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ABSTRACT

Mandibular condylar cartilage functions as the load-bearing, shock-absorbing, lubricating material in temporomandibular joints. Little is known about the precise nature of the biomechanical characteristics of this fibro-cartilaginous tissue. We hypothesized that the fixed charge density associated with proteoglycans that introduces an osmotic pressure inside condylar cartilage will significantly increase the tissue's apparent stiffness. Micro-indentation creep tests were performed on porcine TMJ condylar cartilage at 5 different regions—anterior, posterior, medial, lateral, and central—in physiologic and hypertonic solutions. The intrinsic and apparent mechanical properties, including aggregate modulus, shear modulus, and permeability, were calculated by indentation test data and the biphasic theory. The apparent properties (with osmotic effect) were statistically higher than those of the intrinsic solid matrix (without osmotic effect). Regional variations in fixed charge density, permeability, and mechanical modulus were also calculated for condylar surface. The present results provide important quantitative data on the biomechanical properties of TMJ condylar cartilage.

KEY WORDS: temporomandibular joint (TMJ), condyle head, osmotic pressure, triphasic theory, micro-indentation.

Proteoglycans and Mechanical Behavior of Condylar Cartilage

INTRODUCTION

The fibro-cartilaginous tissue on mandibular condyles functions as an important load-bearing, shock-absorbing, and lubricating material during the physiological activities of temporomandibular joint (TMJ) (Hu *et al.*, 2003; Tanaka *et al.*, 2006). Similar to hyaline cartilage in most diarthrodial joints, the condylar cartilage consists of a fluid phase and a solid phase. The fluid phase, composed of water and dissolved electrolytes, occupies a predominant volume fraction (> 80%) and is responsible for the flow-dependent viscoelasticity in the tissue's mechanical behavior. Collagens and proteoglycans are the essential components of the solid phase, where the proteoglycan macromolecules are enmeshed in a densely woven, strong fibrous collagenous network (Mow *et al.*, 2005). These trapped proteoglycans contain a large number of sulfate and carboxyl groups, fixed along their glycosaminoglycan chains, which become negatively charged in the physiological environment. The density of these fixed charges is known as the fixed charge density. The swelling pressure resulting from the fixed charge density is known as the Donnan osmotic pressure (Donnan, 1924; Maroudas, 1979). This Donnan osmotic pressure, which is the major cause for maintaining cartilage hydration and swelling, plays an important role in the loading support ability of cartilaginous tissue (Donnan, 1924; Maroudas, 1979; Lai *et al.*, 1991; Tanaka *et al.*, 2003). According to the triphasic mixture theory (Mow *et al.*, 1980; Lai *et al.*, 1991), the equilibrium compressive modulus of soft-hydrated charged tissues includes contributions from two sources: the Donnan osmotic effect, resulting from fixed charge density; and the “intrinsic stiffness” of the solid matrix (*i.e.*, the compressive stiffness without charges). Accordingly, the apparent and intrinsic properties of the tissue were defined in the literature to distinguish the properties of the tissue with and without the osmotic effects, respectively (Ateshian *et al.*, 2004; Lu *et al.*, 2004). Recent studies showed that the osmotic pressure can contribute 30-50% of the apparent stiffness of hyaline cartilage (Mow *et al.*, 1998; Huang *et al.*, 2001; Wan *et al.*, 2004).

Several studies have been focused on the dynamic compressive properties of TMJ condylar cartilage (Kuboki *et al.*, 1997; Tanaka *et al.*, 2006). It has been shown that TMJ condylar cartilage deformed significantly less under intermittent compression than under sustained compression (Kuboki *et al.*, 1997). In the present study, micro-indentation tests on TMJ condylar cartilage were performed *in situ*, and the biphasic theory was used to calculate both the intrinsic and apparent mechanical properties of the tissue at 5 different regions (Mow *et al.*, 1989; Athanasiou *et al.*, 1991). The regional fixed charge density values were further obtained according to a triphasic correspondence principle developed recently (Lu *et al.*, 2007). Based on previous studies on articular cartilage in other

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human joints (Mow *et al.*, 2005), we hypothesized that the fixed charge density introduced by proteoglycans would have a significant effect on the mechanical behavior of TMJ condylar cartilage, and that regional differences would exist in compressive stiffness (Hu *et al.*, 2001; Tanaka *et al.*, 2006; Burrows and Smith, 2007).

MATERIALS & METHODS

Porcine TMJ was tested in this study based upon its anatomical similarities to the human TMJ (Bermejo *et al.*, 1993; Herring, 2003). Seven TMJs were harvested from hog heads (Green Village, NJ, USA) within 24 hrs of death. The experiment protocol was approved by the Institutional Animal Care and Use Committee at Columbia University. The TMJ articular disc was maintained as a cover on the condylar head, and the specimen was kept at -80°C until the day of testing. On the day of the experiment, the disc was carefully removed after being thawed at room temperature, and the condylar head was cut from the mandible with a hand saw. A mark at each of the 5 testing points was made on the articular surface with India ink in the anterior, posterior, central, lateral, and medial regions, respectively (Fig. 1A). The sample was then immersed in 0.15 M PBS solution with a protease inhibitor (PI) cocktail (EDTA, 1.8 mM; benzamidine, 5 mM; N-ethyl-maleimide, 7.18 mM; phenylmethylsulfonyl fluoride, 1.39 mM) for 1.5 hrs. Afterward, the cartilage-bone block was mounted on a custom-built micro-indenter device for the first creep test. The specimens were immersed in PBS+PI solution, and the chamber was rotated such that the testing site surface was perpendicular to the cylindrical indenter tip. A rigid porous-permeable indenter tip (diameter, 1.6 mm) was used for all tests (Fig. 1B). At the start of the test, a 0.2-gf-tare load was applied on the cartilage tissue for 15 min, followed by a 2-gf step loading for another 2 ~ 3 hrs to generate the creep curve until equilibrium was attained. After all of the 5 sites were tested, the whole sample was allowed to equilibrate fully in 2 M PBS+PI solution (Lu *et al.*, 2004). A second set of indentation creep tests was performed at the same sites with an identical protocol, although the specimen was then bathed in a 2 M environment. The cartilage thickness at the tested site was measured by the needle penetration method, as described previously (Hoch *et al.*, 1983).

According to the triphasic mixture theory (Lai *et al.*, 1991; Gu *et al.*, 1998), the fixed-charge-density-induced osmotic pressure inside the tissue is close to zero in a hypertonic environment, therefore making negligible contributions to the tissue's compressive stiffness (Flahiff *et al.*, 2002; Chahine *et al.*, 2005). Thus, the compressive loading applied by the indenter in the 2M solution is solely supported by the intrinsic compressive moduli of the solid matrix, *i.e.*, without the fixed-charge-density-induced osmotic effects. Based on this theory, our assumption was that the intrinsic mechanical properties of the TMJ condylar cartilage, aggregate modulus (H_a), Poisson's ratio (ν_s), and solid matrix permeability (k_s) could be extracted by curve-fitting the creep data in the 2M solution by a biphasic program (Mow *et al.*, 1989). In a 0.15 M environment, the indenting load is supported by both the elasticity of solid matrix and the osmotic pressure. Therefore, a biphasic curve-fitting of the creep data in a 0.15 M solution can extract another set of mechanical properties of the tissue, which include the Donnan osmotic effect, defined as apparent properties in the present study. The fixed charge density of the tissue can be calculated by the generalized triphasic correspondence principle with the information of both intrinsic and apparent mechanical properties (Lu *et al.*, 2007).

$$H_a = H_i + \left[\frac{RT(c_0^F)^2}{\phi_0^w \sqrt{(c_0^F)^2 + 4(c^*)^2}} \right],$$

Figure 1. Micro-indentation test on TMJ condyle head. **(A)** Articular surface of TMJ condyle head and the 5 testing sites are indicated. **(B)** The specimen is fixed in a chamber and submerged under PBS+PI solution. The central region is indented by a porous-permeable indenter tip.

H_i and H_a are the intrinsic and apparent aggregate moduli, respectively, c_0^F is the fixed charge density value, c^* is the external bathing solution concentration (0.15 M), R is the universal gas constant, T is the absolute temperature, and ϕ_0^w is the water volumetric fraction of the tissue, for which a uniform value 0.85 was assumed based on previous studies (Nicodemus *et al.*, 2007).

We performed one-way analysis of variance (ANOVA) with Tukey *post hoc* analysis to determine whether any statistical difference existed between the mechanical properties at different regions. A confidence level of 95% was considered significant.

RESULTS

A typical set of indentation creep data from the same testing site on a specimen shows that the mechanical behavior of condylar cartilage varied dramatically in 2 different ionic solutions (Fig. 2). The average equilibrium displacement of the indenter tip in a 2 M solution (0.095 ± 0.040 , mean $\pm$ standard deviation), normalized by cartilage thickness (mm), was significantly larger than that in a 0.15 M solution (0.077 ± 0.034). This indicates that the tissue appeared to be much softer in a hypertonic envi-

Figure 2. Two typical indentation creep curves obtained from the same testing site on a TMJ condyle when tissue is bathed in 0.15 M and 2 M solutions, respectively. The apparent strain is defined as the displacement normalized by tissue thickness. The gap between the 2 curves is due to the Donnan osmotic pressure inside the tissue.

ronment. Significant differences were observed between all of the intrinsic and apparent mechanical properties ($p < 0.001$). The apparent aggregate modulus (0.062 ± 0.027 MPa), shear modulus (0.030 ± 0.013 MPa), and permeability ($0.73 \pm 0.29 \times 10^{-14}$ m⁴/Ns) were all about 20% greater than their corresponding intrinsic values (aggregate modulus, 0.051 ± 0.021 MPa; shear modulus, 0.025 ± 0.011 MPa; permeability, $0.56 \pm 0.21 \times 10^{-14}$ m⁴/Ns). A significant linear relationship ($r^2 = 0.93$) was found between intrinsic and apparent aggregate moduli (Fig. 3A). Similar correlations were also detected between intrinsic and apparent permeability values (Fig. 3B) ($r^2 = 0.66$). Both the intrinsic (0.013 ± 0.03) and apparent (0.03 ± 0.05) Poisson's ratios were close to zero, and the apparent value was significantly higher than the intrinsic value.

Significant differences in the aggregate modulus were detected among the anterior, lateral, and central regions (Fig. 4A). The tissue in the central region had the highest apparent aggregate compressive modulus. The apparent shear moduli in the anterior and posterior regions were significantly lower than that of the lateral region (Fig. 4C). The tissue in the anterior region was significantly thinner than that in the other 4 regions (Fig. 4D).

DISCUSSION

Intrinsic and apparent mechanical properties of TMJ condylar cartilage at different regions were determined by the micro-indentation creep test and biphasic and triphasic mixture theories (Mow *et al.*, 1989; Lai *et al.*, 1991). The aggregate modulus of condylar cartilage was lower than 10% of that of human hip, knee, wrist, or shoulder joint tissues (Mow *et al.*, 2005), while the hydraulic permeability was about 5 times larger. However, the present results are consistent with those reported for TMJ disc and

Figure 3. Significant linear correlations were found between intrinsic and apparent mechanical properties ($n = 35$): **(A)** intrinsic aggregate modulus (mean $\pm$ standard deviation, 0.051 ± 0.021 MPa) and apparent aggregate modulus (0.062 ± 0.027 MPa); **(B)** hydraulic permeability in 0.15 M ($0.73 \pm 0.29 \times 10^{-14}$ m⁴/Ns) and 2 M ($0.56 \pm 0.21 \times 10^{-14}$ m⁴/Ns) solutions.

cartilage in temporal fossa (Kim *et al.*, 2003). Thus, the fibrocartilaginous tissues in TMJ were substantially different from those in other major load-bearing diarthrodial joints. Previous histomorphologic studies on condylar cartilage revealed a very low glycosaminoglycan content in the fibrous zone. Instead, this superficial layer stained rich in collagen, with large collagen bundles running parallel to the articular surface (Burrows and Smith, 2007). Although the mechanical moduli obtained from indentation tests were usually regarded as the average properties through the whole tissue thickness, the superficial tissue layer plays an extra important role when tissue is under indentation (Korhonen *et al.*, 2002). Both experimental measurement and our triphasic indentation analysis (results not shown) showed that the solid matrix deformation under indentation was significantly higher in the top zone (Bae *et al.*, 2006). Therefore, the low compressive modulus of condylar cartilage in the present study can be partially attributable to the lack of proteoglycan content in the

Figure 4. Regional distribution of biomechanical properties (mean ± standard deviation, n = 7). **(A)** Apparent aggregate modulus, **(B)** fixed charge density, **(C)** apparent shear modulus, and **(D)** thickness of cartilage. * and ** indicate statistical significance of $p < 0.05$.

fibrous zone, and its parallel alignment of collagen fibers. Without the proteoglycan macromolecules packed within the collagen fibrous network, the permeability of the ECM also increased dramatically. It has been shown that tissue permeability is inversely related to proteoglycan content (Maroudas *et al.*, 1968). The fixed charge density value calculated from the comparison between the intrinsic and apparent mechanical properties was about 10 times lower than those of human or bovine hyaline cartilage (Mow *et al.*, 2005). This may also be attributable to the lower proteoglycan content in condylar fibrocartilage, especially in the fibrous/superficial zone.

The apparent moduli were significantly higher than the corresponding intrinsic ones, with a significant linear correlation between the intrinsic and apparent values. There is now no doubt that both the osmotic pressure and intrinsic stiffness of ECM play significant and comparable roles in providing the condylar cartilage ability to sustain physiological loading. Previous studies have shown that the osmotic pressure can constitute up to 50% of the total load support in hyaline cartilage (Mow *et al.*, 1998). In the present study, however, the apparent aggregate modulus was only about 20% higher than the intrinsic values. Therefore, the contribution of osmotic effect was less significant in TMJ condylar cartilage than in hyaline cartilage (Lu *et al.*, 2004). More interestingly, the permeability in the physiological condition had an excellent linear correlation with that in the hypertonic condition,

while the apparent value was 15% higher. The osmotic pressure inside the tissue expanded the interspaces between the crimped collagen fibers, which resulted in a larger hydraulic permeability of the more loosely packed solid matrix (*i.e.*, greater pore size).

Regional mechanical moduli, tissue thickness, and permeability variations were observed in the present study. Previous studies on discs indicated an almost linear correlation between proteoglycan content and aggregate modulus when compared in different regions (Kim *et al.*, 2003). In the present study, a similar phenomenon was detected between fixed charge density and aggregate modulus. Both nano-indentation and indentation studies reported that the anterior region exhibited a significantly higher value than the other regions in dynamic complex modulus and storage modulus (Hu *et al.*, 2001; Tanaka *et al.*, 2006). We found the tissue permeability in the anterior region to be lower than in all the other regions. Since the flow-dependent viscosity plays a significant role in the dynamic behavior (Kuboki *et al.*, 1997; Tanaka *et al.*, 2006), a lower hydraulic permeability corresponds with a higher dynamic stiffness of the tissue.

The present data must be interpreted with several caveats. First, it was assumed that the mechanical properties of TMJ condylar cartilage are linear and homogeneous through its depth. Therefore, the biomechanical properties obtained in this study should be interpreted as depth-averaged values, with more contribution from

the superficial layer deformation. Second, non-ideal Donnan osmotic behavior was not considered (Ehrlich *et al.*, 1998). The 2M hypertonic solution cannot absolutely abolish the osmotic pressure inside the tissue. Third, not only does the proteoglycan content increase the compressive stiffness through osmotic mechanism, but also its ultra-structural and molecular interactions with the collagen network may contribute to TMJ cartilage mechanical behavior. Despite these simplifications and limitations, the present results, for the first time, provide valuable information and insights into the biomechanical properties and structure-function relationship of TMJ condylar cartilage.

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