Research paper

Characterization of the linearly viscoelastic behavior of human tympanic membrane by nanoindentation

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A B S T R A C T
Human tympanic membrane (or eardrum) is composed of three membrane layers with collagen fibers oriented in the radial and circumferential directions, and exhibits viscoelastic behavior with membrane (or in-plane) properties different from through-thickness (or out-of-plane) properties. Due to the interaction of bundled fibers and ground substance, these properties could change with locations. In this paper, we use nanoindentation techniques to measure the viscoelastic functions of four quadrants of tympanic membrane (TM). For measurement of in-plane Young's relaxation modulus we fixed a sectioned quadrant of the TM on a circular hole and used a spherical nanoindenter tip to apply force at the center of the suspended circular portion of the specimen. An inverse problem solving methodology was employed using finite element method to determine the average in-plane Young's relaxation modulus of the TM quadrant. Results indicate that the in-plane steady-state Young's relaxation modulus for four quadrants of the TM does not vary significantly. However, a variation of the modulus from 25.73 MPa to 37.8 MPa was observed with measurements from different individuals. For measurement of Young's relaxation modulus in the through-thickness direction a spherical indenter tip was used to indent into different locations on the surface of the TM specimen supported by a substrate. Viscoelastic contact mechanics analysis of the load-displacement curve, representative primarily of the through-thickness stiffness of the TM, was conducted to extract the Young's relaxation modulus in the out-of-plane direction. Results indicate a wide variation in steady-state Young's relaxation modulus, from 2 MPa to 15 MPa, in the through-thickness direction over the TM.

1. Introduction
The human middle ear within the temporal bone includes tympanic membrane (TM) and three ossicular bones (malleus, incus and stapes), suspended and held by middle ear ligaments in an air-filled cavity. The acoustic waves having characteristic amplitude and frequency (20–20 000 Hz) are collected by the external ear canal and induce vibrations of...
the TM, which are further transmitted through the ossicular chain into the cochlea that detects different frequencies of sound (Gan et al., 2004, 2006).

The human TM is composed of three membrane layers: the epidermal layer on the lateral side, middle lamina propria layer, and mucosal layer on the medial side. Lamina propria layer contains bundled collagen fibers oriented in both the radial and circumferential directions (Lim, 1995). The in vitro measurements of elastic modulus of the TM have been reported in the literature. von Békésy (1960) reported Young’s modulus 20 MPa through bending test of the dissected TM strip. Kirikae (1960) measured the Young’s modulus of 40 MPa through longitudinal dynamic test on the TM. Decraemer et al. (1980) conducted tension test and reported modulus of 23 MPa. Fay et al. (2005) presented methods to estimate TM modulus using composite laminate theory under dynamic measurements and reported that the stiffness contribution in TM is mainly due to the presence of collagen fibers. They reported that the stiffness of a particular portion of the TM depends on the density of the fibers in that region, and in actual physiological conditions the stiffness varies in different regions of the TM. Gaihede et al. (2007) conducted in vivo experiments on human eardrum to measure its areal modulus, which is a representative for the homogenized properties of the eardrum. The TM was assumed as homogeneous, having uniform thickness and spherical shape in the deformed state. They reported that the method of estimating areal modulus was sensitive to the area of the original eardrum, and the bending moment had been neglected in the calculation. Cheng et al. (2007) reported the in-plane Young’s relaxation modulus as a function of both time and stress by analyzing the results obtained from tensile relaxation tests on the TM specimens cut primarily along the circumferential direction of a TM. They reported the properties of the TM in the in-plane direction.

The methods for measurements of TM mechanical properties using tensile and bending tests can characterize the TM modulus, and the values obtained are thus homogenized or average values over the portion of a TM sample. However, TM is an inhomogeneous structure with anisotropy in radial, circumferential, and through-thickness directions. Methodology using nanoindentation would be able to characterize viscoelastic relaxation modulus in different directions (Huang et al., 2008). Huang et al. (2008) established methods for measuring linearly viscoelastic properties of human tympanic membrane using nanoindentation. Results were reported for relaxation modulus of the TM in both wet as well as dry conditions with emphasis on the measurement technique on the wet specimen. However in Huang et al. (2008) no thorough measurements were carried out to report the properties in statistical form. In that work, the measurements did not distinguish between different quadrants of the TM, and the results reported were from a small number of tests carried out using localized portions of the TM to demonstrate the measurement technique. In this work, we have carried out extensive nanoindentation tests to report results obtained from multiple TM samples. Moreover, since different regions of the TM within the pars tensa might exhibit different relaxation modulus functions, we have conducted nanoindentation measurements on four quadrants of the TM and reported the results in statistical form.

In physiological conditions, TM is in a conical form with a cone angle of 132° to 137° (Gaihede et al., 2007). Within the TM, the collagen fibers are aligned either along the radial direction or in the circumferential direction, so that nearly orthotropic behavior is present locally. As a simplified case, the TM can be considered as transversely isotropic with the in-plane properties different from that of the out-plane, and these properties could vary at different locations of the TM. Since nanoindentation measures local properties, it can help map TM properties over its entire region, and enable computer model of the middle ear to assign local values of the modulus over the TM. This can help us take a step further in simulating middle ear dynamic behavior (Gan et al., 2004, 2006) comparable with the actual case.

In this paper, we report the results of Young’s relaxation modulus measured on four different quadrants of human TM samples. Separate test setups were used to measure properties in the out-of-plane (through-thickness) (using 3 TM samples) and in-plane directions (using 2 TM samples). Viscoelastic nanoindentation techniques have been established earlier for polymers, for example, by Lu et al. (2003) and Huang and Lu (2006). The methodology adapted for TM specimens has been established by Huang et al. (2008), and for the completeness of this paper the experimental procedure has been described briefly here. The relaxation modulus in the out-of-plane direction is determined using the method developed by Lu et al. (2003), and the in-plane relaxation modulus is determined by solving an inverse problem for a concentrated force applied at the center of a clamped circular plate, using finite element method (FEM).

2. Methods and analysis

2.1. Nanoindentation measurements

An MTS Nano Indenter XP with TestWorks software (version 4.08) was used for nanoindentation on human TM specimens. The nanoindenter had resolutions 0.2 nm in displacement and 50 nN in load. A spherical tip with radius 10 µm was used in all the tests. The TM samples were harvested from fresh-frozen human temporal bones (cadaver ears) through the Willed Body Program at the University of Oklahoma Health Sciences Center. The tympanic annulus was separated from the bony ear canal with malleus attached to the TM. All the TM samples had their outer epidermal layer removed, so that the indenter tip came in direct contact with the lamina propria layer made up of collagen fibers. Measurements were performed within 6 days after obtaining bones, similar to what was used by Cheng et al. (2007).

Fig. 1 shows the medial view of a left ear TM sample. The TM was sectioned into four portions: anterior–superior, anterior–inferior, posterior–superior, and posterior–inferior quadrants following the clinical sectioning procedure (Fig. 2). Each TM specimen was gently stretched by several cycles on a flat substrate for pre-conditioning and flattened before mounting it on a fixture. It is anticipated that this process should not affect much the collagen fiber network inside the
Fig. 1 – An image showing medial view of a typical TM (female, age 82, left ear; annulus damaged on the posterior side).

Fig. 2 – Schematic diagram showing four quadrants of left ear TM (medial view).

TM sample, as no visible shrinkage of the TM specimen was observed. In these procedures, mounting of TM specimen involved placing a portion of the specimen (using forceps) on the orifice (for in-plane testing) or substrate (for out-of-plane testing) after which the TM specimen was not disturbed. For the case of in-plane testing, a thin layer of medium-viscosity epoxy glue was used to glue the TM specimen on the fixture with the location of the glue at a distance away from the circular hole of the fixture as much as possible in each case, and for out-of-plane case, no epoxy glue was used. We have made numerous runs to standardize our procedures so that we have assurance to certain level on the tautness of the specimen to ensure that the specimen is fixed to the substrate. To validate the method for measurements of in-plane properties using infinite element analysis, control tests consisting of in-plane nanoindentation tests and uniaxial relaxation tests on polyethylene film using an Instron 4202 screw-driven material test system were performed. This validation result has been reported in Huang et al. (2008).

In case of out-of-plane testing, a quadrant of the TM sample was placed on aluminum substrate with the mucosal layer facing down (towards the aluminum surface). The nanoindenter tip came in direct contact with the collagen fiber layer (lamina propria). The TM was initially placed over the aluminum substrate and was completely soaked in saline solution (0.9% NaCl, pH 5.6) to ensure that no air remains entrapped in the small TM specimen. The saline solution level was then lowered to allow its meniscus contact the TM periphery, so that the TM remained in wet condition while exposing the TM surface for nanoindentation. During testing, the TM specimen remained in contact with saline solution and the TM can be considered to remain in wet condition since it would sip in the saline from the periphery, and this was visible under the built-in microscope on the nanoindenter.

Fig. 3 shows a schematic diagram of the various layers in pars tensa of the TM (Lim, 1970; Fay et al., 2005). The mucosal layer is very thin (1 to 2 µm, typically made up of 1 to 2 layers of cells) in a healthy TM. The epidermal layer is typically 8 µm and is easy to remove from a TM sample. In order to probe the properties of collagen fibrous layer, the epidermal layer was removed prior to nanoindentation. However, separating the mucosal layer was very difficult and could induce damage to the TM. Hence, we carried out the nanoindentation testing without removing the mucosal layer. In our out-of-plane nanoindentation testing, the effect of compressive displacement induced due to mucosal layer cannot be ruled out completely. We conducted FEM analysis to determine its effect on the measurement result on the collagen layer. During each test, a pre-stiffness (with the resulting pre-load and pre-displacement) was used as a criterion to define the point of contact, which is also the starting point for nanoindentation load–displacement curves. We conjecture that with this procedure the pre-load would compress the less stiff elements such as minute air bubbles if any (to allow it to move to other locations or escape completely) if entrapped. During the actual load–displacement test segment, due to presence of mucosal layer, the result obtained using methodology described in this paper would under-predict the results of collagen layer by less than 5% from the FEM analysis. As far as in-plane testing is concerned, the TM is under biaxial tension state, and the contribution of mucosal layer to the in-plane stiffness (corresponding to the in-plane modulus) can be neglected (Fay et al., 2006).

Nanoindentations were conducted at ∼23 °C. In case of in-plane testing (Fig. 4b), only one nanoindentation test was performed for each quadrant of a TM, which took ∼15 min including the drift correction for the nanoindenter. During this period the saline solution level meniscus was in contact with the TM tissue without visible reduction of saline due to evaporation. However in case of out-of-plane tests (Fig. 4a),
in which several tests were conducted within a batch, it was necessary to ensure that the meniscus stayed in contact with the TM tissue despite the evaporation of saline solution. This was accomplished with the use of relatively large saline-well in the fixture (Fig. 4a). We monitored the saline level to ensure that the TM stayed in saline-soaked condition at the end of every test batch for each sample.

2.2. Viscoelastic analysis for determining the out-of-plane properties

In this method, the viscoelastic solution for an indenter indenting into a substrate is fitted to the nanoindentation data measured in experiment to yield a solution for creep compliance. The Young’s relaxation modulus is then inversed numerically from the creep compliance. The details on the equations and procedures are given as follows.

Lu et al. (2003) have devised a method using viscoelastic analysis to extract the creep compliance of a viscoelastic material from its nanoindentation load–displacement curve. In this method, for spherical nanoindentation under a constant-rate loading history \( P(t) = P_0 H(t) \), with \( P_0 \) being constant and \( H(t) \) Heaviside step function, the analytical solution gives

\[
h_{3/2}(t) = \frac{3(1-\nu)}{8\sqrt{E}} \left[ J_0 + \sum_{i=1}^{N} J_i \right] P(t) - \sum_{i=1}^{N} J_i (P_0 \tau_i) (1 - e^{-\frac{P_0 \tau_i}{P_0 \tau_i}}) \tag{1}
\]

where \( h(t) \) is the indentation displacement as a function of time \( t \), \( R \) is the nanoindenter tip radius, \( \nu \) is the Poisson’s ratio, \( J_0 \) and \( J_i \) are shear creep coefficients and \( \tau_i \) is retardation time of the shear creep compliance, \( J(t) \), given in the generalized Kelvin model

\[
J(t) = J_0 + \sum_{i=1}^{N} J_i (1 - e^{-t/\tau_i}). \tag{2}
\]

The fitting was conducted by maximizing the cross correlation coefficient between analytical nanoindentation load–displacement relationship and the measurement data. This procedure leads to the best-fit parameters, \( J_0, J_i, \tau_i \) which were then used to calculate the shear creep compliance using Eq. (2). The shear creep compliance function represents the linearly viscoelastic behavior of TM independent of the loading rate. Furthermore, in the linearly viscoelastic regime under small deformations, results are independent of stress or strain levels as long as they are infinitesimally small. For analysis of TM in acoustic transmission, the relaxation modulus, \( E(t) \), is needed and can be solved from

\[
\int_0^t E(\tau)(t-\tau) d\tau = 2(1 + \nu)t, \tag{3}
\]

where the Poisson’s ratio, \( \nu \) was assumed constant.

In the viscoelastic analysis we assumed a semi-infinite half-space for the TM specimen. The properties determined would represent the property of the lamina propria. The maximum indentation depth used for viscoelastic analysis to determine viscoelastic properties was restricted to 4 \( \mu \)m so that the effect of substrate can be neglected since the maximum indentation depth was less than 1/15 of the TM thickness (Lu et al., 2006).

2.3. Viscoelastic analysis for determining the in-plane TM relaxation modulus using FEM

For measurements of in-plane viscoelastic properties of the TM, the configuration as shown in Fig. 4(b) was used. The force was applied using a nanoindenter tip at the center of the suspended circular portion of the TM sample. FEM was used to model this viscoelastic nanoindentation problem. An inverse problem was solved to determine the viscoelastic parameters. The procedure for this approach is described herein.

In FEM analysis, the shear relaxation modulus is given by the generalized Maxwell model as,

\[
\mu(t) = \mu_\infty + \sum_{i=0}^{N} \mu_i e^{-t/\tau_i}, \tag{4}
\]

where \( \mu_\infty \) is the steady-state shear relaxation modulus, \( \mu_i \) shear relaxation coefficients, \( \tau_i \) relaxation times, and \( N \) the number of terms in the Prony series.

FEM analysis using ABAQUS, with the use of initial guesses for \( \mu_\infty, \mu_i \) and \( \tau_i \) in Eq. (4), was first conducted to simulate the nanoindentation of a TM specimen (fixed on a circular orifice) by a concentrated force applied at the center of the TM sample. Next, the simulated load–displacement results were compared with the experiment data. An iterative procedure was carried out to maximize the cross-correlation coefficient.
between the experimental and simulated curves and is described here. The choice of parameters is not unique (Knauss and Zhao, 2007) and a variety of set of relaxation times and relaxation coefficients can equally provide the same relaxation function after summing up all the terms on the right hand side of Eq. (4). Thus one is at liberty in prescribing some of the parameters including the relaxation times which are not subject to stringent criteria. We usually allow for one relaxation time per decade of data in the logarithmic scale, within the range of experimental time, and search for the corresponding relaxation coefficients by maximizing the cross-correlation coefficient. In this work, the maximum test-time for in-plane tests in nanoindentation was about \( \sim 300 \) s. With this, we used relaxations times equally spaced on the log-scale, specifically, relaxation times \( \tau_1 = 1 \) s, \( \tau_2 = 10 \) s, and \( \tau_3 = 100 \) s in this case. We have found that \( N = 3 \) [in Eq. (4)] can satisfactorily match the simulation results with nanoindentation experimental curves. We then minimized the difference between simulated and measured load–displacement curves to determine the best-fit relaxation coefficients. For every exponential term in the Maxwell element there is an influence range (on the time scale and influences the relaxation modulus) which covers a time range of about one decade (Knauss and Zhao, 2007). In searching for the relaxation coefficients we tried for several values, to maximize the cross correlation coefficient with the results from nanoindentation experiments. In this process, we started with trial values and adjusted the coefficients for the lower relaxation times first using the short-term nanoindentation load–displacement data, and then moved towards determining the relaxation coefficients corresponding to higher relaxation times.

The best-fit parameters, obtained from the iterative procedure described above, were then used to determine shear relaxation modulus \( \mu(t) \) and the Young’s relaxation modulus of the TM. With the assumption of constant Poisson’s ratio \( \nu \), the Young’s relaxation modulus is,

\[
E(t) = 2(1 + \nu)\mu(t),
\]

and is represented by the generalized Maxwell model as,

\[
E(t) = E_\infty + \sum_{i=0}^{N} E_i e^{-t/\tau_i},
\]

where \( E_\infty \) is the long-term or steady-state uniaxial relaxation modulus, \( E_i \) represents relaxation coefficients, \( \tau_i \) relaxation times, and \( N \) the number of terms in the Prony series. We have validated this technique by measuring the in-plane modulus for polyethylene and comparing it with the relaxation modulus obtained from tensile testing. The results were found to be in reasonable agreement (Lu and Huang, 2007). The method has also been successfully applied for multilayer nanocomposite films (Lu et al., 2006).

For nanoindentation tests using in-plane set-up, there would be penetration of the indenter tip into the specimen surface due to the out-of-plane compliance of the TM sample. This led to an approximate ratio \( E_{\text{solid}}/E_{\text{shell}} = 1.2 \) for the modulus data determined from FEM analysis using solid elements (\( E_{\text{solid}} \)) and shell elements (\( E_{\text{shell}} \)), which was carried out separately (Huang et al., 2008). This factor has been considered in the results presented.

### 3. Results and discussion

#### 3.1. In-plane relaxation modulus

Nanoindentations were conducted on four quadrants of two TM samples (TM0619, female aged 76, left ear; and TM0713, female aged 82, left ear), with an exception for TM0619 whose posterior–superior quadrant was damaged during harvesting. Figs. 5 and 6 show the load–displacement curves obtained from these two TM samples. For in-plane tests on TM samples loading rate of 2.5 \( \mu \)N/s was used with the maximum load reaching \( \sim 0.8 \) mN. The load–displacement curves for different quadrants of the same TM were found to be very consistent. However, the maximum displacement reached for TM0713 (Fig. 6) was higher than that of TM0619 (Fig. 5) under the same force, indicating that the specimen TM0713 was more compliant.

FEM was used to simulate nanoindentation on the TM sample. The FEM model for the TM specimen consisted of 4275 shell elements that can carry both bending and membrane stresses. The TM specimen was considered to have a uniform thickness of 60 \( \mu \)m (thickness of the TM...
Fig. 7a – Correlation of in-plane load–displacement curves obtained from FE analysis and nanoindentation experiment for anterior–superior quadrant for TM samples from female aged 76, left ear (TM0619) (correlation coefficient: 0.999993) and female aged 82, left ear (TM0713) (correlation coefficient: 0.999998).

...with epidermal layer removed). The relaxation modulus obtained using this procedure has inherently considered the effect of loading rates. For viscoelastic properties described by Eq. (4), three terms (N = 3) were used in the Prony series. Fig. 7a shows the results of correlations between load–displacement curves from FEM analysis and nanoindentation measurements. For these two TM samples, correlation coefficients of 0.999993 and 0.999998 were reached, indicating a good agreement between simulation and measurement data. Using Eq. (6) the in-plane relaxation modulus was obtained as

\[ E(t) = 38.1 + 3.05e^{-t} + 1.9e^{-t/10} + 0.76^{-t/100} \text{ MPa} \] (7)

for the anterior–superior quadrant of TM0619, where t is time in seconds; and

\[ E(t) = 27.3 + 2.18e^{-t} + 1.37e^{-t/10} + 0.55e^{-t/100} \text{ MPa} \] (8)

for the anterior–superior quadrant of TM0713.

This procedure was carried out for each of the load displacement curves to find the relaxation coefficients under the same assumed relaxation times. The average relaxation modulus was obtained by averaging the relaxation coefficients corresponding to the same relaxation times. The average in-plane relaxation modulus was obtained as,

\[ E(t) = 37.8 + 2.65e^{-t} + 1.89e^{-t/10} + 1.13^{-t/100} \text{ MPa} \] (9)

for TM0619, and

\[ E(t) = 25.73 + 2.06e^{-t} + 1.29e^{-t/10} + 0.51e^{-t/100} \text{ MPa} \] (10)

for TM0713.

These relaxation modulus functions are plotted in Fig. 7b. The vertical bar value is quantified (±0.52 MPa for TM0619 and ±2.8 MPa for TM0713) as a single number and indicates the deviation from the average. The maximum and minimum values on the vertical bars correspond to the maximum and minimum results on load–displacement curve. Eqs. (9) and (10) imply that the average steady-state values of \( E(t) \) are 37.8 and 25.73 MPa for TM0619 and TM0713, respectively. The steady-state (or long term) value here refers to the value of modulus at time, \( t \to \infty \). The results indicate that the overall stiffness of the TM varies from person to person. The values obtained are close to either the data (40 MPa) reported by Kirikae (1960) or the results (20 MPa) reported by von Békésy (1960).

The results also suggest that the steady-state values for modulus obtained for quadrants of the same TM sample were more or less the same. Before reaching the steady-state values, the Young’s relaxation moduli were observed to decrease with time. For instance at \( t = 1 \) s, the Young’s relaxation moduli values are 41.6 MPa and 28.16 MPa for TM0619 and TM0713, respectively. In Fig. 7b, the vertical bars indicate possible variation (between maximum and minimum) in the values of relaxation modulus due to measured difference in the load–displacement curve obtained from different quadrants belonging to the same TM. This can be explained based on the physiological arrangement of collagen fibers within the TM. Near the outer edge of the TM the density of both types of fibers is almost identical (Fay et al., 2006). However, as one moves radially inwards towards the umbo, the density of radial fibers increases and that of circumferential fibers decreases. As a result the resulting local stiffness is different at different locations over the TM, and this fact is apparent from our results on out-of-plane testing. However, for the sectioned quadrants which were used in this study, even though the density of collagen fiber (and hence the stiffness) may vary locally within the quadrant, the overall stiffness of a quadrant obtained due to structurally similar arrangement of collagen fibers, might be the same. The use of much smaller TM samples for in-plane nanoindentation, sectioned from a quadrant of TM, could give different but more localized stiffness values. Within a quadrant, preparation of the experiment for even smaller samples would be difficult. New techniques are needed for preparation of specimens for measurements of local in-plane modulus.
3.2. Out-of-plane (through thickness) Young’s relaxation modulus of TM

Nanoindentation tests for the measurements of through-thickness properties were conducted at different locations on each of the four quadrants of the TM sample. Three TM samples were tested in this study and specimen information is listed here: male aged 77, right ear (TM0712); female aged 70, left ear (TM0643); and female aged 74, left ear (TM0709). Specimen TM0712 was an exception whose posterior–superior portion of the TM was damaged during harvesting. In all the nanoindentation tests, a constant rate loading history was used. Nanoindentation was made at least 200 μm away from the specimen edge where sectioning was made, so as to possibly rule out the effect of collagen damage in the vicinity of indentation location, and nanoindentations were made roughly 200 μm apart.

The loading rates used were different for different samples due to the limitation of the nanoindenter and were adjusted (based on initial trial indentations) so that the artifacts of decreasing load–displacement slope during the initial portion of the curve can be eliminated (or minimized). This artifact (initial indenter tip acceleration which causes the inertial effect) is caused due to the TM tissue being on the lower side of the capabilities of MTS Nano Indenter XP system. Secondly, due to relatively higher nanoindentation depths there were deviations of the rate of loading (although still constant rate) from the prescribed value. The actual loading rate was calculated from the loading history observed from the output results, and used in analysis to determine the viscoelastic functions. For out-plane tests, the following were the loading rates; for specimen TM079: ~10 μN/s for all, except for posterior–superior which was with ~6 μN/s. Loading rate for TM0643 was ~1.5 μN/s for anterior portions, ~5.5 μN/s for posterior–superior, and 8 μN/s for posterior–inferior. For TM0712 loading rate of ~10 μN/s for posterior–inferior, ~5 μN/s for anterior–inferior and ~1 μN/s for anterior–superior were used. The maximum load was ~0.5 mN for all the tests.

Figs. 8–10 show the load–displacement curves obtained from different quadrants of three TM samples. Since the loading rate is different for different quadrants, the load–displacement could not be compared directly. It should be noted that in the viscoelastic method for determination of relaxation modulus the loading rate has been considered for the corresponding load–displacement curve and the viscoelastic property data is independent of the loading rates used. Thus the relaxation modulus in all the cases can be compared directly (Figs. 11–13). Nanoindentation results are reported for a minimum of 5 different locations on each quadrant, and the variation obtained due to varying stiffness at different locations over the TM is represented using vertical bars in Figs. 11–13. An average of all the relaxation modulus is indicated by a solid line. The long-term value of Young’s relaxation modulus can be calculated from the generalized Kelvin model [from Eq. (2) for creep compliance] as

\[
E(\text{long-term}) = \frac{2(1 + \nu)}{J_0(t \to \infty)} = \frac{2(1 + \nu)}{J_0 + \sum_{i=1}^{N} J_i(1 - e^{-\frac{t}{\tau_i}})}
\]

\[
N
\]

The average Young’s relaxation modulus over time, is calculated from inversion of the average value of creep compliance for that quadrant. The average creep compliance,
in turn, has been calculated by averaging the creep coefficients corresponding to the same retardation times.

The average steady-state values of modulus for each quadrant within the TM samples are reported in Table 1. Results indicated that, in general different quadrants may show different behavior. ANOVA analysis was conducted between different quadrants to probe their behavior considering $\alpha = 0.05$ as the level of significance. We analyzed for the $P$-value between the data-sets, where $P$-value is the probability that the variation between datasets may have occurred by chance. Based on the ANOVA analysis conducted in Microsoft Excel if the $P$-value is greater than 0.05, the two data sets are considered to have similarity. For example, the ANOVA analysis of the results obtained from TM0643 in Table 2(a) showed that the results of different quadrants are from statistically different populations ($P$-value $< 0.05$). Similar results have been shown in the statistics analysis of TM0709 [Table 2(b)] and TM0712 [Table 2(c)] in which majority of the results indicates that different quadrants show different behavior. There were exceptions in which similar behavior was found within anterior quadrants as well as posterior quadrants of TM0709 and inferior quadrants of TM0712. The average steady-state out-of-plane modulus values for all four quadrants were $6.39 \pm 4.58$ MPa for TM0712, $6.7 \pm 2.6$ MPa for TM0643, and $5.96 \pm 1.38$ MPa for TM0709. Additionally, the steady-state values were averaged over the entire membrane (population or the number of tests equals 28 for TM0712 from three quadrants, 50 for TM0643 from four quadrants, and 46 for TM0709 for four quadrants). The ANOVA of the steady-state modulus data obtained from three TM samples indicated that the donors are from the same population. The out-of-plane modulus values are lower than the in-plane moduli because the collagen fibers are oriented within the

![Fig. 9 – Load–displacement curves from out-of-plane nanoindentation tests for samples in saline-soaked condition, for TM from female aged 70, left ear (TM0643).](image)
plane of the TM. However, the difference in the out-of-plane stiffness observed can be a direct indication of variation in modulus due to variation in collagen fiber density over the TM portion.
order of 10 μm, hence there are many collagen fibers (~10 nm diameter) within the indent. Thus, for analysis, the continuum approximation can be considered. The out-of-plane relaxation modulus can be considered as the local average value.

In normal physiological conditions, the TM tissue can be considered to be in the rubbery state (at a temperature much higher than its glass transition). Consequently the Poisson’s ratio can be considered as 0.5. Thus, in the FEM analysis using ABAQUS (version 6.6–1) for shell elements we have used Poisson’s ratio of 0.5. Further, in analysis for extracting viscoelastic properties from the of nanoindentation data, the TM deformation is assumed to be within linearly viscoelastic regime.

In the viscoelastic analysis a semi-infinite half space was assumed for the TM and the viscoelastic properties represent the effective properties of the lamina propria of the TM. In the viscoelastic analysis the Poisson’s ratio is assumed to be 0.5. The analysis presented is restricted to small deformations where the linearly viscoelasticity regime can be assumed. For eardrum in response to the sound wave in the auditory frequency range, the displacement in the eardrum is limited to ±1 μm (Gan et al., 2004). For determining the properties of eardrum under normal service condition, data at small deformations is especially useful. Further investigation of the properties at the large deformation range will be needed to investigate the behavior of eardrum under large deformations for situations such as under blast wave. In the out-of-plane tests the specimens were predominantly in compression, thus the relaxation modulus obtained represents, specifically, the behavior of TM in compression along its out-of-plane direction. On the other hand, during the in-plane testing, the specimen was primarily under tension, and the response obtained, which was used to characterize its relaxation modulus, represents its behavior specifically in tension along in-plane direction.

It should be noted that, the relaxation functions were obtained based on the analysis of a homogeneous, isotropic, linearly viscoelastic material. The human TM, however, is made of a viscoelastic composite so that it is neither homogeneous nor isotropic. The nanoindentation in the through-thickness direction invokes primarily the behavior of the material in the thickness direction. If the material is considered to be transversely isotropic, then the calculated out-of-plane modulus would be dependent on the density of collagen fibers or indirectly on the in-plane value at that location. From our out-of-plane computations on the TM modeled as transversely isotropic elastic structure (not included here) with 10 μm tip radius and 4 μm depth, we arrived at a conclusion that for a pair of out-of-plane and in-plane modulus values of 10 MPa and 40 MPa (as obtained in the results section) the maximum deviation in the calculated out-of-plane modulus would be about –20%/+10% depending on the value used for transverse shear modulus and Poisson’s ratio. In our in-plane analysis, we have considered a relatively simplified analysis procedure by considering the material as isotropic in our analysis. For nanoindentation on the TM fixed at the edge of a circular hole, nanoindentation displacement in the TM is induced primarily by in-plane deformation of the TM specimen suspended over the hole. And thus, in this case the in-plane viscoelastic properties would play a dominant role in the nanoindentation load–displacement relation, so that the assumption on the isotropy in this analysis is not anticipated to affect the in-plane relaxation modulus significantly.

4. Conclusion

The methods of characterizing linearly viscoelastic behavior of tympanic membrane using nanoindentation are sufficiently accurate and robust. We conducted nanoindentation to measure both the in-plane and the through-thickness Young’s relaxation modulus functions on human TM samples obtained from one male and four females aged between 70 and 82. Results from in-plane testing indicate that the viscoelastic effects are pronounced, with in-plane Young’s relaxation modulus changing by ~10% from 1 s to 100 s. The steady-state Young’s relaxation modulus for the specimen from female aged 76 (left ear) was determined to be 37.8 ± 0.52 MPa, and the corresponding value for the specimen from female aged 82 (left ear) was 25.73 ± 2.8 MPa.
Results for the in-plane characterization indicate consistent values of modulus for the four quadrants of each TM. For the TM samples from the same individual, the in-plane steady-state Young's relaxation modulus does not show much variation. However, for TM samples from different individuals the value could show significant variation. The results indicate that the TM modulus in the elderly humans varies from one person to another. The values obtained are close to either the data (40 MPa) reported by Kirikae (1960) or the results (20 MPa) reported by von Békésy (1960).

For measurement of the Young's relaxation modulus in the through-thickness direction, nanoindentations were made on TM specimen with nanoindenter tip in direct contact with the collagen fiber layer. In general, the out-of-plane Young's relaxation modulus is considerably lower than that of the in-plane data. On an average, the out-of-plane relaxation Young's modulus is reduced by 50% from 1 s to 100 s and shows significant viscoelastic effects. At the steady-state, the out-of-plane Young's relaxation modulus varies from 2 MPa to 15 MPa over the TM surface, showing a considerable variation of out-of-plane Young's relaxation modulus with locations. Results in both the in-plane and the through-thickness directions also indicate that linearly viscoelastic constitutive model is appropriate to describe the viscoelastic behavior of the TM under nanoindentation at relatively small displacements.

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References


