Non-Destructive Raman Spectroscopic Serial Monitoring of Cartilage ECM in Response to Disease Modifying OA Drugs

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INTRODUCTION: Articular cartilage extracellular matrix (ECM) is comprised of a robust collagen (COL) fibril network intermeshed with an anionic, glycosaminoglycan (GAG) matrix that retains interstitial water, bestowing the rheological & tribological properties essential to cartilage function. Osteoarthritis (OA) disease progression is characterized by initial GAG loss, reducing tissue stiffness, followed by COL network breakdown and tissue swelling. Chondroprotective and chondroregenerative disease-modifying OA drugs (DMOADs) that mitigate or reverse of OA progression are emerging, but require *in vitro* longitudinal efficacy assessments that include histological, biochemical and mechanical assays to measure the structure, composition, and material properties of cartilage explants, which are laborious, time intensive, and destroy the specimen in a manner incompatible with tissue culture workflows, as well as limit serial repeated measure assessments of the same sample. Furthermore, these efficacy assessments are limited by a lack of standardized criteria and defined outcomes that are clinically relevant *in-vivo* [1]. Raman spectroscopy is an inelastic light scattering technique that reflects the vibrational modes of the biochemical building blocks (amides, sulfates, hydroxyls) of key cartilage constituents: GAG, COL, H_2O. We have developed a contact-free Raman spectral analysis probe to evaluate ECM-specific biomarkers reflective of cartilage composition *in-vitro* for health and disease models [2] using Raman-compatible tissue culture chambers capable of long-term cartilage explant viability. This *in vitro* Raman platform enables non-destructive, real-time, repeated measures of live explant ECM composition in response to mechanical insults, inflammatory cytokines and DMOAD therapies. Here, we validate the ability of our tissue-culture-compatible Raman platform to perform longitudinal, repeated-measures of bulk tissue ECM and site-specific ECM heterogeneities in response to interleukin-1α (IL-1α) and insulin-like growth factor 1 (IGF-1) treatments.

METHODS: *Live Cartilage culture*: Cartilage explants (∅5×3.5mm) were sterilely procured from immature bovine femoral condyles. Explants were cultured in the commercial 4-well plates outfitted with Raman-transparent MgF_2 windows at the bottom of each well. (Fig.1A-B) for 10 days, immersed in untreated culture medium (CTRL) or subjected to 10ng/ml IL-1α ± 100ng/ml IGF-1 treatment (n=3 per group). After flipping the explants such that the Raman probe was directed at the superficial zone, Raman cartilage spectra (30s integration time) were acquired daily through the MgF_2 window using a translating x-y stage to assess the entire explant. Additional site-specific spectra were acquired @ day 8 at the center and periphery of an IL-1α treated sample to examine spatial heterogeneity. *Endpoint analysis*: For comparison to conventional biochemistry, another set of explants (∅3×2mm) were cultured in untreated culture medium and in IL-1α ± IGF-1 (n=20), with explants removed at day 4, 8, 10 for endpoint Raman assessments, biochemical analysis and cell viability assessments. *Raman spectral analysis*: Raman acquisitions were performed using a contact-free probe (RIP-RPB, OceanOptics) consisting of an NIR diode laser (ex=785nm, 84mW), fiber-coupled spectrograph (QEPro, OceanOptics). Multivariate linear regression of the Raman cartilage spectra fingerprint region (800-1800cm^{-1}) was fit to the model: $\mathbf{Cartilage_{spectra}} = GAG_{score}*(\mathbf{GAG_{REF}})+COL_{score}*(\mathbf{COL_{REF}})+H_2O_{score}*(\mathbf{H_2O_{REF}})$, where $\mathbf{GAG_{REF}}$, $\mathbf{COL_{REF}}$ and $\mathbf{H_2O_{REF}}$, are the component spectra of purified reference chemicals and "scores" are regression coefficients reflecting the relative contribution of each constituent to the composite spectra (Fig.1C) [2]. Additional O-H and C-H bond associated biomarkers (OH_{area}, CH_{area}) were extracted from the Raman high wave number region (2800-3800cm^{-1}) [4]. GAG, COL and H2O % weight/volume were calculated from the Raman scores using a data set of ECM phantoms of known chemical composition (Fig. 1D) [3]. *Histological and biochemical assays*: Explants removed at preset endpoints were analyzed for GAG (DMMB) and H_2O (gravimetric) contents. Live/Dead staining was performed on explants cultured in the Raman-compatible chambers.

RESULTS: *Serial Live tissue monitoring of cartilage ECM using Raman:* IL-1α induced ~61% depletion of cartilage GAG over 10 days of tissue culture (Fig.2A). Cartilage treated with IGF-1 partly mitigated (~40%) the IL-1α induced GAG depletion; CTRL cartilage remained unchanged (Fig.2A). Corresponding to GAG depletion, groups subjected to IL-1α displayed increased H_2O content (↑porosity) over time (Fig. 2C). Among treatment groups, COL content changed little over time (Fig.2B). At day 10, GAG content was significantly greater for CTRL explants compared to explants exposed to IL-1α ± IGF-1 ($p<0.01$; Fig. 2D), however IGF-1 treatment mitigated the increase in H_2O content compared no treatment (Fig.2E-F). *Site specific measures*: At day 8, explants exposed to IL-1α exhibited higher GAG content and lower H_2O content at the explant center, relative to the periphery, consistent with spatial transport-limited GAG diffusion out of the explant and increased surface area exposure to IL-1α along the explant periphery (Fig.3A-B). *Biochemical validation*: Raman-derived GAG and H_2O content accounted for 82% and 64% of the variability in assay-measured GAG and H_2O content, respectively ($p<0.01$, Fig.2D). Cells remained viable during long-term culture in the Raman-compatible chambers.

DISCUSSION: This work demonstrates that non-invasive, non-destructive Raman spectroscopy can serially monitor changes in ECM of live cartilage explants in response to inflammatory cytokines and DMOAD therapies. The phantom-corrected Raman-derived GAG and H_2O content accounted for 82% and 64% of the variability in assay-measured GAG and H_2O content, respectively. Serial assessment of these biomarkers revealed significant GAG loss and tissue porosity increase (↑H_2O content) after exposure to IL-1α, that was only partly mitigated by IGF-1. In addition to bulk tissue assessments, the Raman platform could examine spatial gradients in ECM composition allowing analysis and modeling of the transport of IL-1α into the tissue to induce chondrocytes to secrete catabolic enzymes and GAG out of the sample during tissue degradation, as well as the transport of IGF-1 to upregulate ECM production by chondrocytes.

SIGNIFICANCE: This *in vitro* study demonstrates that tissue culture-compatible Raman spectroscopy can non-destructively, non-invasively serially monitor bulk tissue and spatially dependent changes in the ECM of live cartilage explants in response to inflammatory cytokines and DMOAD therapies requisite for preclinical efficacy assessments.

REFERENCES: [1] Kim+ 2022 TAMD; 14. [2] Kroupa+ 2021 JOR; 40:1338-48. [3] Ersland+ 2023 SB^{3}C. [4] Unal+ 2018 Osteoarthr. Cartil.; 27:304-13.

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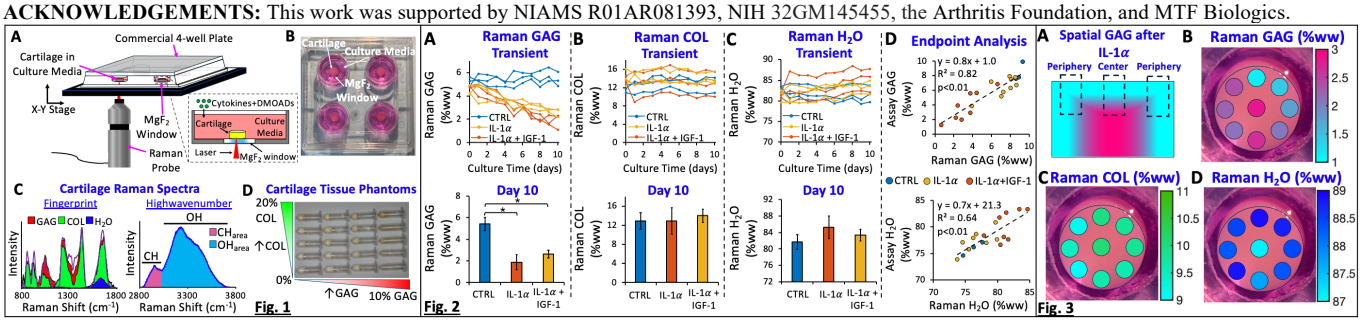

Fig 1: (A) Schematic of live tissue Raman spectral acquisition. **(B)** Cartilage cultured in Raman-compatible tissue culture chambers. **(C)** Representative cartilage Raman spectra and extracted ECM biomarkers. **(D)** Cartilage ECM phantoms of known concentrations for Raman-derived tissue contents. **Fig 2**: **(A-C)** Live cartilage Raman-derived GAG, COL and H_2O content changes over time and at Day 10 for each treatment group, *$p<0.01$. **(D)** Explant Raman-derived GAG and H_2O content vs assay measured GAG and H_2O content. **Fig 3**: **(A)** Schematic of Raman acquisition regions of interest on spatially GAG-depleted explants. Lower GAG is expected for peripheral sites. **(B-D)** Site-specific GAG, COL and H_2O content heterogeneities of IL-1α treated cartilage at Day 8.