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Dynamic Properties of Human Tympanic Membrane Based on Frequency-Temperature Superposition

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Abstract

The human tympanic membrane (TM) transfers sound in the ear canal into the mechanical vibration of the ossicles in the middle ear. The dynamic properties of TM directly affect the middle ear transfer function. The static or quasi-static mechanical properties of TM were reported in the literature, but the dynamic properties of TM over the auditory frequency range are very limited. In this paper, a new method was developed to measure the dynamic properties of human TM using the Dynamic-Mechanical Analyzer (DMA). The test was conducted at the frequency range of 1 to 40 Hz at three different temperatures: 5°, 25° and 37°C. The frequency-temperature superposition was applied to extend the testing frequency range to a much higher level (at least 3800 Hz). The generalized linear solid model was employed to describe the constitutive relation of the TM. The storage modulus E' and the loss modulus E" were obtained from 11 specimens. The mean storage modulus was 15.1 MPa at 1 Hz and 27.6 MPa at 3800 Hz. The mean loss modulus was 0.28 MPa at 1 Hz and 4.1 MPa at 3800 Hz. The results show that the frequency-temperature superposition is a feasible approach to study the dynamic properties of the ear soft tissues. The dynamic properties of human TM obtained in this study provide a better description of the damping behavior of ear tissues. The properties can be transferred into the finite element (FE) model of the human ear to replace the Rayleigh type damping. The data reported here contribute to the biomechanics of the middle ear and improve the accuracy of the FE model for the human ear.

Keywords

middle ear; eardrum; mechanical property; viscoelasticity; complex modulus; dynamic-mechanical analyzer

INTRODUCTION

The human middle ear is composed of the tympanic membrane (TM), three ossicles, suspensory ligaments and muscle tendons. The TM is a thin membrane which separates the ear canal and the middle ear cavity and plays an important role in sound transmission by converting the acoustic waves into the mechanical vibration of the ossicular chain. The mechanical properties of the TM directly affect the efficiency of sound transmission and are essential for the study of the middle ear transfer function. The TM properties are also critical for the accuracy of the finite element (FE) model of the ear.[12]

The mechanical properties of the human TM were first reported by von Békésy in 1960 using a bending test on a rectangular cadaver TM strip.[24] Since then, the elastic modulus of

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the human TM has been measured using various methods.[1, 5–8, 14] There are some studies on the mechanical properties of animal TMs as well, such as those of gerbils.[2, 3] The mechanical properties reported in those cited articles were obtained under the static, quasi-static, or dynamic condition at very low frequencies.

The human TM works under the auditory frequency range of 20 to 20,000 Hz. The dynamic properties of the TM should be more critical and valuable than the static properties. However, the dynamic properties of the TM are very limited in the literature. After the pioneer study at a single frequency of 890 Hz by Kirkae (1960),[15] Luo et al. (2009) used a miniature split Hopkinson tension bar to measure the relaxation modulus of the human TM at high strain rates. They converted the relaxation modulus into the complex modulus in the frequency domain and reported 45.2 – 58.9 MPa in the radial direction and 34.1 – 56.8 MPa in the circumferential direction of the TM from 100 to 2400 Hz.[18] Zhang and Gan (2010) conducted the dynamic test on human TM up to 8000 Hz using a laser Doppler vibrometer to measure the vibration of the TM sample in the strip induced by acoustic driving. The complex modulus was obtained by the inverse-problem solving method using the finite element model.[28] However, the experimental measurement on the complex modulus of the TM sample in the frequency domain is still very limited in the literature.

The Dynamic-Mechanical Analyzer (DMA) is widely used to measure the complex modulus of materials in the frequency domain. However, the current commercial DMA has limited higher frequency access which cannot reach the human auditory frequency range. An approach called the frequency-temperature superposition (FTS) principle using the DMA may be a possible method for measuring the TM dynamic properties over the auditory frequency range. The FTS is an empirical method used to expand the effective frequency scale for viscoelastic measurements on polymers and fibrous composites.[10, 20] The basic theory of FTS considers the viscoelastic behavior of some materials as a function of two principle variables: frequency and temperature. The effect of temperature changes on the viscoelastic properties of the material is equivalent to that of frequency changing.[10]

The FTS was originally developed for amorphous polymers and is applicable to a variety of polymer systems.[22, 23] Recently, researchers have applied the FTS principle to biological tissues. Peters et al. (1997) measured the shear modulus, loss factor and relaxation modulus of bovine brain tissues at 7°–37°C over the frequency range of 0.16 to 16 Hz and extrapolated the dynamic shear modulus for much higher frequencies (up to 1.6 MHz).[21] Chan (2001) measured the viscoelastic properties of the vocal-fold tissue at 5°–37°C over the frequency range of 0.01 to 15 Hz and expanded the equivalent frequency up to about 500 Hz using FTS.[4]

In this paper, we report a recent study on measuring the dynamic properties of human TM using the FTS principle with a DMA. The test was conducted at the frequency range from 1 Hz to 40 Hz at three different temperatures: 5°, 25° and 37°C. The FTS principle was applied to extend the testing frequency range to a much higher level (at least 3800 Hz). The generalized linear solid model was used as the constitutive law for the TM, and the complex modulus of the TM was obtained. The methods and data reported here contribute to the biomechanics of the middle ear and improve the accuracy of FE model for the human ear.

MATERIALS AND METHODS

I. TM specimen preparation

The TM samples were harvested from fresh human temporal bones through the Willed Body Program at the University of Oklahoma Health Sciences Center. A total of 11 TM specimens from six individual donors (five female and one male, age ranged from 64 to 74 with a mean

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value of 68.8) were employed in this study. The tympanic annulus of the TM was first separated from the bony ear canal with the malleus attached and then placed in saline solution (Fig. 1A). A rectangular strip was cut from the posterior or anterior portion of the TM. The specimen had the tympanic annulus attached at both top and bottom sides to maintain the integrity of the membrane. The specimen was treated as a flat rectangular strip for the experiment, and the curvature of the TM was neglected in this study.

The specimen was then laid on the base of a microscope (Olympus SZX12) and fixed to two fixture adapters at both annulus sides using cyanoacrylate gel glue (Loctite). These fixture adapters were connected by two additional plastic bars parallel to the TM specimen along the longitudinal direction. These additional supports were used to stabilize the specimen with the fixture adapters and to avoid any unexpected damage to the TM specimen during the mounting process in DMA. After the specimen was lined up with the grips in the DMA, the additional bars were removed and the initial state with a preload of 0.002 N was set up as shown in Fig. 1B. The images of the specimen were taken by a CCD camera. The length (gap between two fixture adapters) and width of the specimen were measured using the image analysis tools (Adobe Photoshop 7.0) on the acquired images. The results are listed in Table 1. The length of all 11 TM samples ranges from 5.6 to 7.6 mm with a mean of 6.5 mm and S.D. of 0.7 mm. The width ranges from 1.7 to 2.5 mm with a mean of 2.1 mm and S.D. of 0.3 mm. The side image of the specimen was also taken by the CCD camera to measure the thickness of the specimen. The thickness of all samples was measured around 0.06 mm at the central (middle) region of the sample with a resolution of 0.01 mm. Kuyper et al.[16] reported that the thickness of human TM was not uniform and the mean thickness of 0.04 – 0.12 mm was observed in the central region between the umbo and annulus from three TM specimens. In this study, the non-uniformity of the thickness was not taken into account and the thickness of central region of the sample was used.

II. Viscoelastic model of TM specimen

As the first step to study the dynamic properties of TM using FTS, the sample was considered as an isotropic and homogeneous material in this study. The human TM shows typical viscoelastic properties in published papers.[5, 18, 28] To describe the viscoelastic behavior of the TM, the generalized linear solid model[19] was used in this study. Based on this model, the relaxation modulus of the TM is presented as:

$$E(t)=E_0+\sum_{i=1}^{n}E_i \exp(-\frac{t}{\tau_i}) \quad (1)$$

where E_i (i=0,1,…., n) is the relaxation modulus of the i th spring, τ_i is the relaxation time of the i th dashpot.[26] For harmonic analysis, $E(t)$ in the time domain can be converted into the complex modulus in the frequency domain as

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

where $E'(f)$ is the storage modulus, $E''(f)$ is the loss modulus, f is the frequency, and $E'(f)$ and $E''(f)$ can be expressed as:

$$E'(f)=E_0+\sum_{i=1}^{n}E_i\tau_i^2(2\pi f)^2/[1+\tau_i^2(2\pi f)^2] \quad (3)$$

$$E"(f)=\sum_{i=1}^{n}2\pi E_i\tau_i f/[1+\tau_i^2(2\pi f)^2] \quad (4)$$

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

where δ is the phase angle of the complex modulus, and η(*f*) is the loss factor defined as the ratio of the storage modulus to the loss modulus. In this study, we selected two spring-dashpot elements ($n = 2$, giving 5 parameters E_0, E_1, E_2, τ_1,and τ_2) to represent the viscoelastic behavior of the TM. The five parameters will be determined by fitting the generalized linear solid model with the master curve of the complex modulus obtained through the FTS.

III. Dynamic test on TM specimen

In this study, the complex modulus including the storage modulus and the loss modulus of the human TM specimen at three temperatures (5°, 25°, and 37°C) were measured in the DMA (Bose ElectroForce 3200, Eden Prairie, MN). The TM sample was placed inside the temperature-control chamber in the DMA. A thermocouple was placed at 2 cm behind the sample to measure the temperature in the chamber. There was a negative feedback circuit to control temperature stably. The precision of temperature controlling was ±1°C. In the temperature range of 5–37 °C, there is no phase change (freezing) of fluid in tissue cells, and there is no denature of proteins. Thus, the structure of the soft tissue in this temperature range should not change.[4, 21] The temperature of 37°C was chosen as the reference temperature. The TM samples were subjected to the sinusoidal vibrations with small amplitudes at different frequencies. At each frequency *f*, the displacement *d* and force *F* were recorded as the function of time *t*:

$$d=d_0 e^{i2\pi ft} \quad (6)$$

$$F=F_0 e^{i(2\pi ft+\delta)} \quad (7)$$

where σ_0 and E_0 were stress and strain amplitude, respectively. The complex modulus at this frequency *f* was calculated as:

$$|E^*|=\frac{\sigma_0}{E_0}=\frac{F_0/wh}{d_0/l} \quad (8)$$

$$E'=|E^*|\cos\delta \quad (9)$$

$$E"=|E^*|\sin\delta \quad (10)$$

where *w*, *h*, and *l* were the width, thickness and length of the TM specimen, respectively. The test protocol for each TM specimen is described below.

Preconditioning test—It is well known that the stabilized state of biological soft tissue is only reached after preconditioning, a process that load-deformation curves are gradually stabilized during repeated load-unload cyclings on the specimen.[11] In this study the DMA was programmed to perform five cycles of uniaxial preconditioning at a frequency of 0.1 Hz and stretch displacement of 1.0 mm for each specimen before the dynamic test. For all TM

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samples, their stress-stretch curves were almost identical after 5 loading-unloading cycles, which indicated the mechanical properties of TM were stabilized.

Dynamic test—After preconditioning, the dynamic test was conducted at 5°, 25°, and 37°C in sequence. At each temperature, the TM sample was tested at 1, 2, 5, 10, 20, and 40 Hz with the displacement amplitude of 0.2 mm. The sample took a rest for at least 2 minutes for recovering after each run. Note that the sample was kept in physiological moisture by adding saline solution onto its surface between each test. To keep sample in the same moisture condition, the accurate amount of saline solution was controlled by a syringe.

IV. Frequency-temperature superposition (FTS) principle

The FTS principle was first reported in the 1950s,[9, 27] and has become a useful extrapolation technique as applied to polymers, plastics, and composites. The later theories of FTS address a simple relationship between the temperature and frequency (or time) effects on the molecular behaviors of polymers and thus the viscoelastic properties of these materials.[10] The curves of the complex modulus E^* obtained at a relatively low temperature T can be shifted along the frequency axis by a shift factor α_T to a higher temperature T_0 (served as reference temperature). This concept can be expressed by the equation:

$$E^*(T_0, f)=E^*(T, f/\alpha_T) \quad (11)$$

where f is the frequency. The shift factor α_T quantifies the temperature's effect on the material's complex modulus. It is temperature-dependent and needs to obey the Arrhenius equation:

$$\ln \alpha_T=\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right) \quad (12)$$

where T and T_0 are the absolute temperature in Kelvin, E_a is the activation energy for the material, and R is the universal gas constant equal to 8.314 J/mol.K.

Another widely used empirical equation for the FTS principle is the WLF equation, which was first introduced by Williams, Landel and Ferry in 1955:[27]

$$\log \alpha_T=\frac{c_1(T-T_g)}{c_2+T-T_g} \quad (13)$$

where c_1 and c_2 are empirical constants and T_g is the glass transition temperature. The glass temperature is the temperature below which the polymer chain backbone configuration rearrangement stops.[20] Combining the Eqs. (12) and (13) presents the relation between the activation energy E_a, c_1 and c_2:

$$E_a=2.303\, R c_1 c_2 T^2/(c_2+T-T_g)^2 \quad (14)$$

The following procedure for the FTS principle was used to determine the dynamic properties of the TM samples at the higher frequencies: 1) the complex moduli (storage modulus E and loss modulus E") obtained at three different temperatures (5°, 25°, and 37°C) were plotted together as functions of the frequency in logarithmic scale; 2) the complex modulus curves at the lower temperatures were shifted horizontally to the higher frequencies; 3) when the curves were adjacent to each other, the commonly called "master curve" at the reference temperature (37°C) was formed to predict the complex modulus at the higher frequency range; 4) the shift factor α_T and the activation energy E_a were calculated from Eqs. (11) and

(12). Moreover, there are three requirements for the FTS principle to hold:[26] perfect matching of the curve shapes at adjacent regions, same shift factor value for all viscoelastic parameters, and the shift factor obeying the Arrhenius equation or the WLF equation. In this study, these requirements were checked and satisfied.

RESULTS

Dynamic experiments were conducted on 11 TM specimens, and the storage modulus $E'$and the loss modulus E'' of these specimens were obtained over 1 to 40 Hz. Figure 2 shows the typical complex modulus-frequency curves at the different temperatures (5°, 25° and 37°C) obtained from two TM specimens (TM-1 and TM-4). Both the storage modulus and the loss modulus increased with the frequency increasing or temperature decreasing. Larger slopes were found at the lower frequency range for the loss modulus. Other TM samples had the complex modulus-frequency curves similar to that shown in Fig. 2. The inter-subject difference of the E' and E'' curves was observed due to the variation between the TM specimens and the possible error of the experimental measurements.

Following the procedure described in Section-IV of Methods, the complex modulus curves at the lower temperatures were shifted and the master curves created. Figure 3 shows the master curves of the complex modulus at the reference temperature 37°C for specimens TM-1 and TM-4. The complex modulus-frequency curves are generally well matched at the adjacent regions after the horizontal shifts. Thus, the first requirement of the FTS principle is satisfied. The horizontal shift factors are the same for the storage modulus and loss modulus in each specimen, which meets the second requirement of the FTS principle. For specimen TM-1, the storage modulus is 16.5 MPa at 1 Hz and increases to 31.2 MPa at 6120 Hz, while the loss modulus is 0.25 MPa at 1 Hz and 4.7 MPa at 6120 Hz. For specimen TM-4, the storage modulus is 15.8 MPa at 1 Hz and increases to 28.3 MPa at 3920 Hz, while the loss modulus is 0.45 MPa at 1 Hz and 3.7 MPa at 3920 Hz. The shift factors and maximum frequency of the master curves for all 11 TM specimens are listed in Table 2. The mean value of the shift factors from 25° to 37°C is 5.9±0.8. The mean value of the shift factors from 5°C to 37°C is 134.2±37.7. The maximum frequency ranges were from 3800 to 7840 Hz with a mean value of 5371±1506 Hz.

The temperature dependence of the shift factor was tested by fitting experimental data into the Arrhenius equation (Eq.(12)). The activation energy of the TM samples is calculated as the ratio between $R \cdot \ln \alpha_T$ and $\left(\frac{1}{T} - \frac{1}{T_0}\right)$. As an example, Fig. 4 shows the natural logarithmic shift factor $\ln \alpha_T$-temperature curves obtained from specimens TM-1 and TM-4. The relation between the shift factors and the temperature are well matched with the Arrhenius equation (the value of coefficient of determination r^2 was 0.997 for TM-1 and 0.999 for TM-4). The values of r^2 for all TM samples are not less than 0.997, thus the third requirement of the FTS principle was satisfied. The activation energy was obtained as 113.6 kJ/mol for TM-1 and 102.6 kJ/mol for TM-4. Table 2 lists the activation energy of all TM samples, which ranges from 101.0 to 118.3 kJ/mol with a mean value of 109.3±6.5 kJ/mol.

Figure 5 shows the master curves of the complex modulus for all 11 TM samples. The complex modulus generally increases with the frequency increasing except the loss modulus of some specimens decreases a little between 40 to 500 Hz. The storage modulus ranges from 10.3 to 20.9 MPa at 1 Hz. The loss modulus ranges from 0.18 to 0.45 MPa at 1 Hz. The maximum frequency of the master curves for the 11 TM samples was different and ranged from 3800 to 7840 Hz as listed in Table 2. The mean complex modulus was calculated over the common frequency range for the 11 samples (1–3800 Hz). The mean master curves of the storage modulus E’ and the loss modulus E” with S.D. in Fig. 5 were

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also plotted to 3800 Hz. Figure 5 also shows the mean master curves of the storage modulus E' and the loss modulus E" with S.D., which were plotted up to 3800 Hz. The mean storage modulus was 15.1±3.0 MPa at 1 Hz and 27.6±5.1 MPa at 3800 Hz. The mean loss modulus was 0.28±0.1 MPa at 1 Hz and 4.1 ±1.2 MPa at 3800 Hz. The slope of the master curves of the loss modulus decreased to almost zero at 20–100 Hz and then increased at 300 Hz. The larger slope was observed at frequencies below 10 Hz and between 1000–3800 Hz as opposed to other frequency ranges. The change of the slope at certain frequencies relates to the two parameters of relaxation time τ_1 and τ_2.

The generalized linear solid model was used to describe the viscoelastic behavior of the TM samples. As the storage modulus has all five viscoelastic parameters (E_0, E_1, E_2, τ_1, and τ_2) while the loss modulus only has four of them, the experimental data of the storage modulus were first used to fit the theoretical storage modulus-frequency relation (Eq.(3)) to determine the five parameters. The theoretical loss modulus was then calculated from Eq. (4) by substituting the values of the parameters, and the results were compared with the experimental data. As an example, the five parameters for TM-7 were determined as E_0 =11.9 MPa, E_1 =7.0 MPa, E_2 =9.6 MPa, τ_1 =3.85 ms and τ_2 =84.3 µs. Figure 6A shows the storage modulus of specimen TM-7 derived from the generalized linear solid model in comparison with the experimental results. Table 3 lists the viscoelastic parameters determined from all 11 TM samples with the mean values and S.D. The mean experimental complex modulus curves shown in Figure 5 were also fitted by the generalized linear solid model with the five parameters determined as E_0 =15.2 MPa, E_1 =6.3 MPa, E_2 =7.9 MPa, τ_1 =5.14 ms and τ_2 =78.6 µs. The results are shown in Fig. 6B where the theoretical complex modulus curves were compared with the mean experimental data.

We employed the coefficient of determination, r^2, to statistically assess the goodness of fit of the generalized linear solid model to the experimental complex modulus curves over 1 to 3800 Hz. r^2 can be calculated by:

$$r^2=1-\frac{\sum_i (E_i-\hat{E}_i)^2}{\sum_i (E_i-\bar{E})^2} \quad (15)$$

where E_i are the experimentally measured values of complex modulus, $\hat{E}_i$ are the theoretical values of complex modulus derived from the generalized linear solid model, $\bar{E}$ is the mean value of E_i. The r^2 values for all 11 TM samples and the mean experimental curves are listed in Table 3. The results show that the linear solid model agreed with the experimental results reasonably well. For the storage modulus, the smallest value of r^2 was found as 0.960 for sample TM-2, and the mean value was obtained as 0.977. For the loss modulus, the value of r^2 ranged from 0.529 to 0.824 with a mean value of 0.666. The r^2 of the mean complex modulus curves (shown Fig. 6B) were 0.999 and 0.845 for the storage modulus and loss modulus, respectively.

DISCUSSION

I. Comparison with published data

To date, the published mechanical properties of the human TM were obtained mostly from the static or quasi-static test. In this study, the complex moduli of the human TM samples were obtained over the frequency range of 1 to 3800 Hz. The storage modulus obtained at the low frequencies, such as 1 Hz, can be considered comparable to the elastic modulus obtained from the quasi-static test. The mean storage modulus of the 11 TM samples at 1 Hz was 15.1±3.0 MPa from this study. This value is close to or in the range of that reported in

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the literature: 20 MPa reported by von Békésy,[24] 23 MPa by Decraemer et al.,[7] 0.4 – 22 MPa by Cheng et al.,[5] and 17.4 MPa for the posterior portion and 19.0 MPa for the anterior portion reported by Huang et al.[14] Aernouts et al.[1] recently reported the Young's modulus of the human TM from three samples as 2.1, 2.3 and 4.4 MPa, respectively. The storage modulus at 1 Hz obtained in this study is about 5 times larger than the value reported by Aernouts et al. Fay et al.[8] reported the Young's modulus of 30–90 MPa for an isotropic model and 100–400 MPa for an orthotropic model of the human TM, which are much larger than the values reported in this study.

Kirikae[15] measured the Young's modulus of the human TM sample at a single frequency of 890 Hz and reported the value of 40 MPa. In this study, the mean storage modulus at 890 Hz was 22.6±3.9 MPa, which is about half of the valve reported by Kirikae. To our best knowledge, there are two other dynamic tests of the human TM over the auditory frequency range reported in the literature by Luo et al. (2009)[18] and Zhang and Gan (2010).[28] Figure 7 shows the comparisons of the storage modulus and loss modulus obtained in this study (solid lines with square symbols) with Luo et al.'s data (solid lines with triangles) and Zhang and Gan's data (solid line with circles). The results are presented as the mean values with S.D. The TM specimens were normal tissues and assumed as a homogeneous material in all three studies. The common frequency range over 200 Hz in this study was chosen to compare the results from the published studies.

As shown in Fig. 7, the mean storage modulus of the 11 TM samples obtained from this study is lower than that from the other two studies, particularly as being lower than Zhang and Gan's results. The loss modulus shows the different results, and the curve obtained from this study is very close to that of Zhang and Gan's data over the entire frequency range but lower than that from Luo et al.'s study above 400 Hz. The slopes of the storage modulus-frequency curves from the three studies are close to each other over 200–3800 Hz range. However, the slope of the loss modulus-frequency curve in this study is smaller than that in the two published studies. The rationale for the difference in the slope of loss modulus is still not very clear. One possible cause for the difference between this study and the published studies is the different test temperatures. As shown in Fig. 2, the loss modulus has a larger slope at a lower temperature.

The difference of this study from the published studies is probably caused by: 1) the individual variation of the human TM specimens. The human TM samples in the different studies were obtained from donors with different ages, genders and health conditions. The TM samples from the different portions or fiber directions of the TM might have created different mechanical properties. In this study, the TM sample strip was cut along the superior-inferior direction. 2) The physiological condition or moisture level of the TM sample. In this study, we tried to keep the moisture levels to be consistent for all TM samples as we did in the previous studies using the acoustic driving and laser measurement[28] and that used by Luo et al. in the split Hopkinson tension bar test[18]. However, it is not easy to control the moisture level to be similar in different experimental setups. 3) The testing temperature. The temperature had obvious effect on the mechanical properties of the TM as shown in this study. The complex modulus was obtained at the reference temperature of 37°C in this study, while the experiments were conducted at room temperature in other studies. The storage modulus at 37°C is about 15% lower than that at 25 °C. 4) The different experimental methods between this study and the published studies.

The human TM is a multi-layer membrane consisting of the epidermal, collagen fibrous and mucosal layers from the lateral to medial side. The collagen fibers are organized into the matrix of ground substance primarily along the radial and circumferential directions.[17] The radial fibers are thicker and stronger than the circumferential fibers. The arrangement of

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collagen fibers in two layers with different directions makes the TM inhomogeneous and orthotropic. In this study, as the first step in measuring the dynamic properties of the human TM was to use the DMA, the TM was assumed as a homogeneous, isotropic and linear viscoelastic material. This simplification makes the results reported here different from the actual mechanical properties of either the radial or the circumferential fibers in the TM.

In this study, the TM sample strip was cut along the superior-inferior axis, which included both the radial and circumferential fibers. The longitudinal axis of the TM sample strip was along neither the radial nor the circumferential direction of the TM, but more closely to the circumferential direction. The circular fibers provide less stiffness than the radial fibers as reported by Fay et al.[8] Moreover, the pre-stress existing in the intact TM was released when the sample was cut and the stiffness of the TM sample was reduced. Thus, the storage modulus of the TM reported in this study was much lower than that of the intact TM as estimated by Fay et al.[8]

II. Feasibility of FTS on ear soft tissues

The results obtained in this study show that the complex modulus-frequency curves of the human TM samples satisfy all three requirements commented by Ward (1971).[25] Thus, the FTS principle is feasible to measure the dynamic properties of the TM at higher auditory frequencies. This method also has the potential to test other middle ear soft tissues, such as the stapedial annular ligament and the round window membrane. These soft tissues are mainly composed by collagen fibers and can be considered as homogenous materials as well.

Compared to published dynamic testing methods, the advantages of the method developed in this study includes: 1) measurement in the frequency domain with no need of conversion from the time domain or the help of a finite element analysis; 2) no limitation on the tissue sample shape and geometry, unlike the acoustic driving method (Zhang and Gan, 2010) which is only applicable to a thin membrane; 3) the ability to reach higher frequency levels (higher than 3800 Hz) compared to the method using the Hopkinson tension bar.

III. Application of the complex modulus and future studies

The complex modulus of the human TM obtained in this study can be applied into the FE model of the human ear and help improve the accuracy of the model. In almost all published FE models of the human middle ear, a Rayleigh type damping was applied for the soft tissues including the TM.[30] As an example, a value of 0.000075 was used for the β coefficient of Rayleigh damping in Gan et al.'s FE models.[12, 13] A constant elastic modulus and Rayleigh type damping in the FE models make the loss modulus or damping of the TM proportional to the frequency, which makes the damping too high at the higher frequencies. Recently, Zhang and Gan reported a FE model of the human ear with viscoelastic properties for the middle ear soft tissues.[29] The damping or loss modulus was not proportional to the frequency but calculated by equations such as Eqs. (4) and (5) in this study. The application of the viscoelastic properties for the middle ear soft tissues has improved the results at the higher frequencies as shown in Zhang and Gan's model when compared with the previous model.[13]

Future studies will be conducted to measure the dynamic properties of TM samples in diseased conditions, such as otitis media, which is the most common middle ear disease in children, and to measure the dynamic properties of other ear tissues. The relationship between the macro mechanical properties of the human TM sample and the micro fibrous structure will also be studied through experiments or the FE modeling analysis.

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CONCLUSION

In this study, a new method to determine the dynamic properties of the TM samples was developed using the DMA and the FTS principle. The dynamic tests were conducted at frequencies from 1 Hz to 40 Hz at three different temperatures: 5°, 25° and 37°C. The frequency-temperature superposition (FTS) was applied to expand the frequency range to a much higher level (at least 3,800 Hz). The feasibility of the FTS principle was assessed and verified. The generalized linear solid model was used to describe the viscoelastic behavior of the TM samples. The viscoelastic parameters were determined by fitting the model to the experimental results. The storage modulus E' and the loss modulus E" in the frequency domain was obtained from the 11 specimens. The mean storage modulus was 15.1 MPa at 1 Hz and 27.6 MPa at 3800 Hz. The mean loss modulus was 0.28 MPa at 1 Hz and 4.1 MPa at 3800 Hz. The dynamic properties of the human TM obtained in this study provide a better description of the damping behavior of ear tissues, which can be applied into the FE model of the human ear to replace the Rayleigh type damping. The data reported here contribute to the biomechanics of the middle ear and improve the accuracy of the FE model for the human ear.

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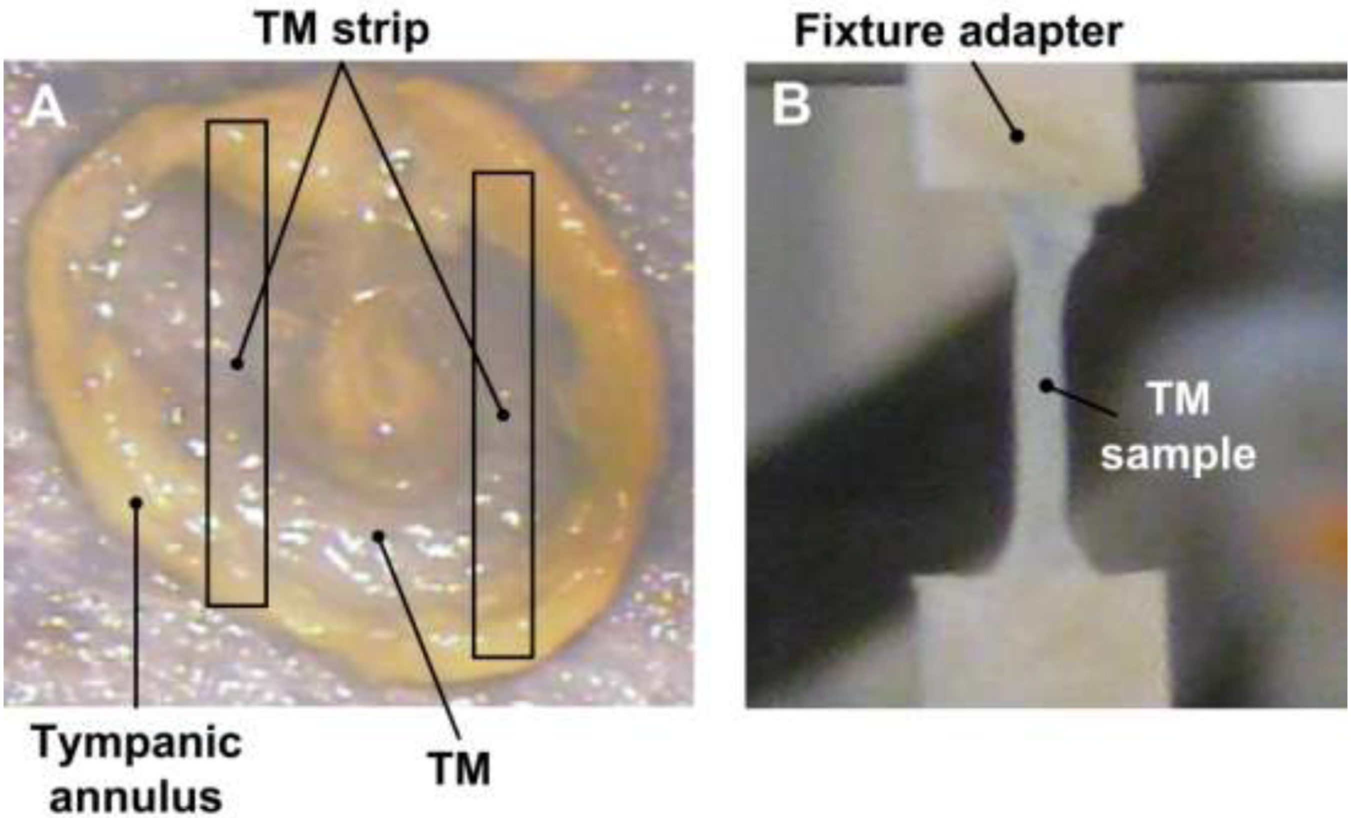

Figure 1.
(A): The TM sample harvested from a temporal bone with the tympanic annulus attached. The TM strip was cut from the posterior or anterior portion of the TM. (B): One TM sample installed in the DMA with plastic fixture adapters.

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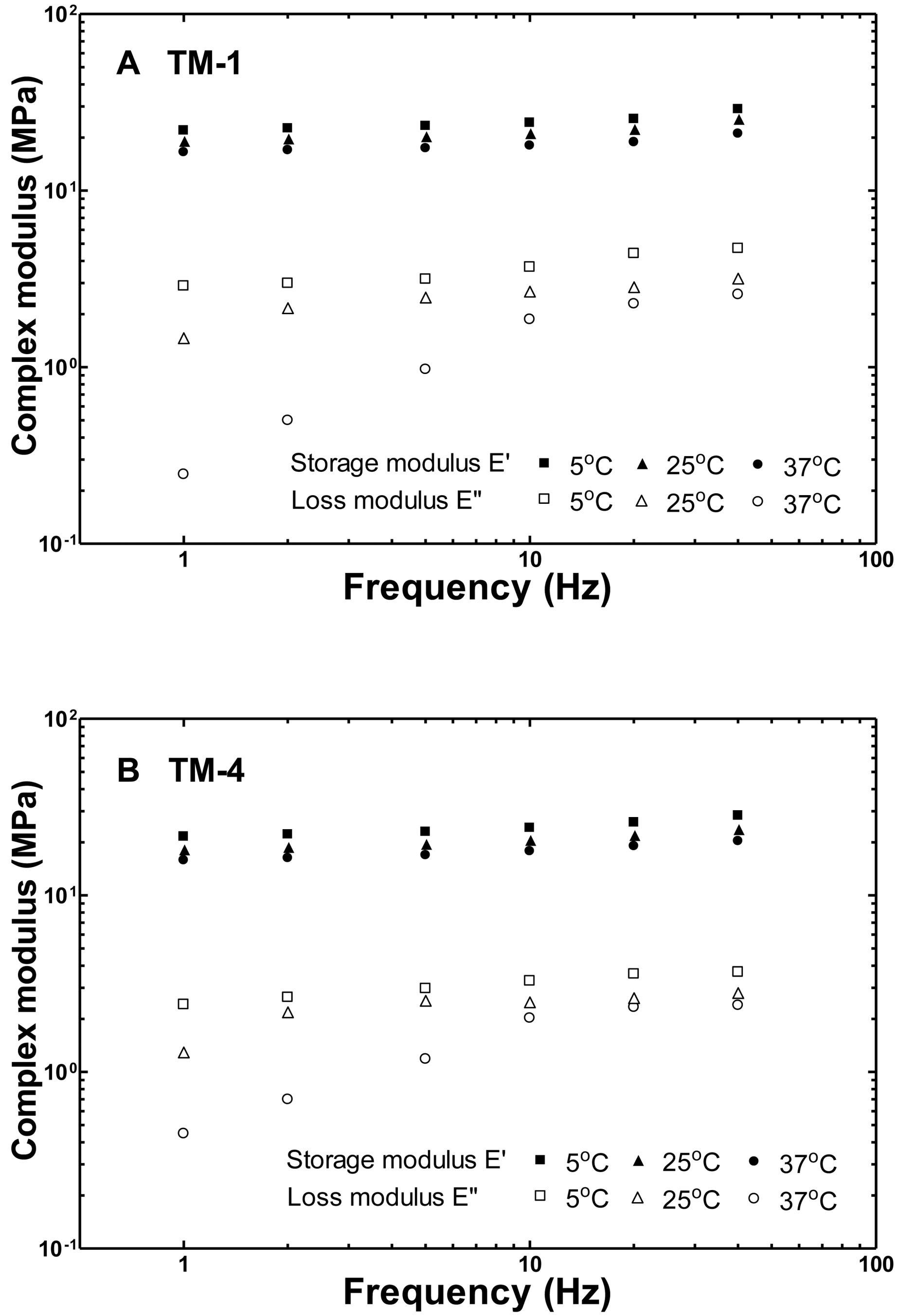

Figure 2.
The complex modulus-frequency curves obtained at the different temperatures (5°C, 25°C and 37°C) from two TM samples: (A) sample TM-1 and (B) sample TM-4.

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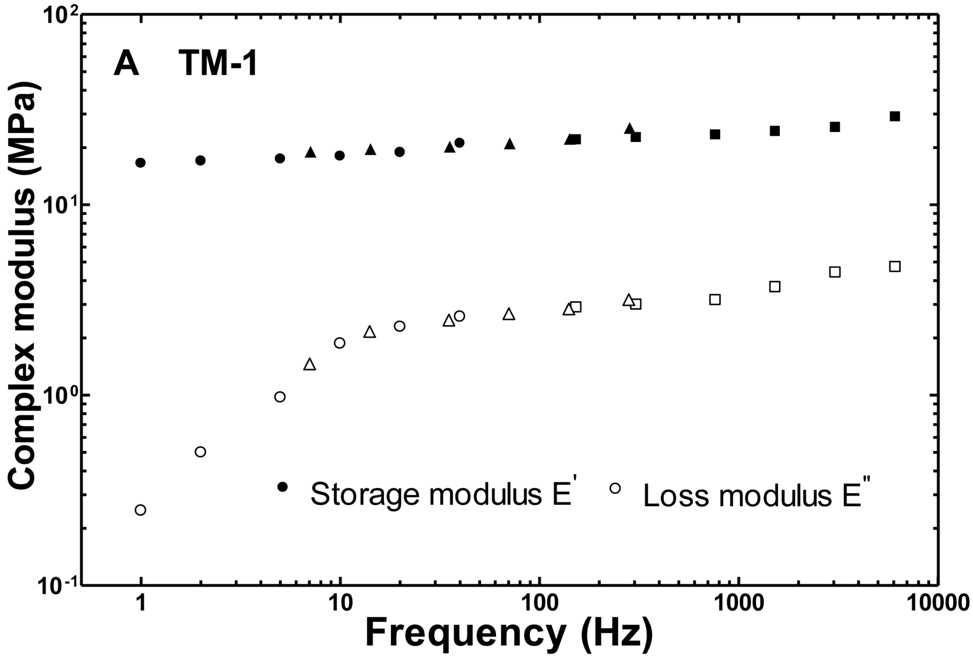

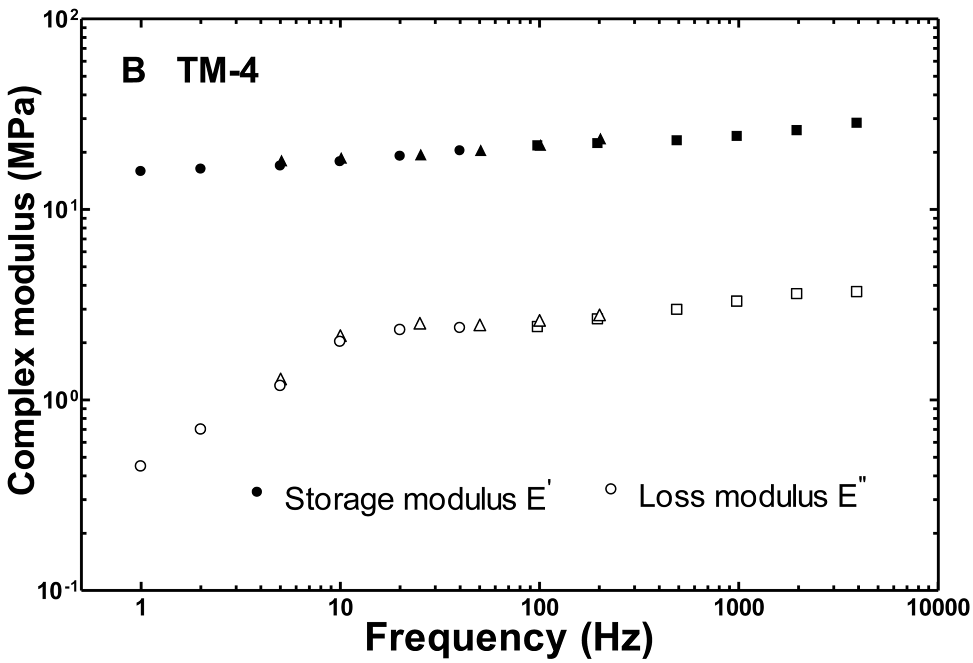

Figure 3.
The master curves of the complex modulus at 37°C obtained from two TM sample: (A) sample TM-1 and (B) sample TM-4.

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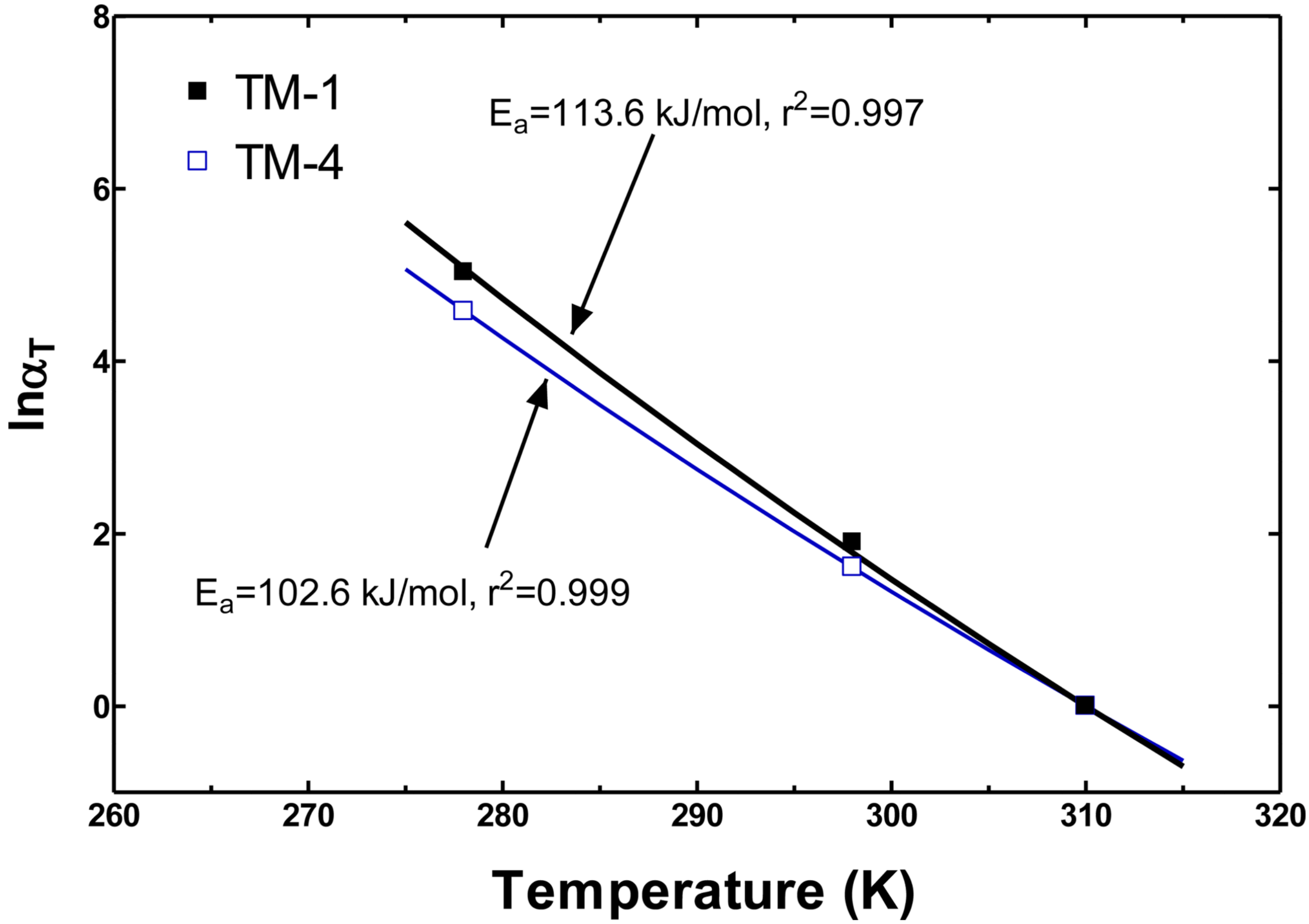

Figure 4.
The fitting of Arrhenius equation (solid lines) to the experimental natural logarithmic shift factor $\ln\alpha_T$ -absolute temperature (in Kelvin degree) curves obtained from samples TM-1 (solid square symbols) and TM-4 (hollow square symbols). The activation energy E_a was obtained as 113.6 kJ/mol for TM-1 and 102.6 kJ/mol for TM-4, respectively.

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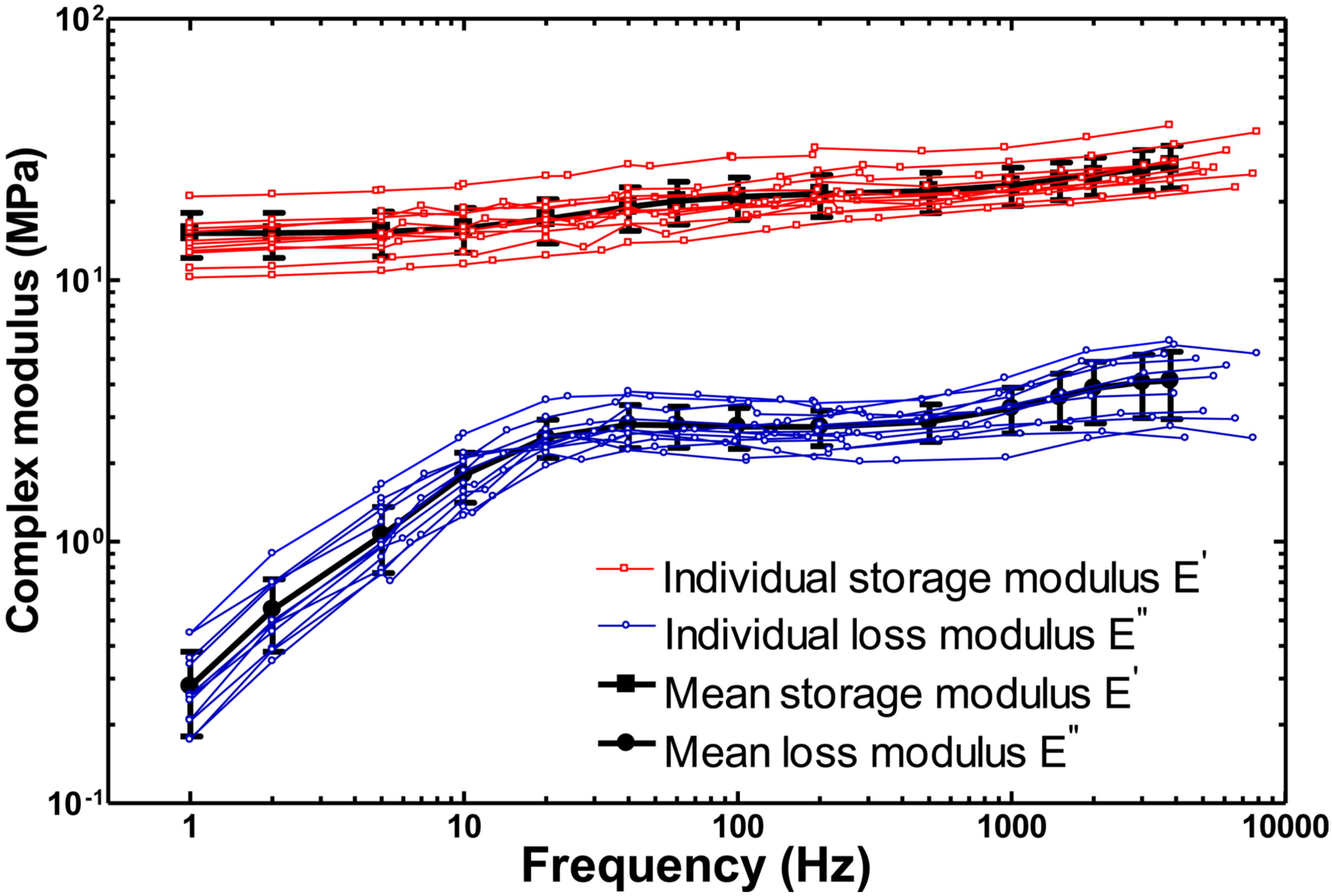

Figure 5.

The master curves of the storage modulus (thin red lines with square symbols) and the loss modulus (thin blue lines with circular symbols) at 37°C from all 11 TM samples and the mean master curves of the storage modulus (thick black line with square symbol) and the loss modulus (thick black line with circular symbol).

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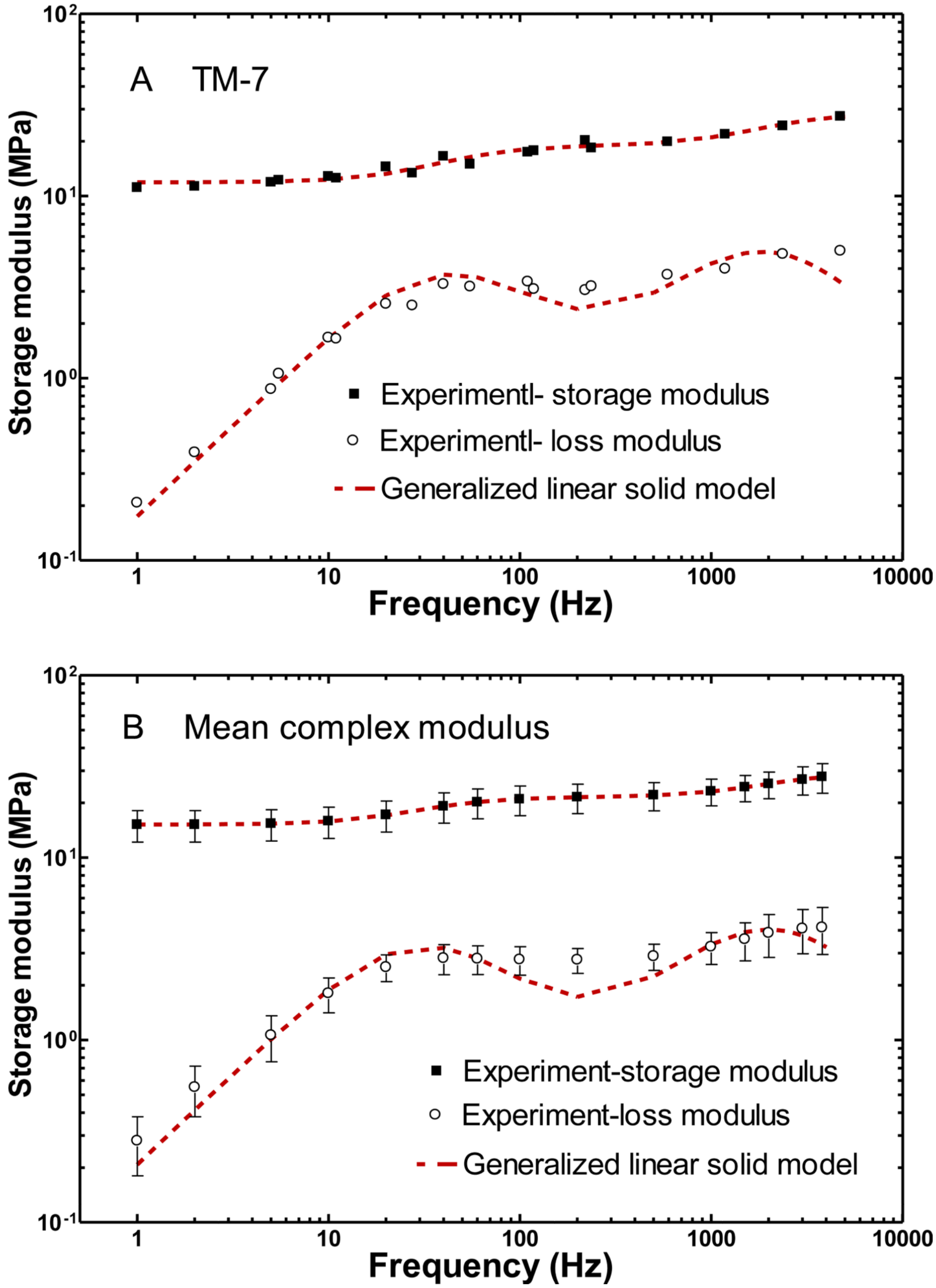

Figure 6.

The theoretical fitting of generalized linear solid model to the experimental complex modulus for (A): sample TM-7 and (B) the mean experimental complex modulus.

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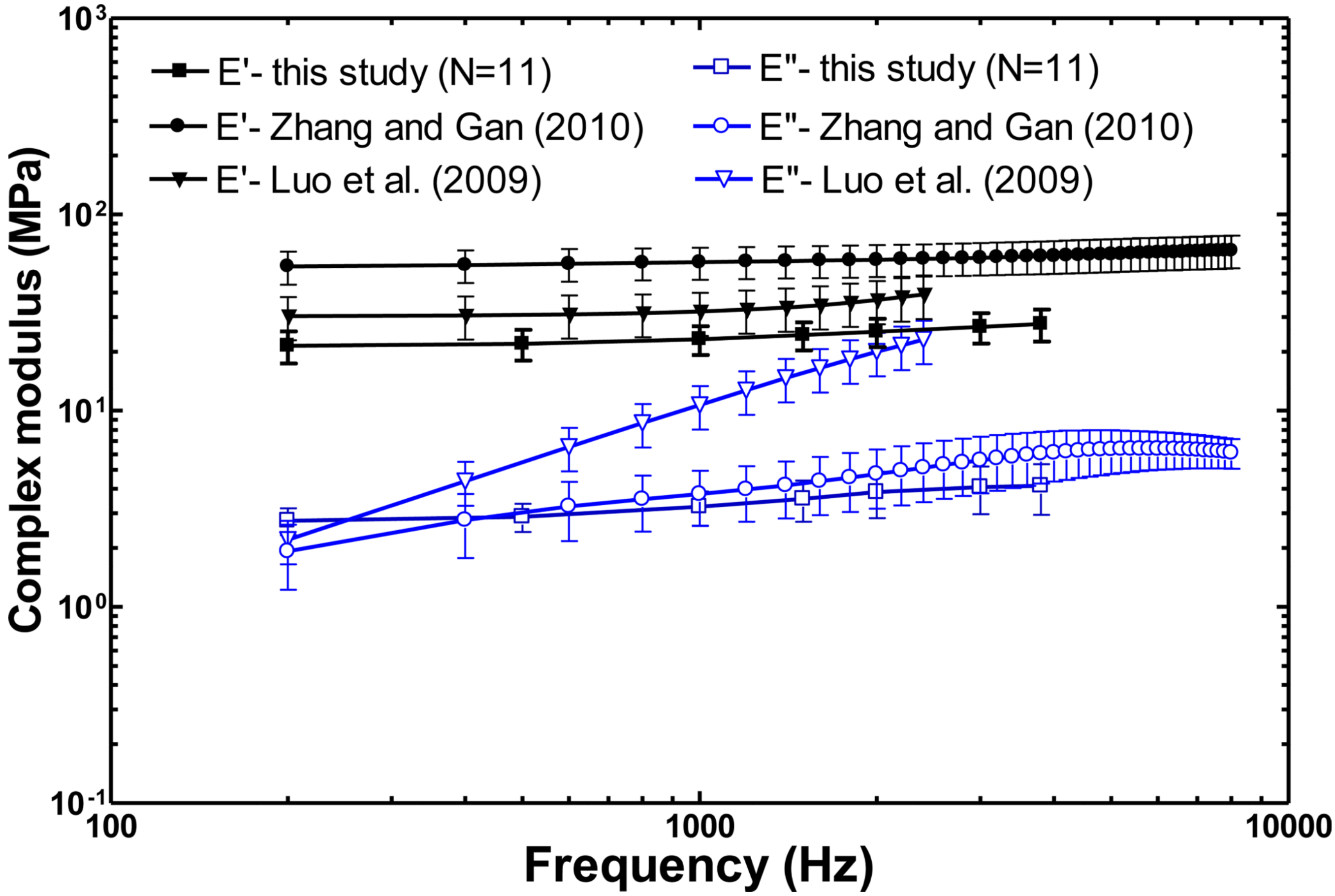

Figure 7.

Comparison of the storage modulus and the loss modulus obtained from this study to the published data by Luo et al. (2009) and Zhang and Gan (2010).

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Table 1

The dimensions of striped human TM samples.

Sample	TM-1	TM-2	TM-3	TM-4	TM-5	TM-6	TM-7	TM-8	TM-9	TM-10	TM-11	Mean±S.D.
Length (mm)	7.6	7.4	6.0	5.6	6.4	7.0	7.1	7.4	5.6	6.0	5.6	6.5±0.7
Width (mm)	2.1	2.5	2.4	2.2	2.0	1.8	1.7	1.8	2.1	2.3	2.2	2.1±0.3

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Table 2

The shift factors, activation energies, and maximum frequency of human TM samples.

Sample	TM-1	TM-2	TM-3	TM-4	TM-5	TM-6	TM-7	TM-8	TM-9	TM-10	TM-11	Mean±S.D.
α_{25} (25 to 37)	6.7	6.0	5.4	5.0	5.0	7.2	5.5	5.8	4.8	7.0	6.4	5.9±0.8
α_5 (5 to 37)	153	126	108	98	90	196	118	137	95	191	165	134.2±37.7
$\ln\alpha_{25}$	1.90	1.79	1.69	1.61	1.61	1.97	1.70	1.76	1.57	1.95	1.86	1.76±0.14
$\ln\alpha_5$	5.03	4.84	4.68	4.58	4.50	5.28	4.77	4.92	4.55	5.25	5.11	4.86±0.28
E_a (kJ/mol)	113.6	109.0	105.2	102.6	101.0	119.1	107.0	110.4	101.7	118.3	114.9	109.3±6.5
Max. freq (Hz)	6120	5040	4320	3920	3600	7840	4720	5480	3800	7640	6600	5371±1506

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Table 3

The viscoelastic parameters of human TM samples (first five rows) and the coefficient of determination r^2 for fitting of storage modulus E’ and loss modulus E” (last two rows).

Sample	TM-1	TM-2	TM-3	TM-4	TM-5	TM-6	TM-7	TM-8	TM-9	TM-10	TM-11	Mean±S.D.
E_0 (MPa)	17.7	15.0	13.6	16.7	14.0	17.0	11.9	13.3	21.6	14.8	10.8	15.1±3.0
E_1 (MPa)	6.3	5.2	4.1	5.1	5.4	9.6	7.0	6.4	8.6	6.1	5.8	6.3±1.6
E_2 (MPa)	9.1	5.7	4.4	7.3	11.5	12.0	9.6	9.1	11.8	4.7	5.6	8.3±2.9
τ_1 (ms)	3.66	6.37	7.36	7.53	9.47	3.98	3.85	6.36	5.95	4.59	3.83	5.72±1.91
τ_2 (μs)	41.7	104.5	152.4	99.8	83.7	47.4	84.3	54.7	74.5	67.3	126.6	85.1±33.9
r^2 for E’	0.970	0.960	0.964	0.972	0.982	0.978	0.973	0.984	0.995	0.984	0.986	0.977±0.010
r^2 for E”	0.669	0.529	0.580	0.545	0.759	0.763	0.824	0.689	0.781	0.602	0.584	0.666±0.104