Quantification of tympanic membrane elasticity parameters from in situ point indentation measurements: Validation and preliminary study

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A B S T R A C T
Correct quantitative parameters to describe tympanic membrane elasticity are an important input for realistic modeling of middle ear mechanics. In the past, several attempts have been made to determine tympanic membrane elasticity from tensile experiments on cut-out strips. The strains and stresses in such experiments may be far out of the physiologically relevant range and the elasticity parameters are only partially determined.

We developed a setup to determine tympanic membrane elasticity in situ, using a combination of point micro-indentation and Moiré profilometry. The measuring method was tested on latex phantom models of the tympanic membrane, and our results show that the correct parameters can be determined. These parameters were calculated by finite element simulation of the indentation experiment and parameter optimization routines.

When the apparatus was used for rabbit tympanic membranes, Moiré profilometry showed that there is no measurable displacement of the manubrium during the small indentations. This result greatly simplifies boundary conditions, as we may regard both the annulus and the manubrium as fixed without having to rely on fixation interventions. The technique allows us to determine linear elastic material parameters of a tympanic membrane in situ. In this way our method takes into account the complex geometry of the membrane, and parameters are obtained in a physiologically relevant range of strain.

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1. Introduction

Finite element modeling is a powerful method for the investigation of middle ear mechanics, (Eiber, 1999; Elkhouri et al., 2006; Koike et al., 2002; Sun et al., 2002), studying the effect of middle ear pathologies, (Gan et al., 2006) and predicting the behaviour of middle ear prostheses (Eiber et al., 2006; Schimanski et al., 2006). The technique allows to take into account the complex shape and boundary conditions of the middle ear structure. Current finite element models are mainly restricted to acoustical sound pressures and low acoustical frequencies, and good data for the mechanical properties of the tympanic membrane are still lacking. It is known, however, that tympanic membrane elasticity has a significant influence on the resulting output (Elkhouri et al., 2006). Furthermore, when one wants to extend finite element models to the quasi-static regime (Dirckx and Decraemer, 2001), effects such as nonlinearity and viscoelasticity of the tympanic membrane can play an important role. On the other hand, when one considers dynamical behaviour, the strain rate dependent tympanic membrane stiffness should be taken into account.

Mechanical properties of the human tympanic membrane were first measured by Békésy in 1960 (Békésy, 1960). He applied a known force on a cut-out strip and measured the resulting displacement. Assuming the sample to be a uniform, isotropic, linear elastic beam with a thickness of 50 µm, he calculated a Young's modulus of 20 MPa. Kirikae measured the 'dynamical' stiffness of a strip of human tympanic membrane in 1960 (Kirikae, 1960). From the system's resonance frequency, he calculated a Young's modulus of 40 MPa based on a thickness of 75 µm. In 1980, Decraemer et al. showed a nonlinear stress–strain curve for human tympanic membrane (Decraemer et al., 1980). In the large strain condition, starting from ±8%, a Young's modulus of 23 MPa was proposed.

In 2005, Fay et al. estimated the elastic modulus of human tympanic membrane based on the elasticity of the collagen fibers and fiber density (Fay et al., 2005). Their data suggested an elastic modulus in the range 100–300 MPa, significant higher than previous values. More recently, new experimental observations of the Young’s modulus of human tympanic membrane were published by Cheng et al. (2007). They conducted a uniaxial tensile test and obtained a stress–strain curve which was in good agreement with that of Decraemer et al. Describing the nonlinearity using the first-order Ogden model, they found $\mu_1 = 0.46$ MPa and $\alpha_1 = 26.76$. In
addition, preliminary stress relaxation tests were performed to study the viscoelastic behaviour of the tissue. Two years later, the same research group characterized more detailed the viscoelastic behaviour by in-plane and out-of-plane nanoindentations and found Young’s relaxation moduli ([Huang et al., 2008; Daphalapurkar et al., 2009]). Their results show an in-plane modulus changing by \(\sim 10%\) from 1 to 100 s with steady-state values of 37.8 N/mm\(^2\) and 25.73 MPa respectively for different samples. The out-of-plane modulus seemed to reduce by 50% from 1 to 100 s with a steady-state value varying from 2 to 15 MPa over the surface. These experiments show a wide variety and it is not straightforward how to interpret the different moduli. Regardless Kirikae’s measurement, all previous mentioned publications report quasi-static behaviour of the tympanic membrane. Very recently, Luo et al. measured the Young’s modulus at high strain rates using a miniature split Hopkinson tension bar ([Luo et al., 2009]). Due to the viscoelastic properties of the membrane, they found that the Young’s modulus increases with the increase in strain rate. However, they only measured up till 2 kHz.

It should also be mentioned that in addition to human tympanic membrane data, a lot of experiments have been performed on tympanic membranes of laboratory animals as well.

It is clear that accurate data for the mechanical properties of the tympanic membrane, both for human and animal samples, cannot be determined using uniaxial tensile tests. At first, when one considers the tympanic membrane to be linear elastic, which is reasonable in the acoustical regime, a Young’s modulus can only be assigned in a physiologically irrelevant strain range starting from \(\pm 8\%\). Moreover, small and long strips with uniform thickness of the tympanic membrane need to be cut to perform uniaxial tensile tests. In general, however, tympanic membrane thickness varies with a factor 4 between periphery and the center ([Kuypers et al., 2005]), making it impossible to cut long uniform samples.

Another parameter that might have an influence on the mechanical behaviour of the tympanic membrane is the presence of prestrain. Bekésy and Kirikae are the only ones who investigated this characteristic, but their results depended on the type of species and were not unambiguous ([Decraemer and Funnell, 2008]). Finally, the pars flaccida has never been examined and properties like anisotropy, inhomogeneity, nonlinearity and viscoelasticity are only poorly studied. These are mechanical properties that may become important in more detailed examinations.

In order to obtain data in more realistic circumstances, we developed a setup to determine tympanic membrane elasticity in situ. The measurement method consists of doing a point indentation perpendicular on the membrane surface; measuring the indentation depth, resulting force and three-dimensional shape data; simulating the experiment with a finite element model and adapting the model to fit the measurements using optimization procedures. In the first part of this paper, the method of measuring and model optimization is validated on a scaled phantom model of the tympanic membrane with known elasticity parameters. In the second part, preliminary results from one rabbit tympanic membrane experiment are shown.

2. Materials and methods

2.1. Phantom model

In the validation experiment, we used rubber from medical gloves, which mainly consists of natural latex. The thickness is \(0.18 \pm 0.02\) mm and the material is isotropic and homogeneous. Like other rubber-like materials, natural latex exhibits very large strains with strongly nonlinear stress–strain behaviour. For this reason, the rubber can be described as a hyperelastic material. A hyperelastic material is typically characterized by a strain energy density function \(W\). A well known constitutive law for rubber-like materials is the Mooney–Rivlin law ([Treloar, 1975]):

\[
W = \sum_{i,j=1}^{N} C_{ij}(l_1 - 3)^i(l_2 - 3)^j + \frac{1}{2} K \ln (J),
\]

with \(N\) the order of the model, \(l_1\) and \(l_2\) the strain invariants of the deviatoric part of the Cauchy–Green deformation tensor, \(C_{ij}\) the Mooney–Rivlin constants, \(K\) the bulk modulus and \(J\) the determinant of the deformation gradient which gives the volume ratio. The invariants are given as:

\[
I_1 = \frac{1}{2} l_1^2 + \frac{1}{2} l_2^2 + \frac{1}{2} l_1^2,
\]

\[
I_2 = \frac{1}{2} \ln^2 J + \frac{1}{2} \ln^2 J,
\]

with \(\lambda_i (i = 1, 2, 3)\) the principal stretches. It is common to assume that rubber materials are incompressible when the material is not subjected to hydrostatic loadings, so that the last term in Eq. (1) will be neglected ([Bradley et al., 2001]). In this study, we will only consider the first-order Mooney–Rivlin equation \(\langle N = 1\rangle\). In this case, Eq. (1) becomes:

\[
W = C_{10}(l_1 - 3) + C_{01}(l_2 - 3).
\]

This low order strain energy function is described by two constants: \(C_{10}\) and \(C_{01}\).

Since the latex rubber is perfectly homogenous in all its physical properties, a uniaxial tensile test was carried out which results in accurate determination of the first-order Mooney–Rivlin parameters.

The phantom model of the tympanic membrane was created by pushing a ‘manubrium shaped rod’ in a flat circular rubber membrane which was constrained at its circumference. In this way, the typical conical tympanic membrane shape was obtained. The diameter of the phantom model was 50 mm and the height was 16 mm, approximately eight times the size of a human tympanic membrane. The phantom model was placed on a translation – and rotation stage, a schematic drawing is shown in Fig. 1. Indentations in and out in a direction perpendicular to the surface membrane were carried out using a stepper motor with indentation depths up till 2 mm. The needle had a cylindrical ending with a diameter of 1.7 mm and indentations were carried out slowly \((\dot{t} = 0.125 \text{ mm s}^{-1})\), meaning that quasi-static behaviour was studied. The points of indentation were chosen in the undermost part.
of the ‘cone’ (the inferior part of the ‘tympanic membrane’) because in the tympanic membrane case, it showed to be experimentally impossible to do indentations in the superior part. By doing the indentation perpendicular to the surface and not too close to the boundary, slippage between the needle and the rubber was avoided. The resulting force was measured with a load cell (Senso-tec Model 31). The exact indentation depth was assessed with an LVDT (HBM KWS3071). All signal processing was done via A/D conversion in Matlab (using NI DAQPad-6015).

Three-dimensional shape data were measured with a projection LCD-Moiré profilometer (Buytaert and Dirckx, 2008; Dirckx and Decraemter, 1989), developed in our laboratory. The device is able to perform topographic measurements with a height resolution of 25 μm on a matrix of 1392 × 1040 pixels. The Moiré shape measurement was used to locate the exact point of indentation and to determine if the membrane is locally at right angle with the indentation needle.

The numerical simulations were performed with the finite element code FEBio, which is specifically designed for biomechanical applications. The rubber membrane was modeled as a first-order Mooney–Rivlin material, triangular incompressible shell elements were used to create a non-uniform mesh, with increased mesh density in the contact areas. The border of the membrane was x, y, z constrained, with x, y and z the axes of a Cartesian coordinate system with the x y plane parallel to the ‘annulus plane’. In the first model steps, the ‘manubrium shaped’ rigid body was moved vertically into the flat membrane using a combination of rigid – and sliding contact. In the following model steps, the perpendicular needle indentation was modeled as a rigid body translation with frictionless sliding contact.

In order to find the first-order Mooney–Rivlin parameters, we optimized the average square force difference defined as

\[ \text{error}_{\text{force}} = \frac{1}{N} \sum_{j=1}^{N} (F_{\text{exp}}(q_j) - F_{\text{mod}}(q_j))^2, \]

in which \( N \) is the number of evaluated points, \( q_j \) the indentation depth, \( F_{\text{exp}}(q_j) \) the experimental force and \( F_{\text{mod}}(q_j) \) the simulated force. The optimization was carried out with a surrogate modeling toolbox (Gorissen et al., 2009; Jones et al., 1998). Using this routine, firstly one has to specify the input domain. Then, a first surrogate model calculated from 24 homogeneously distributed \((C_{10}, C_{01}, \text{error}_{\text{force}})\) data points is built. Afterwards, the model is refined on the basis of a gradient-optimum based sample selection in order to localize very accurately the optimum values.

2.2. Tympanic membrane

The animal model selected for the present study was the New Zealand white rabbit. Animals were sacrificed by injection of 120 mg/kg natrium pentobarbital and temporal bones were removed. Openings were made in the bulla so that visual access was provided from both sides of the tympanic membrane, leaving the tympanic membrane and entire middle ear ossicle chain intact. Total preparation time was always less than 1.5 h. The study was performed according to the regulations of the Ethical Committee for Animal Experiments of the University of Antwerp.

The tympanic membrane was modeled as a linear isotropic homogeneous elastic material which is described with two independent elastic constants: the Young’s modulus \( E \) and Poisson’s ratio \( \nu \). The thickness of the rabbit tympanic membrane varies somewhat along the membrane, however, in this study the membrane was modeled with a uniform thickness. Since no data on rabbit tympanic membrane thickness are available, we used a mean value of 12.5 μm measured for the cat tympanic membrane with confocal microscopy (Kuypers et al., 2006). In forthcoming work, we will add a measured thickness distribution to our model.

The prepared sample was glued on a small tube so it could be placed on a translation and rotation stage in a similar way as in Fig. 1. In a direction perpendicular to the membrane, the indentation needle was moved back and forth from the lateral (external) ear side with a maximal indentation depth up till 400 μm using a piezoelectric actuator (PI P-290) in combination with an LVDT (Solartron) and a feed-back controller unit (PI E-509 E-507). The needle had a cylindrical ending with a diameter of 210 μm. The indentation was carried out in steps of 40 μm with a step time of 4 s, thus quasi-static behaviour was studied. The resulting force was measured with a strain-gage force transducer (SWEMA SG-3). Input and output signals were controlled with a computer via A/D conversion in Matlab (using NI DAQPad-6015).

Three-dimensional shape data were measured from the medial side with our LCD-Moiré profilometer. Because tympanic membrane dimensions are significant smaller than those of the phantom model, the setup was adjusted resulting in a height resolution of 15 μm. Since the tympanic membrane is almost non-reflective, it was coated with a layer of white paint mixed with glycerin to avoid dehydration. Again, the Moiré data were used to determine exact needle position and to verify perpendicular needle positioning.

Moreover, a highly detailed non-uniform mesh was created on the basis of the Moiré shape images. In the needle indentation area and in the manubrium neighbourhood, mesh density was increased. Since in the rabbit case the pars flaccida can geometrically be regarded as almost completely separated from the pars tensa (Fumagalli, 1949), the influence of the pars flaccida can be ignored so that the membrane was modeled as a homogeneous material. The manubrium was modeled as a rigid body that is x, y, z constrained because no measurable motion was observed during indentation (see further). The border of the tympanic membrane was modeled as fully clamped, the perpendicular needle indentation was modeled as a rigid body translation with frictionless sliding contact. Afterwards, in a similar way as described for the phantom model case, the errorforce was optimized in order to determine linear elasticity parameters.

3. Results

3.1. Phantom model

The mean output of the uniaxial tensile test, carried out on three different samples, was \( C_{10} = (43 \pm 5) \) kPa and \( C_{01} = (159 \pm 6) \) kPa. In Fig. 2a, the output of a force-indentation experiment is plotted (line). The indentation was carried out slowly and only a small hysteresis is seen. The associated finite element model is shown in Fig. 2b, with the number of membrane shell elements equal to 4337. In the first model steps, the ‘manubrium shaped rod’ rigid body displacement was applied which results in the typical conical shape. Next, the perpendicular point indentation was applied. The color map represents the effective strain, which rises up to approximately 40% in the indentation area.

The resulting surrogate model is shown in Fig. 3. The evaluated samples are plotted as white points and the color map represents the surrogate model fitted through the errorforce values. A range of minima lying on a linear curve can be seen (dashed line). One can see that as a consequence of the gradient-optimum criterion sample selection, more samples were chosen in the minimum area in comparison to elsewhere. The force-indentation output for three different points of \((C_{10}, C_{01})\) optimum values is plotted in Fig. 2a (different dots). According to this plot, we see that the first-order Mooney–Rivlin equation is able to describe the load curve quite...
well. It is not possible, however, to extract a unique set of Mooney–Rivlin parameters only on the basis of a load curve optimization. Therefore, also a shape analysis was carried out in which the average square difference between model – and experimental deformation (conform Eq. (5)) along a point indentation section was optimized so that the final optima were filtered out of the range of force minima. In Fig. 4, the difference between model and experimental shape data is shown for different Mooney–Rivlin parameters. The final output after shape optimization was $C_{10} = 31 \text{kPa}$ and $C_{01} = 172 \text{kPa}$. The final output for a second indentation experiment was $C_{10} = 33 \text{kPa}$ and $C_{01} = 163 \text{kPa}$.

3.2. Tympanic membrane

Fig. 5 shows an experimentally measured section plot of tympanic membrane deformation during indentation. This experiment shows no manubrium movement, so the manubrium can be regarded as fixed.

Fig. 2. Phantom model: (a) plot of experimental force-indentation data (line) and best models output (different dots), (b) FE model with applied ‘manubrium shaped’ rigid body displacement and needle indentation. The effective strain in the point indentation area after indentation rises up to approximately 40%.

Fig. 3. Phantom model: contour plot with result of $\text{error}_{\text{force}}$ optimization after 119 samples. The samples are plotted as white dots, the color map represents the fitted surrogate model and the resulting range of local minima is highlighted with a dashed line.

Fig. 4. Phantom model: plot of difference between experimental and model deformation data along a point indentation section line for different Mooney–Rivlin parameters. In the case of $C_{10} = 31 \text{kPa}$ and $C_{01} = 172 \text{kPa}$, the difference error is the smallest.

Fig. 5. Rabbit tympanic membrane (right): experimentally measured section plots before and after indentation to show fixed behaviour of manubrium.

The finite element model of the perpendicular point indentation experiment is shown in Fig. 6, with the number of membrane shell elements equal to 5988. The perpendicular point indentation re-
results in an effective strain of approximately 15% in the point indentation area and due to manubrium fixation, no deformation in the opposite membrane part can be seen.

In Fig. 7a, the associated force output of the experiment is plotted (dotted line). When doing slow indentations, only a small hysteresis can be seen. Using the finite element model and the experimental force-indentation data, the surrogate model was calculated. The input interval was set to $E = [20, 40]$ MPa and $\nu = [0.2, 0.49]$, the resulting surrogate model is shown in Fig. 7b. The evaluated samples are plotted as white points and the color map represents the surrogate model fitted through the errorforce values. In total, 118 samples were evaluated to guarantee an accurate determination of the model.

Inspecting the resulting output, one can see that the optimum value for the Young’s modulus $E$ in the Poisson’s ratio interval $\nu = [0.2, 0.45]$ is practically constant: $E \approx 30.4$ MPa. For higher Poisson’s ratio’s, the optimum value decreases. For a Poisson’s ratio $\nu = 0.49$, towards the theoretical upper limit $\nu = 0.5$ that represents incompressibility, the optimum value is $E \approx 26.4$ MPa. In Fig. 7a, the force-indentation curve is plotted for the optimum values with respectively $\nu = 0.2, 0.45$ and $0.49$. The simulated curves show good agreement with the experimental one. For all these optimum values, models show practically no difference in model deformation, so that a shape analysis is not under discussion.

4. Discussion

If large enough samples of a material are available, tensile tests on a strip can be performed to determine elasticity parameters. On small samples like the tympanic membrane, it becomes very difficult to control the exact size and extension of a strip. In this case, it becomes inevitable to perform in situ measurements because specimens are too small and the natural shape of the membranes are complex.

In this paper, a new approach is presented in which elasticity parameters are determined by inverse modeling of an in situ point indentation experiment.

At first, the characterization method was validated on a latex rubber phantom model. Measuring the first-order Mooney–Rivlin elasticity parameters with a uniaxial tensile test, carried out on three different samples, the output was $C_{10} = (43 \pm 5)$ kPa and $C_{01} = (159 \pm 6)$ kPa. Applying the point indentation method, the output of a first point indentation experiment was $C_{10} = 31$ kPa and $C_{01} = 172$ kPa. The output of a second experiment on a different location was $C_{10} = 33$ kPa and $C_{01} = 163$ kPa. One can see that there is a good agreement between the two approaches. However, there is approximately a 25% relative error for the $C_{10}$ values. It is known that the $C_{10}$ parameter has a small influence on the stress–strain curve for strains smaller than 50%. Since effective strains in...
the indentation experiments go only up to approximately 80% and, this apparently high relative error can be argued.

Having validated the method, a preliminary experiment on a right rabbit tympanic membrane was performed. Indentations were carried out slowly, so that quasi-static behaviour was studied. In this first approach, the tympanic membrane was considered to be a linear isotropic homogeneous elastic material. Although we know this is not the best choice, it allows a comparison with previous studies.

From our Moiré shape measurements, we learned that the manubrium does not move under influence of a small point indentation on the tympanic membrane. This is a very important result, as it shows that no complex procedures are needed to fixate the manubrium in order to obtain well defined boundary conditions.

Since there was practically no difference in model deformation for different force-indentation optimum values, we cannot filter out one optimum value for the Young's modulus. In contrast, it will depend on the choice of Poisson's ratio. In most of the middle ear finite element models, a Poisson's ratio in the range \( \nu = [0.3, 0.4] \) is proposed (Elkhouri et al., 2006; Koike et al., 2002; Sun et al., 2002), with a corresponding optimum value of \( E = 30.4 \) MPa. However, it might be argued that the Poisson's ratio should be close to 0.5 because soft tissues are thought to be nearly incompressible. In this case, the corresponding optimum value is \( E = 26.4 \) MPa. In both cases, the optimum values are close to those found in previous studies.

In future work, more point indentation experiments will be performed and a measured tympanic membrane thickness distribution will be added to the finite element models. Simultaneously, we will investigate the behaviour under dynamic load conditions and the influence of nonlinearity, inhomogeneity, anisotropy, viscoelasticity and the possible presence of prestress.

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