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Regional Variation of Bone Tissue Properties at the Human Mandibular Condyle

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Abstract

The temporomandibular joint (TMJ) bears different types of static and dynamic loading during occlusion and mastication. As such, characteristics of mandibular condylar bone tissue play an important role in determining the mechanical stability of the TMJ under the macro-level loading. Thus, the objective of this study was to examine regional variation of the elastic, plastic, and viscoelastic mechanical properties of human mandibular condylar bone tissue using nanoindentation. Cortical and trabecular bone were dissected from mandibular condyles of human cadavers (9 males, 54 to 96 years). These specimens were scanned using microcomputed tomography to obtain bone tissue mineral distribution. Then, nanoindentation was conducted on the surface of the same specimens in hydration. Plastic hardness (H) at a peak load, viscoelastic creep (Creep/ $P_{\max}$), viscosity (η), and tangent delta ($\tan \delta$) during a 30 second hold period, and elastic modulus (E) during unloading were obtained by a cycle of indentation at the same site of bone tissue. The tissue mineral and nanoindentation parameters were analyzed for the periosteal and endosteal cortex, and trabecular bone regions of the mandibular condyle. The more mineralized periosteal cortex had higher mean values of elastic modulus, plastic hardness, and

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viscosity but lower viscoelastic creep and $\tan \delta$ than the less mineralized trabecular bone of the mandibular condyle. These characteristics of bone tissue suggest that the periosteal cortex tissue may have more effective properties to resist elastic, plastic, and viscoelastic deformation under static loading, and the trabecular bone tissue to absorb and dissipate time-dependent viscoelastic loading energy at the TMJ during static occlusion and dynamic mastication.

Keywords

Human mandibular condyle; Temporomandibular joint; Nanoindentation; Viscoelastic; Regional variation

Introduction

Temporomandibular joint disorder (TMJD) is the second most commonly occurring musculoskeletal symptom, affecting up to 25% of Americans [1]. The TMJ is a synovial joint that has a fibrocartilaginous articular disc located between the cartilage of the articular eminence of the temporal bone and the mandibular condyle [2, 3]. As masticatory muscles maintain the integrity of the TMJ, the components of the TMJ are constantly compressed during static occlusion [3–5]. During mastication, muscle contractions associated with TMJ movement can provide cyclic loading in various directions on the articular surface of the joint [4, 6, 7]. More than 10% of TMJD patients have osteoarthritis, which results from erosion of articular cartilage and degeneration of the bony mandibular condyle under daily loading by static occlusion and dynamic mastication [2, 4]. This degeneration can develop when applied loads surpass the mechanical adaptive capacity of the TMJ. As such, it has been suggested that the mechanical properties of the mandibular condyles of the TMJ are the most important factors in determining TMJ osteoarthritis [2, 4, 8]. However, a lack of knowledge exists about characteristics of mandibular condylar bone tissue.

The mechanical properties of bone have been determined mainly by its elastic modulus and fracture strength using static fracture testing [9]. On the other hand, bone is a viscoelastic material in which mechanical properties change over the duration of static and dynamic loading [10–13]. As such, the viscoelastic ability of the mandibular condylar bone to absorb and dissipate the high impact energy of dynamic mastication is critical to maintain mechanical stability of the TMJ. However, few studies have been performed to investigate the viscoelastic response of mandibular bone to dynamic masticatory loading.

Bone tissue consists mainly of water, mineral and collagen components, combinations of which determine its mechanical properties [14]. Many studies indicate that tissue mineral distributions play an important role in determining bone properties at the macro level [10, 11, 15–17]. However, macro level analyses cannot determine the detailed mechanical behavior of bone at the tissue level where microcracks initiate and propagate. Previous studies demonstrated that measured elastic modulus, tissue mineralization and composition of human bone are different between anatomic sites [18, 19]. These results suggest that bone tissue properties are adaptive and vary by functional demands corresponding to specific anatomic sites.

In the current study, we hypothesized that the local mechanical properties of human mandibular condylar bone tissue are not uniform. This hypothesis was tested using nanoindentation, a technique that has been widely used to assess elastic modulus and plastic hardness of bone at the tissue level. Recently, it has also been applied to measure viscoelastic properties of bone tissue at various anatomical locations of different species [20–22]. Thus, the objective of this study was to examine regional variation of elastic, plastic, and viscoelastic mechanical properties of human mandibular condylar bone tissue using nanoindentation.

Materials and Methods

Nine fresh human male cadaveric mandibles (75 ± 15 years) were obtained from the Division of Anatomy's Body Donation Program at The Ohio State University. Only male specimens were included to avoid possible complications due to postmenopausal bone loss and compositional changes, which are common in females. There were no records of temporomandibular joint disorders (TMJD) or gross evidence of it on the specimens. All fresh mandibles were unembalmed and were stored at -21°C until utilization. After soft tissue, with the exception of articular cartilage at the TMJ, was removed from the mandibular bone surface, one condyle from each individual mandible was transversely dissected parallel to the occlusal plane using a low speed saw (Isomet, Buehler, Lake Bluff, IL) under irrigation (Fig. 1a). A total of 7 right and 2 left side condyles were randomly obtained for this study.

Microcomputed tomography

The mandibular condyle specimens were investigated using a three-dimensional (3D) micro-computed tomography (micro-CT) scanner (SkyScan 1172-D, Kontich, Belgium). Scanning and reconstruction voxel sizes were set at $27 \times 27 \times 27 \mu\text{m}^3$. The same scanning conditions were used for all specimens (70 kV, 141 μA , 0.4° rotation per projection, 8 frames averaged per projection, and 120 ms exposure time). The 3D micro-CT images were used to digitally isolate cortical bone (CB) and trabecular bone (TB) regions of the mandibular condyle following a process similar to that described previously [10]. As the first step, non-bone voxels surrounding the periosteal surface of the condylar cortex were removed using a heuristic algorithm introduced in previous studies [10, 23] (Fig. 1b). All bone and non-bone voxels in the cortex and internal trabecular regions of the condyle were maintained in order to mask the entire condylar centrum using a compartmentalizing method [10, 24]. Then, the whole mandibular condylar bone voxels were segmented from non-bone voxels using the heuristic algorithm. The internal region, and therefore trabeculae, was removed to isolate the CB. Subsequently, the TB was isolated by subtracting the CB from the segmented whole condyle. Finally, the CB was digitally separated into the periosteal CB that was defined by 5 voxels inward from the external surface of the cortex and the endosteal CB that was from the endocortical surface to 5 voxels outward (Fig. 1b and Fig. 2c). The distance of 5 voxels is 135 micrometer (5×27 micron voxel size), which could cover the entire distance of interest for nanoindentation (120 micrometer) used in the current study. The open code of Image J software (NIH) was used to manipulate the digital processing of 3D images.

The gray value of each bone voxel, which is proportional to tissue mineral content, was obtained during the segmentation process of micro-CT images (Fig. 2a). Gray value histograms were identified for the whole mandibular condyle (Total), periosteal and endosteal CBs, and TB (Fig. 2b,c). Mean gray values were computed by dividing the sum of gray values by the total number of bone voxels in each region using the gray value histograms of periosteal and endosteal CBs, and TB. The standard deviation (SD) of the gray value histogram was also computed. Low and high gray values (Low₅ and High₅) were determined at the lower and upper 5th percentile values, respectively.

For the descriptive purpose of the human mandibular condyles used in the current study, averaged thickness of CB and architectural parameters of TB including trabecular bone fraction (BV/TV), surface-to-volume ratio (BS/BV), thickness (Tb.Th), number (Tb.N), separation (Tb.Sp), connectivity density (Conn.D), and degree of anisotropy (DA) were digitally measured from the 3D micro-CT image using the morphological code provided by the micro-CT company (CTAn, SkyScan, Kontich, Belgium) (Table 2). The DA was computed by dividing a maximum mean intercept length (MIL1) by a minimum MIL (MIL3). A region of interest for the TB was determined maintaining the same dimension between specimens.

Nanoindentation

Following the micro-CT imaging analyses, the mandibular condyles were dissected further in the anteroposterior direction (Fig. 3). A cubic trabecular bone specimen (5×5×5 mm³) was dissected from the internal trabecular region of the condyle, and a cortex specimen was concurrently obtained from the same mandibular condyle. All dissection was conducted using a low speed saw under irrigation. The top of trabecular bone was located as close as possible to bottom of the endosteal cortical bone while the 3D dimension of trabecular bone region was maintained consistently between specimens. The surfaces of dissected cortical and trabecular specimens were polished in the mediolateral and superoinferior directions, respectively, using silicon carbide abrasive papers with decreasing grit sizes (1200, 2400, and 4000 grits) and Al₂O₃ pastes (1 and 0.3 μm) on soft polishing cloths. Finally, the specimens were sonicated in de-ionized water to remove debris. All polishing steps were performed in wet conditions.

The polished specimens were mounted on a nanoindenter (Nano-XP, MTS, Oakridge, TN) after being glued onto a polycarbonate holder with a fluid drainage system as introduced in previous studies [21, 22, 25]. Indentation regions were determined using a light microscope incorporated within the nanoindenter. Periosteal and endosteal regions of the cortical bone (CB) and trabecular bone (TB) regions were identified (Fig. 3). The periosteal CB was determined within approximately 120 μm from the periosteal surface of condylar cortex, and the endosteal CB was located within approximately 120 μm from the endosteal surface of the condylar cortex. Nanoindentation was performed on the mediolateral direction of the CB and the superoinferior direction of the TB using a pyramidal Berkovich tip for the nanoindenter. All specimens were kept wet during nanoindentation with 0.5 mg/ml solution of gentamicin sulfate (Sigma, St. Louis, MO) using the same operating conditions as used in previous studies [21, 22]. An indentation load rate corresponding to a displacement rate of

10 nm/sec was applied to an indentation depth of 500 nm (Fig. 4). Nanoindentation based elastic modulus (E), plastic hardness (H), static viscoelastic normalized creep (Creep/ $P_{\max}$) and viscosity (η), and dynamic viscoelastic tangent delta ($\tan \delta$) were measured.

The hardness (H) was obtained by Eq. 1.

$$H = \frac{P_{\max}}{A} \quad (\text{Eq. 1})$$

where $P_{\max}$ is the peak indenting load. The indenter contact area (A) was approximated using a shape function of the indented site [26].

Creep developed by the peak loading was measured as the displacement during the 30-second hold period. The traditional viscoelastic Voigt model (Eq.2) was used to fit the displacement-time curve of each indentation.

$$h^2(t) = \frac{\pi}{2} p_{\max} \cot \alpha \left[\frac{1}{E_2} \left(1 - e^{-tE_2/\eta} \right) \right] \quad (\text{Eq.2})$$

where $h(t)$ is creep (nm) as a function of time, α is the Berkovich indenter face angle (65.27°) [27], E_2 (GPa) is an elastic component and η is the indentation viscosity (GPas) term. Thus, the viscosity represents a resistance to viscous deformation. The applied peak load corresponding to the same loading depth (500 nm) varied between indentation sites depending on the intrinsic material properties of heterogeneous bone tissue. As such, the amount of creep was normalized by the peak load (Creep/ $P_{\max}$) to examine the effects of intrinsic bone properties on creep independent of different loading levels.

Tan δ was assessed by continuous stiffness measurements (CSM) that uses the harmonic oscillatory response during the 30-second holding period. Oscillatory force was applied at 45 Hz corresponding to 2 nm of displacement, which is a common operating condition for CSM [28]. Tan δ is computed using Eq. 3.

$$\delta = \tan^{-1} \left(\frac{\omega C}{K - m\omega^2} \right) \quad (\text{Eq. 3})$$

where δ is a phase angle between the force and displacement signals, ω is the oscillation frequency, C is the damping coefficient of indentation contact, K is the spring constant of the contact, and m is the indenter mass. Potential errors in measuring the initial phase angle were recently indicated, so a correction procedure was employed as suggested by Herbert et al. [28]. The initial phase angle was corrected by 1) using CSM data obtained at the relatively constant projected contact area during the holding period, 2) subtracting the average phase angle value of a reference silica (2.517° at 45Hz), and 3) removing contributions from the indenter actuator and the stiffness of the load frame [28].

After the 30-second holding period, the indenter was unloaded with the same corresponding displacement rate of 10 nm/sec. Nanoindentation elastic modulus (E) was measured using Eq. 4 [29].

$$\frac{1}{E_r} = \frac{(1-\nu_s^2)}{E_s} + \frac{(1-\nu_i^2)}{E_i} \quad (\text{Eq.4})$$

where E_r (reduced modulus) is derived from the unloading force-displacement curve slope. E_s is the elastic modulus (E) of the bone tissue sample in the current study. The ν represents Poisson's ratio. For the diamond indenter, values of $E_i = 1141$ GPa and $\nu_i = 0.07$ are typically used. Poisson's ratio for bone was assigned to be 0.3 [30].

Brittleness was also computed by dividing the elastic modulus (E) by the plastic hardness (H), which accounts for spatial elastic deformation prior to permanent damage [31]. This parameter is useful to examine the resistance of a material to fracture because a ductile material has a higher E/H value [32].

Statistical analysis

Nanoindentation measures containing errors in the process of searching for an indentation surface and statistical outliers were removed as indicated in previous studies [22, 33]. Thus, a total of 523 nanoindentation sites were analyzed for each parameter; 179 periosteal CB, 225 endosteal CB, and 119 TB. Repeated measures analysis of variance (RMANOVA) with a post-hoc test (SPSS, IBM) was conducted to test the regional differences of gray values and nanoindentation parameters. Correlations between age and CB thickness and gray level parameters for each region, and between nanoindentation parameters were examined using Pearson's correlation tests. Analysis of covariance (ANCOVA) was used to investigate whether the correlations of elastic modulus with other nanoindentation parameters are different between the regions (periosteal and endosteal CBs, and TB). Significance was set at $p < 0.05$.

Results

Mean values of Mean and Low₅ gray values were not significantly different between the periosteal and endosteal cortical bone (CB) regions of the mandibular condyle ($p > 0.091$) while those of the trabecular bone region (TB) were significantly less than both CBs ($p < 0.034$) (Table 1). Mean values of SD gray values of the periosteal CB were significantly less than the endosteal CB ($p = 0.013$) and marginally less than the TB ($p = 0.06$) while those were not significantly different between the endosteal CB and TB ($p = 0.981$). Mean values of High₅ gray values of the endosteal CB were significantly greater than the periosteal CB and TB ($p < 0.007$) while no significant difference existed between the periosteal CB and TB ($p = 0.647$).

No significant correlations of age with CB thickness and gray value parameters at each region were found ($p > 0.410$). The CB thickness had strong negative correlations with SDs of gray values for the periosteal CB ($r = -0.769$, $p = 0.016$), endosteal CB ($r = -0.860$, $p = 0.003$), and TB ($r = -0.898$, $p = 0.001$).

Most nanoindentation parameters were not significantly different between the periosteal and endosteal CBs ($p>0.116$) except the mean value of brittleness (E/H) that was significantly smaller at the periosteal CB than the endosteal CB ($p=0.037$) (Table 3). The periosteal CB had significantly greater mean values of elastic modulus (E), plastic hardness (H), and viscosity (η) than the TB ($p<0.047$) while those values were not significantly different between the endosteal CB and TB ($p>0.093$). Mean values of E/H and normalized creep ($\text{Creep}/P_{\max}$) were not significantly different between the CBs and TB ($p>0.113$) except for a marginally higher value of $\text{Creep}/P_{\max}$ for the TB than the periosteal CB ($p<0.065$). However, the TB had a significantly higher mean value of $\tan \delta$ than both CBs ($p<0.04$).

All correlations between nanoindentation parameters were significant ($r=0.592\text{--}0.989$, $p<0.001$) (Table 4). Correlations with E/H were not tested because correlations with E and H were separately examined. The positive correlation slopes of E with H and η were not significantly different between regions ($p>0.339$) (Fig. 5). However, the negative correlation slope of E with $\text{Creep}/P_{\max}$ was significantly lower for the TB than the endosteal CB (ANCOVA, $p=0.015$) but not significantly different between other regions (ANCOVA, $p>0.162$). The periosteal CB had significantly lower negative slopes of correlation between E and $\tan \delta$ than other regions (ANCOVA, $p<0.001$) but the correlation slopes were not significantly different between the endosteal CB and TB (ANCOVA, $p>0.826$).

Discussion

The cortical bone regions (CB) of human mandibular condyles had higher gray values, and therefore mineralization, than the trabecular bone (TB) consistent with findings of a previous study that examined bone mineral distribution in human mandibular condyles [34]. Some measured nanoindentation parameters, including modulus (E), hardness (H), and viscosity (η), were higher at the more mineralized periosteal CB than at the less mineralized TB. On the other hand, the TB had greater mean values of dynamic viscoelastic creep ($\text{Creep}/P_{\max}$) and $\tan \delta$ than the periosteal CB. The E , H , and η account for the ability of bone tissue to resist elastic, plastic, and viscous deformations, respectively. The $\text{Creep}/P_{\max}$ represents the capacity of static constant energy absorption and the $\tan \delta$ indicates an ability to dissipate dynamic energy. Thus, the viscosity has the reverse response to the creep under a constant loading. The current findings indicate that tissue properties of the periosteal CB are better able to resist static deformation and those of the TB have a better time-dependent static and dynamic energy absorption and dissipating efficiency.

In the current study, bone tissue mineralization was examined using digitally separated cortical and trabecular portions of the mandibular condyle from micro-CT images, which allowed for analysis of the 3D condylar structure. It has been indicated that distribution of bone mineral content is closely associated with bone modeling and remodeling rates [34, 35]. Bone modeling represents a process of either bone formation or resorption to alter bone shape, while remodeling consists of bone formation following resorption. Osteoblasts (bone forming cells) produce collagen scaffolding in which minerals are progressively deposited [36]. Newly formed bone tissue has less mineral content than pre-existing older bone tissue. Hence, the overall Mean and Low_5 decrease as newly formed bone tissue regions increase resulting from active bone formation, whereas the Mean, Low_5 and High_5 values increase

throughout the mineralization process following bone formation. The SD accounts for the variability of bone tissue mineralization, which can be interpreted as how much the less mineralized new bone tissue forms during bone remodeling and also how much more mineral is added to the pre-existing bone during continuous mineralization following bone remodeling [10, 11]. As such, the similar level of Low₅ between the periosteal and endosteal CBs is likely due to similar new bone formation rates in these regions. On the other hand, the less High₅ of the periosteal CB than the endosteal CB could indicate that the highly mineralized pre-existing cortical bone tissue was more resorbed at the periosteal condyle cortex reducing the variability of tissue mineralization at the periosteal CB. Bone degeneration on the surface of the periosteal CB is likely responsible for this regional difference of tissue mineral distribution where bone is progressively lost due to aging despite being the primary load bearing tissue for static and dynamic loading at the temporomandibular joint (TMJ) [37]. However, no significant correlations of age with CB thickness and each gray value parameter for each periosteal, endosteal, and trabecular bone regions were found in the current study. This result may arise because the number of specimens used in the current *in vitro* study is relatively smaller than that in the previous clinical study which produced significant results [37]. On the other hand, the strong negative correlations between CB thickness and the variability (SD) of gray values for each region suggest that heterogeneity of the bone tissue mineral distribution likely decreases by reducing the CB thickness due to its degeneration. Further clinical studies with larger number of patients are needed to confirm this observation.

Cells responsible for bone remodeling have a greater chance to reach the surface of the porous trabecular bone that has larger surface-to-volume ratio than the compact cortical bone [34, 38]. The active trabecular bone remodeling produces less mineralized new bone tissue, increasing variability while reducing mean tissue mineralization of the TB compared to the periosteal CB, as found in the current study. A finite element model based on the tissue mineral distribution of a human mandibular condyle computed higher strain in the less mineralized trabecular bone tissue than in the cortex during simulation of static loading [39, 40]. It indicated that both the weaker porous architecture and lower tissue mineralization of TB reduced its apparent bone mineral density leading to the more compliant mechanical properties than those found in CB [34]. It has also been observed that higher mineralization provides greater elastic and plastic mechanical properties but endosteal viscoelastic characteristics of bone [11, 21], and variability of tissue mineralization controls viscoelastic creep behavior in trabecular bone [10, 11]. However, these macro level analyses do not provide the detailed mechanical behavior of bone at the tissue level where plastic and viscoelastic damage such as microcracks and time-dependent permanent deformation could propagate.

This is the first study to measure the five parameters (E, H, η , Creep/P_{max}, and tan δ) of elastic, plastic, and viscoelastic mechanical properties at the same site of fresh human mandibular condylar bone tissue using a cycle of nanoindentation. As such, the current results reflect direct associations between the parameters. It has been reported that dehydration and chemical fixation of the bone specimen could cause errors in measures by increasing values of E and H, and decreasing tan δ [41, 42]. Nanoindentation of fresh bone

under wet conditions as used in the current study ensured that these potential errors were avoided. Also, the indentations were performed in the mediolateral direction for the CB and in the superoinferior direction for the TB following principal stress and strain directions on the mandibular condyle as observed in a previous anatomical study of human mandibles [38]. Combined, these experimental conditions provide realistic measures of nanoindentation parameters to characterize mechanical stability of the human mandibular condyle.

It was observed that E, H, and η had positive correlations with tissue mineral content of bone in previous studies [22, 43–45]. The higher mean tissue mineralization of the periosteal CB represents greater chances to indent at specific tissue sites with higher mineral content than in the TB. As a result, the periosteal CB had significantly higher E, H, and η than the TB. The nanoindentation measures at the endosteal CB ranged between those of the periosteal CB and TB. This is not surprising since the endocortex is a transition zone between the TB and CB and trabecularization of the cortex (i.e., intracortical bone loss on the endosteal border) is common in aging individuals. However, no regional differences were found for the positive correlations of E with H and η suggesting that a similar tissue mineralization dependent control mechanism works in determining those properties irrespective of the local regions. It has been elucidated that increased mineralization limits deformation at the molecular level of bone under static loading [46, 47]. As the mineral component mainly bears stress and the collagen component is mainly responsible for deformation, increased mineral density in bone tissue would enhance its ability to resist elastic, plastic, and time-dependent viscous deformation by reducing motion at the mineral and collagen interface under static loading. In light of this mechanism, the high mineralization in the periosteal CB provides tissue properties which are functionally adapted to bear static occlusion loading. However, the lower E/H and higher homogeneity of tissue mineral distribution in the periosteal CB, which decrease toughness of bone tissue [32, 34, 48], would increase the risk of microcrack development leading to subchondral bone degeneration.

Relatively little is known about the viscoelastic behavior of bone tissue compared to elastic and fracture characteristics using nanoindentation. It has been well established that the time-dependent properties of bone tissue are more influenced by collagen components than mineral components [41, 49]. On the other hand, it has also been observed that bone tissue with higher modulus and hardness has lower viscoelastic creep deformation [22] and $\tan \delta$ [41, 49]. Consistent with those observations, the current viscoelastic creep and $\tan \delta$ had negative correlations with E, which account for their higher values at the less mineralized TB than the more mineralized periosteal CB of the mandibular condyle. However, the creep and $\tan \delta$ of TB changed differently from those of periosteal CB with increasing elastic modulus (E), while the E is closely associated with degree of bone tissue mineralization. These findings indicate that a control mechanism causing the regional variation of viscoelastic properties is not fully explained by tissue mineral content. It was previously indicated that a highly ordered, collagen-rich bone tissue has lower $\tan \delta$ compared to a disordered, collagen-poor bone tissue [49]. Newly formed bone tissue has a higher collagen portion but not a fully mature collagen phase [50], which may result in a higher $\tan \delta$ than in pre-existing old bone tissue [41]. Thus, in addition to the amount of mineral and collagen,

other factors such as organization of mineral and collagen components in bone tissue could also be important in determining the regional variation of viscoelastic properties. Further studies to investigate the compositional organization and interaction between mineral and collagen are necessary to understand the detailed mechanism of how those components determine the mechanical properties of bone at the tissue level.

Creep and $\tan \delta$ represent bone's ability to absorb static energy and dissipate dynamic energy, respectively. Thus, the higher variability of tissue mineralization, which provides a greater creep rate of human trabecular bone [11], and the higher values of creep and $\tan \delta$, provide more advantages in absorbing static occlusal loads and dissipating dynamic masticatory loading energy in the TB than the CB. These viscoelastic characteristics of TB could enhance the capacity of its porous architecture to diminish loading energy directly transmitted from the compact CB. Characteristics of the human mandibular condyle presented in the current study provide fundamental information to develop a better treatment plan for osteoarthritis and bone injury at the TMJ. They can also be used to design an effective scaffold to guide bone regeneration and a new implant adapted to the functional demands of the articular mandibular condyle under complicated TMJ loading.

To our best knowledge, there are no nanoindentation studies of human mandibular bone without fixation. Thus, it was not possible to directly compare those properties to other studies utilizing human mandibles. However, the nanoindentation elastic moduli of circumferential lamellar (approximately 9 GPa) and trabecular (8.02 ± 1.31 GPa) bone of human cadaveric fifth lumbar vertebrae are close in magnitude to those of CB and TB in the current study, while those of cortical (higher than 14.53 ± 1.41 GPa) and trabecular (higher than 13.75 ± 1.67 GPa) bone of human cadaveric femur and radius are significantly greater [33]. These comparisons suggest that the mandibular condyle cortex may have similar combinations of lamellar and osteonal bone tissues as observed in the vertebral cortical shell [26], which likely provide its more compliant bone properties than other osteonal cortical bones.

There are limitations that should be indicated in the current study. First, female specimens were not examined despite reports that females are more susceptible to TMJ osteoarthritis than males. Potential complications due to postmenopausal bone loss and associated compositional changes were thus avoided. An effort was also made to exclude the male subjects with known skeletal disorders or treatments (e.g., chemotherapy) that might affect the bone. The sample identified here is meant to be a "normal" representation of the male population. Future work is needed to incorporate females as well as individuals known to be exposed to environmental influences that will affect bone integrity for comparison and will utilize the current data for comparison. Another limitation is that the detailed regional variations for composition and interaction of mineral and collagen at the molecular level of bone were not examined. Changes of these material components of bone tissue are important because they are products of bone modeling and remodeling, which can elucidate how such biological activities control mechanical properties of bone tissue. In particular, direct assessment of the molecular components of bone tissue at the same site of indentation can provide more useful information. Future studies with advanced technologies are required to conduct such an analysis of molecular structures. Finally, it may be indicated that

mechanical analyses of the mandibular condyle at the macro level were not included. Thus, it is not clear how bone tissue properties contribute to the mechanical behavior of the whole condylar structure. A finite element analysis based on the 3D micro-CT image will allow for computation of detailed stress and strain distributions at the tissue level illustrating how they are integrated to estimate the macro level response of the whole mandibular condyle to static and dynamic loading. The elastic, plastic, and viscoelastic mechanical properties of bone tissue measured in the current study can provide more realistic values of bone properties with the heterogeneous finite element model allowing for non-linear time-dependent dynamic simulations.

In conclusion, substantial regional variations of bone tissue mineralization and properties were found in the human mandibular condyle. The mean values of elastic modulus, plastic hardness, and viscosity were greater at the more mineralized periosteal cortex than the less mineralized trabecular bone of the condyle. In contrast, the trabecular bone had higher viscoelastic creep and $\tan \delta$ than the periosteal cortex. These characteristics of bone tissue could be adapted to functional demands for the periosteal cortex to resist elastic, plastic, and viscoelastic deformation under static loading, and for the trabecular bone to absorb and dissipate loading energy at the TMJ during static occlusion and dynamic mastication.

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Highlights

- Bone tissue mineralization and nanoindentation parameters vary at the human mandibular condyle
- Modulus, hardness, and viscosity are greater in the cortex than the trabecular bone tissue
- Trabecular bone has higher viscoelastic creep and $\tan \delta$ than the periosteal cortex
- These characteristics could reflect adaption to functional demands during mastication

Fig. 1.

(a) Isolation of a condyle from a human cadaveric mandible, and (b) the procedure used to identify the condylar centrum in masking region, the trabecular bone (TB) in the centrum, and the cortex (CB). The CB was obtained by subtracting the masking part from the whole condyle. Then, the CB was separated into periosteal and endosteal CBs.

Fig. 2.

(a) Separation of periosteal and endosteal CBs, and TB images from a complete mandibular condyle and detailed voxel gray value distribution (red box) of the micro-CT image. A darker color represents a lesser gray value. (b) Parameters in the gray value histogram and (c) regional variation of gray value histogram obtained from the 3D images as illustrated in Fig. 1.

Fig. 3. Human mandibular condyle dissected in the anteroposterior direction and regions of interest for nanoindentation.

Fig. 4. Five nanoindentation parameters assessed using a cycle of indentation force-displacement curve at the same site.

Fig. 5.

Significant correlations of elastic modulus (E) with (a) plastic hardness (H), (b) viscosity (η), (c) normalized creep ($\text{Creep}/P_{\max}$), and (d) tangent delta ($\tan \delta$). The correlations of E with H and η were pooled between regions if analysis of covariance (ANCOVA) indicated no significant regional effects on the correlations ($p > 0.339$). The correlation equations are shown in each graph ($p < 0.001$ for all).

Table 1

Regional variation of the gray value distribution in the human mandibular condyle (mean±standard deviation).

	Mean	SD	Low ₅	High ₅
Periosteal CB	2767.54 ± 180.41	475.19 ± 54.05	1922.56 ± 232.93	3494.11 ± 187.78
Endosteal CB	2803.60 ± 271.17	511.20 ± 44.34	1980.33 ± 239.08	3648.44 ± 279.71
TB	2565.70 ± 307.60	513.59 ± 37.57	1761.89 ± 268.02	3432.22 ± 309.42

Architectural parameters of the human mandibular condyles used in the current study (mean $\pm$ standard deviation).

Table 2

CB Thickness (mm)	TB					
	BV/TV	BS/BV (mm ⁻¹)	Tb.Th (mm)	Tb.N (mm ⁻³)	Tb.Sp (mm)	Conn.D (mm ⁻³)
0.589 $\pm$ 0.112	0.140 $\pm$ 0.061	25.137 $\pm$ 3.836	0.152 $\pm$ 0.039	0.898 $\pm$ 0.214	0.797 $\pm$ 0.116	4.614 $\pm$ 1.346
						DA
						1.555 $\pm$ 0.122

Table 3

Regional variation of the nanoindentation parameters (mean±standard deviation).

	E (GPa)	H (GPa)	E/H	Creep/P _{max} (nm/mN)	η (GPaS)	tan δ
Periosteal CB	7.63±1.31	0.311±0.057	29.89±14.58	42.19±35.04	19347.58±4323.46	0.069±0.010
Endosteal CB	7.48±3.09	0.204±0.102	48.91±15.62	65.57±35.29	12710.59±8158.26	0.088±0.020
TB	5.11±2.82	0.138±0.069	46.93±15.98	111.85±56.02	8213.70±5056.48	0.114±0.033

Table 4Correlations between nanoindentation parameters ($p < 0.001$ for all correlations).

X	Y	Correlations	r
E (GPa)	H (GPa)	$Y = 0.032X - 0.001$	0.78
	η (GPa S)	$Y = 2102.621X - 1016.414$	0.724
log E (GPa)	log Creep/ $P_{\max}$ (nm/mN)	$Y = -1.288X + 2.603$	0.84
	log tan δ	$Y = -0.429X - 0.766$	0.633
H (GPa)	η (GPa S)	$Y = 66861.225X - 1167.934$	0.942
log H (GPa)	log Creep/ $P_{\max}$ (nm/mN)	$Y = -1.086X + 0.753$	0.983
	log tan δ	$Y = -0.298X - 1.332$	0.61
log Creep/ $P_{\max}$ (nm/mN)	log η (GPa S)	$Y = -1.12X + 5.74$	0.986
	log tan δ	$Y = 0.27X - 1.531$	0.61
log η (GPa S)	log tan δ	$Y = -0.222X - 0.22$	0.571