Research Article

Mechanical Properties of Baboon Tympanic Membrane from Young to Adult

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ABSTRACT

Mechanical properties of the tympanic membrane (TM) play an important role in sound transmission through the middle ear. While numerous studies have investigated the mechanical properties of the adult human TM, the effects of age on the TM's properties remain unclear because of the limited published data on the TM of young children. To address this deprivation, we used baboons in this study as an animal model for investigating the effect of age on the mechanical properties of the TM. Temporal bones were harvested from baboons (*Papio anubis*) of four different age groups: less than 1 year, 1–3 years, 3–5 years, and older than 5 years of age or adult. The TM specimens were harvested from baboon temporal bones and cut into rectangle strips along the inferior-superior direction, mainly capturing the influence of the circumferential direction fibers on the TM's mechanical properties. The elasticity, ultimate tensile strength, and relaxation behavior of the baboon TM were measured in each of the four age groups with a mechanical analyzer. The average effective Young's modulus of adult baboon TM was approximately 3.1 MPa, about two times higher than that of a human TM. The Young's moduli of the TM samples demonstrated a 26 % decrease from newborn to adult (from 4.2 to 3.1 MPa). The average ultimate tensile strength of the TMs for all the age groups was ~2.5 MPa. There was no significant change in the ultimate tensile strength and relaxation behavior among age groups. The preliminary results reported in this study provide a first step towards understanding the effect of age on the TM mechanical properties from young to adult.

Keywords: tensile test, relaxation test, age-related, Young's modulus

INTRODUCTION

The mechanical properties of the tympanic membrane (TM) determine its function and are important in a variety of applications. It is a key parameter for the design of TM grafts for hastening recovery and reducing hearing loss from TM perforations (Luers and Huttenbrink 2016). The mechanical property data can also be applied in finite element (FE) models to simulate sound transmission in a normal ear or ears exhibiting different pathologies (Gan et al. 2007; Gan et al. 2009; Zhang and Gan 2013a).

Mechanical properties of the human TM have been characterized by multiple research groups utilizing different methodologies such as the bending test (von Békésy 1960), tensile test (Kirikae 1960; Aernouts et al. 2012), dynamic test with laser Doppler vibrometry (Zhang and Gan 2010), and split Hopkinson tension bar (Luo et al. 2009). The measurements of the mechanical properties of the human TM have almost entirely been performed on TMs from adults. The only pediatric results were reported in Funnell and Laszlo's (1982) summarization of Békésy's measurements on cadavers obtained from a children's hospital (von Békésy 1960; Decraemer and Funnell 2008). Properties of the infants and young children's TMs are not well studied due to the scarcity of temporal bones from young children. These data, however, are important for understanding how middle ear diseases, such as otitis media, affect the hearing of the pediatric population (Qi et al. 2008;

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Wang et al. 2016; Motallebzadeh et al. 2017b; Motallebzadeh et al. 2017a).

Recently, we reported a study using baboon TM as an animal model to investigate the effect of age on TM mechanical properties using a micro-fringe projection technique (Liang et al. 2018). In that study, the TM's geometries and mechanical properties among 5 different age groups (<1 year, 1 to <2 years, 2 to <3 years, 3 to <5, and >5 years of age) were compared. As a non-human primate, baboons share many characteristics with humans and provide significant research benefits to humans. The aging of the baboon has been well studied with a correlation of 1:3 to that of human (Dormehl et al. 1992). A baboon raised in captivity can live approximately 30 years, which aligns well with a healthy human living approximately 90 years (Luck et al. 2008). The baboon ear has already been extensively utilized for research in ototoxicity (Wright et al. 1987a; Wright et al. 1987b), otoacoustic emissions (Martin et al. 1985), ionomeric prosthesis implants (Geyer and Helms 1997), and immunocytochemical colocalization in the cochlear nuclei (Moore et al. 1996). However, to the best of our knowledge, our recent study (Liang et al. 2018) is the only work investigating the mechanical properties of baboon TM.

In this paper, we used a quasi-static uniaxial tensile test to measure the mechanical properties of the baboon TM. Failure strength and relaxation behavior of baboon TM were also studied. Similar to previous work, baboon TMs were compared based on age groups to investigate the effects of age on the mechanical properties of the TM. The data from the adult baboon group was compared to data from adult human ears measured with the same method (Cheng et al. 2007) to evaluate the similarity between the two species with regard to the TM.

METHODS

Temporal Bone Acquisition

Fresh baboon skulls with an age range of 28 days (0.08 years) to 14 years were obtained from the National Baboon Research Resources at the University of Oklahoma Health Sciences Center (OUHSC). The provided primate tissues used in this study were a means of maximizing data collection from a valuable and limited animal source for researchers. The Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma approved the use of baboon skull tissues under the category of "protocol-exempt" from animals approved and used on other IACUC approval protocols. The temporal bones were harvested from the baboon skulls, packed in dry ice, and moved to the Biomedical Engineering

Laboratory at the University of Oklahoma. The experiments were conducted within 1 week after the arrival of the temporal bones.

Each temporal bone was subsequently examined under a surgical microscope (OPMI-1, Zeiss, Thornwood, NY) to confirm a normal and intact TM. They were stored within a solution of 0.9 % saline and 15 % povidone at 5 °C to maintain their physiological condition before the experiment. In total, 28 ears from fresh temporal bones of 19 animals (14 females and 5 males) were used in this study. The ears were organized into four groups based on the ages of the animals: group 1 (G1) for less than 1 year old, group 2 (G2) for 1 to 3 years old, group 3 (G3) for 3 to 5 years old, and group 4 (G4) for older than 5 years of age. These groups correspond to the human age ranges of 0 to 3, 3 to 9, 9 to 15, and older than 15 years of age, respectively, based on the ratio of 1:3 (Dormehl et al. 1992). The average age for each group was 67 ± 26 days (0.18 ± 0.07 years), 2.2 ± 0.8 years, 4.2 ± 0.5 years, and 9.9 ± 3.5 years for G1, G2, G3, and G4, respectively, as summarized in Table 1. The values preceded by "±" are standard deviations (SDs). The summary of their corresponding approximate human age is also listed in Table 1.

TM Thickness Measurement

The thickness of the TM was measured using optical coherence tomography (OCT). The lateral surface of the TM was first exposed by removing its surrounding bony wall. Subsequently, the temporal bone was placed under the laser beam of the OCT system (Callisto 930 nm OCT Imaging System, Thorlabs, Inc.) where the lateral side of TM surface was aligned normal to the laser for the thickness measurement. The OCT created an image by scanning a series of single points (namely A-scans) along a line, which were combined into the final 2D image (namely B-scans) as shown in Fig. 1(a). To reduce the noise and increase the contrast of the TM image, the A-scans were performed 5 times and B-scans 20 times. The average of these scans created a clear image for the determination of the thickness of the TM with a refractive index of 1.40 (Van der Jeught et al. 2013; Zhou et al. 2013). The final thickness measurement was obtained by averaging the thicknesses of six points along the location where samples were harvested. The thickness at each point was defined by the distance along the direction perpendicular to the TM surface. The typical variation of the thickness at the 6 points for an individual TM was around 10 % of the average. The average thickness for each group is reported in the 4th column of Table 1. The individual thicknesses were used to determine the strain applied to the corresponding TM samples.

TABLE 1
Information of specimen in 4 age groups

Group	Number of specimens	Average age	Approx. human age	Average thickness (μm)	Average length (mm)	Average width (mm)
G1	7	0.18 ± 0.07 years	0.55 ± 0.21 years	25 ± 4	5.64 ± 0.39	1.80 ± 0.28
G2	8	2.2 ± 0.8 years	6.7 ± 2.4 years	27 ± 9	5.75 ± 0.76	1.84 ± 0.23
G3	8	4.2 ± 0.5 years	12.6 ± 1.4 years	23 ± 3	5.83 ± 0.69	1.84 ± 0.23
G4	5	9.9 ± 3.5 years	29.6 ± 10.4 years	24 ± 3	5.68 ± 0.70	1.74 ± 0.28

TM Strip Specimen Preparation and Experimental Setup

Figure 1(b) shows the TM in a temporal bone where the ear canal and surrounding bony wall were surgically removed. The pars tensa (t), pars flaccida (f), and manubrium (*) are shown, and a portion of the annulus (a) is marked with a dashed line. To remove the TM with an intact malleus, the annulus was first separated from the annular sulcus. The incudomalleolar joint (IMJ), the tensor tympani tendon, and the superior, lateral, and anterior ligaments were then severed to release the TM from the temporal bone. The TM was subsequently preserved in the saline solution during transit and further preparation. A rectangular strip was cut from either the posterior or anterior section of the TM for tensile

testing as shown in Fig. 2. The annulus remained intact at the inferior and superior sides of the TM to maintain the integrity of the membrane. The TM was assumed to be a flat rectangular strip, and the curvature of the TM was neglected in this study. Before the experiment, the TM strip specimen was fixed on two aluminum fixture adapters with cyanoacrylate liquid glue (The Original Super Glue Corp.) at both ends under a surgical microscope (Olympus SZX12). Additionally, two plastic support panels were connected in parallel to the strip specimen between the two aluminum adapters. These two plastic panels were used to support the fixture adapters during the mounting process and to avoid any unexpected damage to the TM sample throughout the mounting process. Once the sample was aligned and fixed with the grips of the mechanical analyzer (DMA, Bose

FIG. 1. **a** Example of the B-scan image obtained through OCT imaging on an intact TM within an adult baboon temporal bone. The lateral TM surface that the measurement was aligned to be normal to the laser. To reduce noise of the beam intensity, and the contrast was adjusted to create a clear image of the TM. The OCT creates an image by scanning a single point (A-scans) along a line, which are

combined into the final 2D image (B-scans). The single point A-scans were averaged 5 times and the line scans were average 20 times. The refraction index for the TM is 1.40 and for air is 1.00. **b** Intact TM within the baboon temporal bone, the ear canal, and the surrounding bony structures were removed so that a full view of the TM can be observed

FIG. 2. Typical TM sample after removing from the temporal bone. Strip samples are cut from either the posterior or anterior side of pars tensa indicated by the black square

ElectroForce 3200, Eden Prairie, MN), the support panels were cut, and a preload of 0.002 N was applied to the TM sample. Three parameters were recorded in the static test: elapsed time, displacement, and load at resolutions of 10^{-3} s, 10^{-3} mm, and 10^{-3} N, respectively. The initial state of a mounted specimen was set up as shown in Fig. 3. The gauge length (distance between the two fixture adapters before preload) and width were measured through the microscope. The average dimensions for each age group are listed in Table 1.

Preconditioning

It is well known that the stabilized mechanical state of biological soft tissue can be reached through preconditioning (Fung 2013). In this study, preconditioning was achieved by conducting three loading and unloading cycles on the TM specimens. The specimens were elongated at 0.18 mm/s with a longitudinal stretch ratio of 1.10. Note that the stretch ratio used for preconditioning (1.10) was slightly lower than 1.15 used in the tensile test (see the next section). The value was selected to allow the TM specimen to reach the steady state without damaging the tissue, which was used for mechanical tests of human TM specimens reported by Cheng et al. (2007) and Engles et al. (2017). Figure 4 shows the preconditioning process of one TM specimen with

three cycles of loading and unloading to the maximum stretch of 1.10. As displayed in this figure, the hysteresis loop of the specimen was reduced after three repeating cycles, and the 2nd and 3rd cyclic loading and unloading curves were similar with each other, which indicated the tissue eventually reached a steady state.

Uniaxial Tensile Test

After preconditioning, the specimen was tested with the elongation length set to 15 % of the sample's original length or the maximum stretch ratio at 1.15 which was the same as that used by Cheng et al. (2007) to compare the data obtained in this study with their results in human TMs. The strain rate was approximately 0.018 mm/s, which was the same as that used successfully in the preconditioning process, but lower than that used in Cheng's study (0.1 mm/s). The reason for choosing a lower strain rate was to avoid damage to the specimen. In our preliminary attempts, some samples failed while preconditioning at a strain rate of 0.1 mm/s.

Stress Relaxation Test

The stress relaxation test was further performed to investigate the viscoelastic behavior of the baboon TM. Following previously established methodologies,

FIG. 3. TM strip specimen mounted into DMA. The TM strip specimen was fixed to two aluminum fixture adapters with cyanoacrylate liquid glue (super glue) at both the superior and inferior annulus sides. A preload of 0.002 N was applied to the TM sample.

Six parameters were recorded by the DMA software (WinTest 4.1, Bose, MN): elapsed time, displacement, and load, and each had a resolution of 10–3 s, 10–3 mm, 10–3 N, respectively

a step function of elongation was applied to the TM sample beginning ($t=0$) with a strain rate of 1.8 mm/s to a 15 % elongation of the original length (Cheng et al. 2007). The corresponding stresses, $\sigma(t)$, with the initial stress, σ_0 , at $t=0$ were recorded for a maximum of 200 s, by which time the specimens were mostly or fully relaxed. After 200 s, the data recording was stopped, and the sample was relaxed to its original unstressed state for failure testing. Note that the relaxation test was not repeated for each specimen because of the difficulty in maintaining a consistent hydration level for the specimen over the duration of the prolonged relaxation test.

Data Analysis

The mechanical properties of biological materials can be described by several non-linear material models, such as the Ogden, Mooney-Rivlin, and Yeoh models. For studying rubber-like biological soft tissues, Sarma et al. (2003) showed that from the available models the Ogden model was the most valid and useful to explain the stress-strain behavior of smooth muscle

tissue. In the literature, the Ogden model has been used to predict the behavior of several non-linear and viscoelastic biological tissues such as the skin, brain, and normal human TM (Miller and Chinzei 2002; Sarma et al. 2003; Wu et al. 2003; Cheng et al. 2007). To compare with the mechanical properties measured by micro-fringe projection in our previous work (Liang et al. 2018), the 2nd-order Ogden model was used to analyze the quasi-static experimental data of the baboon TM.

The 2nd-order Ogden model is generally expressed for elongation along a single axis as

$$\sigma = \sum_{i=1}^2 \frac{2\mu_i}{\alpha_i} \left[(1 + \varepsilon)^{\alpha_i-1} - (1 + \varepsilon)^{-0.5\alpha_i-1} \right] \quad (1)$$

where σ is the normal stress, ε is the strain, μ_i are the initial shear modulus, and α_i are the incompressibility parameter (Ogden 1972). In this study, the strain was converted to the stretch ratio λ , which is defined as the ratio of the deformed length to the original length,

FIG. 4. Preconditioning figure of a typical TM specimen. Shown are the first, second, and third cycles. The arrows are pointing at the loading curve

$$\varepsilon = \frac{L}{L_0} \quad (2)$$

and

$$\lambda = \frac{L + L_0}{L_0} = \varepsilon + 1 \quad (3)$$

with this substitution Eq. (1) becomes,

$$\sigma = \sum_{i=1}^2 \frac{2\mu_i}{\alpha_i} (\lambda^{\alpha_i-1} - \lambda^{-0.5\alpha_i-1}) \quad (4)$$

Differentiating Eq. (4) with respect to λ provides

$$\frac{d\sigma}{d\lambda} = \frac{2\mu_1}{\alpha_1} \left[(\alpha_1-1)\lambda^{\alpha_1-2} - \left(\frac{\alpha_1}{2} + 1 \right) \lambda^{-(0.5\alpha_1+2)} \right] \quad (5)$$

which gives the relationship between the tangent modulus $d\sigma/d\lambda$ and the stretch ratio λ .

The stress-stretch relation curves measured during the uniaxial tension test were used to determine the four material constants, μ_i and α_i ($i=1,2$) with the curve fitting software in ANSYS Workbench (ANSYS, Inc., Canonsburg, PA). By substituting μ_1 and α_1 into Eq. (4) and Eq. (5), the constitutive equation of the TM in the Ogden form and the tangent modulus with respect to stretch ratio were determined, respectively. Note that if the displacement is stretched beyond 15 % of the original length, a higher-order Ogden model may need to be used to predict the stress at higher strain values.

Statistical Analysis

To evaluate the statistical significance for the measurement data among different age groups, ANOVA

tests were performed in Excel using Real Statistics Resource Pack (Charles Zaiontz, Real-Statistics.com) with an α value of 0.05. The sample size of each age group (G1, G2, G3, and G4) for tensile tests was 7, 8, 8, and 5, respectively; the sample size of each age group for relaxation and failure tests was 5, 4, 8, and 5, respectively. For single value analysis such as Young's modulus and failure stress, one-factor ANOVA tests were applied on the data and the p value was determined for statistical significance. For analysis of tensile and relaxation data, two-factor ANOVA tests were used. The factors for the tensile data are the age group and stretch ratio, while the factors for the relaxation data are the age group and time. The p values were determined between the age groups for all measurement data. Post hoc analysis, Tukey-Kramer tests were subsequently applied to determine the difference between each pair of age groups, if p values were lower than 0.05".

RESULTS

Figure 5 shows the stress-stretch ratio relationship of the four age groups of baboon TM samples measured from the uniaxial tensile test. All samples were stretched to a maximum stretch ratio of 1.15 or 15 % strain. For some samples, the total stretch ratios were below 1.15 due to the early failure of the sample. These samples were excluded from the relaxation test and the failure test. The stress-stretch ratio curves of all the age groups exhibited a typical non-linear behavior of soft tissues. The slope of the curves continuously increased as the stretch ratio increased indicating a stiffening effect of the tissue materials. This finding indicates that baboon TM follows the behavior of a hyperelastic material. Such a strong non-linear characteristic was similar to TMs from human

FIG. 5. Average tangent modulus for each age baboon group: less than 1 years old (G1), 1 to 3 years old (G2), 3 to 5 years old (G3), and older than 5 (G4) years of age. Error bars represent standard deviation. To obtain the tangent modulus, the experimental stress-

stretch ratio data were fitted into a second-order Ogden hyperelastic model with ANSYS workbench. The parameters were then substituted into the tangent modulus equation to determine the tangent modulus-stretch ratio relationship

and other animal species (Cheng et al. 2007; Liang et al. 2015; Liang et al. 2016).

The stress-stretch ratio relationships were different among groups, and the distinction varied from the low stretch ratio range to the high stretch ratio range. Below a stretch ratio of ~ 1.05 or 5 % strain, the average stresses were relatively linear and the differences among the four groups were small with average stress of 0.14 MPa at 5 %. As the stretch ratio increased to a stretch ratio of ~ 1.075 or 7.5 % strain, the stresses of the 4 groups started to differ significantly and eventually diverged according to the age groups: higher stress for younger ages and lower stress for older ages. G1 had the highest average stress, G2 and G3 had the second and third highest, and G4 had the lowest average stress with values of 1.38 MPa, 1.21 MPa, 1.16 MPa, and 0.90 MPa, respectively. While the lowest average stress decreased with age, the difference between the values for G2 and G3 was trivial. The maximum stress for all the samples at 15 % was 2.1 MPa from G1 and the minimum stress for all the samples at 15 % was 0.65 MPa from G4. To evaluate the significance of the stress-stretch ratio relationship among different groups, two-factor ANOVA analysis was conducted with age group and stretch ratios and as the factors. The p value for the age group was 4.9×10^{-10} , much smaller than the α value. This result indicated that there was a significant difference among the age groups in terms of the stress-stretch ratio relationship. A Tukey-Kramer analysis was further performed for the stretch ratio on 6 pairs from the age groups consisting of G1 vs. G2, G1 vs. G3, G1 vs. G4, G2 vs. G3, G2 vs. G4, and G3 vs. G4. The results of the Tukey-Kramer tests were summarized in Table 2. They showed that there were significant differences between each two age groups except G1 and G2.

For a hyperelastic material, Hook's law can only be applied for a small range of strain. The material

properties defined on the limited range of linear stress-strain relationship is called tangent modulus. In a small initial range of the stretch ratio, Young's modulus is identical to the tangent modulus. To compare with our previous study on baboon TM (Engles 2017; Liang et al. 2018), Young's modulus was determined as the average tangent modulus below a 1.10 stretch ratio or 10 % strain in this study. The values were 4.2 MPa, 4.7 MPa, 4.1 MPa, and 3.1 MPa for G1, G2, G3, and G4, respectively. The single-factor ANOVA test yielded a p value of 0.0837, indicating that within the groups there is no significant difference for the Young's modulus.

By substituting the Ogden material parameters, μ_i and α_i , into Eq. (3), the Ogden model stress-stretch ratio relationships of the four age groups were obtained and plotted in Fig. 5. The average stress of the Ogden model agreed well with the experimental results. The difference between the experimental results and the Ogden model data was less than 10 %. The average μ_1 and α_1 are summarized in Table 3. The range of μ_1 values was from 0.57 to 0.71 MPa and the range for α_1 was from 21.7 to 27.08. The tangent modulus determined from Eq. (4) for all groups were almost identical at low stretch ratio values

TABLE 2

Tukey-Kramer test for the tensile test results on the age group factor q -crit = 3.65

Group 1	Group 2	q -stat	p value	Cohen d
G1	G2	1.31	7.9×10^{-1}	0.12
G1	G3	4.97	2.8×10^{-3}	0.45
G1	G4	8.04	1.5×10^{-7}	0.83
G2	G3	3.79	3.9×10^{-2}	0.33
G2	G4	7.08	5.1×10^{-6}	0.71
G3	G4	3.76	4.1×10^{-2}	0.38

TABLE 3

Average Ogden hyperelastic 2nd-order parameters for all TM samples

Group	μ_1 (MPa)	α_1	μ_2 (MPa)	α_2
G1	0.53	21.39	0.35	21.42
G2	0.58	20.11	0.28	22.57
G3	0.47	21	0.28	21.12
G4	0.45	16.91	0.4	15.9

(<1.05) with a median of 3.9 MPa for the four groups. Above stretch ratio of 1.05, they diverged: G1 was highest, G2 and G3 were equal at 2nd highest, and G4 was the lowest average tangent modulus with 40.10 ± 8.15 , 28.24 ± 2.46 , 29.32 ± 3.18 , and 18.49 ± 2.19 MPa, respectively.

Figure 6 shows the stress relaxation behavior of the TM obtained from the four age groups of baboon TMs. The normalized stress relaxation function $G(t)$ in the y-axis is defined as the ratio between the stress $\sigma(t)$ at time t and the initial stress σ_0 . The initial strain rate used for this study was 1.8 mm/s, which was 10 times the strain rate used in the uniaxial tensile test. The mean initial stresses σ_0 for G1, G2, G3, and G4 were 1.61 MPa, 1.35 MPa, 1.22 MPa, and 0.83 MPa, respectively. The relaxation curves of the four groups followed a similar pattern. The stress decreased quickly with time in the beginning and then reached a relatively stable state, but the curves were still decreasing after 200 s. Within 1 s, 10 % of the stress relaxed, 20 % of the stress relaxed at 5 s, the stress relaxation gradually approached an asymptote after 100 s, and finally, 25 % of the stress relaxed. The relaxation times for G1–G4 age groups to relax 10 % of the stress were 2.1 s, 1.0 s, 1.8 s, and 1.0 s, respectively, and to relax 20 % of the stress were 72.1 s, 76.7 s, 40 s, and 20.0 s, respectively. The mean stress of each group after relaxation was 1.26 MPa,

1.06 MPa, 1.02 MPa, and 0.62 MPa for G1, G2, G3, and G4, respectively. The change of the stress with time under the constant stretch indicates that the baboon TM is a typical viscoelastic material. Two-factor ANOVA tests were performed with the age group and the time as the factors. It yielded a p value of 0.061, showing that within the groups there is no significant difference. This indicates that the differences of relaxation among the ages group are not as significant as what is shown for the stress-stretch ratio relationship.

After all tests were completed, the samples were stretched at a constant rate of 0.1 mm/s until failure. Table 4 lists the ultimate stress or failure stress and stretch ratio for each group of baboon TMs. The mean failure stretch ratio and the mean failure stress for all the TM samples were 1.22 and 2.5 MPa, respectively. The failure mode of the TMs was nearly identical for the four groups of TMs. The breaking location of all the TMs was at the middle section as shown in Fig. 7. The separation of the specimen was primarily in the ground substance with the broken circumferential fibers. This observation was similar to the failure mode in a miniature split Hopkinson tension bar experiments of human TM strip specimens harvested along the circumferential direction (Luo et al. 2009). A single-factor ANOVA test was performed on the failure stresses of the four age groups. The p value is 0.69, indicating that the failure stress/stretch ratio does not show a significant difference among the four age groups.

DISCUSSIONS

Contribution of the Study

The effect of age on mechanical properties of the TM is of interest not only because it is related to the degradation of hearing function, but also because it is

FIG. 6. Normalized mean stress relaxation for each group. The normalized stress relaxation function $G(t)$ in y-axis is defined as the ratio between the stress at time t and the initial stress

TABLE 4

Average stretch ratio and stress at ultimate stress or failure stress for all TM samples		
TM specimen	Failure stretch ratio λ	Failure stress (MPa)
G1 ($n = 5$)	1.21 ± 0.06	2.57 ± 0.66
G2 ($n = 4$)	1.26 ± 0.05	2.56 ± 0.46
G3 ($n = 8$)	1.21 ± 0.05	2.47 ± 0.82
G4 ($n = 5$)	1.21 ± 0.05	2.40 ± 1.06
Average	1.22 ± 0.05	2.50 ± 0.75

pertinent to properly understand the ear diseases that are prevalently diagnosed within certain age groups. Otitis media, for instance, is a middle ear disease that is mostly diagnosed in young children (Cunningham et al. 2012; Monasta et al. 2012). Despite its importance, the TM mechanical properties of young children are mainly limited to morphological study (Ruah et al. 1991), estimation from skin tissue based on the structural similarity between skin and TM (Motallebzadeh et al. 2017b), or model fitting (Wang et al. 2016). The present study provides TM mechanical properties of a non-human primate, baboon, from ages ranging from 0.18 to 10 years, which associates with the human age from 3 months to 42 years old. These data allow for the investigation of the age effect on the mechanical properties of the TM from infancy to middle age and potentially provide descriptions of the degradation of the human TM and from the pediatric hearing disease.

Age Effect on TM Mechanical Properties

To the best of our knowledge, the only published direct measurements of the age effect on the TM's elastic properties is our previous paper on the baboon's TM mechanical properties (Liang et al.

2018) at different ages using micro-fringe projection. The present study is a continuation of our previous study. The average Young's modulus measured with micro-fringe projection was higher than that measured with the uniaxial tensile test in the present study. Note that in preparation for the tensile test measures, the strip was cut along the superior-posterior direction. The material properties measured in the present tensile tests highly depend on the density of the collagen fibers of the TM oriented in the circumferential direction. On the other hand, micro-fringe projection measurements describe the overall mechanical properties of the TM. While there are limitations for both tensile tests and micro-fringe projection, both show similar trends for the changes in the mechanical properties of the TM with age: the TM becomes slightly stiffer after the baboon reach ~ 1 year old. Its stiffness then remains stable up to 5 years old. After 5 years of age, the overall stiffness of the TM starts to decrease.

In addition to the elastic properties, the viscoelastic properties of the baboon TMs in terms of stress relaxation curves are also presented in this work (Fig. 6). The stress for all groups decreased with time but reached a relatively stable state after 100 s. The rate at which the stress relaxes was relatively fast at the beginning. The stress changes over time under constant stretch indicated that the baboon TM at any age range remains a viscoelastic material. The mean initial and relaxed stress for each age group of TMs demonstrated a consistent decrease from a young age to adult. This again confirms the conclusion from the tensile tests that the elasticity of the baboon TM decreases with an increase in age. However, the average and standard deviation of normalized stress relaxation from each

FIG. 7. Typical failure patterns of TM tensile specimens in tensile test

group do not result in a significant difference according to the ANOVA analysis. Furthermore, the relaxation time of the TMs remained almost unchanged from young age to adult (Fig. 6) which implied that the viscous behavior of the TM does not alter for the range of ages under investigation.

The mechanical properties of the TM are controlled by its microstructure. In the through-thickness section of the TM, a multi-layer fiber structure can be identified consisting of distinct layers varying in density, thickness, composition, and arrangement in different regions. In detail, the TM is a tri-laminar membrane with an inner mucosal epithelial layer on the medial side, an outer epidermal layer on the lateral side, and an intermediate fibrous layer in the middle connective lamina propria. The lamina propria is a composite structure composed of collagen ground substance and aligned fibers arranged in radial and circumferential directions (Lim 1970). The elastic behavior of soft tissues, such as the TM, is dominated by the collagen and elastic fibers (Akhtar et al. 2011). Decrease of collagen production is generally considered the cause of chronologically aged soft tissue (Varani et al. 2006). For human TM, Ruah has reported that the fibrous layers become thinner and looser as the TM ages (Ruah et al. 1991). Because the extracellular matrix proteins in collagen are generally long-lived, fracture, fatigue, and damage of these macromolecular assemblies continuously accumulate over time in aging populations. Such a deterioration of the fibrous lamina propria may not affect the TM elasticity at low stretch ratio level as reflected by the Young's modulus. It, however, can lead to a notable decrease of the TM elasticity at a high stretch ratio.

Compared to the change in elasticity, the change in viscoelasticity was less apparent. The relaxation time, which is determined by the viscosity of the material, remained nearly identical among the age groups. The viscous behavior of the TM may be mainly related to the mucosal layer, epidermal layer, and ground substance of the lamina propria. One possible reason is that the components and structure of these layers or the ground substance in fibrous layers remain unchanged through the aging process. This could result from the continuous replacement of ground substance materials (Vollandri et al. 2011). However, how much these soft tissue components contribute to the mechanical behavior of the TM is still undetermined. Further investigation into the microstructure of the aged baboon TM is needed to understand the correlation between age-related changes in structural and molecular tissue components and the changes in overall TM mechanical properties.

Comparison to Human TM

TM mechanical properties for human children are not available due to the inaccessibility of pediatric temporal bones. Baboon TMs were used in this study for the investigation into the age-related change of TM mechanical properties due to the genetic similarity between baboon and human. To elucidate the relationship between the adult baboon TM and human TM, the stress-stretch ratio relationships for both groups were plotted in Fig. 8. It should also be noted that mechanical properties of the adult human TM were reported with numerous methods including tensile test (Kirikae 1960; Decraemer et al. 1980), indentation (Daphalapurkar et al. 2009; Aernouts et al. 2012), static pressure (Gaihede et al. 2007), and holographic methods (De Greef et al. 2014). The results vary with the methods used to apply the force or load on TM samples for measuring the quasi-static properties. Most of the published Young's modulus values were 20–40 MPa as reported by von Békésy (1960), Cheng et al. (2007), and Daphalapurkar et al. (2009). Lower values were also reported from the measurement of the intact TM samples. For instance, Young's modulus was reported as 3 MPa from the indentation test by Aernouts et al. (2012) and 6.9 MPa from the static pressure test by Gaihede et al. (2007).

To be consistent, the present tensile test results of baboon TM were compared with the human data measured with a similar method. These data were obtained from a previous study and consisted of 11 human cadaver subjects with an age range from 51 to 92 years old of which 5 were male and 6 were female (Cheng et al. 2007). In the present study, 5 female adult baboon subjects were investigated, and the age range was 6 to 14 years, which is approximately 18 to 42 years old for human based on the age scaling factor. As is shown in Fig. 8, the average adult baboon is slightly higher than the published human data. However, if additional baboons are tested that are sufficiently older than in the current study, it is possible that the stress-stretch ratio relationship will continue to follow the trend observed in Fig. 8 and align with published human data (Cheng et al. 2007).

Figure 9 shows the relationship between the stress relaxation behavior of the TM in the adult baboon and published adult human (Cheng et al. 2007). Cheng et al. reported that the stress relaxation behavior was as follows: “within 1 sec, 10% of the stress is relaxed; at 5 sec, 20% of the stress is relaxed; after 50 sec, the stress relaxation gradually tends stable and finally, on average, 35% of the stress is totally relaxed.” The adult baboon TM relaxed at a relatively similar rate, but less of the stress was relaxed at each time point.

FIG. 8. Comparison of stress-stretch ratio relationship between human TM and baboon TM

Mechanical properties of the baboon TM and those of the human TM exhibit significant similarities for adults. Thus, baboon is potentially a useful animal model for studying the age effect on human TM. This study focused on the mechanical properties obtained from quasi-static tensile test and relaxation test from young to adult baboon TMs. However, an additional study can be done to measure the dynamic properties of TM tissue in the auditory frequency range for different baboon groups. This will allow for a better understanding of how the acoustic function of the TM changes with age across the frequency range of hearing. This will be the next phase of work.

Limitation

Quasi-static or dynamic test on a strip sample is a classical measurement technique that has been commonly used in various soft tissues composed of collagen fibers including the human TM (Kirikae 1960; von Békésy 1960; Decraemer et al. 1980; Cheng et al. 2007; Luo et al. 2009; Zhang and Gan 2013b). As a standard measurement technique, it was used in the present study for comparison of baboon TM material

properties among age groups. The procedures for sample preparation were consistent for all the age groups. The specimens were harvested from either the anterior or posterior side of the TM, and the uniaxial tensile tests were performed along the superior-inferior direction of the specimens. The TM was considered as an isotropic and homogeneous material. The results from baboon TM samples from different age groups provided important information for understanding the effect of age on the mechanical behavior of non-human primates.

However, the TM is a multi-layer membranous tissue with collagen fibers along the radial and circumferential directions. Considering the specimens were cut along the inferior-superior direction, the results mostly captured the effect of the circumferential direction fibers of the TM. Fay et al. (2005) used a composites layer theory (CLT) to interpret the mechanical testing on TM tissues at the micro-level. The determination of the Young's modulus of the radial and circumferential fiber layers requires prior knowledge of the TM composition and the fiber density distribution, which is lacking in the present study. Therefore, the results in this work should be

FIG. 9. Comparison of stress relaxation between human TM and baboon TM

considered as a general comparison of TM mechanical behaviors among different age groups.

CONCLUSIONS

In summary, the preliminary results showed that the elastic properties of TM decreased as baboons aged. The stress-stretch ratio relationship obtained from uniaxial tension tests showed that Young's modulus of adult baboons was significantly lower than the pediatric age range. Relaxation test also supported the hypothesis that the TM became less elastic from newborn to adult. This finding is consistent with our previous measurement using micro-fringe projection. However, the viscous behavior and the ultimate strength of the TM did not exhibit significant differences within the studied age range. Because of the well-known scaling of 3 to 1 in age between baboon and human, the observation from the animal results provides evidence for the speculation that elastic properties of the human TM decrease with age. The mechanical properties of the baboon TM can also potentially be used to fill the gap of unavailability of the TM mechanical properties for newborn to childhood age humans, which will improve the study and treatment of pediatric middle ear diseases.

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