Nanomechanical Properties of Murine Temporomandibular Joint Cartilaginous Tissues

Basak Doyran¹, Xingyu Chen², Qing Li¹, Eiki Koyama³, Hyun-Duck Nah³, X. Lucas Lu², Lin Han¹.
¹School of Biomedical Engineering, Sciences and Health Systems, Drexel University, Philadelphia, PA, USA, ²Department of Biomedical Engineering, University of Delaware, Newark, DE, USA, ³Department of Surgery, The Children’s Hospital of Philadelphia, Philadelphia, PA, USA.

Disclosures:  B. Doyran: None. X. Chen: None. Q. Li: None. E. Koyama: None. H. Nah: None. X. Lu: None. L. Han: None.

Introduction: Temporomandibular joint (TMJ) cartilaginous tissues, including the articular condyle and disc, provide important biomechanical functions of load bearing, shock absorbing and lubrication [1-3]. Murine models were recently shown as a novel tool to investigate the developmental biology and osteoarthritis pathology of TMJ [4,5]. Current evaluation of murine TMJ cartilaginous tissues is, however, mainly based on histological, histochemical and gene expression analyses [4,5]. While these assays can detect OA-associated phenotype in mice TMJ, they do not provide a direct functional measure to the mechanical properties of TMJ tissues. Limited by its small volume and irregular shape, there is little understanding of the mechanical properties of murine TMJ tissues even in healthy, wild-type mice. Without this fundamental knowledge, it is difficult to unveil the function-related mechanical changes of TMJ tissues during OA. Recently, the advance in nanotechnology enabled direct mechanical quantification of murine cartilaginous tissues such as knee cartilage [6] and meniscus [7]. Toward this end, to provide a mechanical knowledge basis for TMJ OA murine studies, this study quantified the mechanical properties of TMJ condyle cartilage and articular disc in skeletally mature mouse using atomic force microscopy (AFM)-based nanoindentation.

Methods: TMJ articular disc and condyle head were harvested from mature 12-week old male C57BL/6J mice (n ≥ 6). Tissues were preserved in phosphate buffer saline (PBS, IS = 0.15 M, pH = 7.4) with protease inhibitors (Pierce) at 4°C for less than 24 hours before the test. Following our previously established procedures on murine knee cartilage [6], nanoindentation was performed on the central region of condyle cartilage, and both tubercle and condyle sides of the TMJ disc using a spherical tip (R ≈ 5 μm, nominal spring constant k ~ 7.4 N/m) and a Dimension Icon AFM (BrukerNano) (Fig. 1). For the disc, mechanical heterogeneity related to each of these regions (center, center to peripheral and lateral peripheral) was also quantified. For each tissue and region, indentation was carried out up to ~ 1 μm maximum depth at 10 μm/s rate for at least 10 different locations. Effective indentation modulus, $E_{ind}$, was calculated from the loading portion of force-indentation depth curves using Hertz model with correction for the finite sample thickness (~ 30 μm at the center of the disc) [8].

Results: For all the tested specimens, the mandibular condyle and tubercle side of the disc showed significantly higher $E_{ind}$ than the condyle side of the disc (n ≥ 6 via Mann-Whitney U test, Fig. 2). In addition, for both sides of the articular disc, $E_{ind}$ was found to vary significantly with the location, where the lateral peripheral region had significantly higher $E_{ind}$ than the central region (Fig. 3, Kruskal-Wallis test followed by Tukey-Kramer multiple comparison).

Discussion: This study is the first to quantify the mechanical properties of articulating joint tissues of murine TMJ. Using AFM-based nanoindentation, we observed significant variation in $E_{ind}$ of different TMJ soft tissues, where the articular condyle showed significantly higher moduli than the disc. With the
indentation depth limited to ≤ 1 µm, AFM-nanoindentation was able to detect the local heterogeneity in mechanical properties of the ~ 30 µm thick murine articular disc. The heterogeneity was found on both sides of the disc (Fig. 2) and different regions on each side (Fig. 3). Unlike the hyaline cartilage in the knee, the TMJ condyle surface is composed of type I collagen-based fibrocartilage, and proteoglycans are mostly concentrated in the middle-deep zone [9]. The $E_{\text{ind}}$ measured here could mostly reflect the mechanical properties associated with the type I collagen fibrils on condyle surface. Similarly, proteoglycan is also present at very low concentration in TMJ disc, and could also have negligible contribution to the measured $E_{\text{ind}}$. The micromechanical heterogeneity associated with these tissues are most likely associated with variations in the assembly of collagen fibril-based ECMs. Our ongoing studies are probing the nanostructure of collagen fibrils in TMJ tissues to provide structural insights into the observed mechanical heterogeneity.

While porcine TMJs are known to bear the closest structural similarity to human TMJ [10], murine model provides a unique platform to study the developmental biology and OA pathogenesis of TMJ tissues. Previous studies have shown the early onset of TMJ OA-like changes in mice lacking type II [11] or XI collagen [12], biglycan/fibromodulin [4] and/or Prg4 (lubricin) [5]. Due to the fact that the mechanical properties are directly related to the biomechanical function of TMJ tissues, and could be more sensitive to macroscopic morphological signs measured by histology, our study clearly suggested that nanoindentation could be a valuable tool to study the mechanical properties of TMJ cartilaginous tissues.

Using the mechanical properties measured on normal murine TMJ here as the benchmark, our long term goal is to establish a nanomechanical standard that can be used for early detection and functional evaluation of TMJ OA-associated symptoms in mice.

**Significance:** This study quantified the heterogeneous micromechanical properties of murine TMJ tissues for the first time. Methodology and knowledge learned here will create a new platform to study the structure-mechanics relationships of TMJ cartilaginous tissues and their mechanical degradation during OA.
Fig. 1 Typical indentation force versus depth curves at 10 µm/s on articular condyle and both sides of articular disc. Data shown are mean ± SEM of indentation curves from one sample each (≥ 10 locations), and solid lines are associated Hertzian fits.
Fig. 2 Average $E_{ind}$ of murine articular condyles and tubercle (t) /condyle (c) sides of articular discs ($n \geq 6$ mice, mean ± SEM, *: $p < 0.05$ via Mann-Whitney U test).
Fig. 3 Regional difference tubercle side and condyle side of the articular disc ($R \approx 5 \mu m$, mean ± SEM at ≥ 6 locations on one sample each, *: $p < 0.05$ via Kruskal-Wallis test followed by Tukey-Kramer multiple comparison). The trends are consistent on additional animals tested.