

Relationship between Microscale Shear Modulus, Composition, and Structure in Porcine, Canine, and Human TMJ Cartilage

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Introduction: Temporomandibular Joint (TMJ) condylar cartilage is a tissue that can withstand decades of repetitive daily loads^[1,2]. This unique tissue has a fibrous surface layer that is distinct from hyaline cartilage in other joints of the human body^[3,4]. In many models of TMJ cartilage disease and degeneration, surgery is performed on young (i.e. skeletally immature) animals, due to the ease of surgical access^[5,6,7,8]. As such, the relevance of these models to naturally occurring disease in species such as dogs^[9] and humans is unclear. Notably, TMJ cartilage has a zonal structure and associated variations in mechanics^[10]. However, the extent to which these zones and their mechanics are similar between these species and quantification of the relationship between tissue composition and structure to the local mechanics is not fully understood. Therefore, the aims of this work were to (i) investigate the zonal characteristics of the local shear mechanics and (ii) identify the quantitative relationship between tissue composition and structure to the local shear mechanics in porcine, canine, and human TMJ cartilage.

Methods: Porcine TMJ condyles (Schrader Farms), canine TMJ condyles (CARE Tissue Exchange program, Cornell College of Veterinary Medicine), and IRB approved human cadaver TMJ condyles (Weill Cornell Medicine) were obtained. All samples were observed to be macroscopically normal and free of disease. Full thickness cartilage explants (4mm diameter) were obtained from the condyles. Samples were bisected in the posteroanterior direction into hemicylinders and were mechanically tested using a previously established method using confocal elastography^[11]. Confocal micrographs obtained were post analyzed to measure depth dependent intensity which is a surrogate of the extracellular matrix (ECM) content in ImageJ^[12]. Furthermore, Fast Fourier Transform (FFT) was performed with a custom MATLAB code by moving a region of interest per pixel over a horizontal sliding window through the depth of the tissue on the image plane^[13] (**Fig. 1**). Orientation index (OI) represents the relative strength of fiber orientation, and the angle represents the orientation of the fibers where 0° is parallel to the surface. Statistical analysis was performed in RStudio using Estimated marginal means of linear trends.

Results: Local shear modulus shows a non-linear relationship through the depth of the cartilage. The relative zonal thickness between the three species of the hypertrophic zone [IV] and mature zone [III] were similar. However, human TMJ cartilage lacked the proliferative [II] and fibrous [I] zones which resulted in overall reduction in total thickness (**Fig 1A**) as well as canine tissue lacked the fibrous (I) zone. Shear modulus range ($10^5 \sim 10^7$ Pa) of the three species were similar in regions closer to the subchondral bone (Zones [IV] and [III]). Relationships between shear modulus with ECM content, orientation index, and angle were fit to a log-linear model (**Fig. 1B**). ECM content, plotted against the depth-dependent shear modulus showed a linear fit which represented that small changes in composition has magnitudes of changes in shear modulus. Furthermore, the slope of the three species compared against each other were not statistically significant except for canine and porcine ($p < 0.05$). Correlation between shear modulus and OI showed that high fiber alignment had lower shear modulus which was mainly observed in the surface regions of all three species. The slope of the three species showed no statistical difference except canine and porcine ($p < 0.05$). Correlation of shear modulus and the angle of alignment did not show any significant correlation which aligns with previous findings^[14].

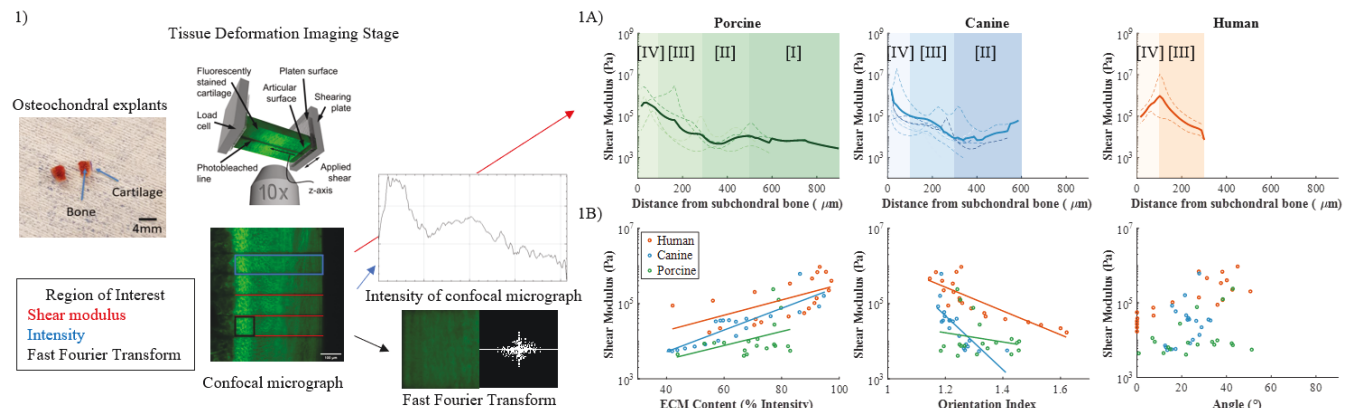


Figure 1. Schematic of experimental process of obtaining shear modulus, intensity, and OI (FFT). 1A) Zonal divided local shear modulus. Shaded regions represent tissue zones. 1B) Correlation plots of shear modulus with intensity, orientation index, and angle.

Discussion: Results suggest that porcine, canine, and human TMJ cartilage have similar zonal characteristics of the local shear mechanics. However, the relationship with the ECM content with the local shear mechanics of the tissue show that there exists a difference in elevation of magnitude between the three species. Furthermore, ECM content, which in TMJ cartilage is mainly collagen, has shown to positively correlate with shear modulus which states that composition plays a role to the underlying properties of the local shear mechanics. Elevation in shear modulus with respect to ECM content poses that human and canine tissue have higher stiffness than relatively young, skeletally immature porcine tissue. Several factors can contribute to this elevation including increase of collagen crosslinking as a result of aging^[15] and also collagen fiber thickness, which is unknown between species. However, these factors are not captured with the current analysis methods performed. Overall, this study provides insight that young (i.e. skeletally immature) porcine TMJ cartilage has lower composition related shear mechanics compared to species (canine and human) that are susceptible to naturally occurring disease and degeneration.

Significance: Porcine, canine, and human TMJ cartilage show similar local shear modulus profiles for zones closer to the subchondral bone. However, the difference seen in the correlation between composition and shear mechanics suggest that although porcine models are a widely used for TMJ research, it does not fully represent naturally occurring disease and degeneration that are clinically more relevant to canine and human tissue.

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