Research Article

Surface Motion of Tympanic Membrane in a Chinchilla Model of Acute Otitis Media

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ABSTRACT

The conductive hearing loss caused by acute otitis media (AOM) is commonly related to a reduction of the tympanic membrane (TM) mobility in response to sound stimuli. However, spatial alterations of the TM surface motion associated with AOM have rarely been addressed. In this study, the TM surface motion was determined using scanning laser Doppler vibrometry (SLDV) in a chinchilla model of AOM. The AOM was established by transbullar injection of nontypeable *Haemophilus influenzae*. The TM surface vibration was measured in control (uninfected) animals and two AOM groups of animals: 4 days (4D) and 8 days (8D) post inoculation. To quantify the effect of middle ear pressure in those infected ears, the SLDV measurement was first conducted in unopened AOM ears and then in middle ear pressure released ears. Results showed that middle ear infection generally reduced the TM displacement across the entire surface, but the reduction in the umbo displacement over the time course, from 4 to 8 days post inoculation, was less than the reduction in the displacement at the center of each quadrant. The presence of middle ear fluid shifted the occurrence of traveling-wave-like motion on the TM surface to lower frequencies. The observation of the spatial variations of TM surface motion from this study will help refine our understanding of the middle ear sound transmission characteristics in relation to AOM.

Keywords: tympanic membrane, middle ear, acute otitis media, conductive hearing loss, scanning laser Doppler vibrometer

INTRODUCTION

The mechanical process of sound wave transmission from the tympanic membrane (TM) to the stapes via the ossicular chain is the first stage in conveying auditory information to the brain. In addition to an accumulation of fluid and a buildup of negative pressure in the middle ear cavity, pathological changes induced by otitis media (OM) may lead to alterations in anatomical structures and mechanical properties of the TM and middle ear soft tissues (Rovers et al. 2004; Guan and Gan 2013; Wang et al. 2015; Yokell et al. 2015). These changes in structures, material properties, and middle ear environment in OM ears significantly impact the TM and ossicular chain motion in response to sound and ultimately affect sound energy transmission to the inner ear (Ravicz et al. 2004; Guan et al. 2014; Wang and Gan 2016). While there are various models describing TM function, our understanding of TM function in relation to middle ear disease remains incomplete due to the complexity of TM motion.

The umbo velocity or displacement is a common experimental measure to assess middle ear abnormality (Stasche et al. 1994; Gan et al. 2006; Dai and Gan 2008; Jakob et al. 2009; Nakajima et al. 2012), and several studies have been conducted on animal models to investigate how OM affects the umbo motion. Utilizing a guinea pig model of acute otitis media (AOM), Guan and Gan (2013) studied the

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effects of middle ear pressure (MEP), middle ear effusion (MEE), and ossicular structure changes on the umbo motion. Thornton et al. (2013) reported changes of the umbo velocity and cochlear microphonic response in chinchillas when the middle ear was filled with different volumes of silicone oil. Their results showed the umbo velocity was reduced and cochlear microphonic threshold was elevated with increasing amounts of middle ear fluid, and a greater reduction of the umbo velocity occurred at high frequencies. These results were consistent with the previous studies in temporal bones by Ravicz et al. (2004) and in guinea pigs by Guan and Gan (2011). In order to address the factors contributing to conductive hearing loss during the course of a middle ear infection, Guan et al. (2014) quantified the roles of MEP, MEE, and structural changes of the ossicular chain by measuring TM mobility loss in a chinchilla model of AOM at two phases of the disease (4 and 8 days post inoculation). Although previous studies evaluated changes of the TM mobility induced by middle ear diseases by means of the umbo vibration, how the TM vibration varies across different regions of the TM surface during the course of an infection is not well understood.

Full-field motion measurement of the TM can provide simultaneous vibration data from a large number of points on the membrane surface, which has the benefit of showing both the spatial and temporal behaviors of the TM vibration in response to sound stimuli. The term “full-field” herein means that the measurement is performed over the entire visible area of the TM. Measurement of the entire TM surface motion has been used to explore the relationships between the structure and acoustic function of the middle ear in animals (Khanna and Tonndorf 1972; Wada et al. 2002; Rosowski et al. 2009) and human cadaveric temporal bones (Tonndorf and Khanna 1972; Cheng et al. 2010; Burkhardt et al. 2014). Rosowski et al. (2009, 2011) measured the full-field motion of the TM, and demonstrated that the displacements of TM surface in response to sound result from a combination of lower-order modal response patterns and traveling waves. The full-field vibration measurements of the TM have also been utilized to analyze middle ear mechanics in diseased and reconstructed ears (Huber et al. 2001; Aarnisalo et al. 2009; Rosowski et al. 2011). Aarnisalo et al. (2009) and Rosowski et al. (2011) evaluated the effect of tympanoplasty on the full-field motion of the TM by stroboscopic holography. Ulku et al. (2014) used stroboscopic holography to determine the sound-induced motion of the TM in human temporal bones with reconstructed ossicular chains and compared the performance differences between the different ossicular reconstruction techniques. Although there

is an ongoing argument about how different portions of the TM contribute to sound conduction via umbo motion, a TM motion altered from the normal spatial response patterns may interrupt the efficient conduction of sound energy through the TM. Several studies have been performed to evaluate the effects of OM on the entire TM vibration. Zhang et al. (2014) investigated the effects of middle ear fluid on TM surface motion in human cadaver ears by using scanning laser Doppler vibrometry (SLDV). Their studies showed that the middle ear liquid resulted in a decrease of the overall displacement amplitude and in more complicated deflection shapes. Recently, we investigated the TM motion in normal, AOM, and otitis media with effusion (OME) ears of guinea pigs by using SLDV (Wang et al. 2016), and an effort was made to evaluate the influences of AOM and OME on the TM surface motion. Although various studies have been performed to measure the TM motion in OM ears, a few important issues not addressed in previous studies are: how the entire TM motion varies across the OM time course, and how MEP and MEE alter the TM vibration patterns.

AOM is one of the most common infectious diseases in young children and can result in hearing loss (Minovi and Dazert 2014). However, neither the features of overall TM vibration nor the physical mechanisms involved have been clearly determined (Ravicz et al. 2004; Rovers et al. 2004; Guan et al. 2014). By measuring the vibration patterns of the TM surface, we are able to establish a direct relationship between the temporal and spatial characteristics of the TM motion and the AOM-induced mechanical changes in the middle ear. The purpose of this study is to investigate features of the TM motion in AOM ears by utilizing SLDV and distinguish the changes of TM mobility in response to sound during the course of AOM. This is a step toward understanding how the MEP and MEE affect the TM mobility as well as hearing loss.

METHODS

Animal Preparation

Eight adult chinchillas 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 were evaluated to be free from disease at the beginning of the study.

These chinchillas were divided into three groups: untreated control group, AOM group with 4 days (4D) post bacterial challenge, and AOM group with 8 days (8D) post challenge. The control group included two animals (4 ears) without any treatment

in either ear. Each of the 4D and 8D groups included three animals. Five ears in each AOM group were measured with the complete process described below. Thus, we reported data from these five ears for each group. Following the procedure described by Morton et al. (2012) and Guan et al. (2014), AOM was established by transbullar injection of nontypeable *Haemophilus influenzae* strain 86-028NP in both ears. Under general anesthesia with ketamine (10 mg/kg) and xylazine (2 mg/kg), 0.3 ml bacterial suspension containing 3000 CFU was injected into the superior bulla bilaterally using a 1-cm³ syringe with a 26 gauge needle. After the bacterial challenge dose was administered, daily otoscopic examination was conducted.

Experimental Protocol

On the 4th or 8th day post-inoculation, animals were anesthetized with a mixture of ketamine and xylazine as described above. Supplemental anesthesia was administered as required to maintain areflexia. The body temperature of the chinchilla was maintained throughout the experiment at approximately 38 °C by placing the chinchilla on a thermo-regulated heating blanket.

The pinna and cartilaginous portion of the external ear canal were removed to expose the bony ear canal. Following the surgical process, the TM was examined under a light microscope to identify signs or symptoms of AOM such as hyperemic TM and purulent effusion in the middle ear. Then MEP was measured by using a wideband tympanometer (Model AT235h, Interacoustic, MN). Detailed methods used to calibrate and measure wideband tympanometry for the chinchilla were described by Guan (2014). Briefly, the diameter at the entrance of the bony part of chinchilla ear canal was 4–5 mm. The system was calibrated with a set of two rigidly terminated tubes with an inner diameter of 4.5 mm prior to data collection (Keefe and Simmons 2003). The measurement probe with a commercial tip (outer diameter of 8 mm) was pressed to the bony rim of the ear canal and held by hand to ensure a good probe seal. Using click stimuli, wideband tympanometry was performed at 60 different frequencies between 0.25 and 8 kHz while air pressure in the ear canal swept from 200 to –300 daPa. A bandpass energy absorbance tympanogram averaged over a frequency range from 0.38 to 2 kHz was used to define a bandpass tympanometric peak pressure (TPP) (Liu et al. 2008). In this study, the TPP measured by the wideband tympanometer was used as an estimate of the MEP. This estimate could be influenced by the direction of the pressure sweep. Liu et al. (2008) reported that in adult human ears, the TPP shifted positively with ascending sweeps and negatively with

descending sweeps. In the AOM ears with negative middle ear pressure, the absorbance-based estimate of TPP may be lower than its true value.

Upon the completion of tympanometry measurement, the lateral bony wall of the ear canal was widely opened until the surface of the TM was easily visible from the lateral view. The lateral surface of the TM was painted with a suspension of 3 % TiO₂ powder (Acros Organics, NJ) in saline to increase the amount of backscattered light from the translucent TM. The painting has no significant effect on the motion of the TM (Rosowski et al. 2009). The effect due to the added mass of the painting material on TM motion is a point of later discussion.

The measurement of TM motion in AOM ears was performed in two experimental stages: an intact bulla containing both MEP and MEE (AOM-1) and a bulla with MEP released from the middle ear (AOM-2).

The energy reflectance and sound-induced TM velocity in the AOM-1 condition was measured first with the middle ear intact. Afterwards, the skin of the superior bulla was partially removed and a small hole (1 mm in diameter) was drilled into the superior portion of the bulla to release the MEP. After sealing the opening with dental cement (PD-135, Pac-Dent, CA), the measurement of stage AOM-2 was performed immediately.

After completion of the SLDV measurement at the AOM-2 stage, the hole in the superior portion of the bulla was reopened and enlarged to 4 mm in diameter. A tube was inserted into the middle ear cavity through the hole. A 1-cm³ syringe was connected to the end of the tube outside the bulla, and the MEE was aspirated manually from the cavity into the syringe. The aspiration process was repeated as necessary until no additional fluid could be drained from the tympanic cavity. The amount of the effusion in each infected ear was recorded.

The untreated control ears were prepared in the same manner as described by Guan et al. (2014). To exclude the effect of MEP in anesthetized animals, a small hole of 1 mm diameter was made in the top of the bulla bilaterally to release possible MEP. Upon completion of the pressure release, the holes were sealed with dental cement.

Scanning Laser Doppler Vibrometry Measurement

A SLDV (PSV-400, Polytec Inc., Irvine, CA) was used to measure the TM surface vibration. A schematic of the experimental set-up used for this study is shown in Fig. 1. A chirp stimulus with a frequency range of 0.1 to 10 kHz was delivered onto the TM through a sound delivery tube. The duration of each chirp was 80 ms, and the stimulus level varied between 70 and 85 dB SPL. A probe microphone (Model ER-7, Etymotic

FIG. 1. Schematic diagram of the experimental setup with a scanning laser Doppler vibrometer (SLDV) for measuring TM motion in chinchilla ear

Research), placed about 1–2 mm from the edge of the TM, was used to monitor the sound pressure level. Background noise measurements were performed by recording the TM velocities in the absence of an excitation source. The signal-to-noise ratios at the umbo and at the centers of the four quadrants were calculated. Because noise increased at low frequencies, the measured data below 0.4 kHz were not used. The signal-to-noise ratios were greater than 15 dB at the frequencies above 0.4 kHz.

In the present experimental setup, the angle between the laser beam and the plane of the tympanic annulus was visually estimated to be approximately 60–70°. Figure 2a shows a photograph of an exposed TM surface taken from the same viewing angle as the laser beam, and Fig. 2b shows the scanning grid with measurement points which were set and aligned on the TM live video image in the PSV 8.8 software (PSV 8.8, Polytec Inc., Irvine, CA). The TM was not entirely visible because of the curved shape of the ear canal

and the structure of the TM attached to the canal bony wall on the inferior side. The number of measurement points over the exposed TM surface ranged from 260 to 270 with 265 being the most common number. The scanning process usually took about 10–12 min. Due to a lack of quantitative surface normal information, no angle correction was performed in this study.

The laser beam was steered by a built-in video camera and a computer-driven mirror system. During the scanning, the laser beam moved point-by-point in the grid of discrete points. The vibration velocity of each point was recorded sequentially by SLDV, and the displacement results were further computed and analyzed over the frequency range of 0.1 to 10 kHz with the PSV 8.8 software. The displacement magnitude and phase distributions on the TM surface were drawn by a linear interpolation of the values at neighboring grid points.

FIG. 2. **a** Picture of the exposed TM sample. **b** The typical scanning points assigned on the TM surface. The red outline roughly shows the connection of the TM to the manubrium of the malleus, and the edge of the TM

RESULTS

Middle Ear Pressure and Fluid in AOM Ears

In 4D AOM ears, a slightly yellow effusion was evident. In 8D AOM ears, the TM appeared hyperemic and opaque, and a yellow-colored MEE was observed. The MEP at the AOM-1 stage was measured by wideband tympanometry prior to the measurement of TM vibration. The pressure of 4D AOM ears ranged from -115 to -180 daPa, and the mean and standard deviation (SD) was -142 ± 30 daPa. In 8D AOM ears, the MEP of two ears could not be quantified because they exhibited a flat tympanogram. The pressure of the remaining 8D AOM ears ranged from -60 to -100 daPa (mean $\pm$ SD = -80 ± 20 daPa). The volume of MEE in 4D AOM ears ranged from 0.25 to 0.80 ml (mean $\pm$ SD = 0.52 ± 0.21 ml). The effusion level in the ears from the 4D group was below the umbo except in the ear with an effusion volume of 0.8 ml. In the 8D AOM group, the volume of MEE ranged from 0.71 to 0.80 ml (mean $\pm$ SD = 0.78 ± 0.04 ml). In the 8D AOM ear, the effusion covered the majority of the TM.

TM Deflection Pattern in Normal and AOM Ears

Figures 3, 4, and 5 show the typical deflection and phase contours of TM motion measured in three ears: a control ear (No. 12-3-10-R), a 4D AOM ear (No. 12-3-9-R), and an 8D AOM ear (No. 12-3-4-L). In each figure, at the top the anterior (Ant.) and superior

(Sup.) orientations of the TM are indicated. The displacement results at frequencies 1, 4, and 8 kHz are presented from the left-hand column to the right-hand column. For each AOM ear, TM motion is shown for the two experimental stages (AOM-1 and AOM-2), and the displacement magnitude is normalized by the input sound pressure. The displacement phase is described relative to that of the input sound pressure. The deflection and phase contours at the experimental stages are displayed by corresponding rows with the scale bar at the bottom.

For the non-infected control ear in Fig. 3, the deflection contours of the TM look relatively simple at 1 kHz. In general, the vibration magnitude of the inferior portion was larger than that of the superior portion, and the entire TM surface moved in phase. The maximal displacement magnitude was close to 180 nm/Pa. As the frequency increased to 4 kHz (column 2), the deflection pattern exhibited some changes with two displacement peak regions emerging at the anterior-posterior portion of the TM with a maximal value of 100 nm/Pa. The phase difference across the TM surface was less uniform at 4 than at 1 kHz. At 8 kHz, the displacement pattern became more complicated with multiple peaks, and the peak displacement of the TM decreased with a maximal value of about 20 nm/Pa. The phase map shows that the TM surface was not vibrating in phase and had relatively sudden half-cycle phase changes between the adjacent regions. In other control ears, the maximal displacement magnitudes varied from

FIG. 3. Typical displacement magnitude and phase distributions of the TM lateral surface measured by SLDV in a control ear (12-3-10-R) at frequencies of 1, 4, and 8 kHz. The displacement is normalized with respect to the input sound pressure

FIG. 4. Typical displacement magnitude and phase distributions of the TM lateral surface measured by SLDV in a 4D AOM ear (12-3-9-R) at frequencies of 1, 4, and 8 kHz at experimental stages AOM-1

and AOM-2. Each of the three columns describes data taken at one frequency. The displacement is normalized with respect to the input sound pressure

112 nm/Pa to 386 nm/Pa at frequencies below 1 kHz, and the sites of the peak displacement were in the inferior regions. Additionally, two or more displacement peak regions appeared at frequencies of 1.3 to 1.5 kHz. Below these frequencies, the TM surface moved in phase. At high frequencies, the patterns of motion became more complicated, and the number of displacement peaks and the maximal displacement magnitude varied from sample to sample.

For the 4D AOM ear at the AOM-1 stage shown in Fig. 4, the deflection patterns of the TM motion exhibited a few differences when compared to the control ear. A major displacement peak occurred in the inferior portion of the TM at 1 kHz, and the maximal magnitude was approximately 110 nm/Pa. The second displacement peak was observed at the anterior-superior quadrant and occurred at a different phase angle from the major displacement peak. At

4 kHz, the displacement contour became more complex in the inferior portion of the TM. The displacement level was reduced with a maximal value of about 10 nm/Pa. At 8 kHz, it can be observed that the magnitude of TM displacement was reduced further for most of the surface, and the phase contour showed half-cycle phase changes in the adjacent regions. For the 4D AOM ear at the AOM-2 stage (a bulla with MEP released) shown in Fig. 4, the peak displacement magnitude was 150 nm/Pa at 1 kHz, which was slightly larger than that of AOM-1. At 4 kHz and higher frequencies, the TM displacement pattern did not change much when compared to AOM-1. However, the release of MEP resulted in numerous differences in the phase maps.

The deflection and phase contours in an 8D AOM ear is shown in Fig. 5. At the AOM-1 stage from 1 to 8 kHz, multiple displacement peaks were observed on

FIG. 5. Typical displacement magnitude and phase distributions of the TM lateral surface measured by SLDV in an 8D AOM ear (12-3-4-L) at frequencies of 1, 4, and 8 kHz at experimental stages AOM-1

and AOM-2. Each of the three columns describes data taken at one frequency. The displacement is normalized with respect to the input sound pressure

the TM surface, and the overall displacement level was much lower than in the control ears. The patterns of the phase contours show that the TM surface was no longer moving in phase, and the phase difference across the TM exhibited approximately half-cycle changes. Regarding AOM-2 stage of the 8D AOM ear, the patterns of the TM displacement were generally similar to those at stage AOM-1. At 1 and 4 kHz, the peak displacement increased in the superior region of the TM. At 8 kHz, the peak displacement decreased in the anterior region, and the maximum displacement was in the posterior-

inferior quadrant. The overall phase distributions across the TM had less similarity between the two stages, indicating that release of MEP affected the displacement phase in the majority of the TM.

In other 4D ears, multiple local magnitude peaks could be observed on the TM surface even at a frequency of 0.4 kHz, and the TM surface was not vibrating in phase. For example, at 1 kHz, ear 12-3-9-L had two displacement peaks at the AOM-1 stage while more than five local magnitude peaks appeared at the AOM-2 stage. At 4 kHz, the maximum TM displacement of ear 12-3-9-L was 5 nm/Pa for the AOM-1

FIG. 6. Mean displacement magnitude at the umbo and four quadrants in the control ears, 4D AOM, and 8D AOM ears. **a** Schematic representation of the umbo and the selected four quadrant

locations on the TM. **b–f** the normalized displacement curves at the umbo and four quadrants measured by SLDV. The error bars indicate the standard deviations

stage, and 8 nm/Pa for the AOM-2 stage. At 8 kHz, the largest displacement observed on the TM was 2 nm/Pa for the AOM-1 stage, and 5 nm/Pa for the AOM-2 stage. These maximum displacement magnitudes of ear 12-3-9-L were lower than those of ear 12-3-9-R. Regarding the TM motion in 8D AOM ears, at 1 kHz, it was observed that more than three peaks were moving out of phase for all 8D ears, and the location of the maximum displacements varied among specimens. As frequency increased above 1 kHz, the pattern of the TM motion appeared more variable from specimen to specimen. However, it was not possible to capture the fine motion features at high frequencies because only about 265 points were measured across the entire TM.

Change of TM Movement at Selected Locations due to AOM

The displacements at the umbo and four selected locations were extracted from the measured surface motion of the TM. Figure 6a is a schematic showing the selected locations on the TM: the umbo was selected at the tip of the TM manubrium and Q1 is the location selected at the center of the anterior-superior quadrant. The angle between the line from the umbo to Q1 and the horizontal chord is close to 45°. Similarly, Q2, Q3, and Q4 were selected at the posterior-superior quadrant, posterior-inferior quadrant, and anterior-inferior quadrant, respectively. These locations corresponded to specific grid points

FIG. 7. Mean displacement magnitude of the umbo and four quadrants in the 4D AOM ears, comparing TM displacement variations before and after release of middle ear pressure. The error bars indicate the standard deviations

of vibration measurement. The SLDV provided the coordinates of each measuring grid defined on the imaging plane. The distance between the umbo and the centers of the four quadrants ranged from 1.2 to 2.0 mm on the imaging plane, which was calculated based on the coordinate values of the measuring grids.

Figure 6b–f compares the mean displacement–frequency curves with standard deviations at the umbo and four selected locations. Each figure displays the measured curves of the control, 4D, and 8D AOM ears at the AOM-1 stage. As shown in Fig. 6b, the umbo displacements of the AOM ears were lower than those of the control ears. At the four quadrant centers, the mean displacements of AOM ears were also lower than those of control ears

except for the Q1 location at frequencies below 1 kHz and the Q4 location at frequencies around 5 kHz. The displacements of 4D ears were greater than those of 8D ears at the frequencies above 2 kHz except for the Q3 location at frequencies of 5 to 8 kHz. The AOM-induced change of the TM displacement varied at different locations. It was also observed that the differences of mobility at the umbo and the four quadrant centers between the control and 4D AOM-1 ears were generally larger than the differences between the 4D and 8D ears, except for Q3 and Q4 in a few frequency intervals. This observation agrees with that reported by Guan et al. (2014) on the umbo mobility changes in infected chinchilla ears. They found that the TM mobility in AOM ears is affected by three factors: the

middle ear pressure, effusion, and ossicular changes. Thus, the AOM-induced MEP, MEE, and ossicular structural change at an early infection stage contribute to the reduction of TM surface displacement in a different manner from those at a later infection stage.

Comparison of TM Displacement Between AOM-1 and AOM-2 Stages

To evaluate the effect of MEP of AOM ears on the TM vibration, we quantitatively compared the TM displacements of 4D ears at the AOM-1 stage with

those at the AOM-2. Figure 7 shows the mean displacements of the umbo and the four specific locations at the AOM-1 and AOM-2 stages of 4D ears. In general, the release of MEP resulted in a displacement increase at low frequencies as observed from Fig. 7a–e. In particular, the umbo displacement had an increase of 6 dB at frequencies below 1 kHz, and the maximal increase occurred at Q4 with an average value of 14 dB at frequencies between 0.4 and 1 kHz. This observation indicates that MEP affects TM motion at low frequencies even in the presence of MEE.

Figure 8 compares the TM displacements between 4D and 8D ears at the AOM-2 stage. The TM

FIG. 8. A comparison of mean displacement magnitude of the umbo and four quadrants between 4D and 8D AOM ears after release of middle ear pressure. The difference between 4D AOM-2

and 8D AOM-2 indicates the effect of middle ear effusion and structural changes on the TM displacement from 4 to 8 days post inoculation. The error bars indicate the standard deviations

displacement differences in the inferior quadrants (Q3 and Q4) were generally larger than those in the superior quadrants at low frequencies, but at high frequencies the TM displacement differences in the anterior quadrants (Q1 and Q4) were larger than those of posterior quadrants. Among the four quadrant locations, Q4 had the largest displacement difference with an average of -13 dB from 4D to 8D. The difference of the mean umbo displacements between 4D AOM-2 and 8D AOM-2 ears was small at frequencies below 3 kHz.

Comparison of Frequency Dependence of TM Displacements at Selected Locations

Figure 9 is a plot of the comparison between the umbo displacement and the four quadrant displacements in control ears, 4D and 8D AOM ears at two experimental stages. As shown in Fig. 9, at the most frequencies, the umbo displacement was generally lower than the four quadrant displacements for the control, 4D and 8D AOM ears. In control ears (Fig. 9a), the displacement curves of the umbo and

FIG. 9. Displacement magnitude of the umbo and the centers of the four quadrants in control, 4D AOM, and 8D AOM ears. **a** Displacements of the umbo and four quadrants in control ears. **b** Displacements of the umbo and four quadrants in 4D AOM-1 ears. **c**

Displacements of the umbo and four quadrants in 4D AOM-2 ears. **d** Displacements of the umbo and four quadrants in 8D AOM-1 ears. **e** Displacements of the umbo and four quadrants in 8D AOM-2 ears

four quadrant centers had similar variations and showed a dip at a frequency of 2.6 kHz. For the 4D ears at the AOM-1 stage (Fig. 9b), with increasing frequency, the umbo displacement exhibited an overall decreasing trend at frequencies below 6 kHz with a local peak observed at 2.25 kHz. In contrast, the mean displacement curves at quadrant centers stayed fairly constant below 3 kHz but with multiply minor peaks and then began to decrease up to 6 kHz. Although the displacement fluctuations at the four quadrant centers were more evident, there was no obvious local peak in the Q1, Q2, and Q4 curves at 2.25 kHz. For the 4D ears at the AOM-2 stage in Fig. 9c, the mean umbo displacement showed a gradual decrease with increasing frequency below 6 kHz, and the mean displacements at the four quadrant centers showed a similar decreasing trend as the umbo except at frequencies below 0.5 kHz.

For the 8D ears at the AOM-1 stage (Fig. 9d), the umbo curve exhibited an overall decrease with increasing frequency up to 8 kHz. The displacement curves of the four quadrant centers contained a few minor peaks at multiple frequencies, but the majority showed a decreasing trend in frequencies below 5 kHz. Additionally, the displacement curves of quadrant centers showed a local peak at 5.5 kHz which was not present in the umbo curve. At frequencies higher than 8 kHz, the displacement curves became more complicated, showing a few local peaks. After release of MEP, as shown in Fig. 9e, all curves remained similar and showed an overall decreasing trend as frequency increased to 5 kHz. At frequencies higher than 5 kHz, the displacement curves showed less consistency between the umbo and the quadrant centers. As observed in Fig. 9, the displacements at different locations on the TM generally showed a simplified decrease as the frequency increased in the AOM ears. The frequency dependence of the TM motion at different locations was more similar in the infected 8D ears than that in the infected 4D ears.

Effect of AOM on TM Vibration Modes

Figure 10 illustrates temporal variations in the displacement of the TM surface by showing the displacement at six successive instants of time within a cycle during 1 kHz stimulation in a control and an 8D AOM ear (as reconstructed from measurements of magnitude and phase at each grid point). At the top of the figure, the orientation of the TM is indicated. The maps of displacement magnitude corresponding to six different phase angles (0° , $1/6$, $1/3$, $1/2$, $2/3$ and $5/6$ cycles or 0° , 60° , 120° , 180° , 240° , and 300°) are displayed in three columns. For the control ear (column 1), the displacement patterns show that the

position of local displacement peak did not change with phase angle, indicating that the vibration mode of the TM was dominated by a standing-wave-like motion or modal motion (Rosowski et al. 2011). As frequency increased to 2 kHz (not shown), a traveling-wave displacement component in the TM could be observed. A combination of modal and traveling-wave motions contributed to the complicated surface motion of the TM. Similarly, in other control ears, it was observed that the location of the displacement peak started varying with phase at a frequency of approximately 2 kHz.

In AOM ears with unopened bulla (AOM-1, column 2 of Fig. 10), it was found that the local displacement peak of the TM moved with phase angle variation even at 1 kHz. For example, a local peak, indicated by an arrow, in the interior portion of the TM shifted along the circumferential direction when phase angle varied from 180° to 300° . This suggested the presence of a traveling-wave displacement component in the TM of AOM ears at 1 kHz. The vibration patterns of the TM surface in AOM-2 (column 3 of Fig. 10) also contained an obvious traveling-wave component. When the phase angle varied from 0° to 180° , the local displacement peak, indicated with an arrow in column 3, traveled from the anterior-superior quadrant to the anterior-inferior quadrant. As the frequency increased, more complex surface motions continued to emerge as combinations of traveling and standing waves. Furthermore, the obvious traveling-wave component was observed at frequencies between 0.6 and 0.85 kHz in the remaining 4D AOM ears, and 0.4 to 0.7 kHz in the 8D AOM ears. After release of MEP, the frequency where the traveling-wave motion occurred did not change. In addition, there were two kinds of phase variations across the TM surface in Figs. 3, 4, and 5: gradual phase changes and relatively sudden half-cycle phase changes. The gradual phase changes are suggestive of traveling waves along the TM surface, while the abrupt half-cycle phase changes are consistent with modal standing waves (Cheng et al. 2013). Therefore, the phase variations in Figs. 4 and 5 also demonstrate that the traveling-wave component of the TM motion occurred in combination with modal-like vibrations in AOM ears at the frequency of 1 kHz.

DISCUSSION

Comparison with Previous Studies of TM Motion in Chinchilla

Umbo displacements in normal chinchilla ears have been reported in previous studies (Ruggero et al. 1990; Thornton et al. 2013; Ravicz and Rosowski 2013; Guan et al. 2014). In this study, the umbo displace-

FIG. 10. TM deflection pattern measured by SLDV in the control and the 8D AOM ears at six different phases along an oscillation cycle at a frequency of 1 kHz. Column 1 shows displacement maps of the control ear; column 2 shows displacement maps of 8D AOM

ear before release of middle ear pressure (AOM-1); column 3 shows displacement maps of 8D AOM ear after release of middle ear pressure (AOM-2)

ments extracted from the TM surface motion of control ears (shown in Fig. 6b) were about two times lower than those reported by Ruggero et al. (1990) and Guan et al. (2014). It should be noted that, in the present study, the angle of the laser beam relative to the tympanic annulus plane was estimated to be approximately 60–70°. Because of the conical shape of the TM, the normal direction of the TM surface varies with location. At a given measurement point, the angle between the surface normal and the laser beam was difficult to determine due to a lack of quantitative surface normal information. Thus, angle correction could not be meaningfully performed on the experimental data. This could introduce a system-

atic error into the measured displacement. Based on the geometry data of the chinchilla TM reported by Vrettakos et al. (1988), we calculated the cone angle of the chinchilla TM to be about 133°. Due to the angle of the laser beam relative to the tympanic annulus plane being approximately 60–70°, the inferior quadrants of the TM were nearly perpendicular to the laser beam, but the superior quadrants, including the umbo, were on the order of 45° relative to the measuring light path. Therefore, the conical shape of the chinchilla TM caused the vibration amplitudes of the umbo and the superior portion of the TM to be underestimated. Similarly, a recent report by Rosowski et al. (2013) using opto-electronic

computer-assisted holography also showed that the absolute value of the umbo displacements was a factor of three times smaller than the mean chinchilla umbo displacements reported by Ruggero et al. (1990). Therefore, one of the possible explanations for the umbo displacement difference between the previous work and this study may be attributed to the angle correction and experimental setup used in the TM surface motion measurement.

Regarding vibration patterns or modes of the normal chinchilla TM, Rosowski et al. (2009) performed an analysis of the TM surface motion by time-averaged holograms. Although the spatial resolution of time-averaged holograms is much higher than that in the present study, the motion features of the TM surface at low frequencies were captured in this study. We found that a simple TM motion pattern occurred at low frequencies, and the standing wave dominated TM motion at frequencies below 2 kHz. Additionally, the entire TM surface of control or normal chinchillas moved in phase with the largest motion being in the posterior region at 1 kHz (Fig. 10). Similar patterns were observed in human and cat TMs (Guinan and Peake 1967; Rosowski et al. 2009; Cheng et al. 2010). However, in this study, the relatively small number of measurement points (about 265) meant that it was possible to capture only motion features no less than 0.4 mm in spatial size. The observation of the TM motion features at high frequencies depends on high spatial resolution of the measuring technique. Thus, the fine structures of TM motion could not be determined in this study, and the high frequency displacement patterns looked rather simplistic compared to the patterns with multiple local minima and maxima reported by Rosowski et al. (2009, 2013). In order to capture the complex motion at high frequencies, a more refined arrangement of measurement points on the TM would be needed.

Guan et al. (2014) reported that both 4D and 8D AOM resulted in reduction of the umbo displacements across all frequencies, and the displacements at 8D ears did not differ significantly from that of 4D ears at frequencies below 1.5 kHz. In this SLDV measurement, the mean umbo displacement of 4D AOM ears was 12 dB lower than that of control ears, and the 4D and 8D AOM ears did not exhibit significant difference in umbo displacements below 7 kHz as shown in Fig. 6b. The discrepancy between the umbo displacement here and those of Guan et al. results is most likely due to the smaller MEE volume in this study. Guan et al. measured a difference of MEE volume between 4D and 8D ears of 0.4 ml, while here we measured a difference of MEE volume between 4D and 8D of 0.26 ml. Because the TM movement becomes more mass-dominated at higher frequencies, the large difference of MEE volume between 4D and 8D AOM ears in Guan's study resulted in a larger displacement difference observed at high frequencies.

Effect of AOM on TM Movement

Comparing the TM motion patterns of the untreated control ears to the AOM ears, three basic trends were observed: (1) traveling-wave-like motion occurred in AOM ears at lower frequencies than in control ears, (2) the TM displacement in response to sound stimulation in AOM ears was generally reduced, and (3) AOM resulted in alteration of the frequency dependence of TM displacement.

In normal chinchilla ears, the sound-induced TM surface vibration exhibited standing-wave-like motion at frequencies below 2 kHz, and a combination of traveling-wave-like motions and modal motions at higher frequencies. The measured results from both AOM-1 and AOM-2 ears indicated that the traveling-wave-like motion component occurred at frequencies between 0.4 and 0.8 kHz in combination with modal-like motions. After the MEP was released (AOM-2), the traveling-wave component could still be observed in both 4D and 8D AOM ears at these low frequencies. This suggests that the traveling-wave component occurring in the low frequency range is fundamentally due to the presence of MEE, rather than the MEP. The traveling-wave motion at the low frequencies is a result of fluid-structure interaction between the TM and MEE. Such a traveling-wave motion component induced by AOM may affect the temporal properties of sound energy transmission from TM to ossicular chain.

Release of MEP had a significant effect on the TM displacements of AOM ears. In 4D ears as shown in Fig. 7, an average 6.7 dB increase at Q1 and Q2 locations was observed. At Q3 and Q4 locations, a mean displacement increase of 10 dB occurred below 2 kHz. These results indicate that the displacement increase in the inferior portion of the TM was higher than that in the superior portion owing to the release of MEP. The effects of MEP variation on the umbo motion and middle ear input admittance have been investigated (Guinan and Peake 1967; Wever and Lawrence 1954; Lee and Rosowski 2001). These earlier results demonstrated that increased middle-ear static pressure can reduce middle-ear sound transmission or admittance at low frequencies. In our present study, the increase of TM displacement upon releasing static MEP was not uniformly distributed over the four quadrants of the TM. This shows that the TM vibrations at various locations have different sensitivities to static MEP.

Figure 8 compares the TM displacement in 4D and 8D AOM ears after the MEP was released. The displacement differences between 4D and 8D ears at the AOM-2 stage may be attributed to the effects of MEP and infection-induced middle ear structural changes on the TM motion during the course of

AOM (Guan et al. 2014). The displacement differences observed at the Q3 and Q4 locations were larger than those at the Q1 and Q2 locations even at low frequencies. This indicates that the development of AOM also affects the TM motion at low frequencies even if MEP is released.

Considering the spatial variations of the TM displacement shown in Fig. 9a, the displacement variations at the umbo were consistent with those at the centers of the four quadrants, suggesting a strong spatial dependence of TM motion in normal ears. In the unopened 4D and 8D AOM ears (AOM-1 stage), the displacements of the umbo and quadrant centers contained a series of minor peaks, but those peaks occurred at different frequencies at the different locations. Such differences in the peak frequencies suggest a weakened spatial dependence of TM displacements in the 4D and 8D AOM ears. Figure 9c shows that release of MEP leads to more consistent variations in the umbo and quadrant displacements in 4D AOM-2 ears than in 4D AOM-1 ears (Fig. 9b) at frequencies below 5 kHz. This observation indicates that MEP is a primary factor contributing to the altered spatial dependence of the TM motion in 4D AOM ears. For 8D AOM ears, the displacements at the four quadrants showed fluctuations that were different from those of the umbo at high frequencies even if MEP was released. Although it is not completely clear how different portions of the TM motion couple to the umbo, the displacement fluctuations of the umbo and quadrant centers in AOM ears occurring at various frequencies indicate that spatial dependence of the TM motion is altered by AOM. This is consistent with AOM having different effects on the frequency dependence of the transfer function at the umbo. Therefore, the altered spatial dependence of the TM motion in AOM ears may interrupt the efficient conduction of sound energy through the middle ear. Apart from the reductions in TM mobility, MEE also conducts a portion of sound energy and alters input to the round window (Thornton et al. 2013), and MEP also acts on the round window membrane and alters the compliance of the membrane. As a result, the presence of MEP and MEE restricts the motion of the round-window membrane. This in turn may reduce the mechanical response level of the inner ear. Therefore, it would be valuable to establish the relationship between the variations in AOM-induced TM displacement and the inputs to the inner ear in future studies.

Factors Affecting Measurement Results

To increase the light reflection from the membrane, the lateral surface of the TM was lightly painted with a suspension of TiO_2 powder in this study. It should be

noted that the TM vibration was measured at a series of discrete points, so a relatively thin and discontinuous coating could be employed in this study. The effects of TiO_2 measured by Rosowski et al. (2009) suggested that the paint-induced changes of stapes motion occurred at the higher frequencies, indicating that the TiO_2 painted on the TM mainly led to an increased TM mass. To investigate the effect of painting material on TM vibration, the finite element model of the chinchilla ear developed previously (Wang and Gan 2016) was used to calculate the TM displacement change caused by applying a layer of material (TiO_2). It has been assumed that the physical interactions of discrete TiO_2 particles are low enough that the coating contributes to mass but not to stiffness. The mass density of TiO_2 powder was set to be 4230 kg/m^3 . The painted layer was first assumed to be equivalent to a $1\text{-}\mu\text{m}$ thick layer applied on the lateral surface of the TM, and the total weight of the painted layer was 0.325 mg . Figure 11 shows the changes of the TM displacement at the umbo and the centers of the four quadrants across frequency from 0.2 to 10 kHz . At frequencies below 1.5 kHz , the maximum displacement change was only 0.38 dB due to the paint. At high frequencies, the displacement level of the painted TM was nearly 1 dB lower than that of the unpainted TM. When the thickness of the painting layer was assumed to be equivalent to $2 \mu\text{m}$, the displacement curves had similar patterns to those of $1 \mu\text{m}$ thickness, but the maximum displacement change was -2.5 dB , occurring at the posterior-inferior quadrant as shown in Fig. 11. In addition, the painted TM did not produce observable alterations to the basic patterns of the TM displacement. The TiO_2 particles are about 0.4 to $0.6 \mu\text{m}$ in diameter, and we used approximately two to five times the particle size to simulate the painted layer. The computational results suggest that the influence of a painted material on the TM motion is relatively small when the equivalent thickness of the painted layer is less than $2 \mu\text{m}$.

In anesthetized animals, static pressure changes in the middle ear cavity are another source affecting the TM displacement measurements. At the AOM-2 stage, the bullae were resealed following release of MEP. Resealing the bulla possibly caused build-up of MEP for the anesthetized animals (Guinan and Peake 1967). In investigating the effect of closing the bulla of anesthetized cats, Guinan and Peake (1967) discovered that the middle ear transmission slowly decreased within 10 min (in their Fig. 18) after the bulla was closed, but middle ear transmission decreased more than 20 dB after the bulla was closed for 60 min . In an earlier experiment, we conducted some preliminary observations in two ears of anesthetized chinchillas to assess the build-up of MEP. In one ear,

FIG. 11. Simulated displacement changes of the TM painted with TiO_2 . The sound-induced displacements at the umbo and centers of four quadrants were calculated using a finite element model. The

ratio of displacements between the painted TM and the unpainted TM has been converted to decibels. The painted layer was assumed to be equivalent to 1 and 2 μm thick, respectively

there was no MEP change in 60 min of anesthesia, which indicated that a negative MEP did not build up in this animal. In the other chinchilla ear, the middle ear cavity maintained a constant pressure following a MEP variation from -45 to -165 daPa in the first 60 min of anesthesia. After releasing MEP by a small hole drilled into the bulla and resealing the hole, the MEP did not show any change within 10 min. In the present study, the SLDV measurement was performed about 45–60 min after initiating anesthesia because of surgical preparation, wideband tympanometry measurement, and setup for SLDV. While the measurements of AOM-2 stage were conducted, we managed to keep the measurement time of each ear to less than 10 min following release of MEP to overcome the possible effect of MEP variation. The measured TM displacement appeared reasonable when comparing the data of AOM-2 stage with those of AOM-1 stage. Additional tests with a vent tube placed in the bulla to prevent static pressure changes may be used to improve this measurement in the future.

In this study, the TM surface motion in the presence of MEE was measured, but the area of TM covered by MEE was not quantitatively identified in the experimental animals. There were individual variations in the amount of MEE between the ears. Additionally, in the present study, we did not measure TM motion after the MEE was aspirated from the middle ear cavity. It is commonly believed that the TM motion changes induced by MEE are volume- and frequency-dependent. In order to quantitatively analyze the vibration characteristics of the TM in relation to the amount of MEE, we need to better define the small features present in the TM motion in future studies. Moreover, other measurements such as auditory brainstem response (ABR) or cochlear microphonics will be helpful in identifying the contribution

of TM motion changes to the conductive hearing loss caused by AOM.

CONCLUSION

Measurements of full-field TM motion were performed by using SLDV in normal and AOM chinchillas. The results showed that AOM reduced the movement of the TM across the entire surface. The reduction in the umbo displacement over the time course, from 4 to 8 days post inoculation, was less than the reduction in the displacement at the centers of the four quadrants. The frequencies where a traveling-wave component of the TM motion occurred were lower in AOM ears than in normal ears. Release of middle ear pressure did not change the frequencies where traveling-wave motion occurred.

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