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Dynamic Properties of Round Window Membrane in Guinea Pig Otitis Media Model Measured with Electromagnetic Stimulation

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Abstract

The round window, one of two openings into the cochlea from the middle ear, plays an important role in hearing and is known to be structurally altered during otitis media. However, there have been no published studies systematically describing the changes in biomechanical properties of the round window membrane (RWM) that accompany bacterial otitis media. Here we describe the occurrence of significant changes in the dynamic properties of the RWM between normal guinea pigs and those with acute otitis media (AOM) that are detectable by electromagnetic force stimulation and laser Doppler vibrometry (LDV) measurements. AOM was induced by transbullar injection of *streptococcus pneumoniae* into the middle ear, and RWM specimens were prepared three days after challenge. Vibration of the RWM induced by coil-magnet coupling was measured by LDV over frequencies of 0.2–40 kHz. The experiment was then simulated in a finite element model, and the inverse-problem solving method was used to determine the complex modulus in the frequency domain and the relaxation modulus in the time domain. Results from 18 ears (9 control ears and 9 AOM ears) established that both the storage modulus and loss modulus of the RWM from ears with AOM were significantly lower than those of RWM from uninfected ears. The average decrease of the storage modulus in AOM ears ranged from 1.5 to 2.2 MPa and the average decrease of the loss modulus was 0.025 to 0.48 MPa. Our findings suggest that middle ear infection primarily affects the stiffness of the RWM due to the morphological changes that occur in AOM ears. We also conclude that the coil-magnet coupling method for assessment of RWM function may provide a valuable new approach to characterizing the mechanical response of the RWM when reverse driving is selected for middle ear implantable devices.

Keywords

round window membrane; *Streptococcus pneumoniae*; complex modulus; laser vibrometry; finite element model; otitis media

INTRODUCTION

The round window is one of the two openings into the cochlea from the middle ear. The round window membrane (RWM) vibrates with a phase opposite to the vibration of the

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stapes at the oval window, the other opening of the cochlea to the middle ear. Mechanical properties of the RWM affect cochlear fluid motion and, thus, the movement of the basilar membrane where the hair cells are located.

The RWM serves as a barrier between the middle ear cavity and cochlea and plays an important role in middle ear and cochlear mechanics of normal and diseased ears (Paparella et al., 1983; Nomura, 1984; Hellstrom et al., 1997; Nordang et al., 2001). Acute otitis media (AOM), one of the most common infectious diseases in young children, is caused by bacterial or viral infection of the middle ear and is characterized by middle ear inflammation and fluid accumulation. AOM causes morphological and material property changes of middle ear tissues including the tympanic membrane and RWM, and it results in conductive hearing loss. Animal experiments also show that inflammatory mediators and bacterial products from the middle ear can enter the inner ear via the RWM and cause inner ear damage (Ikeda and Morizono, 1988; Goycoolea and Lundman, 1997; Yoon and Hellstrom, 2002; Shachern et al., 2008).

The RWM consist of three layers: the outer epithelium facing the middle ear, a layer of connective tissue, and an inner epithelium adjacent to the inner ear (Goycoolea and Lundman, 1997). The connective tissue layer contains collagen fibers, other elastic fibers, and fibroblast and provides the main structural support for the RWM. The RWM is thicker at the edge and becomes thinner towards the center. This thickness change produces a slight convexity toward the scala tympani (Carpender et al., 1989). The mean thickness of the human RWM is approximately 70 μm (Goycoolea and Lundman, 1997). The RWM of animals (e.g., rodents such as the rat, chinchilla, guinea pig) generally is much thinner than that of humans. For example, RWM mean thickness is 11.5 μm in rats (Yoon and Hellstrom, 2002) and 11–14 μm in chinchillas (Goycoolea et al., 1988).

Changes in the thickness and ultrastructure of the RWM associated with otitis media have been observed both in human patients and in experimental animal models (Sahni et al., 1987; Goycoolea, 1995; Yoon and Hellstrom, 2002). Yoon and Hellstrom reported that the thickness of rat RWMs increased 3-fold by the third day post challenge with *Streptococcus pneumoniae* when compared to uninfected control animals. The RWM exhibited severe changes, characterized by inflammation in the connective tissue layer with edema and invasion of inflammatory cells. An increased thickness of the RWM in chinchillas following administration of various pneumococcal proteins also has been reported by Schachern et al. (2008). However, no prior studies have documented changes in the mechanical properties of the RWM associated with otitis media. The present study was undertaken to determine if such changes actually occur and, if so, to quantify the magnitude of their effects.

A long term goal of our research is to establish a new method to characterize the mechanical response of the RWM when reverse driving on the RWM is selected for middle ear implantable hearing devices. Toward that end we recently developed a new technique to measure the dynamic properties of ear tissues, including the human eardrum and the RWM (Zhang and Gan, 2010; 2012). This technique is based on the use of acoustic driving to deform ear tissue specimens and the measurement of the outcome effects by the laser Doppler vibrometry (LDV). An inverse-problem solving method then is used to derive the complex modulus over the frequency domain. Our approach is similar to the coil-magnet driving or reverse driving used to measure dynamic responses for implantable middle ear devices (Colletti et al., 2009; Beltrame et al., 2009). Prior to our current investigation we conducted a preliminary study to evaluate the effects of electromagnetic force stimulation on the RWM (Nakmali et al., 2010). Here we report the first complete description of the dynamic properties of the RWM in both normal guinea pigs and those with bacterial AOM

based on electromagnetic force stimulation and LDV measurement over frequencies from 0.2–40 kHz.

METHODS

A. AOM model and RWM specimen preparation

A-1 Creation of AOM model—Adult Hartley guinea pigs (n=18) of mixed gender weighing between 280–380 g were utilized in this study. The study protocol was approved by the IACUC of the University of Oklahoma and met the guidelines of the National Institutes of Health. Prior to bacterial challenge, all animals were free of middle ear disease as evaluated by otoscopic examination.

Guinea pigs were randomly divided into two groups: Control – no treatment to either ear (5 animals for 9 control ear samples) and AOM – left ear only inoculated with *Streptococcus pneumoniae* (9 animals for 9 AOM ear samples). In our preliminary study, several right ears showed mild cross-infection after bacteria inoculated in the left ear. Thus, the right ears of the inoculated animals were not used as the control ears. Specimens from one animal of each group were used for histology and specimens from one animal of each group (control and AOM) were used to obtain scanning electron microscope (SEM) images of the RWM.

The AOM model was created by injection of *Streptococcus pneumoniae* type 3 (Sp3) suspension into the left middle ear following the procedure reported by Dai et al. (2009). Briefly, under general anesthesia, the skin of the mastoid area was disinfected with povidone-iodine and a transbullar injection of about 0.1 ml Sp3 suspension at a concentration of $1.3\text{--}2.4 \times 10^6$ CFU/ml (ATTC 6303, Manassas, VA) was performed using a 1cc syringe with 26 gauge needle under a surgical microscope. The right ear was untreated.

Three days post inoculation, the animals were anesthetized with ketamine (40 mg/kg) and xylazine (10 mg/kg). The tympanic membrane (TM) was examined under otoscopy to determine the color of the TM as well as the presence of an effusion in the middle ear. The middle ear compliance and pressure were then measured using a multiple-frequency tympanometer (MAICO MI24, MAICO Diagnostics, Eden Prairie, MN) to confirm the presence of AOM in the left ear. In control ears, the TM was visualized to confirm normal color without an effusion in the middle ear cavity. Then, the animals were euthanized by an overdose of ketamine (100 mg/kg) and xylazine (20 mg/kg) and the bullas were harvested to prepare the RWM samples for mechanical tests as well as the histology and SEM studies.

A-2 Preparation of RWM specimen for dynamic testing—Nine ears from the control group and nine left ears from the AOM group were used for preparing RWM specimens. The bulla or temporal bone was trimmed by removing the bulla bony wall and cut into a block containing the malleus-incus complex, stapes, cochlea, and promontory (Fig. 1A). Then, a cut at the inferior edge of the footplate created a flat surface to open the view of RWM from the scala tympani. The round window niche was visualized just next to the lower edge of the footplate and the bone near the niche was removed to expose the RWM from both sides: the middle ear or lateral side and the cochlear or medial side. The membrane was shown intact and attached to the bony wall (Fig. 1B). Note that a magnet bead was placed on the RWM as shown in Fig. 1B.

Specimens were examined under a microscope to verify that the RWM was not damaged during the preparation. A magnetic microbead shaped like a disc 250 μm in diameter and 125 μm in thickness with a mass of 0.03 mg (Fair-Rite Products Corp., NY) was placed on the medial side at the center of the RWM. The bead was held in place by the moistened

mucosal tissue on the RWM. Figure 1B shows the photograph of a RWM specimen with the microbead taken by a CCD camera. The RWM has an elliptical shape with the long axis (a) and short axis (b). Tables 1 and 2 list the dimensions of a and b measured from each specimen using the image analysis tools (Adobe Photoshop 7.0) in control and AOM ears, respectively. The average long and short axes for control RWM were 1.28 and 0.86 mm, respectively; the average long and short axes for AOM ears were 1.34 and 0.85 mm, respectively. The average mass for control RWMs was 0.041 mg and for AOM samples was 0.098 mg.

B. Experimental setup

A laser Doppler vibrometer (HLV-1000, Polytech PI, Tustin, CA) was used to measure the vibration of the RWM induced by electromagnetic force stimulation, a technique developed in the mid-90s to measure viscoelasticity of cell membrane and cytoplasm (Bausch et al., 1999; Feneberg et al., 2004). Figure 2 shows a schematic diagram of the experimental setup and indicates that the vibration of the magnet at the center of RWM was measured by LDV along the vertical direction. The electromagnetic coil made from the wire (AWG 30) of 500 turns and the high μ steel core (Carpenter Technologies Inc., IN) had the inductance of 2.41 mH and resistance of 3.9 Ohm. The coil was placed directly under the RWM to vibrate the bead attached to the membrane. The distance between the coil and bead was maintained at 1 mm. This minimum distance was confirmed under a microscope to ensure that no movement of the bead occurred secondary to the static attraction force between the steel core and magnet bead, and to verify the small static force would not affect the measurement. The coil was driven by the dynamic signal generator/analyzer (DSA, HP35670A, Agilent Tech., CO) and the power amplifier (B&K 2718, Denmark) and an AC current of 16.7 mA was delivered to the coil sweeping from 0.2 to 40 kHz. The laser beam was focused onto the microbead, and the vibration of RWM was acquired by the DSA and recorded on a computer for further analysis. The vibration amplitude of the RWM was directly calculated from the voltage output of the laser vibrometer velocity decoder. Note that it is difficult to keep the force acting between the magnet and coil constant over the frequency range. However, in this study the magnetic bead, the coil, and the distance between the bead and coil were maintained the same for each sample test and the driving force was mainly determined by the current delivered to the coil (Fung, 1993; Ethiraj, 2003).

It is well known that the stabilized mechanical state of biological soft tissue is only reached after preconditioning in which the loading-unloading process has been repeated for at least 3–5 cycles prior to the mechanical test (Fung, 1993). Because of the extremely small size and fixed bony boundary of the RWM specimen, it was difficult to carry out standard preconditioning procedure in a mechanical testing system. As an alternative method, cyclic hydraulic pressure was applied onto the specimen surface by using saline solution in a 1 cc syringe. The peak pressure was estimated around 10 Pa. Movement of RWM was observed under a surgical microscope. This process was repeated for 5 cycles to reach the stabilized state for specimen. Note that the RWM specimen was maintained under moist conditions by spraying saline solution onto the middle ear side surface.

C. Finite element modeling of dynamic tests

Dynamic tests of the RWM specimens were simulated as finite element (FE) models in ANSYS (ANSYS Inc, Canonsburg, PA). The FE model-based inverse-problem solving method was used to determine dynamic properties of the RWM in control and AOM ears.

C-1 FE model of dynamic test—Each RWM specimen was simulated as a FE model of the elliptical membrane with long axis a and short axis b as listed in Tables 1 and 2 for the control and AOM samples, respectively. The thickness of the RWM changed from 30 μ m at

the center to 50 μm at the edge for control RWM based on the published data. For the RWM in AOM, the thickness varied from 80 μm at the center to 100 μm at the edge based on the published data and our measurements on histological sections of the RWM specimens. The magnetic microbead was modeled as a disc with diameter of 250 μm and thickness of 125 μm . Nine FE models of normal RWM and nine models of AOM specimens were created. Figure 3A shows the planar view (medial side) of the FE model of one RWM sample for dynamic test with the magnetic driving force applied at the microbead at the center. The lateral view of the model for the control specimen with fixed boundaries is shown in Fig. 3B and the lateral view of the model for AOM specimen with partially fixed boundaries is shown in Fig. 3C.

The RWM was meshed by 6000 hexahedral elements (type Solid 185) and the convergence analysis showed that this element number was adequate to reach accurate results. The density of the RWM was assumed to be 1200 kg/m^3 , the same as that of the tympanic membrane (Zhang and Gan, 2010). The microbead was meshed by 1600 hexahedral elements (type Solid 45). The density and elastic modulus of the bead was set as 5000 kg/m^3 and 210 GPa, respectively. Displacements of the nodes on boundaries between the RWM and magnet were coupled in the FE model and there was no relative motion between the RWM and magnet surface. As shown in Figs. 3B and 3C, based on observations in histological sections (displayed in Fig. 5), the normal RWM was fully clamped at the edge without free displacement, while only one third of the edge or boundary nodes was clamped for the RWM in AOM situation. The driving force of 0.03 μN applied onto the magnet surface was calculated through the electromagnetic analysis in ANSYS using the method developed in our lab (Ethiraj, 2003). All RWM specimens were considered to be isotropic linear viscoelastic materials, and material properties are described in the next section.

It is noticed that the boundary condition of the RWM would affect the FE model-derived results. A simple support of the tympanic membrane was reported in gerbil middle ear model by Buytaert et al. (2011). However, the boundary condition of the RWM used for this study was based on observations in histological sections. If the boundary condition changed from fully clamped to simple support, an increase of elastic modulus of the RWM was expected.

C-2 Material constitutive law for RWM specimen—Biological soft tissues usually show viscoelastic properties, and their dynamic behaviors are time or frequency dependent (Fung, 1993). The standard linear solid model (Machiraju, et al., 2006) was used to describe the viscoelastic behavior of RWM. The relaxation modulus of RWM is 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 $t = \infty$, $(E_0 + E_1)$ is the initial elastic modulus at time $t = 0$, and τ_1 is the relaxation time. For harmonic analysis, $E(t)$ can be converted into 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, ω is the angular frequency, and $E'(\omega)$ and $E''(\omega)$ can be expressed as:

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

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

or

$$\eta(\omega) = \tan \delta = E''(\omega) / E'(\omega) \quad (5)$$

where δ is the phase angle of the complex modulus, $\eta(\omega)$ is the loss factor, defined as the ratio of the loss modulus to the storage modulus. The frequency-dependent storage modulus $E'(\omega)$ and loss factor $\eta(\omega)$ are applied into FE model as the material properties of the RWM.

C-3 Theoretic basis for vibration measurement—Classic vibration theory of thin membrane was used to analyze the motion of the RWM specimen, similar to that used in our previous study on tympanic membrane (Zhang and Gan, 2010). Briefly, the forced vibration of the RWM induced by driving force or pressure at the center is governed by:

$$\nabla^2 w + \frac{\bar{m}}{\alpha E^* h} \frac{\partial^2 w}{\partial t^2} = P \quad (6)$$

where ∇ is the Laplacian operator, w is the transverse displacement of the membrane, $\bar{m}$ is the mass per unit area, P is the pressure force applied onto the RWM, h is the thickness, α is a coefficient related to the RWM dimension, and E^* is the complex modulus as shown in Eqs. 2–5. For harmonic vibration, the primary resonance frequency ω_n can be expressed as

$$\omega_n = \sqrt{\frac{\beta E'(\omega_n)}{\bar{m}}} \quad (7)$$

where β is a coefficient related to the RWM dimension and Poisson's ratio. Eq. (7) shows that the resonance frequency ω_n is proportional to the square root of the storage modulus E' . This provides the theoretical basis for mechanical properties of the RWM to be determined by measuring the vibration resonance.

Moreover, when $\omega = 0$ (static deformation state), the displacement at the center of RWM, w_0 becomes

$$w_0(0) = \frac{P_0}{\alpha E'(0)} = \frac{P_0}{\alpha E_0}, \quad (8)$$

where P_0 is the amplitude of pressure force applied on the membrane; when $\omega = \omega_n$ (resonance),

$$w_0(\omega_n) = \frac{P_0}{\alpha E'(\omega_n)} \frac{E'(\omega_n)}{E''(\omega_n)} = \frac{P_0}{\alpha E'(\omega_n)} \frac{1}{\eta(\omega_n)}. \quad (9)$$

Thus, the displacement amplification ratio R , defined as the displacement at resonance frequency over the displacement at static state, can be expressed as

$$R = \frac{w_0(\omega_n)}{w_0(0)} = \frac{E_0}{E'(\omega_n)\eta(\omega_n)} \quad (10)$$

Equations (7–10) show that the resonance frequency ω_n , displacement amplitude at static state $w_0(0)$ (approximately using the amplitude at 200 Hz), and displacement amplification ratio R are all related to frequency-dependent storage modulus E' and loss factor η of the RWM. Finally, three parameters, E_0 , E_1 , and τ_1 (Eqs. 3–5) can be determined through the measurements of ω_n , $w_0(0)$, and R . However, it is difficult to derive the theoretical solution by solving equations above. In this study, the FE modeling and inverse-problem solving method were used to determine the parameters (E_0 , E_1 , and τ_1) and the complex modulus E^* .

C-4 Inverse-problem solving method and validation of measurements on RWM

—The inverse-problem solving approach was used to determine three parameters, E_0 , E_1 , and τ_1 , and finally to obtain the complex modulus of the RWM. The FE model of each RWM sample was used to verify these parameters as well as the complex modulus by comparing the FE model-derived displacement-frequency curve with the experimental measurement curve in three characteristics: the resonance frequency ω_n , displacement amplitude at low frequency $w_0(0)$, and displacement amplification ratio R . A brief description of the optimization process includes four steps: 1) The initial values of 1.0 MPa, 0.8 MPa and 30 μ s were selected for E_0 , E_1 , and τ_1 , respectively, based on the published value range (Zhang and Gan, 2012). 2) The storage modulus $E'(\omega)$ calculated by Eq. (3) and loss factor $\eta(\omega)$ by Eq. (5) were inputted into the model. 3) Harmonic analysis of the FE model was then conducted from 200 Hz to 40 kHz with an interval of 50 Hz. Vibrations of the central node of the RWM induced by magnetic driving force were calculated from the FE model. 4) The model-derived displacement-frequency curve was compared with the experimental curve for each specimen. The correlation coefficient between the two sets of data was calculated. If the coefficient met the set value, the process was ended and the parameters of E_0 , E_1 , and τ_1 were determined. If the correlation coefficient did not meet the set value, E_0 , E_1 , and τ_1 were then adjusted based on Eqs. (7–10), and the iteration process was repeated from step 2). Through this process, the values of E_0 , E_1 , and τ_1 were optimized and determined. Finally, E_0 , E_1 , and τ_1 were substituted into Eqs. (3–5) to obtain the complex modulus and loss factor of the RWM as functions of frequency.

To validate that the inverse-problem solving method is capable of providing reliable parameters for the tested RWM, we performed the same experiment and FE modeling process as described in the above sections on aluminum foil (Reynolds Wrap, Richmond, VA). The aluminum sample had a diameter of 6.3 mm, thickness of 0.018 mm, and density of 2700 kg/m³. In addition to a single magnet located at the center of the foil, we placed the magnet bead at several different locations along the aluminum sample's radius. The experimental measurements showed that the resonance frequency was around 3400 Hz for the foil with the magnet at different locations and the phase curves were all flat over frequencies of 100 Hz to 40 kHz. The inverse-problem solving with the FE model of the aluminum foil sample resulted in an elastic modulus of 48 GPa that was close to 46.7 GPa of elastic modulus of the foil obtained from the DMA testing machine (Bose ElectroForce 3200, Eden Prairie, MN). Thus, it was confirmed that the electromagnetic stimulation with LDV measurement and the inverse-problem solving method are reliable to measure viscoelastic parameters for the RWM samples.

RESULTS

A. Observation of morphological changes of RWM in AOM

The SEM images obtained from a control specimen and an AOM specimen of the RWM are shown in Fig. 4 with the view from the middle ear side. The RWM in the control ear (Figs. 4A and 4B) shows the smooth outer epithelial surface (Fig. 4A). The thickness of the epithelial layer is thin and the core connective tissue layer clearly demonstrates the fibrous structure (Fig. 4B). The RWM in the AOM ear (Figs. 4C and 4D) is much thicker than that in control ear. There is an obvious increase in the thickness of the outer epithelial layer of the AOM ear.

Figure 5 shows the histological section images collected from the control (Fig. 5A) and AOM (Fig. 5B) ears. Note that only a partial RWM of the control ear was shown in Fig. 5A. The average thickness of the control RWM was about 0.04 mm (0.03–0.053 mm) and the average thickness of AOM RWM was about 0.092 mm (0.08–0.11 mm). The thickness of RWM in AOM was 2 to 3-fold greater than that of the control, which is consistent with the observation reported in AOM ears of the rats (Yoon and Hellstrom, 2002). It is also noticed that the RWM was thicker peripherally than centrally. The convex shape of the membrane towards the scala tympani was clearly demonstrated in AOM ears.

B. Experimental measurement data and FE modeling results

Figure 6 shows the displacement amplitude-frequency curves measured from nine control RWM specimens (Fig. 6A) and nine AOM specimens (Fig. 6B). In control group, each specimen has a prominent displacement peak at a resonance frequency between 1482 and 1935 Hz. The displacement at low frequencies (below 500 Hz) ranged between 5 and 12 nm (except for one sample). The resonance frequency $f_n = \omega_n/2\pi$ and displacement amplification ratio R of each RWM specimen are listed in Table 1. The mean resonance frequency was 1686 ± 180 Hz. The displacement amplification ratio R ranged between 3.8 and 9.6 with a mean value of 6.6 ± 1.9 .

In the AOM group, each specimen has a peak displacement at a resonance frequency between 1266 and 1696 Hz which is lower than the resonance frequency measured from control samples. The displacement at low frequencies (<500 Hz) also ranged from 5 to 12 nm. Table 2 lists the resonance frequency and amplification ratio R of each RWM specimen. The mean resonance frequency was 1452 ± 163 Hz for AOM samples. The displacement amplification ratio R was ranged between 4.3 and 9.7 with a mean value of 6.5 ± 2.0 .

FE models were created to simulate the dynamic experiments of the RWM specimens in control and AOM groups. As an example, Fig. 7 shows the results from two specimens in each group (control, upper; AOM, lower). As shown in this figure, the displacement amplitude-frequency curves derived from the FE models (dashed lines) and measured from the experiments (solid lines) match well in the resonance frequency, peak amplitude, and the amplitude at low frequencies. Specimens Control-4 and Control-7 had experimental resonance frequencies at 1504 and 1884 Hz, respectively. The resonance frequency of the corresponding FE model was at 1500 and 1900 Hz, respectively. Similarly, specimens AOM-2 and AOM-7 had experimental resonance frequencies at 1335 and 1671 Hz, while the corresponding model resulted in resonance frequencies of 1350 and 1650 Hz. In general, the FE modeling results correspond well with the experimental data over the frequency range, particularly near the resonance frequency. Some very small peaks at lower frequencies (for example, at 1 kHz of AOM-7) were not considered as the primary resonance peak. The curves from the FE model were obtained under ideal conditions and had no such artificial peaks. The potential resources of these peaks may result from the sound distortion or the experimental setup.

Three parameters E_0 , E_1 , and τ_1 for each RWM specimen in control and AOM groups were determined through the inverse-problem solving with the FE model and are listed in Tables 1 and 2. In the control group, the value of E_0 ranged from 1.35 to 2.22 MPa, E_1 ranged from 0.90 to 1.65 MPa, while τ_1 ranged from 18 to 24 μ s. In AOM group, the value of E_0 ranged from 0.27 to 0.47 MPa, E_1 ranged from 0.16 to 0.36 MPa, while τ_1 ranged from 20 to 25 μ s. These results show that the values of E_0 and E_1 for AOM specimens are lower than that for control specimens, but τ_1 values are in the similar range for both AOM and control samples. There are some differences between the specimens for each parameter within both groups, which may be due to individual variation between the RWM specimens (physiological conditions, geometric dimensions) and the variation between the experimental setups.

C. Dynamic properties of the RWM in AOM ears

Based on parameters E_0 , E_1 , and τ_1 obtained from each specimen, the storage modulus E' , loss modulus E'' , and loss factor η of all specimens in control and AOM groups were calculated and displayed across the frequency range of 0.2 to 40 kHz in Fig. 8. Considering the E' and E'' values for AOM specimens much smaller than that for controls, we used different scales for E' and E'' in control and AOM groups. As shown in the upper panel of Fig. 8, the storage modulus increased with frequency for all specimens in control and AOM groups with a similar pattern of variation with frequency. The storage modulus was almost constant at the frequency below 1 kHz, and slightly increased at 1–3 kHz. The increasing rate of storage modulus reached a maximum at frequency of 3–10 kHz, and then decreased from 10 to 40 kHz.

The change of loss modulus with frequency shows a different behavior from the storage modulus as displayed in the middle panel of Fig. 8. There was a peak loss modulus for RWM specimens in both control and AOM groups at the frequency of 7–9 kHz. As frequency decreased to 100 Hz or lowest, the loss modulus decreased and approached to zero. As frequency increased to 40 kHz, the loss modulus decreased to an average of 0.24 MPa for control and 0.05 MPa for AOM. Variations in both storage modulus and loss modulus for RWM specimens in control and AOM ears shared similar patterns, but were centered around different values as demonstrated in Fig. 9.

The bottom panel of Fig. 8 displays the loss factor η for each specimen in control and AOM groups. A peak value was observed between the frequencies of 4.5–6.5 kHz in both groups. The peak value of nine control RWM specimens ranged from 0.38 to 0.21 and the peak value of nine AOM specimens ranged from 0.35 to 0.23. It shows that the peak value of loss factor η was in the same level for control and AOM samples, which indicates that the dampening behavior of the RWM does not change much in AOM ears.

Mean values of the complex modulus and loss factor with the S.D. obtained from nine controls and nine AOMs are displayed in Fig. 9. Figure 9A shows that the mean storage modulus from control samples was 1.65 ± 0.31 MPa for frequencies below 1 kHz and 2.84 ± 0.38 MPa at 40 kHz. The storage modulus increased rapidly for frequencies over 3 kHz. The mean storage modulus for AOM samples was 0.3 MPa for low frequencies ($f < 1$ kHz) and increased to 0.6 MPa at 40 kHz.

Figure 9B shows that the mean loss modulus reached a peak at 8 and 7 kHz for control and AOM specimens, respectively. The maximum loss modulus was 0.61 ± 0.12 MPa for control and 0.14 ± 0.04 for AOM. The value decreased to 0.22 and 0.05 MPa at 40 kHz for control and AOM, respectively. The loss modulus was much smaller than the storage modulus for both control and AOM samples, particularly for controls, which suggests that the RWM may primarily demonstrate an elastic behavior. As shown in Fig. 9C, a minor change of the maximum mean loss factor from 0.28 to 0.31 was observed at the frequency around 5 and 6

kHz between the control and AOM specimens. The loss factor for AOM samples was slightly larger than that for control at frequencies of 1–10 kHz.

In addition to the complex modulus in the frequency domain as displayed in Figs. 8 and 9, we used the parameters listed in Tables 1 and 2 to calculate the relaxation modulus in the time domain using Eq. (1). Figure 10 shows the mean relaxation modulus of nine specimens with the S.D. in control (solid line) and the mean relaxation modulus of nine specimens in AOM (dashed line). The initial modulus for control (at time $t=0$) was 2.8 MPa and the static modulus (at time $t=\infty$) was 1.7 MPa. The RWM specimens in AOM had much lower relaxation modulus compared to control samples. The initial modulus for AOM was 0.6 MPa and the static modulus was 0.3 MPa. Moreover, there was a rapid drop of the initial modulus in the first 50 sec for control samples. The AOM samples also show the drop of the initial modulus within 50 sec, but at a really small relaxation modulus level.

DISCUSSION

I. Dynamic measurement on RWM: Electromagnetic driving vs. acoustic driving

In this study, a new measurement method using the electromagnetic force stimulation and laser vibrometry over the auditory frequencies to determine the dynamic properties of RWM in normal and infected (AOM) ears was developed. The experimental measurements were associated with the inverse-problem solving method, and each experiment was simulated in a FE model of the specimen with individual dimensions and attached microbead at the center of the RWM. The mass effects of the bead and specimen were included in the FE model for calculating the mechanical properties of RWM and the results were validated using an aluminum foil sample of known elasticity parameters. Thus, the good agreement between the experimental data and FE modeling results as shown in Fig. 7 suggests that the complex modulus over frequency domain obtained for each RWM sample is reliable.

Another measurement method for dynamic properties assessment of tympanic membrane tissue over the auditory frequency range using acoustic force stimulation has also been reported recently by Zhang and Gan (2010; 2012). The acoustic pressure driving associated with the inverse-problem solving technique was used to measure dynamic properties of human RWM samples (Zhang and Gan, 2012). The measurement concept of acoustic driving is similar to the present study of electromagnetic driving to determine the complex modulus of the guinea pig RWM. Either acoustic driving or electromagnetic force stimulation can be used to apply the load on the tissue sample without direct contact to the tissue. The acoustic driving applies the pressure load on the sample surface, and the electromagnetic driving applies a concentrated force on the sample. Since the loading used in these two approaches does not overstretch the materials or affect the material structure, these two methods should be acceptable for measuring dynamic properties of soft tissues, particularly of ear tissues over the auditory frequency range.

To understand whether different results would be derived from these two measuring methods and further validate the electromagnetic stimulation method, we used the acoustic driving method on several RWM samples after the electromagnetic stimulation tests, following the procedure reported by Zhang and Gan (2012) (data not shown). We found that the complex modulus of the guinea pig RWM measured from acoustic driving was in a range similar to that obtained from electromagnetic stimulation. The comparison study suggested that the electromagnetic stimulation and acoustic driving both can be used to measure dynamic properties of the membrane soft tissues over the auditory frequency range. These two methods for measuring the material properties of RWM are based on the same principle of vibration theory and the material constitutive law to derive the dynamic properties of tissues.

The method of using electromagnetic stimulation to determine the RWM mechanical properties in normal and diseased ears provides an additional choice for dynamic tests of ear tissues if the sound delivery system and acoustic isolated booth are not available. In particular, the setup for the coil-magnet coupling on the RWM used in this study is similar to the electromagnetic or reverse driving for the middle ear implantable hearing devices. When the device actuator such as the floating mass transducer (FMT) of Soundbridge (Colletti et al., 2006; Kiefer et al., 2006) is attached to the RWM, it is not clear what mechanical response of the RWM would be induced by the implantable devices. Thus, the method reported in this paper may initiate a new direction to investigate the relationship between the middle ear implantable devices and RWM under the reverse driving method.

II. Comparison with human RWM – Control case

To better understand the RWM dynamic properties measured in this study, we compared the results obtained in control or normal ears of guinea pigs with the data of normal human RWMs reported by Zhang and Gan (2012). Note that the human data were obtained from acoustic stimulation as the loading source. Figures 11A and 11B show the comparison of the complex modulus, including the storage and loss modulus, between the human and guinea pig RWMs over the frequency range of 200 Hz – 10 kHz. The dashed lines represent the results from human tissues (mean $\pm$ S.D.) and the solid lines display the results from guinea pig controls (mean $\pm$ S.D.) with results cut off at 10 kHz. As can be seen in Fig. 11, the storage modulus of human RWM is overall significantly higher than that of guinea pig RWM, and the loss modulus of human RWM is higher than that of guinea pig at frequencies less than 6 kHz. The loss modulus curves from human and guinea pig crossover at 6 kHz and the loss modulus of human RWM is lower than that of guinea pig at frequencies over 6 kHz.

As shown in Fig. 11C, the relaxation modulus of the human RWM was overall significantly higher than that of the guinea pig, including the initial modulus (at time $t=0$). However, the time to reach a full relaxation state was similar at 200 μ s. It is also noted that the storage modulus for guinea pig continued to increase beyond 10 kHz, but the storage modulus for human RWM peaked at 10 kHz. The loss modulus for the guinea pig RWM peaked at a frequency higher than that for the human. Those observations are consistent with the fact that the auditory frequency range for guinea pig (100 Hz – 40 kHz) is much higher than that for a human (100 Hz – 10 kHz).

III. Contribution of this study and future work

Dynamic properties of the infected tissue, RWM in AOM ear, over the auditory frequency range were measured for the first time in this study. The results from 18 ears (nine ears each in control and AOM groups) show that the complex modulus (both storage modulus and loss modulus) of RWM in AOM ears was reduced across frequencies of 0.2 to 40 kHz. The mechanical changes of RWM in AOM ears were mainly caused by the morphological or microstructural changes in the infected tissue as observed through the SEM and histology section images. These findings add new knowledge regarding biomechanics of the ear in this diseased state. This study also demonstrated that the new approach to measure mechanical properties of the RWM by using electromagnetic force stimulation on the membrane is similar to the reverse driving of the middle ear implantable hearing devices. This measurement technique with FE modeling provides new research options to characterize mechanical response of the RWM activated by the implantable devices.

It is also noticed that the thickness of the RWM used for FE model in the inverse-problem solving was based on the published data and histology sections for all RWM samples in this study. The real thickness of RWM specimen varies with the samples, which may affect the

complex modulus derived from the FE model. To estimate the error induced by the thickness variation on complex modulus of the RWM, we created the FE models of normal RWM with different thickness. It was found that when the thickness increased by 15% of the normal value, the model-derived E_0 and E_1 decreased about 25%; while the thickness decreased by 15%, the model-derived E_0 and E_1 increased about 35%. The relaxation time τ_1 did not change significantly in both cases. Thus, these estimations may give an approximate error range for the complex modulus induced by the tissue thickness variation.

In addition to the contribution of experimental measurement method, the dynamic properties of the normal and infected RWM tissues obtained in this study can be employed into the FE model of the guinea pig ear to predict the middle ear function change in AOM. The guinea pig model is currently under investigation in our lab. The FE modeling of middle ear function change in otitis media is likely to provide new insight into the mechanism of otitis media-induced conductive hearing loss. It is noted that this study was focused on the vibration at one point, the center of the RWM, to derive the parameters in FE analysis. At high frequencies, the RWM probably has complex vibration mode under the coil-magnet coupling. The effect of the membrane vibration mode will be considered in the future studies.

CONCLUSION

In this study, an experimental approach to measurement of the dynamic properties of RWM in normal and AOM ears was developed. Vibrations of the RWM specimen induced by electromagnetic force stimulation were measured by laser Doppler vibrometry over the frequency range of 0.2–40 kHz. The experiments on RWM specimens were simulated in FE models, and good agreement between the experimental measurements and FE modeling results was observed in all RWM specimens. Dynamic properties of the RWM were derived by the inverse-problem solving method and presented as the complex modulus in the frequency domain and relaxation modulus in the time domain. The results from 18 ears (nine ears each in control and AOM groups) showed that the storage modulus and loss modulus of infected RWM were both significantly lower than that of normal RWM over 0.2 to 40 kHz. The average decrease of the storage modulus in AOM ears ranged from 1.5 to 2.2 MPa (or 78% to 88% of the control value) and the average decrease of the loss modulus was 0.025 to 0.48 MPa (or 75% to 81% of the control). Our new findings suggest that *Streptococcus pneumoniae* infection of the middle ear leads to functional changes in the RWM resulting from its decreased stiffness due to the morphological changes in AOM ears. Finally, this method of coil-magnet coupling on the RWM may open a new approach to characterizing the mechanical response of the RWM when the reverse driving is selected for the middle ear implantable devices.

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References

- Beltrame AM, Martini A, Prosser S, Giarbini N, Streitberger C. Coupling the Vibrant Soundbridge to Cochlea Round Window: Auditory Results in Patients With Mixed Hearing Loss. *Otology & Neurotology*. 2009; 30:194–201. [PubMed: 19180678]

- Buytaert JAN, Salih WHM, Dierick M, Jacobs P, Dirckx JJJ. Realistic 3d computer model of the gerbil middle ear, featuring morphology of bone and soft tissue structures. *JARO*. 2011; 12:681–696. [PubMed: 21751073]
- Bausch AR, Möller W, Sackmann E. Measurement of local viscoelasticity and forces in living cells by magnetic tweezers. *J Biophysical*. 1999; 76:573–579.
- Carpenter AM, Muchow D, Goycoolea MV. Ultrastructural studies of the human round window membrane. *Arch Otolaryngol Head Neck Surg*. 1989; 115:585–590. [PubMed: 2706104]
- Colletti V, Soli SD, Carner M, Colletti L. Treatment of mixed hearing losses via implantation of a vibratory transducer on the round window. *International journal of audiology*. 2006; 45:600–8. [PubMed: 17062502]
- Colletti V, Carner M, Colletti L. TORP vs round window implant for hearing restoration of patients with extensive ossicular chain defect. *Acta oto-laryngologica*. 2009; 129:449–52. [PubMed: 19191049]
- Dai, C.; Li, W.; Gan, RZ. Change of cochlear mechanics in acute otitis media and otitis media with effusion. Association for Research in Otolaryngology (ARO) - Midwinter Meeting; Baltimore, MD. 2009. p. 984
- Ethiraj, R. Master Thesis. University of Oklahoma; 2003. Bench testing and computer modeling of electromagnetic coupling for a hearing implantable device.
- Feneberg W, Aepfelbacher M, Sackmann E. Local microviscoelasticity of the apical surface of human umbilical vein endothelial cells (HUVEC) with confluent monolayers. *J Biophysical*. 2004; 87:1338–1350.
- Fung, YC. Biomechanics: Mechanical Properties of living Tissues. 2. Springer; New York: 1993.
- Goycoolea MV. Oval and round window membrane changes in otitis media in the human. *Acta Otolaryngol (Stockh)*. 1995; 115:282–285. [PubMed: 7610823]
- Goycoolea MV, Lundman L. Round window membrane. Structure function and permeability: A review, *Microsc Res Tech*. 1997; 36(3):201–211.
- Goycoolea MV, Muchow D, Martinez GC, Goycoolea HG, Goycoolea CV, Schachern P, Knight W. Permeability of the human round-window membrane to cationic ferritin. *Arch Otolaryngol Head and Neck Surg*. 1988; 114:1247–1251.
- Hellstrom S, Eriksson PO, Yoon YJ, Johansson U. Interactions between the middle ear and the inner ear: Bacterial products. *Ann N Y Acad Sci*. 1997; 830:110–119. [PubMed: 9616671]
- Ikeda K, Morizono T. Changes of the permeability of round window membrane in otitis media. *Archives of Otolaryngology - Head & Neck Surgery*. 1988; 114(8):895–897. [PubMed: 3390334]
- Kiefer J, Arnold W, Staudenmaier R. Round window stimulation with an implantable hearing aid (Soundbridge) combined with autogenous reconstruction of the auricle - a new approach. *ORL Journal for Oto-Rhino-Laryngology and Its Related Specialties*. 2006; 68:378–85. [PubMed: 17065833]
- Machiraju C, Phan AV, Pearsall AW, Madanagopal S. Viscoelastic studies of human subscapularis tendon: relaxation test and a weichert model. *Comput Methods Programs Biomed*. 2006; 83:29–33. [PubMed: 16824643]
- Nakmal, D.; Zhang, X.; Gan, RZ. Measuring dynamic properties of round window membrane by electromagnetic force stimulation. Proceedings of the BMES 2010 Annual Meeting; Austin, TX. 2010.
- Nordang L, Anniko M. Hearing loss in relation to round window membrane morphology in experimental chronic otitis media. *ORL J Otorhinolaryngol Relat Spec*. 2001; 63(6):333–340. [PubMed: 11713421]
- Nomura Y. Otological significance of round window membrane, *Adv. Otorhinolaryngol*. 1984; 33:1–162.
- Paparella MM, Schachern PA, Choo YB. The round window membrane: otological observations. *Ann Otol Rhinol Laryngol*. 1983; 92(6 Pt 1):629–634. [PubMed: 6660755]
- Sahni RS, Paparella MM, Schachern PA, Goycoolea MV, Le CT. Thickness of the human round window membrane in different forms of otitis media. *Arch Otolaryngol Head Neck Surg*. 1987; 113(6):630–634. [PubMed: 3566946]

- Schachern P, Tsuprun V, Cureoglu S, Ferrieri P, Briles D, Paparella M, Juhn S. The round window membrane in otitis media: effect of pneumococcal proteins. *Arch Otolaryngol Head Neck Surg.* 2008; 134(6):658–662. [PubMed: 18559736]
- Yoon YJ, Hellstrom S. Ultrastructural characteristics of the round window membrane during pneumococcal otitis media in rat. *J Korean Med Sci.* 2002; 17(2):230–235. [PubMed: 11961309]
- Zhang X, Gan RZ. Dynamic Properties of Human Tympanic Membrane - Experimental Measurement and Modeling Analysis, *Int. J Experimental and Computational Biomechanics.* 2010; 1(3):252–270.
- Zhang X, Gan RZ. Dynamic properties of human round window membrane in auditory frequencies. *Med Eng Phys.* 2012;10.1016/j.medengphy.2012.05.003

Research Highlights

- Dynamic properties of RWM measured with electromagnetic force and laser vibrometer
- FE modeling and inverse-problem solving to determine complex modulus of RWM
- Preparation and morphological observation of RWM in normal and AOM ears
- Description of viscoelastic properties of RWM over frequency and time domains
- Reduction of complex modulus in AOM ears compared with the normal ears

Figure 1.

(A) A picture of the RWM specimen with the malleus-incus complex, stapes, round window, and cochlea. (B) Enlarged RWM specimen image (Control-9) with the magnetic bead placed at the center of the RWM. The shape of RWM was described in elliptic with the long axis “a” and the short axis “b”.

Figure 2.
The schematic of experiment setup with the electromagnetic force stimulation and vibration measurement by laser Doppler vibrometry (LDV) for dynamic test on RWM specimen.

Figure 3.

The FE models of dynamic experiments on RWM specimens with the bead attached at the center. (A) The planar view (medial side, Control-4) of the FE model; (B) the lateral view of the model for control specimen; (C) the lateral view of the model for AOM specimen (AOM-2).

A and B: control

C and D: AOM

Figure 4. SEM images of the RWM obtained from the control (A and B) and AOM (C and D) samples. (A) Image of the outer epithelial surface (the middle ear side) of a control RWM; (B) Enlarged image of the area marked in Fig. 4A, which shows the fibrous structure of the core connective tissue layer; (C) Image of the outer epithelial surface of an AOM RWM; (D) Enlarged image of an area marked in Fig. 4C, which shows the thicker epithelial layer covering the core connective tissue layer.

A: control
B: AOM

Figure 5. (A) A histological section image of the RWM in control ear; (B) A histological section image of the RWM in AOM ear, which shows the increase of RWM thickness in AOM ear compared with that in control ear.

Figure 6.
The displacement amplitude-frequency curves measured from the experiments of nine control RWM samples (A) and nine AOM samples (B) over the frequency range of 0.2–10 kHz.

Figure 7.

The FE modeling results of displacement amplitude (dashed lines) obtained from two RWM models in control (the upper panel) and two RWM models in AOM (the lower panel) in comparison with the corresponding experimental curves (solid lines).

Figure 8.

The complex modulus determined from nine RWM specimens in control ears (the left column) and nine RWM specimens in AOM ears (the right column) over the frequency range of 0.2–40 kHz. The top panel displays the storage modulus E' , the middle panel shows the loss modulus E'' , and the bottom panel displays the loss factor η .

Figure 9.

The mean complex modulus of nine control RWM specimens and nine AOM specimens with standard deviations (S.D.). (A) Storage modulus E' ; (B) Loss modulus E'' ; (C) Loss factor η .

Figure 10.

The relaxation modulus in time domain (mean $\pm$ S.D.) of nine control samples and nine AOM samples obtained from the complex modulus of all samples in frequency domain.

Figure 11.

Comparison of the complex modulus (E' and E'') and relaxation modulus $E(t)$ determined from the guinea pig control samples in this study with those obtained from normal human RWMs (Zhang and Gan, 2012). (A) Storage modulus E' ; (B) Loss modulus E'' ; (C) Relaxation modulus $E(t)$.

Data from control ears (thickness 30 μm at the center and 50 μm at the edge). The first four rows list dimensions, resonance frequency f_n and vibration amplification ratio R of RWM specimens. The last three rows list the viscoelastic parameters of RWM specimens determined from FE modeling analysis using the inverse-problem solving method.

Table 1

Specimen	Con-1	Con-2	Con-3	Con-4	Con-5	Con-6	Con-7	Con-8	Con-9	Mean $\pm$ S.D.
Long axis a (mm)	1.36	1.29	1.23	1.31	1.32	1.27	1.26	1.29	1.20	1.28 $\pm$ 0.05
Short axis b (mm)	0.91	0.86	0.82	0.89	0.90	0.87	0.84	0.85	0.80	0.86 $\pm$ 0.04
f_n (Hz)	1482	1635	1696	1504	1482	1671	1884	1935	1884	1686 $\pm$ 180
R	5.9	3.8	7.7	6.5	6.8	4.0	6.6	8.4	9.6	6.6 $\pm$ 1.9
E_0 (MPa)	1.35	1.45	1.50	1.44	1.45	1.55	2.04	2.22	1.87	1.65 $\pm$ 0.31
E_1 (MPa)	1.14	1.65	0.90	1.20	1.15	1.62	1.32	1.18	0.94	1.23 $\pm$ 0.26
τ_1 (μs)	22	24	20	20	20	25	21	19	18	21.0 $\pm$ 2.3

Data from AOM ears (thickness 80 μm at the center and 100 μm at the edge). The first four rows list dimensions, resonance frequency f_n and vibration amplification ratio R of RWM specimens. The last three rows list the viscoelastic parameters of RWM specimens determined from FE modeling analysis using the inverse-problem solving method.

Table 2

Specimen	AOM-1	AOM-2	AOM-3	AOM-4	AOM-5	AOM-6	AOM-7	AOM-8	AOM-9	Mean $\pm$ S.D.
Long axis a (mm)	1.30	1.36	1.33	1.34	1.40	1.37	1.35	1.30	1.30	1.34 $\pm$ 0.04
Short axis b (mm)	0.90	0.84	0.86	0.85	0.87	0.82	0.82	0.81	0.86	0.85 $\pm$ 0.03
f_n (Hz)	1266	1335	1482	1296	1696	1315	1671	1438	1566	1452 $\pm$ 163
R	5.8	5.4	4.3	4.8	6.2	9.7	5.3	7.9	9.2	6.5 $\pm$ 2.0
E_0 (MPa)	0.27	0.30	0.36	0.28	0.47	0.26	0.40	0.29	0.39	0.34 $\pm$ 0.07
E_1 (MPa)	0.27	0.30	0.35	0.30	0.35	0.16	0.36	0.20	0.22	0.28 $\pm$ 0.07
τ_1 (μs)	22	22	25	24	21	20	21	21	20	21.8 $\pm$ 1.7