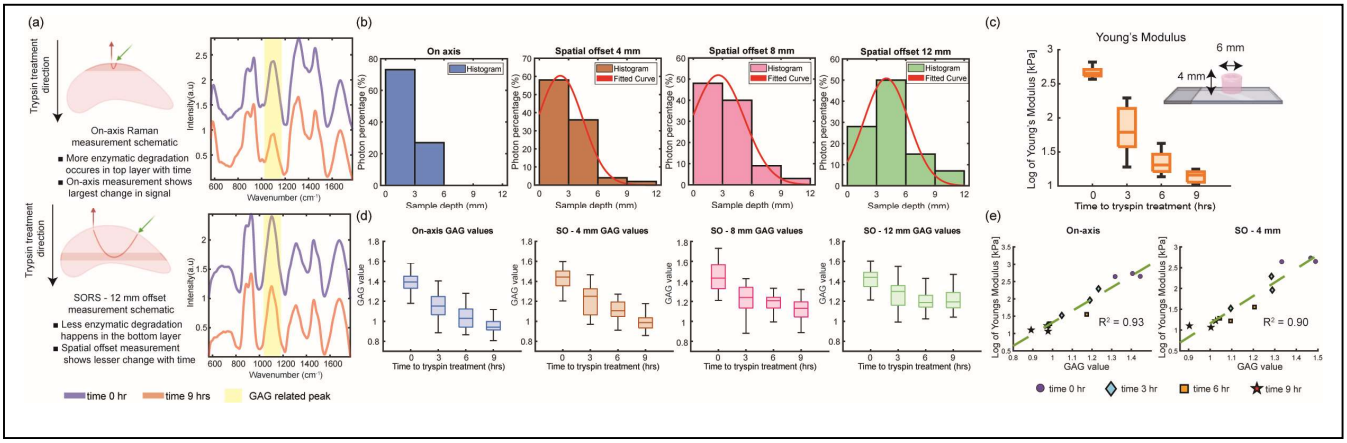

Figure 1: (a) Figure explaining the reason for differential GAG signal at different spatial offset Raman measurement at different timepoint in SORS along with the corresponding mean Raman spectra. (b) Photon distribution vs. sample depth plot for different spatial offsets. (c) Log of cartilage Young's modulus at different trypsin treatment timepoint (N = 6-8). (d) GAG value for different spatial offset at different trypsin treatment timepoint highlighting that more degradation is occurring in the top layer and less degradation happens in the bottom layer (N = 3). (e) Correlation between GAG values measured with Raman spectroscopic measurements and log of Young's modulus (kPa) measured through compression testing for on-axis, and a spatial offset of 4 mm.