

Microscale Structure and Composition Predict Depth Dependent Shear Modulus of Temporomandibular Joint Condylar Cartilage

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INTRODUCTION: Temporomandibular joint condylar cartilage (TMJ) is a unique tissue that withstands repetitive daily loads over the course of decades.^{1,5} Despite containing four different layers, (fibrous surface, proliferative, mature and hypertrophic)⁵, the mechanics of the TMJ cartilage are not fully understood and therefore is in need for further investigation. Previous studies show the depth-dependent shear modulus mechanics of porcine TMJ cartilage have shown modulus differences depending on anatomic locations^{2,7}. To further investigate this theme, we used mature TMJ cartilage from canines, which is a species known to develop disease and degeneration. Notably, this spatial variation in structure and composition of canine TMJ cartilage has not yet been quantified. The aims of this work were (i) investigate the depth dependent shear modulus between anatomic locations in canine TMJ cartilage as well as (ii) analyze the relationship between the depth dependent mechanical properties and composition.

METHODS: Canine TMJ condyles were obtained from Cornell College of Veterinary Medicine. Full thickness cartilage explants (4mm diameter) were obtained from two anatomic locations of the condyle (Central lateral (CL) and Central medial (CM)). Samples were bisected in the posteroanterior direction into hemicylinders and were mechanically tested using a previously established method³ using confocal elastography. Videos obtained were analyzed using FFT with a custom MATLAB code where orientation index (OI) which represent the relative strength of fiber orientation and the angle of orientation of the fibers were calculated by moving a region of interest per pixel over a horizontal sliding window through the depth of the tissue on the image plane⁴. Furthermore, depth dependent intensity which is proportional to relative matrix density⁶ were obtained from 5-(4,6-dichlorotriazinyl)aminofluorescein (5-DTAF) stained images from ImageJ. For statistical analysis, a two-way ANOVA was performed to analyze differences in anatomic locations and Pearson's correlation was used to analyze between shear modulus, DTAF staining intensity for relative protein concentration, orientation index of the collagen fiber organization and angle of orientation in R studio.

RESULTS: The confocal micrograph of DTAF stained samples show progressive non-linear increase in intensity throughout the depth of the canine TMJ cartilage. The measured shear modulus shows a non-linear relationship through the tissue depth. Furthermore, there was no statistically significant difference between the CL (n=6) and CM (n=4) anatomic regions from a two-way ANOVA (p-value = 0.649). The depth dependent intensity taken showed a non-linearity that resembles the depth dependent shear modulus. OI and angle of orientation obtained from FFT show that the fibrous zone (depth ~25%) is tangentially aligned (angle closer to 180°) with a stronger strength (high OI > 1.3) as represented in Figure 2. Relationship between shear modulus and intensity was fit to $y = 1325.2 \exp(0.0555x)$ with $R = 0.907$ on a log-linear scale and had a linear correlation with $R = 0.838$, p-value = 7.41×10^{-6} ($y = 3.96 \times 10^3 x - 1.79 \times 10^5$). Shear modulus and angle did not show any significant correlation. However, shear modulus and OI was fit to $y = 3 \times 10^{12} \exp(-14.38x)$ with $R = 0.794$ on a log-linear scale and showed a negative linear correlation with $R = -0.680$, p-value = 0.00136 ($y = -8.25 \times 10^5 x - 1.11 \times 10^6$).

DISCUSSION: The aims of this study were to investigate the depth dependent mechanics of canine TMJ cartilage within different anatomic locations and the relationship between the structural properties and composition. There was no anatomic variation in shear modulus between CL and CM regions, which can be accounted by the explants both being located centrally of the condyle. Depth dependent intensity, a surrogate of the extracellular matrix, represents the relative protein content of the tissue. The linear variation between the intensity scales with the log scale shear modulus (Figure 3A). Interestingly, small changes in intensity lead to orders of magnitude changes in the shear modulus which is consistent with previous measurements¹. Furthermore, the weak correlation between the shear modulus and angle of orientation also corresponds to previous measurements¹. The negatively correlated relationship between the OI and shear modulus represents that at high OI the shear modulus is low, which corresponds to the tangentially aligned fibers sliding at the surface under shear.

SIGNIFICANCE: Overall results suggest that the microscale structural and compositional properties have a significant effect to the depth dependent shear modulus of the TMJ cartilage.

REFERENCES: 1. Silverberg, et al. 2014, 2. Tanaka, et al. 2014, 3. Buckley, et al. 2008, 4. Boys, et al. 2019, 5. Kuroda, et al. 2009, 6. Morikawa, et al. 2016 7. Gologorsky, et al. 2020

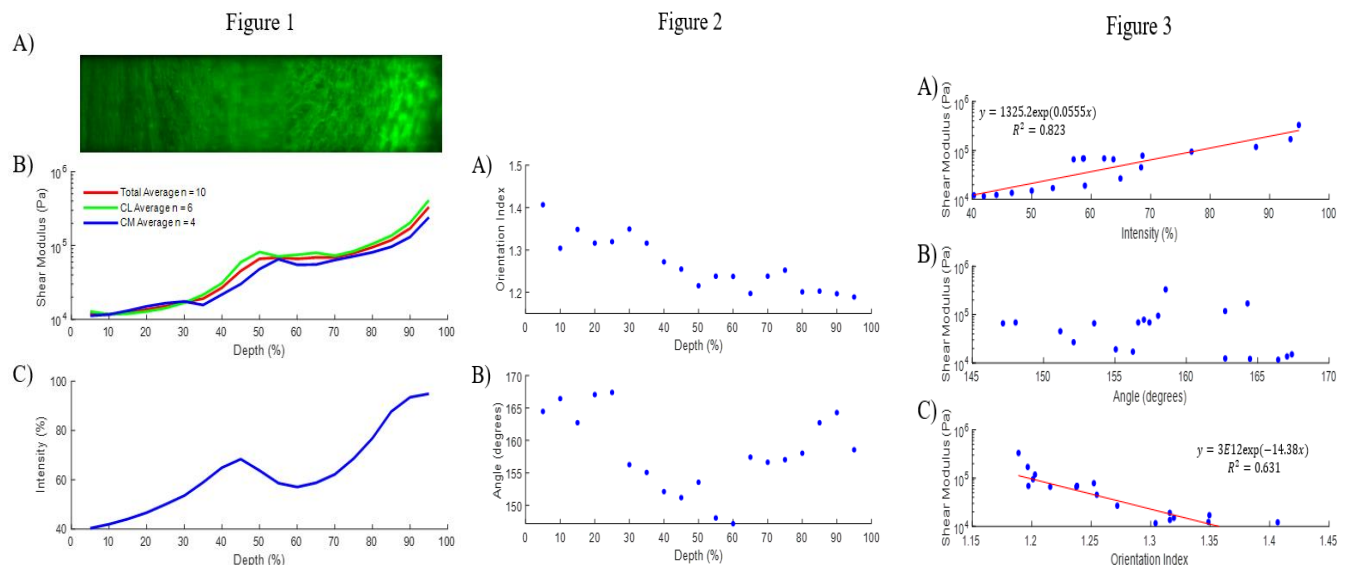


Figure 1: A) DTAF stained confocal image. B) Average depth dependent shear modulus values of different anatomic zones and their averaged value. C) Depth dependent DTAF intensity values of the cartilage. Figure 2: A) Depth dependent orientation index of the cartilage. B) Depth dependent angle of orientation of the cartilage. Figure 3: A) Correlation plot of shear modulus and intensity. B) Correlation plot of shear modulus and angle of orientation. C) Correlation plot of shear modulus and orientation index.