Non-Destructive Spatial Mapping of GAG Loss in Articular Cartilage Using Confocal Raman Spectroscopy

Tianyu Gao[1], Alexander Boys[1], Lara A. Estroff[1], Lawrence J. Bonassar[1]
[1]Cornell University, Ithaca, NY
tg365@cornell.edu

Disclosures: Tianyu Gao (N), Alexander Boys (N), L. A. Estroff (N), L. J. Bonassar (N)

INTRODUCTION: Decrease of glycosaminoglycans (GAGs) in cartilage is a common symptom in the early stages of several degenerative diseases, like osteoarthritis (OA). Raman spectroscopy is a non-destructive technique that can resolve the local biochemical compositions in articular cartilage[1,2]. The analysis could be applied while cartilage is still hydrated and cells are alive. Previous studies have shown that compositional change in native cartilage can be detected and could also quantitatively relate to the local concentration of extracellular matrix components[3]. However, tracking GAG loss using Raman spectroscopy has not been reported. In this study, we show a method to track proteoglycan reduction in cartilage by confocal Raman mapping and validate it by comparing Raman maps to histology images.

METHODS: Cartilage samples (5mm in diameter) were harvested from 1-3-day-old neonatal bovine tibial plateaus. The fully digested group was soaked in 0.25% trypsin and incubated for 2 hours[4]. In another group, the samples were soaked in trypsin with various concentrations (0.25%, diluted by 1, 250, and 400 times) and incubated for 0.5 hours at 37°C. The samples were then rinsed by PBS at 4°C for 1 hour. Each sample was identically cut into 2 pieces. One half was directly fixed, and the other half was saved for Raman analysis. The bottom of Raman samples was embedded into agarose gel to prevent movement. Samples were mounted in the confocal Raman microscope to enable measurements along the cut face of the sample (cross-section scan) or from the articular surface, where depth-dependent information was gathered by changing the working distance from the articular surface (depth scan). All the samples were submerged in PBS (phosphate buffered saline) during data collection. Confocal Raman spectra were collected using a 532nm laser with a 62mW power. Spectra were normalized to the maximum intensity of their -OH stretching peaks (2850-3750 cm^{-1}), then cropped, baselined and fitted by 3 reference spectra (Fig. A). CS fitting coefficient maps were generated via WITec Project 5 software. Fixed samples were soaked into formalin then 70% alcohol, both for 24 hours. Samples were decalcified, sectioned, and stained with Alcian blue to assess proteoglycan distribution.

RESULTS: The histology image for native cartilage (Fig. B) demonstrates a relatively low GAG level at the surface, with a higher GAG concentration at the depth, which is consistent with previous publications[5]. The Raman map of chondroitin sulfate (CS) coefficients on the same sample (Fig. C) shows similar results. Both histology and Raman results for fully digested cartilage (Fig. D-E) indicate there is extremely low GAG concentration throughout the tissue. 250x diluted trypsin treated sample shows a narrow region at the edge with low GAG composition, demonstrated both in the Raman map and the histology image (Fig. F-G). Linear scans from cross section (Fig. H) show a higher GAG concentration in deep zone of native cartilage and demonstrate low proteoglycan levels for fully digested samples. Linear depth scans (Fig. I) indicate similar results. However, spectra of depth scan become noisier below 300μm and could not be used for reference fit. Depth mapping of 400x diluted trypsin treated sample (Fig. J) shows a region about 100μm with low CS composition at the top and the side edge of cartilage samples. Similar patterns were observed from the cross-section maps (Fig. K).

DISCUSSION: This study shows that proteoglycan reduction can be measured by confocal Raman imaging. High-resolution images of GAG contents recapitulate distribution seen in histology. We demonstrate that depth scans taken from the articular surface allow mapping the biochemical composition within 300μm, enabling subsurface detection of GAG content depletion. This study used trypsin digested bovine cartilage as a model for GAG loss. Chemical signatures for actual OA could be more complex. Future work includes Raman scans on clinical canine cartilage with osteoarthritis.

SIGNIFICANCE: This study demonstrates the non-destructive measurement of GAG reduction in cartilage after trypsin treatment. Also, it allows detection of subsurface compositional changes by scanning at the articular surface. Combined, we are well-positioned to develop *in vivo* imaging for osteoarthritis.

REFERENCES: [1] Gamsjaegar+ *J Raman Spectr* 2014 [2] Akiva+ *JACS* 2016 [3] Bergholt+ *Biomaterials* 2017 [4] Bonassar+ *Arthritis Rheum.* 1995 [5] Silverberg+ *Biophys J* 2014

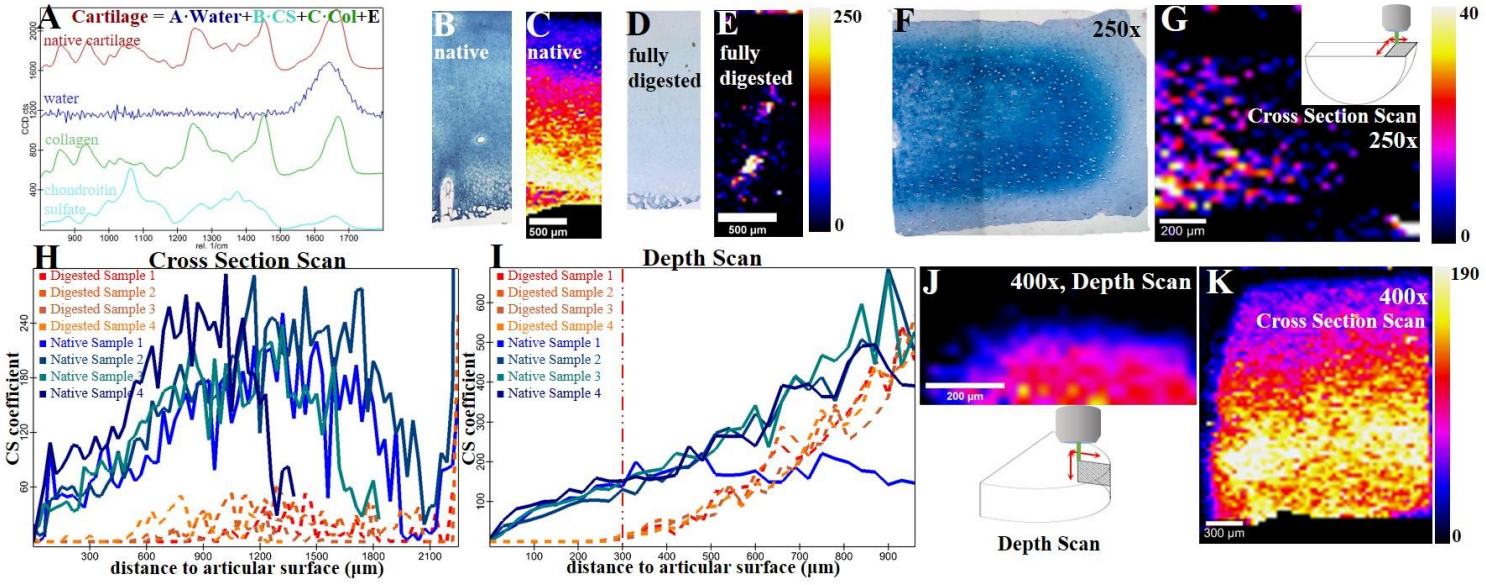

Figure 1. A) Raman spectra at the fingerprint area (800-1800 cm^{-1}) for native cartilage and three references. B) Histology image for native cartilage. C) CS coefficients map for the same area of native cartilage. D) Histology image for digested cartilage. E) CS coefficients map for the same area of digested cartilage. F) Histology image for cartilage digested by 250x diluted trypsin. G) CS coefficients map for the same area of 250x diluted trypsin treated sample. H) CS coefficients distribution for 4 native cartilage samples and 4 fully digested samples, scanned from cross section. I) CS coefficients distribution for 4 native cartilage samples and 4 fully digested samples, from depth scans. J) CS coefficients map for 400x diluted trypsin treated sample, from a depth scan. K) CS coefficients map for the same 400x diluted trypsin treated sample, from a cross-section scan.