# Raman Spectroscopic Needle Probe Provides Real-time Biomarkers of Temporomandibular Joint Cartilage Composition Predictive of Tissue Function

**Jacob J. Giering[1], Erik E. Ersland[1], Javier Garcia[1], Henry Brisebois[1], Thomas P. Schaer[2], Daniel E. Perez[3], David J. Chang[4], Mark W. Grinstaff[1], Mads S. Bergholt[5], Brian D. Snyder[6], Michael B. Albro[1]**

[1]College of Engineering, Boston University, Boston, MA, USA, [2]Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA, [3]Department of Oral and Maxillofacial Surgery, University of Texas Health Science Center, San Antonio, TX, USA, [4]Department of Oral and Maxillofacial Surgery, Tufts Medical Center, Boston, MA, USA, [5]Centre for Craniofacial and Regenerative Biology, King's College London, London, UK, [6]Department of Orthopaedic Surgery, Boston Children's Hospital, Boston, MA, USA.

**Disclosures:** None

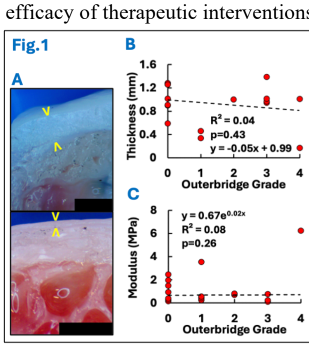
**INTRODUCTION:** The temporomandibular joint (TMJ) is a specialized diarthrodial joint that permits both hinge and sliding movements of the mandible. Its biomechanical function depends on a smooth gliding surface provided by articular cartilage and a fibrocartilaginous disc that distributes compression and shear loads during mastication [1]. The extracellular matrix (ECM) of TMJ cartilage is composed of anionic glycosaminoglycans (GAGs), which impart compressive load support through interstitial fluid retention, and a fibrillar collagen network (COL), which imparts tensile strength and structural integrity. Analogous to the knee, trauma, internal derangement, joint instability, ligamentous deficiency, skeletal malalignment incites mechanical overloading → injury → osteoarthritis of the TMJ (TMJ-OA) that afflicts 8%–16% of the US population. Characterized by degradation of the condylar cartilage and disc, loss of tissue ECM and progressive tissue erosion compromises tissue lubricity and load support, leading to pain and loss of function. TMJ diagnostics rely on visual arthroscopic grading schemes (Outerbridge [OB] scoring) that reflect macroscopic changes in tissue morphology, but do not provide insight into changes in tissue composition governing the material properties of the tissues fundamental to TMJ performance [2]. As such, a diagnostic modality capable of quantitatively assessing cartilage composition *in vivo* would address a critical gap in TMJ disease management. 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 developed a needle probe for Raman spectral analysis that measures ECM-specific biomarkers indicative of changes in cartilage composition responsible for the tissue's material properties [3,4]. In this study, we evaluate the capability of a needle-based arthroscopic Raman probe to quantify cartilage thickness and measure the relative biochemical composition of explanted TMJ condylar cartilage predictive of tissue mechanical function. To establish clinical feasibility, we performed Raman probe measures of TMJ cartilage *in situ* arthroscopically on a cadaveric human jaw to evaluate Raman as a diagnostic modality to "optically biopsy" the tissues comprising the TMJ to aide decision-making whether to repair or replace the TMJ.

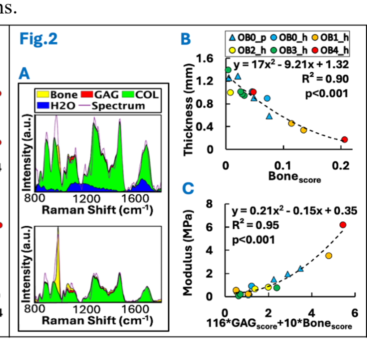
**METHODS:** Discarded human TMJ condyles (n=12) procured during arthroplasty, exhibited a range of degenerative changes as portrayed by OB grade: OB0 (n=2), OB1 (n=4), OB2 (n=2), OB3 (n=3), and OB4 (n=1). Five porcine TMJ condyles without visible degeneration (OB0) were procured to assess healthy tissues. For each condyle, Raman spectra were collected from the chondral surface using a handheld arthroscopic probe optimized for intra-articular deployment, consisting of an NIR diode laser (ex=785nm, 500mW), fiber-coupled spectrograph (Ibsen), and needle tips (∅1mm and 2mm sapphire ball lenses). Spectral analysis was performed using a multivariate linear regression approach, where $\mathbf{Cart_{spectra}}$, the cartilage composite fingerprint spectrum (800-1800cm$^{-1}$), was modeled as a linear combination of reference spectra for ECM constituents GAG, COL, $H_2O$ and subchondral bone by the model: $\mathbf{Cart_{spectra}}=GAG_{score}*(\mathbf{GAG_{REF}})+COL_{score}*(\mathbf{COL_{REF}})+H_2O_{score}*(\mathbf{H_2O_{REF}})+Bone_{score}*(\mathbf{Bone_{REF}})$, where $\mathbf{GAG_{REF}}$, $\mathbf{COL_{REF}}$, $\mathbf{H_2O_{REF}}$, and $\mathbf{Bone_{REF}}$ are the component spectra of purified reference chemicals for each constituent. The regression-derived "scores" portray the relative contributions of GAG, collagen, water, and bone to the TMJ condyle cartilage Raman spectra for each sample. To evaluate mechanical function, indentation testing was performed to determine cartilage elastic modulus. Tissue thickness was measured from cross-sections under a dissecting microscope (Fig.1A). In addition, Raman spectra were acquired arthroscopically on a cadaveric human condyle and disc *in situ* to evaluate feasibility and spectral fidelity in the surgical environment.

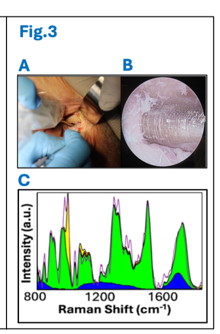
**RESULTS:** Macroscopic OB grade did not correlate with either elastic modulus ($R^2$=0.08; Fig.1C) or cartilage thickness ($R^2$=0.04; Fig.1B), underscoring the need for a non-destructive diagnostic to measure tissue composition and structure that account for tissue function. The multivariate regression model portraying the condylar cartilage composite fingerprint spectrum accounted for 88±3% of the variation in Raman spectra (Fig.2A). Raman-derived compositional biomarkers predicted cartilage structure, composition, and function: $Bone_{score}$ accounted for 90% of the variation in thickness of the chondral layer ($R^2$ = 0.90; Fig.2B); a linear combination of $GAG_{score}$ and $Bone_{scores}$ accounted for 95% of the variation in modulus ($R^2$ = 0.95; Fig.2C). These relationships were applicable for both the human degenerated TMJ and healthy porcine TMJ samples. Furthermore, Raman spectra collected arthroscopically from the cadaveric TMJ condyle and disc exhibited comparable quality to spectra acquired *ex vivo* from excised samples (Fig.3). This demonstrates the technical feasibility of using our Raman needle probe during arthroscopic procedures, with no significant loss of signal fidelity or diagnostic capability.

**DISCUSSION:** We demonstrate the clinical feasibility of using Raman spectroscopy as a diagnostic modality to derive compositional biomarkers that portray cartilage material properties. Raman-derived bone score accounted for 90% of the variation of cartilage thickness and Raman-derived biomarkers accounted for 95% of the variation in tissue modulus, reflecting changes in tissue structure and composition critical to the health and function of the TMJ. The ability to obtain high-quality, real-time Raman spectra i*n situ* through an arthroscopic portal establishes the feasibility of using the needle probe as a diagnostic adjunct during TMJ arthroscopy that can be readily integrated into surgical workflows, enabling quantitative assessment of TMJ health. Beyond diagnostics, Raman spectroscopy can serve as a valuable tool for monitoring responses to disease-modifying osteoarthritis drugs and regenerative therapies in clinical trials.

**SIGNIFICANCE:** This study highlights the limitations of conventional arthroscopic grading schemes such as Outerbridge, which only reflect gross surface morphology but fail to characterize cartilage composition and thickness critical to joint function. These results support the use of Raman spectroscopy as a minimally invasive adjunct to TMJ arthroscopy to provide real-time quantitative assessment of TMJ cartilage composition and structure essential to joint function, facilitating clinical decision-making whether to replace the TMJ and to evaluate tissue response to chondroprotective and/or chondroregenerative strategies. Real time Raman spectroscopy can transform clinical practice by enabling objective diagnosis, monitoring disease progression, and evaluating the efficacy of therapeutic interventions.




**ACKNOWLEDGEMENT**: This work was supported by NIH R01AR081393.

**REFERENCES:** [1] Lee YH 2024 J Korean Dent Sci. 17(1):14-35. [2] Martin-Granizo R+ 2018 J Craniomaxillofac Surg (46):82-89. [3] Kroupa KR+ 2021 JOR. [4] Kazemi et. al. 2023 ORS Ann. Mtg (#339).

**Fig.1**: **(A)** Representative cross sections of TMJ condyle explants. Yellow carrots indicate boundary of chondral layer. Black scale bars = 1mm. **(B)** Outerbridge grade vs tissue thickness. **(C)** Outerbridge grade vs elastic modulus. **Fig.2: (A)** Representative Raman stacked area plots depicting cumulative contribution of GAG, COL, $H_2O$, and bone to tissue spectra for thick (top) and thin (bottom) chondral layers. **(B)** Multivariate polynomial regression between $Bone_{score}$ vs cartilage thickness. Subscript 'p' denotes porcine, 'h' denotes human. **(C)** Multivariate polynomial regression between a linear combination of Raman biomarkers ($GAG_{score}$ and $Bone_{score}$) vs elastic modulus. **Fig.3: (A)** Raman probe entry portal into TMJ during arthroscopy in human cadaver. **(B)** Arthroscopic Raman probe in contact with TMJ articular disk. **(C)** Representative Raman stacked area plot depicting cumulative contribution of GAG, COL, $H_2O$, and bone to tissue spectra of TMJ retrodiscal tissue obtained arthroscopically.