Dynamic Properties of Tympanic Membrane in a Chinchilla Otitis Media Model Measured With Acoustic Loading

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*Otitis media is the most common infectious disease in young children, which results in changes in the thickness and mechanical properties of the tympanic membrane (TM) and induces hearing loss. However, there are no published data for the dynamic properties of the TM in otitis media ears, and it is unclear how the mechanical property changes are related to TM thickness variation. This paper reports a study of the measurement of the dynamic properties of the TM in a chinchilla acute otitis media (AOM) model using acoustic loading and laser Doppler vibrometry (LDV). AOM was created through transbullar injection of *Haemophilus influenzae* into the middle ear, and AOM samples were prepared 4 days after inoculation. Vibration of the TM specimen induced by acoustic loading was measured via LDV over a frequency range of 0.1–8 kHz. The experiment was then simulated in a finite element (FE) model, and the inverse-problem solving method was used to determine the complex modulus in the frequency domain. Results from 12 ears (six control and six AOM) show that the storage modulus of the TM from AOM ears was on average 53% higher than that of control ears, while the loss factor was 17.3% higher in control ears than in AOM ears at low-frequency ($f < 1$ kHz). At high-frequency (e.g., 8000 Hz), there was a mean 40% increase in storage modulus of the TM from AOM compared to control samples. At peak frequency (e.g., 3 kHz), there was a 19.5% increase in loss factor in control samples compared to AOM samples. These findings quantify the changes induced by AOM in the chinchilla TM, namely, a significant increase in both the storage and loss moduli. [DOI: 10.1115/1.4030410]*

Keywords: acute otitis media, tympanic membrane, chinchilla, complex modulus

1 Introduction

The TM, or eardrum, is a soft tissue membrane separating the ear canal from the middle ear. The TM plays an important role in the transmission of sound pressure in the ear canal into mechanical vibration of the ossicular chain, which is transported into the cochlea. Changes in the structure and mechanical properties of the TM directly affect the sound transmission and result in conductive hearing loss.

Otitis media is an infectious disease of the middle ear characterized by abnormal middle ear pressure, fluid accumulation in the middle ear, and middle ear tissue structural changes [1,2]. Both morphology and mechanical properties of the TM are changed in otitis media ears. These changes may disrupt the transmission of sound and cause conductive hearing loss. Investigating the variations of mechanical properties of the TM in otitis media ears will assist in understanding the mechanisms of disease-induced hearing loss and morphological changes of the ear.

The mechanical properties of soft tissues are determined either statically or dynamically. In static or quasi-static testing, a tissue sample is subjected to a fixed stimulus such as stress-strain testing of the human TM with a material testing system (MTS) or nanoindentation [3,4]. Dynamic tests, involving varied stimuli, such as testing under different frequencies, have been performed on the human TM using acoustic driving from a speaker over a frequency range and the dynamic-mechanical analyzer (DMA) with frequency-temperature superposition [5,6]. Dynamic properties of the guinea pig round window membrane (RWM) have been

determined by inducing vibration over a frequency range through electromagnetic stimulation of a magnetic microbead [7].

To measure dynamic properties of the TM in otitis media ears, an animal model becomes the unique choice for the measurement approach because the otitis media cannot be naturally created in human temporal bones. Chinchilla has been used as a common animal model in auditory research for developing AOM model through transbullar injection of bacteria such as *Haemophilus influenzae* [2,8–11]. The infection induced by *H. influenzae* in the chinchilla middle ear causes middle ear pressure, middle ear effusion, and structural changes in the ear tissues as reported by Guan et al. [2]. However, there is a lack of study of the changes of dynamic-mechanical properties of the TM in chinchilla AOM ears.

A technique has been developed for measuring dynamic properties of ear membrane tissues using acoustic loads over a range of frequencies while measuring the deformation with LDV [5]. The inverse-problem solving method is used along the FE modeling to determine the complex modulus of the ear tissue over the frequency domain. This technique has been applied to the human TM and RWM [5,6]. In this paper, we report the current study on dynamic properties of the chinchilla TM in both normal and AOM ears using a similar approach: acoustic loading and LDV measurement associated with the inverse-problem solving method. The goal is to determine the changes in the mechanical properties of the TM induced by AOM in chinchilla ears. The results provide important data for the biomechanical change in the middle ear during infectious disease.

2 Methods

2.1 AOM Model and TM Specimen Preparation

2.1.1 Creation of AOM Model. Adult chinchillas (*Chinchilla lanigera*, $n = 10$) of mixed gender weighing between 600 and

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Fig. 1 Chinchilla TM. Box indicates region that was excised and tested.

Fig. 2 Experimental setup for acoustic driving dynamic testing of TMs

780 g were included in this study. The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma and met the guidelines of the National Institutes of Health. All animals had disease-free middle ears before inoculation, as determined by otoscopic examination.

The chinchillas were divided into two groups, four in the control group and six in the AOM group. The animals of the control group were left untreated and both ears were used for testing. In the AOM group, the diseased state was produced through trans-bullar injection of *H. influenzae* 86-028NP suspension in the left ear following the procedure described by Guan et al. [2] and Morton et al. [11] on chinchillas. Under general anesthesia (10 mg/kg ketamine and 2 mg/kg xylazine), 0.3 ml of a bacterial suspension containing 3000 CFU of *H. influenzae* was injected into the superior bulla using a 1 cc syringe with a 26 gauge needle. Daily otoscopic examinations were performed after the administration of the challenge dose.

On the fourth day postinoculation, each animal was anesthetized and had its middle ear pressure and compliance measured with a multiple-frequency tympanometer (MAICO MI24, MAICO Diagnostics, Eden Prairie, MN) to confirm the presence of AOM. Control ears were similarly tested to verify the absence of infection. Following the tympanometry testing, each control and AOM animal was euthanized in preparation for harvesting the TM samples.

2.1.2 Preparation of TM Specimen. After euthanasia, TM control specimens ($n = 6$) and AOM specimens ($n = 6$) were prepared. The auditory bullae of euthanized chinchillas were surgically removed from their skulls. Each bulla was opened, and the bone surrounding the tympanic annulus was drilled away, separating the TM with undamaged annulus from the rest of the bulla. The malleus was left intact, and samples were immediately placed in saline solution. A 5×2.5 mm rectangular strip was cut from the posterior or anterior part of the TM as shown in Fig. 1, with the tympanic annulus intact on both the top and bottom in order to maintain structural integrity. The specimen was treated as a flat rectangular strip for the experiment, and the curvature of the TM was neglected in this study. A laser reflective tape (3M Co., St. Paul, MN) with an area of 0.5 mm^2 and a mass of 0.04 mg was attached to the center of each specimen to serve as the laser target for LDV measurements of TM sample vibration.

2.2 Experimental Setup for LDV Measurement. Figure 2 is a schematic diagram of the experimental setup with LDV to measure dynamic properties of the TM. Each specimen was mounted in the MTS (TestResource, MN) with two fixture adapters which were connected by a pair of plastic bars parallel to the longitudinal direction of the TM specimen, one on either side. The bars ensured that the adapters could not move and damage the TM sample during the mounting process. The TM specimen was then fixed lengthwise to the adapters using cyanoacrylate gel glue (Loctite) and mounted in the MTS. After the adapters and TM sample were lined up with the grips in the MTS, the plastic supports were removed. Images of each specimen were taken by a CCD camera. The length (gap between two fixture adapters) and

Table 1 Dimensions, resonance frequency, amplification ratio, and viscoelastic parameters of the control specimens including the mean with standard deviation

	CON 1	CON 2	CON 3	CON 4	CON 5	CON 6	Mean $\pm$ SD
Length (mm)	4.0	5.4	5.0	5.0	5.2	5.1	5.0 ± 0.5
Width (mm)	2.5	2.7	2.6	2.4	2.4	2.5	2.5 ± 0.1
ω_n (Hz)	580	360	420	360	360	290	395 ± 100
R	5.7	8.6	8.1	9.7	10.6	6.4	8.2 ± 1.9
E_0 (MPa)	366	370	286	301	176	356	309 ± 74
E_1 (MPa)	560	496	404	376	417	500	459 ± 71
τ_0 (μ s)	33	34	32	32	35	30	33 ± 2

Table 2 Dimensions, resonance frequency, amplification ratio, and viscoelastic parameters of the otitis media specimens including the mean with standard deviation

	AOM 1	AOM 2	AOM 3	AOM 4	AOM 5	AOM 6	Mean $\pm$ SD
Length (mm)	5	4.9	5.3	5.5	5.7	5.7	5.4 ± 0.3
Width (mm)	2.6	2.8	2.8	2.7	2.7	2.6	2.7 ± 0.1
ω_n (Hz)	660	660	740	650	590	580	647 ± 58
R	6.7	6.5	7.8	7.9	7.3	5.4	6.9 ± 0.9
E_0 (MPa)	381	354	593	528	495	482	472 ± 90
E_1 (MPa)	434	401	614	597	614	720	563 ± 122
τ_0 (μ s)	37	38	32	34	36	40	36 ± 3

Fig. 3 (a) Lateral view of the FE model showing TM (right), end of delivery tube (left), acoustic elements (surrounding), and boundary conditions (triangles). (b) Plane view of the FE model showing TM (center) with boundary conditions (triangles). Note that the mesh shown here is coarser than that used in the actual models for visualization purpose.

Fig. 4 Histology sections of (a) control chinchilla TM and (b) chinchilla TM after 4 days of otitis media infection with external ear canal (EEC) and middle ear cavity (MEC) labeled

width of the specimen were measured through image analysis software (ADOBE PHOTOSHOP 7.0). The dimensions of each sample are listed in the first two rows of Tables 1 and 2 for control and AOM, respectively.

The acoustic load applied on the lateral side of the sample was delivered from a speaker through a sound delivery tube at 1 mm away from the TM. The sound signals (pure tones) were generated by the dynamic signal analyzer (DSA, HP 35670A, CA) and amplified by the power amplifier (B&K 2718, Norcross, GA). A probe microphone (ER-7C, Etymotic Research, IL) was attached to the sound delivery tube to monitor the sound pressure level (SPL) at the surface of the TM. The laser beam from an LDV (HLV-1000, Polytech PI, Tustin, CA) was focused on the reflective tape to measure the sample vibration induced by acoustic loading.

It is well known that the stabilized state of biological soft tissue is only reached after preconditioning, in which stress–strain curves are gradually stabilized through repeated loading–unloading cycles [12]. In this study, the preconditioning for each specimen was performed by the MTS with five cycles of uniaxial stretching at 0.05 mm/s and a stretch ratio of 10%. Saline solution was sprayed onto the lateral side of the sample in order to maintain physiological conditions. Following the preconditioning, an acoustic load of 80 dB SPL over the frequency range of 100–8000

Hz was applied at the center of the TM specimen and the vibration of the sample was recorded by LDV.

2.3 FE Model of Dynamic Testing. Dynamic testing of each specimen was simulated in an FE model in ANSYS (ANSYS, Inc., Canonsburg, PA) using acoustic–structure coupled analysis. The inverse-problem solving method was applied to determine the dynamic properties of the TM sample using the experimental data.

2.3.1 FE Model. Figure 3 shows both side and plane views of the model. The TM was modeled as a rectangular plate with length and width as shown in Tables 1 and 2 for control and AOM specimens, respectively. The thickness was determined via histology sections of normal and AOM TMs. Figure 4 displays typical histology sections of a normal TM and an AOM TM 4 days post-infection. The average thickness for control TMs was about 15 μm and for AOM TMs it was 26 μm . The TM was assumed to be an isotropic material with a density of 1200 kg/m³ and Poisson's ratio of 0.3.

The TM sample was meshed with four-node tetrahedral solid 185 elements in ANSYS. The air surrounding the TM was meshed with four-node tetrahedral fluid 30 elements within a 0.1 m radius sphere of air around the TM. This sphere was meshed with a maximum element edge size smaller than 0.007 m in order to follow the rule of thumb of six elements per shortest acoustic wavelength for acoustic scattering ($1/6 \times c/f = 1/6 \times 343 \text{ m/s}/8000/\text{s} = 0.0071 \text{ m}$) [5,13]. Air properties were 1.21 kg/m³ density and 0.007 boundary admittance coefficient. To simulate sound absorbance within an infinite open field, the boundary surface of the sphere was covered in three-node triangular fluid 130 elements. The surface of acoustic elements contacting the solid TM elements was flagged as a fluid–structure interface with acoustic pressure coupled to the structure as force.

Sound pressure of 80 dB SPL over the frequency range of 100–8000 Hz was applied on acoustic nodes 1 mm away from the surface of the TM, representing the position of the end of the sound delivery tube. The displacement at the center of the TM model was calculated through harmonic analysis in ANSYS.

2.3.2 Constitutive Equation of TM Specimen. The standard linear solid model or Wiechert model was used to describe the TM viscoelastic behavior, as shown in Fig. 5 [5,14]. The relaxation modulus of the TM can be expressed as

$$E(t) = E_0 + E_1 \exp\left(-\frac{t}{\tau_1}\right) \quad (1)$$

where E_0 is the relaxed elastic modulus at time $t = \infty$, E_0 plus E_1 is the initial elastic modulus at $t = 0$, and τ_1 is the relaxation time. For harmonic analysis, $E(t)$ can be converted into the complex modulus E^* in the frequency domain as

$$E^*(\omega) = E'(\omega) + iE''(\omega) \quad (2)$$

where $E'(\omega)$ is the storage modulus, $E''(\omega)$ is the loss modulus, and ω is the angular frequency. $E'(\omega)$ and $E''(\omega)$ can be expressed as

Fig. 5 Standard linear solid model with $n=3$ parameters E_0 , E_1 , and τ_1

$$E'(\omega) = E_0 + \frac{E_1 \tau_1^2 \omega^2}{1 + \tau_1^2 \omega^2} \quad (3)$$

$$E''(\omega) = \frac{E_1 \tau_1 \omega}{1 + \tau_1^2 \omega^2} \quad (4)$$

with the loss factor $\eta(\omega)$, the ratio of loss modulus to storage modulus, as

$$\eta(\omega) = \tan(\delta) = \frac{E''(\omega)}{E'(\omega)} = \frac{E_1 \tau_1 \omega}{E_0 + (E_0 + E_1) \tau_1^2 \omega^2} \quad (5)$$

where δ is the phase angle of the complex modulus, $E'(\omega)$ is entered into the model as the frequency dependent elastic modulus, and $\eta(\omega)$ as the damping factor.

It is noted that the three parameter model in Eq. (1) was used to fit viscoelastic properties in this study. A higher order model can provide better expression for the complex behavior of the viscoelastic materials in the frequency domain. However, the number of material constants selected depends on the available experimental data.

2.3.3 Inverse-Problem Solving Method. The inverse-problem solving method was used with these equations to determine three parameters E_0 , E_1 , and τ_1 and to finally obtain the complex modulus of the TM. The displacement–frequency curve derived from each FE model was compared to the experimental curve obtained from the corresponding sample. The primary features compared between the FE model-derived displacement–frequency curve and the experimental measurement curve were the resonance frequency ω_n , the amplitude of displacement at low-frequency (e.g., $f=100$ Hz), and the displacement amplification ratio R of peak amplitude to low-frequency amplitude.

The optimization process for determining the FE model-derived curve comprises four steps, as previously performed by Gan et al. on the RWMs of guinea pigs [7]. Briefly, the initial values of 1.0 MPa, 0.8 MPa, and 30 μs were first chosen for E_0 , E_1 , and τ_1 . Then, the storage modulus $E'(\omega)$ was calculated from these parameters using Eq. (3) over the frequency range tested, and the loss factor $\eta(\omega)$ was calculated using Eq. (5). These values were input into the FE model. The third step was to run a harmonic analysis on the FE model from 100–8000 Hz to determine the vibrations of the central node of the TM resulting from the sound pressure stimulus. Finally, the displacement–frequency curve from the model was compared to the experimental data. If the fit was not sufficiently close in regard to ω_n , the displacement at 100 Hz, or R , new values for the three parameters would be calculated based on the methods of Gan et al. [7].

3 Results

3.1 Morphological Changes of TM in AOM. The morphological changes of the TM in AOM ears were studied using histological sections and scanning electron microscopy (SEM) images. Figure 4 shows histology sections of a control TM and an AOM TM. Significant thickening and inflammation are visible in the AOM tissue, with the thickness of the TM being approximately double that of the control TM. SEM images obtained from the control and AOM TMs are shown in Fig. 6. The surface of the control TM is smoother than that of the infected one. The fibrous structure on the inside of the tissue is visible in both samples through slits that were cut while preparing the samples for SEM imaging. The AOM TM is visibly thicker than the control TM.

The length and width of each sample are listed in the first two rows of Tables 1 and 2 for control and AOM samples, respectively. The length of control samples ranged from 4.0 to 5.4 mm with a mean of 5.0 ± 0.5 mm, and the width ranged from 2.4 to 2.7 mm with a mean of 2.5 ± 0.1 mm. The AOM samples ranged in size from 4.9 to 5.7 mm long with a mean of 5.4 ± 0.3 mm and

Fig. 6 SEM images of (a) control chinchilla TM and (b) chinchilla TM after 4 days of otitis media infection

2.6 to 2.8 mm wide with a mean of 2.7 ± 0.1 mm. Tissue density was not measured, but was assumed in both control and AOM samples to be 1200 kg/m^3 .

3.2 Experimental Measurement and FE Modeling Results.

The experimental results of displacement amplitude over the frequency range obtained from all control samples ($n = 6$) are shown

Fig. 7 Vibration amplitude versus frequency curves obtained from dynamic tests on (a) control group and (b) otitis media group

in Fig. 7(a) and those from AOM samples ($n = 6$) are shown in Fig. 7(b). The resonance frequency ω_n and the amplification ratio R (ratio between the amplitude at the resonance frequency and the amplitude at the lowest frequency measured) measured from control and AOM samples are listed in Tables 1 and 2, respectively. The average ω_n of control specimens was 395 ± 100 Hz, lower than the average ω_n of 647 ± 58 Hz for the AOM samples. The amplification ratio R averaged 8.2 ± 1.9 for the control ears and 6.9 ± 0.9 for the AOM ears. There is a clear delineation between the control and AOM states with displacement being significantly higher and resonance frequency being lower in the control specimens compared to the AOM specimens.

FE models were created to simulate the dynamic experiments of the TM samples for all control and AOM ears. Figure 8 shows the results from four TM samples (two for control and two for AOM), including the experimental measurement curves (solid lines) and model-derived displacement amplitude–frequency curves (broken lines). A fit was considered to be good if the level at low frequencies, the resonance frequency, and the amplitude at the resonance frequency matched between the experimental and model curves. Tables 1 and 2 list three parameters E_0 , E_1 , and τ_1 at which good fit between model and experiment was found for each sample. E_0 ranged from 176 to 370 MPa in the control ears and from 354 to 593 MPa in the AOM ears. E_1 fell between 376 and 560 MPa for control tissues and 401 and 720 MPa for AOM tissues. τ_1 ranged from 30 to 35 μs for control specimens and from 32 to 40 μs for AOM specimens. The average values of E_0 , E_1 , and τ_1 were 309 MPa, 459 MPa, and 32.7 μs , respectively, for control samples. In AOM samples, the average E_0 , E_1 , and τ_1 values were 472 MPa, 563 MPa, and 36.2 μs , respectively. Variance in these parameters between control and AOM could be due to differences in the geometry of the samples or possible variations in the setup.

3.3 Dynamic Properties of the TM in Control and AOM Ears.

Based on the viscoelastic parameters for each specimen, the storage modulus $E'(\omega)$, loss modulus $E''(\omega)$, and loss factor $\eta(\omega)$ over the frequency range of 100–8000 Hz of individual specimens in control and AOM were calculated and displayed in Fig. 9. The mean curves of $E'(\omega)$, $E''(\omega)$, and $\eta(\omega)$ are shown in Fig. 10 with standard deviation bars. As displayed in Fig. 9, the storage modulus increased with increasing frequency after remaining nearly constant at low frequencies of 100–1000 Hz. After that point, the storage modulus increased at a fairly steady rate over the frequency range studied.

Unlike the storage modulus, the loss modulus curves (middle panels of Fig. 9) do not increase over the entire frequency range. While the loss modulus did increase with frequency before reaching a peak at approximately 4000–6000 Hz, after that point the loss modulus decreased with increasing frequency. This trend was evident in both the control and AOM ears, although the loss

Fig. 8 Comparison of experimental results to modeling curves for the frequency dependent displacement amplitude for $n = 2$ control samples and $n = 2$ AOM samples

Fig. 9 Dynamic properties of control ($n = 6$) and AOM ($n = 6$) chinchilla TMs over the frequency range of 100–8000 Hz. Storage modulus for control (a) and AOM (b) animals, loss modulus for control (c) and AOM (d) animals, and loss factor for control (e) and AOM (f) animals.

Fig. 10 Mean storage modulus (a), loss modulus (b), and loss factor, and (c) of chinchilla TMs with error bars

modulus peaked slightly earlier in AOM TMs than it did in control TMs. The loss factor, as shown in the bottom panels of Fig. 9, behaved similarly to the loss modulus.

At 100 Hz, the storage modulus for control samples ranged from 176.2 to 370.2 MPa. For AOM samples at the same frequency the storage modulus ranged from 354.2 to 593.2 MPa. At 8000 Hz, the storage modulus ranges for the control samples and AOM samples were 491.2–776.7 MPa and 668.7–1059.22 MPa, respectively. On average, the storage modulus was 163.1 MPa higher in AOM case than in the control at 100 Hz and 259.9 MPa higher at 8000 Hz as shown in Fig. 10.

The loss modulus ranged from 7.6 to 11.6 MPa at 100 Hz in control samples. The loss modulus of AOM samples at 100 Hz ranged from 9.6 to 18.1 MPa. The peak loss moduli ranged from 188.0 to 279.8 MPa in the control specimens and 200.3 to 371.0 MPa in the AOM specimens. On average, the loss modulus was 3.4 MPa higher in the AOM samples at 100 Hz. At peak, the

loss modulus averaged 50.2 MPa higher in the AOM specimens compared to the control as shown in Fig. 10.

The loss factors at 100 Hz ranged from 0.025 to 0.052 for the control samples and from 0.021 to 0.037 for the AOM samples. The loss factors at their peaks ranged from 0.416 to 0.645 in the controls and from 0.363 to 0.473 in AOM. At both points, the loss factor decreased in the AOM compared to the control. The loss factor was 17.3% higher in control ears than in AOM ears at low frequency ($f < 1000$ Hz) and 19.5% higher in control samples compared to AOM samples at its peak ($f = 3000$ Hz) as observed in Fig. 10.

Overall, the storage modulus and loss modulus both increased in AOM tissues compared to the control samples. The loss factor decreased in the AOM specimens compared to the controls, showing that the increase in storage modulus due to AOM was relatively larger than the increase in loss factor.

4 Discussion

4.1 Comparison of TM Modulus Values. The complex modulus of the chinchilla TM over the frequency range of 100–8000 Hz was determined in this study. The mean storage modulus in the control group was 309.4 MPa at 100 Hz and 643.4 MPa at 8000 Hz. Zhang and Gan conducted the dynamic test on human TM with the same testing method as the present study [5]. They reported that the mean storage modulus was 54.34 MPa at 200 Hz and 65.54 MPa at 8000 Hz. Subsequently, they performed DMA testing with the frequency–temperature superposition method, and the mean storage modulus was 15.1 MPa at 1 Hz and 27.6 MPa at 3800 Hz. Luo et al. used a miniature split Hopkinson tension bar and found that the Young’s modulus of human TM increased from 45.2 to 58.9 MPa in the radial direction and from 34.1 to 56.8 MPa in the circumferential direction for strain rates in the range of 300–2000/s [15]. Overall, the storage modulus of chinchilla TM is about ten times larger than that of the human TM when an isotropic TM model was employed.

Aernouts and Dirckx used the micro-indentation measurement to estimate the Young’s modulus of gerbil TM, considering an isotropic elastic membrane [16]. They reported that the Young’s modulus varied between 71 and 106 MPa. Fay et al. presented various estimates using three different methods and used dynamic measurements on the cat TM along with a composite shell model [17]. The composite shell model gave best fittings in the Young’s modulus range of 100–220 MPa posterior and 200–400 MPa anterior. Alternately, Fay et al. estimated the fibril packing elastic modulus in cat TM to be 290–580 MPa [17]. Compared with all those published data, the elastic modulus or storage modulus of chinchilla TM reported in this study approaches the fibril packing elastic modulus of the cat TM.

The modulus values of the chinchilla TM obtained in this study are significantly higher than the values reported by Zhang and Gan [5] for human TMs and Aernouts and Dirckx [16] for gerbil TMs and are only close to those in Fay et al. [17] for cat TMs. The following considerations may explain this difference. First, chinchilla TM has different ultrastructure and fiber composition compared to human TM [18]. The chinchilla TM is much thinner than the human TM. This has resulted in a higher elastic modulus compared with the human TM. Second, the dynamic modulus in the present study was obtained from a very fine FE mesh with much larger element numbers than those used in the human TM model by Zhang and Gan [5]. The coarse FE mesh can lead to a decreased elastic modulus.

Interspecies variations of the TM exist in its thickness and size. The thickness of human TM ranges approximately from 40 to 120 μm , and a mean thickness of 70 μm was used in Zhang and Gan’s paper to derive the modulus. The thickness of chinchilla TM is 15 μm in this study. The average diameter of the chinchilla TM is around 8.32–8.53 mm, corresponding to about 90% of the

human TM diameter. For a circular plate of this size, the primary resonance frequency is

$$\omega_0 \propto \frac{\sqrt{Et}}{a^2} \quad (6)$$

where E is elastic modulus, a is the diameter, and t is the thickness. Considering the hearing range of chinchillas is close to that of humans and assuming the same primary resonance frequency for both TMs, the ratio of elastic modulus of chinchilla TM to the human TM is: $(a_1/a_2)^4(t_2/t_1) = (0.9)^4 \times (70/15)^2 = 14.29$. This ratio resembles the result from comparing the chinchilla TM storage modulus and the human TM published by Zhang and Gan [5]. This brief calculation may suggest that the complex modulus of chinchilla TM measured in this study is reasonable.

4.2 Effects of AOM on Mechanical Properties of TM.

Dynamic modulus of the chinchilla TM over the frequency range of 100–8000 Hz was determined for AOM ears. On average, the storage modulus at 100 Hz was 163.1 MPa higher than the control, and the loss modulus was 3.4 MPa higher than the control. At 8000 Hz, the average increase in storage modulus of AOM versus control was 259.9 MPa, and the average increase in loss modulus was 34.2 MPa. Overall, the storage modulus and loss modulus both increased in AOM tissues compared to the control.

Recently, Guan et al. utilized the chinchilla AOM model to quantify the factors affecting the TM mobility loss in AOM ears [2]. The TM displacement of control ears was higher than that of AOM ears with the middle ear pressure released and middle ear effusion removed. The residual TM mobility loss at low frequencies suggested that AOM induced middle ear structural changes and increased the middle ear stiffness. The residual TM mobility loss observed in chinchilla AOM ears seems to be consistent with the present data with the increase of TM stiffness in AOM.

Mechanical properties of the middle ear tissues were obtained mostly from normal ears in the literature. Recently, dynamic properties of the infected RWM in AOM ears were measured by Gan et al. [7] for guinea pig and by Wang et al. [19] for chinchilla. The results from both guinea pig and chinchilla RWMs in AOM ears showed that the resonant frequency of the RWM decreased compared to that in normal ears. This led the storage modulus and loss modulus of RWM in AOM ears to be lower than that of normal ears, in contrast to the increase of dynamic modulus of the TM specimen in AOM ears. The reason for such a different response in change of dynamic modulus between the TM and RWM in AOM ears is still unclear. However, it should be noted that the boundary conditions for samples in experiments were different between the TM and RWM. The RWM was kept intact and attached to the bony wall at its periphery, which means that factors such as the additional deformation caused by AOM were included and the mechanical properties were observed from whole RWM structure. Because the TM sample strip was cut from the posterior or anterior portion, the residual deformation or relaxation due to AOM existing in the intact structure was released when the sample was cut, and the mechanical properties of the TM sample represented the material behaviors only. In future experiments, inclusion of the intact TM should be accounted for in AOM ears to evaluate the effect of AOM on dynamic properties of the intact TM.

4.3 Model Parameters. In middle ear tissues, the general curves of the storage modulus $E'(\omega)$ and loss modulus $E''(\omega)$ as functions of frequency are probably more complex than those given in Fig. 9 [6]. The generalized Maxwell model or Wiechert model consisting of a large number of material parameters could account for this complex behavior of the tissue. However, in this study, we measured the vibration at the central point of the TM sample using a single LDV, and only the first resonance peak in the response curve was observed. The resonances at high

frequencies ($f > 1$ kHz) could not be captured, and the data at higher frequencies were limited. As a result, the inverse-problem solving approach was performed using the first or primary resonance characteristics based on the three-parameter model.

It is difficult to evaluate the accuracy of the material properties at high frequencies in this study due to the lack of directly measured data. Using the generalized Wiechert model with more material constants would not necessarily ensure the derivation of more meaningful results. The three parameter model (E_0 , E_1 , and τ_1) employed for data analysis appears to be good for at least the first approximation. It is worth noting that the model and curve fitting method selected for the inverse approach affect the estimation of the viscoelastic constants, and could result in a discrepancy at higher frequencies. Despite its limitation, the proposed approach is good enough to describe at least the primary resonance characteristics of the material behavior as shown in Fig. 8. The measurement of multiple points at high frequencies could obtain multiple resonance frequencies and displacement patterns, and provide more complete data for calculation of the frequency dependent properties of the TM. To this end, the multiple-constant model may improve the estimation of the complex modulus.

5 Conclusions

The dynamic properties of the chinchilla TM in both normal and AOM ears were reported in this study. Sound pressure over a frequency range from 100 to 8000 Hz was used as a driving force for the TM specimen, and LDV was used to measure the resulting vibrations. FE models were constructed for TM samples and the inverse-problem solving method was applied to derive the complex moduli of the TM over the frequency domain.

Results from 12 ears (six control and six AOM) show that the storage modulus of the TM from AOM ears was on average 53% higher than that of control ears while the loss factor was 17.3% higher in control ears than in AOM ears at low-frequency ($f < 1000$ Hz). At a high-frequency (e.g., 8000 Hz for storage modulus and peak frequency for loss factor), there was a 40% increase in storage modulus of the TM from AOM compared to control samples and a 19.5% increase in loss factor in control samples compared to AOM samples. These findings quantify the changes induced by AOM in the chinchilla TM, namely, a significant increase in the storage modulus and decrease in the loss factor.

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