Medical Engineering and Physics 000 (2016) 1–10

Contents lists available at ScienceDirect

Medical Engineering and Physics

journal homepage: www.elsevier.com/locate/medengphy

Dynamic property changes in stapedial annular ligament associated with acute otitis media in the chinchilla

Brooke M. Hitt, Xuelin Wang, Rong Z. Gan*

School of Aerospace and Mechanical Engineering and Biomedical Engineering Center, University of Oklahoma, Norman, OK 73019, United States

ARTICLE INFO

Article history:
Received 31 May 2016
Revised 26 October 2016
Accepted 5 December 2016
Available online xxx

Keywords:
Stapedial annular ligament
Acute otitis media
Chinchilla
Complex modulus
Frequency-temperature superposition
Dynamic mechanical analyzer

ABSTRACT

Located at the end of the ossicular chain, the stapedial annular ligament (SAL) serves as a closed yet mobile boundary between the cochlear fluid and stapes footplate. It is unclear how SAL properties change with acute otitis media (AOM). This paper reports the measurements of SAL dynamic properties in chinchilla AOM model using dynamic mechanical analyzer (DMA) and frequency-temperature superposition (FTS) principle. AOM was analyzed in two infection groups: 4 days (4D) and 8 days (8D) post induction. SAL specimens were measured using DMA at three temperatures: 5, 25, and 37°C. To extend the testing frequencies to higher levels, FTS principle was employed. Then generalized Maxwell model was utilized to define the constitutive equations of the SAL. The complex shear moduli were obtained from seven samples of control, 4D, and 8D groups. Results show that the storage and loss shear moduli of SALs decreased due to AOM. The storage moduli for 4D and 8D ears were similar below 100 Hz, and the loss modulus for 4D was significantly larger than 8D across the entire frequency range. This study reports data that contributes to ear biomechanics and improves understanding on the effects of AOM in middle ear tissues.

1. Introduction

Connecting the base of the stapes to the oval window of the ear is the stapedial annular ligament (SAL), a ring of fibrous tissue which provides a mobile boundary between the cochlear fluid and stapes footplate. SAL enables the stapes to transfer vibration into the cochlear fluid, stimulating the sensory hair cells in the cochlea which communicate with the brain to process hearing [1]. SAL is mainly composed of mature elastic fibers as shown in histological and transmission electron microscopy (TEM) images [2,3,4].

Due to its anatomical location, SAL is a particularly important component in modulating acoustic-mechanical transmission to the cochlea from the middle ear. Recently, there have been several studies on the mechanical properties of SAL reported using human temporal bones [5,6,7]. Gan et al. [5] used a quasi-static uniaxial tension test to measure the shear modulus of human SAL and demonstrated that it is a typical viscoelastic material with hysteresis, a non-linear stress-strain relationship, and stress relaxation function. The shear modulus changed from 3.6 to 220 kPa as the shear stress increased from 2 to 140 kPa. Kwacz et al. [8] measured the static stiffness of human SAL via atomic force microscopy and reported that SAL is a linear elastic material for deflections up to approximately 100 nm with a stiffness of approximately 120 N/m and an elastic modulus of approximately 1.1 MPa. Lauxmann et al. [9] investigated the nonlinear stiffness properties of the human SAL and measured the stiffness value as 1050 N/m. These studies provide important data for understanding SAL mechanical behavior in response to force or deformation. However, the SAL works across the auditory frequency range up to 20 kHz and the dynamic properties of the ligament largely determine sound transmission from the middle ear to the cochlea. There is only one recent publication about human SAL dynamic properties by Zhang and Gan [7]. They reported a storage shear modulus of 31.7 kPa at 1 Hz and 61.9 kPa at 3.76 kHz and a mean loss shear modulus of 1.1 kPa at 1 Hz and 6.5 kPa at 3.76 kHz.

Middle ear diseases cause alterations in the morphological and mechanical properties of middle ear tissues, including the tympanic membrane (TM), round window membrane (RWM), and the SAL, as proven in previous investigations with acute otitis media (AOM), an infective disease of the middle ear [10,11,12]. To measure variation of any mechanical properties in AOM ears, an animal model is necessary for experimental approach because AOM cannot be induced properly in cadaver temporal bones. Recently, an AOM model was created in the chinchilla and the changes in mechanical properties of TM and RWM were reported by Yokell et al. [13] and Wang et al. [14]. For the SAL, however, biomechanical changes in pathological ears have never been reported. It is also unknown how the mechanical properties of the SAL vary as

* Corresponding author: fax. 1-405-325-1088.
E-mail address: rgan@ou.edu (R.Z. Gan).

http://dx.doi.org/10.1016/j.medengphy.2016.12.003
1350-4533/

the AOM infection progresses. The degree of inflammatory response changes during AOM development, but how this affects the mechanical properties of SAL across the infection period remains unclear [13,14]. Therefore, further investigation of SAL in AOM ears is needed to understand the mechanism of AOM-induced hearing loss.

The aim of this study is to determine the mechanical properties of the SAL and variation of these properties in different phases of AOM in chinchilla ears. Following the method developed by Zhang and Gan [7,15], a control and two treatment groups that characterize relatively early and later distinct stages of infection, 4 days (4D) and 8 days (8D) post AOM induction, were measured using dynamic mechanical analyzer (DMA) at frequencies from 2 to 40 Hz at 5, 25, and 37 °C. To spread the testing frequencies to higher levels which will better represent the auditory frequency range of both the chinchilla and human, frequency-temperature superposition (FTS) principle was employed. [7]. Next, generalized Maxwell model was later utilized to define the constitutive equations regarding the SAL. The complex modulus, comprised of storage shear modulus G' and loss shear modulus G'', was attained from both normal and AOM ears. All data reported here contributes to scientific knowledge of biomechanics of the middle ear.

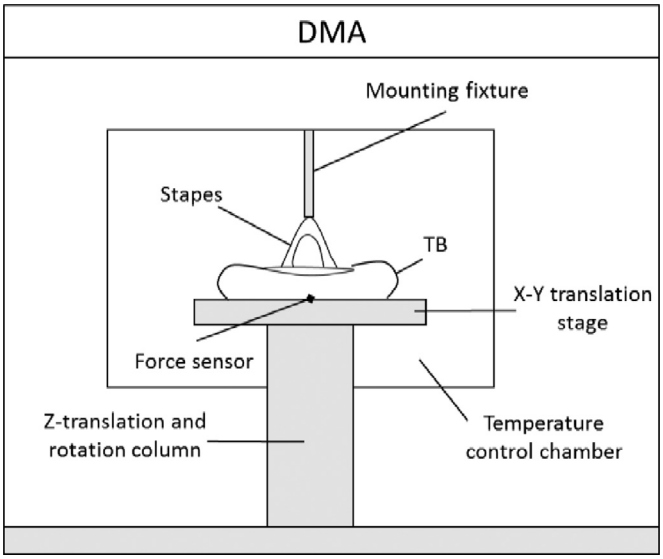

Fig. 1. The schematic of the experimental setup for dynamic test of the SAL specimen in DMA. The stapes head was affixed to the mounting fixture, and the SAL was located where the stapes met the temporal bone (TB).

2. Methods

2.1. SAL specimen preparation and experimental setup

2.1.1. Chinchilla AOM model

This study included twelve adult chinchillas (*Chinchilla lanigera*) measuring between 600 and 780 g. The protocol was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma and meets the guidelines of the National Institutes of Health. All animals had healthy, disease-free ears before AOM inoculation according to otoscopic examination.

Twelve chinchillas were separated into a control group of four animals and an AOM group of eight animals which was further divided into two groups: the 4D group of four animals and 8D group of four animals. AOM infection was induced via transbullar injection of *Haemophilus influenzae* 86-028NP suspension bilaterally following the method described by Morton et al. [16]. Under general anesthesia [ketamine (10 mg/kg) and xylazine (2 mg/kg)], 0.3 ml of a bacterial suspension (3000 CFU) was injected into the superior bulla of each ear using a 26 gauge needle on 1 cc syringe. The control group remained untreated, and daily otoscopic examination was performed on all chinchillas.

At the 4th or 8th day post-inoculation, each animal was anesthetized and the TM was microscopically or otoscopically examined to confirm the presence of AOM before being euthanized in order to harvest the bullae. Similarly, Control ears were examined to confirm the absence of infection before sacrifice.

2.1.2. SAL specimen preparation

After euthanasia, both bullae from each animal were excised from the skull. After opening the superior bulla and removing both the TM and malleus-incus complex, each temporal bone was cut into an approximate 2 cm x 2 cm x 1.5 cm cube to reveal the SAL, stapes, and adjacent bony wall following the procedure described by Gan et al. [5]. Near the oval window, the scala vestibule wall was opened to release cochlear pressure and saline solution was applied to the underside of the footplate to help preserve physiological conditions.

2.1.3. Experimental setup

Fig. 1 is the experimental setup schematic for dynamic testing of SAL specimens in DMA and is similar to that reported by Zhang and Gan [7] using DMA measurements in human SALs. Briefly, the bony cochlea portion of the sample was secured onto the X-Y translational platform and positioned inside a temperature chamber 2 cm away from a thermocouple. A negative feedback circuit was installed to maintain a specified temperature with precision of ± 1 °C. The SAL specimen was aligned in the analyzer (ElectroForce 3200, Bose, Eden Prairie, MN) by adjusting the Z-translational column and X-Y translational stage under a surgical microscope (Zeiss, OPMI 1-FC). As shown in Fig. 1, a titanium partial ossicular-replacement prosthesis (PORP, Gyrus, ENT, LLC, Memphis, TN) served as a mounting fixture for the peak of the stapes, which was carefully attached to the PORP with cyanoacrylate gel glue, none of which reached the SAL.

2.2. Measurement of specimen dimensions

Since the SAL was hidden between the bony cochlear wall and the stapes footplate, dimensions were taken after testing when the footplate was removed from the oval window, as described by Gan et al. [5]. A digital CCD camera captured still images of the oval window and stapes. Fig. 2A depicts the image and dimensions of the stapes footplate length L1 and width L2, while Fig. 2C depicts the oval window length L3 and width L4. Previous histology on the human middle ear suggests that the SAL is thicker in the anterior than in the posterior [17,18]. However, due to technique limitation, accurate in vivo measurement along the entire perimeter of the footplate to measure this is not currently possible. For simplification, SAL thickness was assumed to be uniform in this study. Therefore, the width or length difference between the oval window and stapes footplate, L4–L2 or L3–L1, was calculated and the average value was taken for SAL thickness, d. Potential errors due to this assumption were estimated in a previous study on static properties of human SAL [5].

SAL height h was assumed equal to the footplate edge height and was the average of measurements taken at three different locations (posterior, anterior, and middle areas) from an image, as shown in Fig. 2B. Similarly, the inner rim perimeter of the footplate was calculated from a transferred binary image. SAL dimensions of all specimens are listed in Table 1 with average and standard deviation (SD) for the (A) control, (B) 4D, and (C) 8D groups.

Please cite this article as: B.M. Hitt et al., Dynamic property changes in stapedial annular ligament associated with acute otitis media in the chinchilla, Medical Engineering and Physics (2016), http://dx.doi.org/10.1016/j.medengphy.2016.12.003