Structure-Function Relationships of Temporomandibular Retrodiscal Tissue

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Abstract

It is estimated that 2% to 4% of the US population will seek treatment for temporomandibular joint (TMJ) symptoms, typically occurring with anterior disc displacement. The temporomandibular retrodiscal tissue (RDT) has been postulated to restrict pathologic disc displacement. To elucidate RDT function, understanding regional RDT biomechanics and ultrastructure is required. No prior biomechanical analysis has determined regional variations in RDT properties or associated biomechanical outcomes with regional variations in collagen and elastin organization. The purpose of this study was to determine direction- and region-dependent tensile biomechanical characteristics and regional fibrillar arrangement of porcine RDT. Incremental stress relaxation experiments were performed on 20 porcine RDT specimens, with strain increments from 5% to 50%, a ramp-strain rate of 2% per second, and relaxation periods of 2.5 min. Tensile characteristics were determined between temporal and condylar regions and anteroposterior and mediolateral directions. RDT preparations were imaged using second-harmonic generation (SHG) microscopy for both collagen and elastin. Young's modulus showed significant differences by region ($P < 0.001$) and strain ($P < 0.001$). Young's modulus was <1 MPa from 5% to 20% strain, before increasing from 20% to 50% strain to a maximum of 2.9 MPa. Young's modulus trended higher in the temporal region and mediolateral direction. Instantaneous and relaxed moduli showed no significant difference by region or direction. Collagen arrangement was most organized near the disc boundary, with disorganization increasing posteriorly. Elastin was present at the disc boundary and RDT mid-body. Porcine RDT demonstrated region- and strain-dependent variations in tensile moduli, associated with regional differences in collagen and elastin. The small tensile moduli suggest that the RDT is not resistive to pathologic disc displacement. Further biomechanical analysis of the RDT is required to fully define RDT functional roles. Understanding regional variations in tissue stiffness and ultrastructure for TMJ components is critical to understanding joint function and for the long-term goal of improving TMJ disorder treatment strategies.

Keywords: temporomandibular joint, tensile biomechanics, second-harmonic generation microscopy, tissue ultrastructure, collagen and elastin network, stress relaxation

Introduction

It is estimated 2% to 4% of the US population will seek treatment for temporomandibular symptoms (Haskin et al. 1995). Radiological evidence suggests temporomandibular joint (TMJ) degenerative changes occur in half the population older than 50 y, increasing to 85% by age 75 y (Lawrence 1987; Moskowitz 1988; Peyron and Altman 1992; Haskin et al. 1995). Approximately 30% of all temporomandibular joint disorders (TMJDs) involve degenerative changes to the TMJ disc (Ahmad et al. 2009; Schiffman et al. 2010), typically including anterior disc displacement (Larheim et al. 2001; Dias et al. 2012). The temporomandibular retrodiscal tissue (RDT) has been postulated to restrict pathologic anterior disc displacement during jaw movement (Osborn 1985; Eriksson et al. 1992). However, RDT biomechanics and ultrastructural arrangement are poorly understood, with no determination of physiologic regional variations in tensile properties or fiber organization.

The RDT is located posterior to the TMJ disc and is divided into temporal, intermediate, and condylar regions (Rees 1954; Scapino 1983, 1991; Kino et al. 1993; Piette 1993; Wilkinson

and Crowley 1994; Christo et al. 2005). The temporal RDT attaches the temporal bone to the superior aspect of the disc, while the condylar RDT attaches the posterior condylar surface to the inferior aspect of the disc (Rees 1954; Piette 1993; Christo et al. 2005). The intermediate RDT is highly innervated and vascularized (Scapino 1991; Wilkinson and Crowley 1994). During jaw motion, the RDT experiences tension as it is pulled anteriorly by the disc over the mandibular condyle (Osborn 1985; Scapino 1991; Scapino 1997; Tanaka et al. 2002). RDT functions have been postulated, including limiting anterior disc displacement, supported by pathologic disc

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Figure 1. Left: High-resolution T1-weighted 7 T magnetic resonance imaging of an intact porcine temporomandibular joint (TMJ) in the sagittal plane. The retrodiscal tissue (RDT) (bounded) is divided into temporal, intermediate, and condylar regions. Right: Intact RDTs were dissected into temporal and condylar regions. AP, anterior-posterior; ML, medial-lateral; SI, superior-inferior.

displacement following induced RDT injuries (Eriksson et al. 1992).

Few studies have fully investigated RDT in vitro biomechanical properties, with literature limited to a single study on human cadaveric RDT in tension (Kang et al. 2000) and single studies each on bovine RDT in unconfined compression (Tanaka et al. 2002) and tension (Tanaka et al. 2003). However, bovine and human TMJs have significant structural and functional dissimilarities—principally, the bovine TMJ is designed for large transverse translations (Herring 2003) and is significantly larger relative to humans (Kalpakci et al. 2011). The porcine TMJ has been identified as the most similar nonprimate animal model of the human TMJ (Herring et al. 2002; Herring 2003) and is frequently implemented for biochemical and biomechanical analysis (Detamore and Athanasiou 2003; Nickel et al. 2004; Kuo et al. 2011; Shi et al. 2013; Wu et al. 2015; Matuska et al. 2016). To our knowledge, there are no reports on the tensile characteristics of porcine RDT and no reports comparing the RDT to the TMJ disc following similar protocols.

Current literature provides an incomplete description of the ultrastructural arrangement of the RDT, limited to macroscopic observations (Cascone et al. 1999). It has been reported that the RDT is a fibrous connective tissue, composed primarily of collagen, with the temporal RDT also containing numerous elastin fibers (Scapino 1991; Piette 1993; Wilkinson and Crowley 1994; Christo et al. 2005). Understanding regional variations in collagen and elastin fiber arrangement is critical, given that regional differences in fibrillar arrangement contribute strongly to regional tensile properties in the TMJ articular disc (Detamore and Athanasiou 2003; Wright et al. 2016).

Given the incomplete RDT biomechanical and ultrastructural description, the purpose of this study was to determine 1) direction- and region-dependent tensile biomechanical characteristics and 2) regional fibrillar arrangement of the porcine RDT. Tensile biomechanical characteristics were determined from RDT preparations in anteroposterior and mediolateral directions, from the temporal and condylar regions. RDT specimens

were tested following an incremental stress relaxation protocol similar to reports for human and porcine TMJ discs (Detamore and Athanasiou 2003; Wright et al. 2016). Condylar and temporal RDT preparations were imaged using second-harmonic generation (SHG) microscopy to determine ultrastructural organization (Chen et al. 2012; He et al. 2013). We hypothesized significant regional (condylar vs. temporal) and directional (anteroposterior vs. mediolateral) differences in tensile biomechanical response for the TMJ RDT, associated with regional differences in collagen and elastin organization and orientation. Regional variations in tensile biomechanical outcomes and fibrillar organization were hypothesized given reported compositional differences between layers and a hypothesized principle loading direction.

Methods

Specimen Preparation

Twenty intact TMJs were excised en bloc bilaterally from 10 male Yorkshire swine (aged 6–8 mo) obtained from a local slaughterhouse within 4 h postmortem and frozen at -20°C until use. Intact porcine TMJs were imaged using a high-resolution 7 using a T1 fast low angle shot (FLASH) 3-dimensional sequence, with fat suppression: flip angle = 20° , repetition time (TR) = 50 ms, echo time (TE) = 5.5 ms, slice thickness = 1 mm, slice matrix = 240×320 , field of view (FoV) = 45×60 mm, in-plane resolution = 0.1875×0.1875 mm, average = 1. The images (Fig. 1, left) confirmed the health of the TMJs.

Following magnetic resonance imaging (MRI), intact TMJs were dissected and RDTs were isolated, carefully preserving discal attachments. After removal, intact RDTs were dissected into temporal and condylar regions (Fig. 1, right). One specimen per region was dissected from each RDT (Fig. 2, top left and right). From right TMJs, RDT preparations were further dissected anteroposteriorly in the temporal region (AP-T) and mediolaterally in the condylar region (ML-C), while RDT preparations from left TMJs were further dissected mediolaterally in the temporal region (ML-T) and anteroposteriorly in the condylar region (AP-C). RDT specimens had a nominal width of 4 mm and nominal thickness of 2.5 mm.

Biomechanical Testing

RDT specimens were rigidly gripped by custom machined clamps, using sandpaper to prevent micro-slippage (Fig. 2, bottom left). Test specimens were clamped on excess tissue, outside of the nominal grip-to-grip distance. Anteroposterior specimens had a nominal grip-to-grip length of 10 mm, and mediolateral specimens had a nominal grip-to-grip length of 5 mm. Incremental stress-relaxation experiments were performed with strain increments of 5%, 10%, 15%, 20%, 30%, 40%, and 50%, applied with a ramp-strain rate of 2% strain per second, with relaxation periods of 2.5 min (Fig. 3, top left) (Tanaka et al. 2003). Biomechanical tests were performed using a Bose tensile testing machine (ElectroForce 3220; TA Instruments) in a custom temperature-controlled water bath at 37°C .

Biomechanical Outcomes

RDT tensile biomechanical characteristics were determined by region (temporal and condylar) and direction (anteroposterior and mediolateral) for Young's modulus, instantaneous modulus, and relaxed modulus from constructed stress-strain plots (Fig. 3, bottom left). Young's modulus was calculated as the slope of the best-fit line during the ramp-loading phase for each strain increment. Instantaneous and relaxed moduli were calculated as the slope of the best-fit lines through the local maxima and minima, corresponding to the local peaks of the stress-strain curve and end of each stress relaxation phase, respectively (Detamore and Athanasiou 2003; Wright et al. 2016). Biomechanical analysis was performed using MATLAB (MATLAB R2016a; The MathWorks).

Tissue Imaging

RDT tissue preparations were imaged using SHG microscopy (Chen et al. 2012; He et al. 2013). RDT preparations were fresh, hydrated, and never fixed or stained. RDT preparations were placed on a 35-mm glass-bottom dish (MatTek) and imaged near the 1) disc boundary, 2) RDT mid-body, and 3) posterior end of the RDT. Each location was imaged twice consecutively, once for collagen and once for elastin. Collagen was imaged with an excitation wavelength of 860 nm and emitted signal collected in the 420- to 460-nm range, while elastin was imaged with an excitation wavelength of 740 nm and emitted signal was collected in the 420- to 460-nm range (Wagnieres et al. 1998; Zipfel et al. 2003). Color composites of the collagen and elastin networks were created (ImageJ 1.50i; US National Institutes of Health). SHG microscopy was performed using a Fluoview 1200 MPA (Olympus), with 30× oil immersion objective lens (UPLSAPO, 30XSIR; Olympus). Image resolution was 1,024 × 1,024, with a field of view of 423 × 423 μm .

Statistical Analysis

Statistical modeling assessed differences in instantaneous modulus and relaxed modulus among RDT region (temporal and condylar) and direction (anteroposterior and mediolateral), as well as differences in Young's modulus among disc region, direction, and strain increment (5%, 10%, 15%, 20%, 30%, 40%,

Figure 2. Retrodiscal tissue specimen preparation, dissection, and set-up for mechanical testing. **Top left:** Retrodiscal tissue (RDT) samples were dissected from intact porcine temporomandibular joints (TMJs). The discal attachments were preserved, with single specimens dissected from each RDT region. **Right:** Either an anteroposterior or mediolateral specimen was dissected from the temporal and condylar region, depending on joint side. **Bottom left:** Specimens were tested in a custom enclosed water bath, with each specimen (center) gripped by custom machined clamps using fine grit sandpaper to prevent micro-slippage.

Figure 3. Tensile biomechanical testing protocol, outcome definitions, and Young's Modulus, Instantaneous Modulus, and Relaxed Modulus results. **Top left:** Biomechanical tests were performed following an incremental stress-relaxation protocol with strain increments of 5%, 10%, 15%, 20%, 30%, 40%, and 50%, applied with a ramp-strain rate of 2% strain per second, followed by a constant strain relaxation periods of 2.5 min. **Bottom left:** Retrodiscal tissue tensile biomechanical parameters were determined from constructed stress-strain plots for Young's modulus, instantaneous modulus, and relaxed modulus. **Top right:** Young's modulus for each direction and region combination at every strain increment. Horizontal bars below chart indicate significant pairwise contrasts. **Bottom right:** Instantaneous modulus and relaxed modulus for each direction and region combination. AP-C, anteroposteriorly in the condylar region; AP-T, anteroposteriorly in the temporal region; ML-C, mediolaterally in the condylar region; ML-T, mediolaterally in the temporal region.

and 50%). Linear mixed-effects models for each outcome variable incorporated these predictors, with random effects for donor to accommodate within-donor correlation. Where significant

Figure 4. Second-harmonic generation microscopy images of preparations from the condylar retrodiscal tissue (RDT) (left) and temporal RDT (right). RDT preparations were imaged near the 1) disc boundary, 2) RDT mid-body, and 3) posterior end of the RDT. Each location was imaged twice consecutively, once for collagen (top row) and once for elastin (center row), and color composites (bottom row; collagen in red, elastin in green) of the collagen and elastin networks were created. Images were acquired normal to the temporal and condylar RDT surfaces, with the tissue in an unloaded condition. Each square image has a side length of 423 μm , with pixel density per image of $1,024 \times 1,024$.

differences in direction or region were detected, paired *t* tests were used to evaluate contrasts: pairwise within the anteroposterior (AP-T vs. AP-C) and mediolateral (ML-T vs. ML-C) directions and pairwise within the temporal (AP-T vs. ML-T) and condylar (AP-C vs. ML-C) regions. The linear mixed-effects model determined pairwise differences among the 7 strain increments for Young's modulus, with Holm adjustment for multiple comparisons. Descriptive statistics are reported as mean $\pm$ standard deviation, with a sample size of 10. Statistical differences are reported at $P < 0.05$. Analyses were performed in SPSS (version 23.0; SPSS, Inc.).

Results

Young's Modulus

Young's modulus, adjusted for strain, showed significant differences between temporal and condylar regions ($P < 0.001$), with no significant difference between anteroposterior and mediolateral directions ($P = 0.418$). No pairwise contrasts (AP-T vs. AP-C, ML-T vs. ML-C, AP-T vs. ML-T, AP-C vs. ML-C) were significant, but mediolateral specimens trended to have higher Young's modulus compared with anteroposterior specimens, and temporal specimens trended to have higher Young's modulus compared with condylar specimens. In the mediolateral direction, average Young's modulus was 1.08 ± 1.63 MPa for temporal RDT specimens and 0.65 ± 0.75 MPa for condylar RDT specimens. In the anteroposterior direction, average Young's modulus was 0.94 ± 1.15 MPa for temporal RDT specimens and 0.58 ± 1.31 MPa for condylar RDT specimens.

For Young's modulus, strain effects adjusted for direction and region were significant ($P < 0.001$) (Fig. 3, top right). Average Young's modulus, adjusted for direction and region, was small from 0% to 15% strain: 0.10 ± 0.15 MPa at 5%

strain, 0.16 ± 0.21 MPa at 10% strain, and 0.29 ± 0.34 MPa at 15% strain. Average Young's modulus, adjusted for direction and region, increased from 20% to 50% strain: 0.48 ± 0.57 MPa at 20% strain, 0.76 ± 0.92 MPa at 30% strain, 1.51 ± 1.55 MPa at 40% strain, and 2.40 ± 1.62 MPa at 50% strain.

Instantaneous Modulus

Instantaneous modulus showed no significant difference by region ($P = 0.867$) or direction ($P = 0.165$) (Fig. 3, bottom right). Temporal specimens trended to have higher instantaneous modulus compared with condylar specimens in both anteroposterior and mediolateral directions. In the mediolateral direction, instantaneous modulus was 0.97 ± 0.84 MPa for temporal RDT specimens and 0.48 ± 0.28 MPa for condylar RDT specimens. In the anteroposterior direction, instantaneous modulus was 0.82 ± 0.41 MPa for temporal RDT specimens and 0.61 ± 0.63 MPa for condylar RDT specimens. There were no observable trends between anteroposterior and mediolateral directions for instantaneous modulus.

Relaxed Modulus

Relaxed modulus showed no significant differences by region ($P = 0.808$) or direction ($P = 0.101$) (Fig. 3, bottom right). Temporal specimens trended to have higher relaxed modulus compared with condylar specimens in both anteroposterior and mediolateral directions. In the mediolateral direction, relaxed modulus was 0.36 ± 0.30 MPa for temporal RDT specimens and 0.20 ± 0.08 MPa for condylar RDT specimens. In the anteroposterior direction, relaxed modulus was 0.34 ± 0.20 MPa for temporal RDT specimens and 0.22 ± 0.19 MPa for condylar RDT specimens. There were no observable trends between anteroposterior and mediolateral directions for relaxed modulus.

Tissue Imaging

SHG microscopy revealed variations in collagen and elastin fiber arrangement in the temporal and condylar regions (Fig. 4). In the temporal RDT near the disc boundary, tightly packed collagen fibers were arranged in large bundles anteroposteriorly oriented. In the temporal RDT mid-body, bundles became increasingly disorganized, while at the posterior end, collagen bundles transitioned to interwoven fibers. Thick anteroposteriorly oriented elastin fibers were present in the temporal RDT near the disc boundary, while in the mid-body, many small branching elastin fibers were observed. In the condylar RDT, collagen fibers were arranged in well-organized sheets. In the condylar RDT mid-body, collagen fibers were loosely organized

Table. Tensile Biomechanical Properties (Young's Modulus, Instantaneous Modulus, and Relaxed Modulus) for TMJ RDT and TMJ Disc and Disc Attachment Reported in the Literature and from the Present Study.

Study	Loading Profile	Young's Modulus	Instantaneous Modulus	Relaxed Modulus
Tanaka et al. (2002) Compression RDT Bovine (n = 10)	10% and 20% strain @ 1.04 mm/s	1.5 MPa	—	—
Kang et al. (2000) Tension RDT Human (n = 4)	Strain to failure @ 0.02 mm/s	4.4 MPa	—	—
Tanaka et al. (2003) Tension RDT Bovine (n = 10)	0.5, 1.0, 1.5, and 2.0 MPa @ 10% strain/s	4.3 MPa	—	—
Present study Tension RDT Porcine (n = 10)	5%, 10%, 15%, 20%, 30%, 40%, and 50% strain @ 2% strain/s	@ 50% strain: AP-T: 2.8 MPa AP-C: 2.5 MPa ML-T: 2.9 MPa ML-C: 1.6 MPa	AP-T: 1.0 MPa AP-C: 0.9 MPa ML-T: 0.9 MPa ML-C: 0.5 MPa	AP-T: 0.4 MPa AP-C: 0.3 MPa ML-T: 0.3 MPa ML-C: 0.2 MPa
Murphy et al. (2013) Tension Disc attachments Porcine (n = 24)	Strain to failure @ 1% strain/s	AP: 4.7 MPa ML: 5.8 MPa	—	—
Detamore and Athanasiou (2003) Tension disc Porcine (n = 6)	Strain to failure in 5% increments @ 6 mm/min	—	AP-CENT: 28.6 MPa ML-CENT: 1.0 MPa	AP-CENT: 18.5 MPa ML-CENT: 0.6 MPa
Matuska et al. (2016) Tension disc Porcine (n = 12)	Strain to failure @ 1% strain/s	AP-MED: 65 MPa AP-CENT: 140 MPa AP-LAT: 45 MPa	—	—
Wright et al. (2016) Tension disc Human (n = 12)	5%, 10%, 15%, 20%, and 30% strain @ 1% strain/s	AP-CENT: 16 MPa ML-CENT: 9 MPa	AP-CENT: 10 MPa ML-CENT: 6 MPa	AP-CENT: 5 MPa ML-CENT: 3 MPa

AP-C, anteroposteriorly in the condylar region; AP-CENT, anteroposteriorly in the disc center; AP-T, anteroposteriorly in the temporal region; ML-C, mediolaterally in the condylar region; ML-CENT, mediolaterally in the disc center; ML-T, mediolaterally in the temporal region; RDT, retrodiscal tissue; —, parameter not defined by the indicated study

in thick anteroposterior bundles, while at the posterior end, thick disorganized collagen bundles were observed. In the condylar RDT, elastin was only observed near the disc boundary.

Discussion

Porcine RDT tended to be stiffer in the temporal region in tension, potentially associated with subtle differences in RDT ultrastructure between temporal and condylar regions. Temporal specimens had higher Young's modulus, instantaneous modulus, and relaxed modulus compared with condylar specimens. Region and strain effects were significant for Young's modulus, confirming hypothesized regional differences in biomechanical response, but differences in magnitude were small. The small tensile moduli and generally disorganized ultrastructural arrangement potentially suggest the RDT is not capable of resisting pathologic anterior disc displacement.

High-resolution MRI confirmed RDT divisions suggested by Rees (1954), with distinct temporal and condylar regions, separated by a loosely organized intermediate zone. SHG microscopy of fresh unstained RDT preparations revealed variations in collagen and elastin ultrastructure between temporal and condylar RDT regions and with increasing distance from the TMJ disc. Collagen fibers were most organized near the disc boundary, principally anteroposteriorly oriented. As distance from the disc increased, fiber organization decreased, with the temporal collagen network retaining a higher degree

of fiber organization compared with the condylar RDT. Elastin was present near the disc boundary and in the temporal RDT mid-body. Regional collagen and elastin fiber organization is critical to understanding the loading environment of the RDT.

Porcine RDT material properties were similar to those reported, with significant differences from the TMJ disc. Compared with the RDT biomechanical literature (Table), Young's modulus was lower than bovine and human RDT but was of similar magnitude. It should be considered that comparisons between the present study and the literature (Kang et al. 2000; Tanaka et al. 2003) are complicated by potential differences between intact RDT specimens and RDT specimens dissected into direction and regional preparations. Comparing the RDT to the TMJ disc, under similar test protocols, tensile moduli were an order of magnitude higher for the disc (Detamore and Athanasiou 2003; Matuska et al. 2016; Wright et al. 2016), with the posterior discal attachments providing an intermediately stiff stress-distribution boundary between the soft RDT and strong and stiff posterior band of the TMJ disc (Murphy et al. 2013). Porcine RDT ultrastructural arrangement demonstrated significantly less organization than reported for the TMJ disc (Detamore and Athanasiou 2003; Wright et al. 2016). Near the disc boundary, RDT fibers were most organized, distributing stresses from the significantly stiffer disc. Given the generally loose and disorganized nature of the RDT, it was not possible to determine differences between anteroposterior and mediolateral directions, contrary to hypothesized strong directional differences.

It has been postulated the RDT limits pathologic anterior disc displacement (Osborn 1985; Eriksson et al. 1992), which would require the RDT to possess some inherent stiffness. Given its low tensile moduli and generally loose and disorganized ultrastructural arrangement, it is unlikely the RDT mechanically resists pathologic disc displacement. Young's modulus was less than 1 MPa until 40% strain, remaining low even at 50% strain, the anticipated physiologic strain limit (Kang et al. 2000). In comparison, the load-bearing temporomandibular disc has a tensile modulus upward of 75 MPa (Beatty et al. 2001; Matuska et al. 2016). It should be considered, however, that the present study is unable to definitively conclude that the RDT is incapable of preventing pathologic disc displacement based solely on its low tensile modulus and loose and disorganized ultrastructural arrangement. SHG microscopy images of the RDT tissue were taken in an unloaded condition, and it is probable that under tensile loads, collagen alignment increases. It is likely, however, that fiber organization under tensile loading is marginal, with the RDT continuing to demonstrate less fiber organization compared with the TMJ disc, which maintains its highly organized ultrastructural arrangement even in unloaded conditions (Detamore and Athanasiou 2003; Wright et al. 2016). It should also be considered that at large strain increments, the RDT does show stiffening, and in the present study, specimens were not tested to failure. Other hypotheses for RDT functions have been reported, including acting as a volumetric compensatory device for limiting anterior disc displacement (Scapino 1991; Wilkinson and Crowley 1994), but biomechanical tests have not yet been performed. It has also been reported that the RDT provides nutrients and water to TMJ tissues through a hydrostatic pump mechanism (Wilkes 1978; Kino et al. 1993; Molinari et al. 2007). During joint motion, as the jaws are opened and the RDT is pulled anteriorly, negative pressure develops within the RDT (Cascone et al. 1999). Similarly, during jaw closure, positive pressure develops within the RDT as the RDT is pressed into a thick sheet behind the condyle. With the pressure fluxation during jaw articulation, the large venus plexus in the intermediate RDT is filled with blood during jaw opening, which is discharged when the jaws are closed (Wilkes 1978; Cascone et al. 1999). The temporal and condylar regions then serve as a periphery connecting the RDT to its surrounding hard tissues.

Limitations with the present study include comparison of tensile characteristics between studies complicated by differences in test methods. Kang et al. (2000) used an incremental loading protocol with very small strain increments (up to 6%) prior to performing a strain-to-failure experiment. At the 50% strain increment in the present study, Young's modulus was approximately 3 MPa, compared with 4.4 MPa for cadaveric human RDT. Bovine RDT response to tensile loading showed a similar Young's modulus to human RDT response (Kang et al. 2000; Tanaka et al. 2003), but significant differences in TMJ size, joint structure, and functional mechanisms complicate comparisons and clinical or modeling implementation. Some previous reports (Kang et al. 2000; Tanaka et al. 2003) also report viscoelastic characteristics of the RDT, but an

incremental viscoelastic model has yet to be comprehensively developed. It has been reported that the porcine TMJ is the closest animal model to human in composition and mechanical function, but differences do exist. Significantly, the porcine TMJ lacks a postglenoid wall (Herring et al. 2002), but it is anticipated that RDT function is similar between porcine and humans, based on similarities in joint articulation and tissue composition (Herring et al. 2002; Herring 2003; Kalpakci et al. 2011). Given the large variation and comparatively small differences between contrasts, region- and direction-dependent differences were unable to be statistically determined for instantaneous and relaxed moduli. Despite similarities in magnitude for Young's modulus, instantaneous modulus, and relaxed modulus, regional variations in collagen and elastin fiber arrangement were observed throughout the temporal and condylar RDT. We believe understanding RDT anisotropy is critical for developing high-fidelity TMJ models; however, it is possible that specimen preparation altered RDT biomechanical response and potentially contributed to the large variability given the loose, irregular fiber arrangement.

In conclusion, porcine RDT demonstrated region-dependent variations in tensile stiffness over a range of physiologic strains, associated with regional differences in collagen and elastin fiber arrangement. The small tensile stiffness suggests that the RDT is not mechanically resistive to pathologic anterior disc displacement, but determination of functional mechanisms requires further study. Functional roles, including acting as a pressure compensatory device and nutrient pump, should also be investigated. Understanding the stiffness and structural properties of TMJ components is critical to improving our understanding of TMJD development and progression and for the long-term goal of developing improved treatment strategies.

Author Contributions

M.C. Coombs, H. Yao, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; J.M. Petersen, contributed to design, data acquisition, analysis, and interpretation, drafted the manuscript; G.J. Wright, contributed to design and data acquisition, drafted the manuscript; S.H. Lu, contributed to design and data analysis, drafted the manuscript; B.J. Damon, contributed to conception and data acquisition, drafted the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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