Published in final edited form as:

J Mech Behav Biomed Mater. 2013 January ; 17: 137–151. doi:10.1016/j.jmbbm.2012.08.005.

Pipette aspiration applied to the characterization of nonhomogeneous, transversely isotropic materials used for vocal fold modeling

S. Weiß^{a,*}, S.L. Thomson^{b,c}, R. Lerch^a, M. Döllinger^d, and A. Sutor^a

^aChair of Sensor Technology, Friedrich-Alexander University, Erlangen, Germany

^bDepartment of Mechanical Engineering, Brigham Young University, Utah, USA

^cGraduate School in Advanced Optical Technologies (SAOT), Friedrich-Alexander University, Erlangen, Germany

^dDepartment of Phoniatics and Paediatric Audiology, University Hospital Erlangen, Erlangen, Germany

Abstract

The etiology and treatment of voice disorders are still not completely understood. Since the vibratory characteristics of vocal folds are strongly influenced by both anatomy and mechanical material properties, measurement methods to analyze the material behavior of vocal fold tissue are required. Due to the limited life time of real tissue in the laboratory, synthetic models are often used to study vocal fold vibrations. In this paper we focus on two topics related to synthetic and real vocal fold materials. First, because certain tissues within the human vocal folds are transversely isotropic, a fabrication process for introducing this characteristic in commonly-used vocal fold modeling materials is presented. Second, the pipette aspiration technique is applied to the characterization of these materials. By measuring the displacement profiles of stretched specimens that exhibit varying degrees of transverse isotropy, it is shown that local anisotropy can be quantified using a parameter describing the deviation from an axisymmetric profile. The potential for this technique to characterize homogeneous, anisotropic materials, including soft biological tissues such as those found in the human vocal folds, is supplemented by a computational study.

Keywords

Vocal fold modeling; Pipette aspiration technique; Material characterization

1. Introduction

Because speech is the primary mode of human communication, individuals suffering from the effects of voice disorders (e.g., hoarseness) are limited in their quality of life [1]. There are many issues related to the origin, prevention and treatment of voice disorders that are not

© 2012 Elsevier Ltd. All rights reserved.

*Corresponding author: sandra.weiss@lse.eei.uni-erlangen.de (S. Weiß).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

yet fully understood. Figure 1 shows a schematic view of the human larynx, including the vocal folds. Sound for typical voiced speech is initiated by flow-induced vibration of the vocal folds. Because the vocal folds are central to voice production, analyzing their properties and oscillatory characteristics is useful for exploring these challenging issues.

Two important factors that play a central role in governing vocal fold vibratory response include tissue properties and geometry. Consequently, studying these factors is an important component of voice research [2, 3]. The vocal folds are comprised of multiple layers of different types of tissues, the biomechanical properties (stiffness, viscosity, etc.) of which vary spatially and between individuals [4].

Two descriptions of vocal fold tissue layers are commonly used in the literature. In one description the vocal folds are described as having two layers, a stiff body layer and a flexible cover layer [4]. In this functional description the cover is the region in which the bulk of the vocal fold vibration occurs. The other description is anatomical and distinguishes the layers based on histological sections. The layers include a very thin epithelium, a superficial layer of lamina propria (SLLP) (sometimes called Reinke's space) [5], a vocal ligament (intermediate and deep lamina propria layers), and a thyroarytenoid muscle (M. Vocalis). This anatomic structure is shown in Fig. 1. Generally the cover is thought to consist of the epithelium and SLLP layers, with the body consisting of the ligament and body layers.

Analyses of excised human vocal folds show that the body is the thickest layer (about 7 mm thick) and consists of muscle fibers [4]. The SLLP layer is thinner (about 0.5 mm thick) and very flexible and includes an extracellular matrix with loose elastin and collagen fibers [6]. The epithelial layer is assumed to have a high modulus of elasticity, but is only a few cells thick [5]. The ligament (about 1 mm thick) is comprised of bundles of elastin fibers and spiraling collagen fibers, with a higher density of collagen fibers than the SLLP layer. The fibers are primarily oriented in the anterior-posterior direction, leading to a transversely isotropic material behavior [7, 8].

1.1. Synthetic modeling

In addition to studying vocal fold properties and geometry, experiments are often performed to study the mechanics of vocal fold flow-induced vibration (e.g., [9, 10]). With respect to reliable measurement methods to determine these mechanics, reproducible specimens are needed that withstand several investigations without changes in their material properties. For that reason, synthetic vocal fold models are fabricated.

One recent class of synthetic vocal fold models consists of cast models of polyurethane [11] or silicone [12], the general stiffness characteristics of which are similar to those of real biological tissue. Variations of this cast vocal fold model concept can be found in the literature, including: homogeneous one-layer models [11, 13, 14, 15, 16, 17], two-layer models consisting of a stiff body and a flexible cover layer [18, 19, 20], as well as a recent multi-layer model representing the body, ligament, SLLP, and epithelium layers of the vocal folds [21].

However, these models have only used isotropic materials even though some real vocal fold tissue layers also exhibit transversely isotropic behavior. For this reason, a first approach to mimicking the transversely isotropic behavior of the vocal ligament in a vocal fold model was demonstrated in [22]. In that study a two-layer body-cover model was fabricated and tested. A stiff body layer was covered with a flexible cover layer. Curled elastic fibers were embedded in the cover layer, oriented in the anterior-posterior direction. The flow-induced model responses at different strains showed an increase in fundamental frequency with

increasing length due to material stiffening. This has also been observed in the human vocal folds [23, 24]. Consequently, by embedding fibers in silicone rubber, the material response of the human vocal folds is better replicated. Hence, continued development of vocal fold models with transversely isotropic materials is needed for more realistic vocal fold model responses.

1.2. Vocal fold material property characterization

To compare synthetic material properties with those of real tissue, and to assist in the further development of synthetic materials that more closely resemble real tissue, measurement methods for characterizing the mechanical properties of both synthetic and real tissue are required. Several approaches for determining the mechanical properties of both artificial and real vocal folds have been published and are here classified into three categories: (1) methods to determine the static material properties of isotropic materials, (2) those that quantify the dynamic material behavior in general, but are not able to differentiate between multiple layers, and (3) spatially-resolved measurements. Some examples of these are reviewed below.

The static material response can be analyzed by means of simple tensile tests with an ergometer [9, 25, 26, 27, 28, 29]. In these types of studies, excised canine or human vocal folds have been tested and their static elasticity modulus values have been determined. This measurement technique is useful for the description of the static behavior, but does not provide information about the dynamic characteristics.

Because the primary human voice signal spans a wide range of frequencies (e.g., 78 to 698 Hz for males [30]), and since vocal fold tissue material properties are frequency dependent [8], there is a need for methods to quantify the dynamic material behavior. A rod resonance apparatus [31, 32] was used to measure the frequency-dependent Young's modulus of viscoelastic materials. Furthermore, in [33, 34], the measurement with a so-called vibration transmission analyzer was demonstrated to estimate the frequency-dependent parameters of single-layer silicone samples used for vocal fold models with the aid of an inverse method [35, 36]. These methods provide dynamic information, but require homogeneous specimens of known geometry; consequently, not all types of real tissue can be investigated. For layer-specific investigations, rheometer systems were used in [8, 37, 38] to measure, for example, the shear modulus of an excised human vocal fold cover layer. Although this method is capable of determining the material properties over a range of frequencies, the approach requires tissue that has been cut from the vocal fold and relatively large specimens that preclude fine spatial resolution.

Consequently, there is need for non-destructive spatially-resolved measurement methods that can be applied *in vivo*. One approach is the "indentation" method capable of determining spatially-resolved material properties of biological samples [39]. A small indenter tip is pushed into the specimen's surface, and by measuring the contact force and the tissue displacement, the Young's modulus can be calculated. This method has been used to estimate the Young's modulus of silicone rubber specimens used for vocal fold modeling [40], of human skin [41], of porcine and human liver tissue [42, 43], and of excised vocal fold covers [44]. Furthermore, anisotropy in biological tissue for implantable heart valves has been investigated using this technique [45]. This measurement method is therefore useful for the spatially-resolved characterization of the static behavior, but does not provide information about frequency-dependent properties.

A promising method for acquiring spatially-resolved, frequency-dependent material property measurement data is the pipette aspiration technique. This method of determining the mechanical material parameters of soft tissues was first reported in [46]. In that study, a

pipette was placed on the specimen's surface and by applying static suction, the specimen was aspirated into the pipette. By measuring the maximum aspiration displacement as a function of the aspiration pressure, the local stiffness was estimated. This technique has been applied to the determination of the local stiffness of aortas [47], the viscoelastic parameters of aortic valve interstitial cells [48], of other human organs (e.g., livers and kidneys) [49], and of free lipid membranes [50]. Ohashi et al. [51] introduced the use of the approach to analyze anisotropic mechanical properties of blood vessels under biaxial stretch. In a layer-specific analysis of multi-layer biomaterial samples, Zhao et al. [52] demonstrated the potential of this method to determine superficial layer stiffness. These studies show the suitability of the pipette aspiration technique for material property investigations of both synthetic and biological soft tissues. Zoerner et al. [53] extended the capability of this technique to include a fluctuating pressure, thereby enabling frequency-dependent measurements. This was done by replacing the static suction pressure in the original setup of [46] with a fluctuating pressure. In their study, the Young's modulus of isotropic silicone samples used for vocal fold modeling at specific frequencies within the human frequency range was calculated.

These studies show the potential for the pipette aspiration technique to be used to obtain spatially-resolved material property measurements of both synthetic and biological tissue within the frequency range of human speech. However, to date no studies dealing with the characterization of transversely isotropic synthetic materials used for vocal fold modeling using this technique have been published. The purpose of this paper is to report such data. The fabrication of several inhomogeneous, transversely isotropic silicone specimens with different amounts of anisotropy is described. Similar to the work of [22], elastic fibers were embedded in the silicone rubber to produce specimens that exhibit a transversely isotropic material behavior. Because the human vocal fold ligament is transversely isotropic and the vocal fold replicas investigated in [22] showed similarities with real vocal folds, the present investigation serves as a basis for further studies towards a more realistic vocal fold model. Moreover, by measuring displacement profiles at different positions of the inhomogeneous transversely isotropic specimens under strain, empirical data are provided that demonstrate the suitability of the pipette aspiration technique for characterization of these soft specimens. A finite element simulation is used to provide an analytical basis for extending the pipette aspiration technique to the characterization of homogeneous transversely isotropic materials.

2. Materials and methods

2.1. Specimen preparation

Our specimens were fabricated using the two-part addition-cure silicone rubber *Ecoflex 0030* (Smooth-On, Inc., Easton, Pennsylvania). This material exhibits stiffness properties similar to the human vocal folds and has often been used for vocal fold modeling [12, 20, 21]. This silicone rubber consists of equal amounts of the two subcomponents, denoted parts A and B. In order to reduce the stiffness of the cured silicone a variable amount of silicone thinner is added. In this study, two different mixtures, one with three parts thinner (here denoted 1:1:3) and one with four parts thinner (1:1:4) were used. This is comparable to the amounts that have previously been used in vocal fold models.

To create multiple silicone samples of the same size, a mold of another stiffer silicone rubber (*Smooth-Sil 950*) was produced. Cuboid specimens measuring 50 mm × 50 mm × 10 mm were cast in this mold (see Fig. 2).

The silicone rubber itself is isotropic. As some layers of human vocal fold tissue are transversely isotropic, with the stiffer direction aligned with the anterior-posterior direction [5], there is a need to increase the silicone sample stiffness in one direction. Following [22],

we embedded fiber bundles in the top layer of our silicone sample to mimic the ligament structure that consists of bundles of elastin fibers and spiraling collagen fibers. The fiber bundles, close-up views of which are shown in Fig. 2 and also in Fig. 12, were obtained by unweaving elastic polyester fabric. The mean diameter and elastic modulus of four fiber bundles were measured. The mean diameter, determined by imaging stretched fibers under a microscope, was $332.5 \mu\text{m} \pm 15 \mu\text{m}$. Moreover, one single fiber was extracted from the bundle and found to have a diameter of about $13 \mu\text{m}$. A tensile test apparatus (*Instron 3342*, Boston, Massachusetts) yielded a static Young's modulus estimate of $358.4 \text{ MPa} \pm 96 \text{ MPa}$.

The fiber bundles were positioned in the mold with nearly equivalent prestrain by inserting them into between 12 and 48 approximately equidistant cuts along opposing edges. The liquid silicone mixture was poured into the mold and allowed to cure for at least four hours.

The specimen was mounted in an apparatus that allowed stretching in the anterior-posterior direction (see right in Fig. 2). As shown in the figure, a layer of a stiffer 1:1:1 mixture of silicone rubber was attached to each side of the specimen to attach the specimen to the apparatus. The 1:1:1 mixture was glued to two aluminum plates using silicone rubber adhesive (*Sil-Poxy*). One of these plates had two threaded holes near the upper corners, into which threaded rod was screwed. At the other plate, countersinks ensured footing of the screws. By turning the threaded rod, the distance between the plates could be increased, causing the fiber bundles to straighten and increasing the overall specimen stiffness in the fiber direction. This setup allowed for a strain range from 0% to 30%.

2.2. Measurement setup

The pipette aspiration setup, shown in Fig. 3, was based on that of [53]. In our study, an aluminum annulus-shaped pipette was used, the bottom surface of which was gently pressed against the surface of the specimen. The contact force, measured by a force sensor (Burstner *8432-10*, Gernsbach, Germany), was kept constant for all investigations.

To enable measurements at different frequencies, a sinusoidal pressure was generated. A pistonphone with a stroke of 50 mm and an inner diameter of 30 mm was mounted on an electromechanical shaker (*TIRA vib S 504*, Schalkau, Germany). The mechanical oscillation induced by the shaker was transmitted to the lobe of the piston-phone, the frequency and amplitude of which were controlled via the automation software LabVIEW®. The pistonphone was connected to a gauge head by a flexible tube, allowing for pressure fluctuation to be generated within the pipette. The pressure amplitude was monitored by an electret microphone (Sennheiser *KE4-211-2*, Wedemark-Wennebostel, Germany) and controlled. A laser scanning vibrometer (Polytec *PSV 300*, Waldbronn, Germany) was also controlled via LabVIEW®. The vibrometer measured the out-of-plane velocity at a grid of measurement points on the surface of the specimen within the area enclosed by the pipette at a prescribed frequency.

2.3. Experimental procedure

Displacement profiles of specimens with different fiber densities were investigated. Unless otherwise noted, the specimens consist of silicone rubber in a 1:1:3 mixture. For all experiments a pipette with an inner radius of $r = 1.5 \text{ mm}$ was used. The contact force was maintained at 0.25 N. The pressure amplitude in the gauge head was kept constant at 20 Pa. The specimen was covered with a thin coating of a titanium dioxide powder to increase the laser reflection on the specimen's surface. The specimen was oriented under the pipette as shown in the top view of Fig. 3. The fiber direction coincided with the y -direction of the area enclosed by the pipette (see Fig. 3). This measurement area contained 73 measurement

points that were radially distributed over the pipette diameter with the center point matching the center of the pipette region.

The out-of-plane velocity of each measurement point at 120 Hz was saved in an ASCII-File. The data was afterwards converted to a corresponding displacement. Data postprocessing was performed in MATLAB® to generate and analyze the displacement profiles over the area enclosed by the pipette.

2.4. Simulation

Simulations were performed to (1) explore the measurement results using a simplified model of the experimental setup, and (2) determine whether the present measurement technique could conceivably be used to characterize anisotropy in vocal fold tissue. The latter was required because the experimental specimens were - in contrast to real tissue - locally nonhomogeneous.

2.4.1. Inhomogeneous computational model—A three-dimensional finite element model was defined based on the experimental setup; Fig. 4 shows the model geometry. The model was prepared using the ANSYS® preprocessor and solved using the finite element software package CFS++ (Coupled Field Simulation) [54]. The pipette ($r_{in} = 1.5$ mm) was modeled as the end of an annulus placed on top of a cuboid sample that had an edge length of 50 mm and a total height of 10 mm. The fiber bundles embedded in the silicone specimens were modeled as five cylinders with a diameter of 0.335 mm and a length of 50 mm. These cylinders were oriented in the y -direction and positioned 0.4 mm beneath the specimen surface. The gap between adjacent fibers was set to 0.99 mm.

Thus the size, position and spacing between the fibers were comparable to the experimental specimen with a fiber density of $0.72 \frac{\text{fibers}}{\text{mm}}$. The center fiber passed the pipette center so that the simulation represented the measurement above a single fiber. The three-dimensional model was comprised of 293561 linear quadrilateral elements and 416925 nodes. There were 6962 nodes on the aspiration surface. The pipette wall and the side and bottom surfaces of the cuboid specimen were fixed in all directions. A harmonic simulation was performed with a frequency of 120 Hz. A fluctuating pressure with an amplitude of 20 Pa was applied to the aspiration area.

The silicone was modeled with a Poisson's ratio of 0.49 and a Young's modulus of 5.2 kPa corresponding to the static elasticity modulus determined by tensile tests (e.g., [55]). For the fiber regions a Poisson's ratio of 0.4 was chosen and the elasticity was set to 358 MPa (the latter based on the fiber bundle tensile tests).

2.4.2. Homogeneous computational model—As mentioned above the specimens exhibited anisotropic behavior but were not homogeneous. However, on a macroscopic scale of interest (e.g., approximately on the order of 0.1 mm and greater), most vocal fold tissue can be treated as being locally homogeneous. Simulations were therefore performed to determine whether the present measurement technique could conceivably be used to characterize local homogeneous anisotropy in vocal fold tissue. Since synthetic, inhomogeneous, anisotropic material of the same order of stiffness as the human vocal folds was not readily available, we used a three-dimensional finite element model based on the experimental setup. The model geometry was similar to that shown in Fig. 4, but entirely comprised of a homogeneous material. This three-dimensional model included 169085 linear quadrilateral elements and 219231 nodes. There were 19881 nodes on the aspiration surface. The boundary conditions were the same as for the inhomogeneous model. A transversely isotropic material tensor $\mathbf{C}_{\text{trans-iso}}$ was chosen. In general, a transversely isotropic material with the x - z -plane as the plane of isotropy must satisfy the following

relations between the Young's moduli E_i in i -direction, the shear moduli G_{ij} in the i - j -plane, and the Poisson's ratios ν_{ij} :

$$\begin{aligned}
 E_{\text{iso}} &:= E_x = E_z \\
 E_t &:= E_y \\
 \nu_{\text{iso}} &:= \nu_{xz} = \nu_{zx} \\
 \nu_{\text{ti}} &:= \nu_{yx} = \nu_{yz} \\
 \nu_{\text{it}} &:= \nu_{xy} = \nu_{zy} \\
 \frac{\nu_{\text{ti}}}{E_t} &= \frac{\nu_{\text{it}}}{E_{\text{iso}}} \\
 G_{\text{iso}} &:= G_{xz} = G_{zx} = \frac{E_{\text{iso}}}{2 \cdot (1 + \nu_{\text{iso}})} \\
 G_t &:= G_{yz} = G_{zy} = G_{yx} = G_{xy}
 \end{aligned} \quad (1)$$

The simplified compliance matrix $\mathbf{S}_{\text{trans-iso}}(E_{\text{iso}}, E_t, \nu_{\text{iso}}, \nu_{\text{ti}}, G_t)$ for a transversely isotropic material with the x-z-plane as the plane of isotropy [56] has the form

$$\mathbf{S}_{\text{trans-iso}}(E_{\text{iso}}, E_t, \nu_{\text{iso}}, \nu_{\text{ti}}, G_t) = \begin{pmatrix} \frac{1}{E_{\text{iso}}} & -\frac{\nu_{\text{ti}}}{E_t} & -\frac{\nu_{\text{iso}}}{E_{\text{iso}}} & 0 & 0 & 0 \\ -\frac{\nu_{\text{ti}}}{E_t} & \frac{1}{E_t} & -\frac{\nu_{\text{ti}}}{E_t} & 0 & 0 & 0 \\ -\frac{\nu_{\text{iso}}}{E_{\text{iso}}} & -\frac{\nu_{\text{ti}}}{E_t} & \frac{1}{E_{\text{iso}}} & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{G_t} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{G_{\text{iso}}} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{G_t} \end{pmatrix}. \quad (2)$$

The material tensor $\mathbf{C}_{\text{trans-iso}}$ finally is related to the compliance matrix by

$$\mathbf{C}_{\text{trans-iso}} = \mathbf{S}_{\text{trans-iso}}^{-1}. \quad (3)$$

The five independent material parameters were chosen such that the overall material stiffness was somewhat similar to that of the experimental specimens. Because of the fundamental differences in material definitions, the simulation results cannot be directly related to the experimental results or to the inhomogeneous model simulation result. Rather, they provide an example of the expected pipette aspiration technique output when used to test a general, homogeneous, transversely isotropic material. The chosen parameter values are summarized in Table 1. The elasticity in the y-direction (transversely isotropic direction), E_t is 1000 times higher than E_{iso} in the isotropy plane (x-z-plane). The shear modulus in the y-z-plane and x-y-plane, G_t , was assumed to be nearly one third of the Young's modulus E_t in y-direction (as is the case for isotropic materials). The Poisson's ratios ν_t and ν_{iso} were set to 0.4 and 0.49, respectively.

2.5. Case studies

This study included seven different, but closely related investigations. All contributed to the common goals of (1) demonstrating the capability of the pipette aspiration technique to characterize the displacement response of a transversely isotropic material, and (2) applying this technique to explore the anisotropy of a material fabrication process intended for subsequent incorporation in synthetic vocal fold models. These case studies, along with the corresponding sections in which their results are discussed, were focused on:

- The suitability of the measurement technique to characterize inhomogeneous, transversely isotropic materials (Sec. 3.1)
- The influence of the measurement position on measured displacement profiles (Sec. 3.2)

- The density of fibers embedded in the specimen, important because the ligament of the human vocal folds is composed of many collagen and elastic fiber bundles [3] (Sec. 3.3)
- The influence of silicone material stiffness on displacement profiles (Sec. 3.4)
- The influence of specimen strain on measured displacement profile (Sec. 3.5)
- The repeatability of the results (Sec. 3.6)
- The simulated response of both an inhomogeneous and a homogeneous, transversely isotropic material, underscoring the potential of the pipette aspiration technique for the characterization of both homogeneous and inhomogeneous anisotropic materials (Sec. 3.7)

3. Results

3.1. Demonstration of concept

We first provide data to demonstrate that the pipette aspiration technique is suitable for interrogating spatially-resolved, frequency-dependent displacement fields of non-homogeneous transversely isotropic material samples. This is done by comparing the displacement profiles of an isotropic material sample with that of a transversely isotropic sample, both at 30% strain and consisting of silicone rubber in a 1:1:3 mixture. The pipette was placed against the center of the top surface of the isotropic specimen (i.e., at the position illustrated in Fig. 3). The displacement results for the isotropic sample and two corresponding profile cross sections in orthogonal x - and y -directions are shown in Fig. 5. Also shown in Fig. 5 are results obtained using a transversely isotropic specimen with 12 fibers. The fibers were aligned with the y -direction. The pipette was positioned on the specimen, centered directly above one fiber.

The displacement profile of the isotropic sample, Fig. 5(a), is axisymmetric, as evidenced by the matching profile cross sections. The maximum displacement of approximately $3.5 \mu\text{m}$ was found at the centerpoint and the displacement monotonically decreased towards the inner edge of the pipette. In contrast, the profile cross sections of the locally anisotropic sample, Fig. 5(b), exhibited significant variations. The displacement profile of the cross section aligned with the fiber direction was approximately parabolic and thus resembled that of the isotropic sample, but with reduced displacement. The displacement profile in the direction perpendicular to the fiber differed significantly. For stretched fibers the effective stiffness of the specimen near the fibers was increased, resulting in a lower displacement in the vicinity of the fibers. This caused the profile cross section perpendicular to the fiber to exhibit a local minimum directly above the fiber. The influence of strain is further discussed below (Sec. 3.5).

To facilitate quantitative comparison between cases, we here introduce a numerical parameter, A^* , to describe the deviation of the displacement field from that of an axisymmetric profile caused by transverse isotropy. The parameter is a ratio of the areas of the profiles in orthogonal directions. For the profile cross section perpendicular to the fiber direction, we consider the displacements in the x - z -plane for $y = 0$. Integrating this measured displacement profiles, signified as $d(x, 0)$, over the diameter of the pipette with radius r , one obtains

$$A_x = \int_{-r}^{+r} d(x, 0) dx. \quad (4)$$

The corresponding relationship for the y -direction integrated profile can be written as

$$A_y = \int_{-r}^{+r} d(0, y) dy. \quad (5)$$

The ratio of these two areas, A^* , is a measure of the deviation of the profile from axisymmetry, that is,

$$A^* = \frac{A_y}{A_x}. \quad (6)$$

Isotropic specimens yield $A^* = 1$ (with some small deviation possible due to measurement error). For the isotropic sample profiles in Fig. 5(a), $A^* = 1.01$. For the case with the measurement on top of the fiber, Fig. 5(b), the integrated area perpendicular to the fiber direction, A_x , is greater than that in the fiber direction, A_y , leading to a value of $A_{on}^* = 0.86$.

3.2. Influence of measurement position

The profiles at two different positions on the locally anisotropic sample with 12 fibers at 30% strain were considered: first, with the pipette centered directly above a fiber oriented in the y -direction, and second, with the pipette placed approximately equidistantly between two adjacent fibers. These profiles were compared with those from an isotropic specimen. The top view of each measured profile within the measurement area enclosed by the pipette is illustrated in Fig. 6.

While the profile of the isotropic specimen (Fig. 6(a)) shows concentric circles about the centerpoint, the profiles of the transversely isotropic specimen are somewhat elliptically-shaped. The top view of the displacement profile of the measurement centered above one fiber (Fig. 6(b)) clearly demonstrates the local minimum in the center and the peaks on either side of the x -axis. The elliptical displacement profile between two fibers (Fig. 6(c)) is due to the influence of the fibers located outside of the aspiration area with this fiber density. By stretching the transversely isotropic material, the effective stiffness near the fiber increased, resulting in a lower displacement in the area of the fibers. As a result, the profile aligned with the x -direction is narrower than that aligned with the fiber direction. This can also be seen in the corresponding anisotropic specimen profile cross sections shown in Fig. 7. Moreover, the overall displacement is reduced by the fibers. Since the embedded fibers are much stiffer than the silicone rubber itself, the displacement is decreased in the area of the fibers. The A^* -parameter for the measurement between two adjacent fibers was $A_{between}^* = 1.10$, that for measurement above a single fiber was $A_{on}^* = 0.86$. Thus, with the current orientation, the material response above a fiber yielded an A^* value less than 1, whereas that between the fibers yielded an A^* value greater than 1.

3.3. Influence of increasing fiber density

Studies to investigate the influence of fiber density on displacement profiles and on A^* are here discussed. The fiber density, ρ_f , is the ratio of the fiber number, N_f , to the total sample width, $w_s = 50$ mm. Specimens with 12, 24, 36, and 48 fibers were investigated, corresponding to fiber densities of 0.24, 0.48, 0.72 and $0.96 \frac{\text{fibers}}{\text{mm}}$, respectively. Further increases in fiber number were not feasible using this setup because of the narrow spacing between adjacent fibers.

The specimens at 30% strain were investigated at two positions, on a fiber and between fibers, and the corresponding A^* values were calculated. The profile cross sections perpendicular to the fiber direction are plotted in Figure 8 and the A^* values are listed in Table 2.

Figure 8 shows that the increase in fiber density caused a decrease in displacement. This was attributed to increased specimen stiffness in the fiber direction. Furthermore, the higher the fiber density, the smaller the spacing was between the fibers. This resulted in a steeper slope of the profile cross sections towards the pipette wall since the influence of the fibers within the measurement area also increased. The smaller spacing of the fibers with increasing fiber density can be seen in the x -direction profiles (Fig. 8(b)), primarily in the shallower slope near the pipette wall.

The figures show that as more fibers were embedded in the specimen, the deviation from an axisymmetric profile became greater. This can also be seen in the A^* values for the different fiber densities (cf. Table 2). The values for the measurements where the pipette was centered on a fiber, A_{on}^* , were decreasing with increasing fiber density, as the effective stiffness near the fiber increased, resulting in a lower displacement in the area of the fibers. By quadrupling the fiber density from 0.24 to 0.96, the A_{on}^* decreased from 0.86 to 0.71. By contrast, $A_{between}^*$ for the measurements between adjacent fibers increased from 1.10 to 1.38 with increasing fiber density.

3.4. Influence of mixing ratio

In this section, specimens fabricated using a more flexible silicone mixture are compared with the above results. A mixing ratio of 1:1:4 was used. The static Young's modulus of this mixture was determined by tensile tests to be about 3 kPa. Specimens with a fiber density of $0.72 \frac{\text{fibers}}{\text{mm}}$ (36 fibers) and $0.96 \frac{\text{fibers}}{\text{mm}}$ (48 fibers) were fabricated. Strains of 30% were applied to the specimens and the specimens were investigated with regard to their displacement profiles for different measurement positions.

Figure 9 shows the profile cross sections perpendicular to the fiber direction for investigations where the pipette was placed above a fiber (Fig. 9(a)) and between adjacent fibers (Fig. 9(b)) for the two mixing ratios. The results are compared to the those presented in Sec. 3.3 with specimens out of a 1:1:3 mixture. In general, a more flexible specimen leads to higher displacements. The maximum displacements of the profiles of the specimens out of a 1:1:4 mixture are about $0.5 \mu\text{m}$ greater than those of the stiffer 1:1:3 mixture. Moreover, the local minimum in the cross sections of Fig. 9(a) is more distinctive for the 1:1:4-specimens because the regions without fibers are pulled further into the pipette. The profiles are also consistent with the above-mentioned observation that a higher fiber density decreases the overall displacement.

Table 3 summarizes the calculated A^* values. The results are consistent with the previous figure. The decrease in stiffness of the sample results in smaller A_{on}^* and higher $A_{between}^*$ values.

3.5. Influence of strain

The investigations discussed above were all conducted with specimens at 30% strain. In this section we discuss the influence of strain on A^* . The 1:1:3-specimen with a fiber density of $0.72 \frac{\text{fibers}}{\text{mm}}$ was used. One measurement point above a fiber towards the center of the specimen was marked with a black dot. The pipette was centered on the marked fiber of the unstretched specimen, the displacement profile was measured, and A^* was calculated. The strain of the specimen was increased to 10%, the pipette position was adjusted to remain

centered on the mark, the measurement procedure was repeated, and the respective A^* was determined. A^* values for 20% and 30% were similarly obtained.

Figure 10 presents the profile cross sections perpendicular to the fiber direction as a function of increasing strain. It can be seen that the displacement decreased with increased strain. Moreover, there is no local minimum in the profile of the unstretched sample. The local minimum becomes more distinctive with increasing strain, showing that by stretching the fiber, the effective stiffness near the fiber increases.

The corresponding A^* for the different strains are summarized in Table 4. Increasing strain also decreased the A^* -measure of asymmetry in the profile cross sections. The value for the unstretched case of nearly one indicates an almost isotropic behavior.

3.6. Repeatability

To quantify the repeatability of the fabrication process and the measurement procedure, five 1:1:3-specimens with a fiber density of $0.72 \frac{\text{fibers}}{\text{mm}}$ were prepared. These are denoted cases M1 – M5. The specimens were fabricated using the same process but at different times, attempting to maintain the same degree of fiber prestrain, silicone mixture, and fiber depth. After curing, the specimen was marked above a fiber towards the middle of the sample with a black dot. The pipette was centered on the mark of the unstretched specimen and the displacement profile was measured. This measurement was repeated for two other points along the same fiber, both 10 mm away from the centered mark. The corresponding A_{on}^* was determined from the displacement results, thus each specimen yielded three different A_{on}^* values in the unstretched case. This was repeated for 10%, 20% and 30% strains, respectively, yielding 12 A_{on}^* values for each specimen.

Figure 11 shows a histogram of all calculated A_{on}^* values for the measurements at three positions along one fiber of each of the five investigated specimens M1 – M5 for different strains. Each bar group summarizes the A_{on}^* values at the same measurement point with increasing strain. For all 15 measurement points (three per specimen), the A_{on}^* values decreased with increasing strain, consistent with the previously presented results. In the unstretched case all specimens show an A_{on}^* of approximately one (first bar of each bar group). The measurements at three positions along one fiber (distance of 10 mm between adjacent measurement positions) of the same specimen are shown by the couples of three bar groups for each specimen M1 – M5. More precisely, the first three bar groups show the results for the measurements performed on specimen M1, the following three bar groups those of the investigations on M2 and so on. The A_{on}^* values from the three different positions on a given fiber are consistent, demonstrating the repeatability of the measurement procedure.

The mean values as well as the standard deviations of A_{on}^* were calculated for the five samples at the three different positions. Table 5 summarizes the results for the different strains. The decrease of the mean A_{on}^* values to about 0.74 associated with the increase in strain to 30% is also in agreement with the above results.

The standard deviations are very small for strains up to 10%, and slightly greater for higher strains. This, along with observed differences in A_{on}^* values shown in Fig. 11, is primarily attributed to changes in the manual fabrication process. This can be seen in microscopic photographs of specimen M4 shown in Fig. 12. The left picture (Fig. 12(a)) shows a top view of the unstretched specimen. Unequal spacing between neighboring fibers is evident. As a result, the sample's inhomogeneity due to the embedded fibers is not precisely the same for the different specimens and different measurement positions. Moreover, because

the fiber prestrain was not directly measured, the actual strain of the fibers would have differed somewhat. A third factor that should be taken into account is potential variation in depth of the embedded fibers which is displayed in the cross section of a specimen (Fig. 12(c)). Notwithstanding these fabrication-related differences that show that the consistency of the fabrication process needs to be improved, the consistent trends observed in the histogram data indicate that this measurement method and the corresponding A_{on}^* value calculations form a reasonable approach for characterizing the local anisotropy of these material specimens.

3.7. Results of finite element simulation

In this section, the results of the computational studies introduced in Sec. 2.4 are discussed. The mechanical behavior at a frequency of 120 Hz was analyzed with respect to the profile cross sections in orthogonal x - and y -directions. The cross sections of both models are displayed in Fig. 13(b) and (c), respectively.

The cross sections in Fig. 13(a) show the experimental result for a specimen with a fiber density of $0.72 \frac{\text{fibers}}{\text{mm}}$ as the inhomogeneous model in Fig. 4 was based on that configuration. The cross sections in Fig. 13(b) were obtained from the simulation of the inhomogeneous model with the pipette positioned directly above the fiber. As is the case with the experimental results, the profile line perpendicular to the fiber direction (solid line) exhibits a local minimum. The profile line in y -direction (along the fiber, dashed line) shows a nearly uniform displacement over the bulk of the pipette diameter. The corresponding A^* value is 0.83.

In comparison to the experimental result in Fig. 13(a), the absolute displacements of the numerical result (Fig. 13(b)) are higher, but of the same order of magnitude. Moreover, the profile line in the fiber direction from the experimental result is not nearly as uniform. These differences are attributed primarily to the following three factors. First, a contact force of 0.25 N was used in the experiments, whereas the specimen surface in contact with the pipette wall was fixed in the simulations. This simplification was done in order to avoid modeling the pipette itself as well as the contact between the pipette and the specimen. Second, the specimens in the experiments were stretched to a strain of up to 30% to straighten the initially curved fiber bundles. In the simulations, the fibers were initially straight cylinders. Consequently, the experimental displacements were lower than the numerical calculated ones. Third, the material parameters in the simulation were frequency-independent. However, Rupitsch et al. [33] showed that the elasticity of this silicone material increases with frequency. Since a stiffer material would likely result in lower displacements [46], this is consistent with lower displacements in the experimental result. Despite these differences, the simulation produced measured profile cross sections that exhibited promising qualitative agreement with those observed in the experiments.

In the following, the results of the simulations conducted to predict the general behavior of homogeneous transversely isotropic materials investigated with pipette aspiration are presented. This simulation was also performed to determine whether one could expect to observe similar types of responses as those that have been discussed here, i.e., A^* different than one. The resulting profile cross sections are shown in Fig. 13(c). Differences in cross sections can be seen. The slope of the profile in the isotropy direction (solid line, x) is less steep near the center but steeper near the pipette wall than the profile in the transverse isotropy direction (dashed line, y). The cross-sectional area of the profile in the isotropy direction was greater than that in the transverse isotropy direction, and the A^* was calculated to be 0.93. This demonstrates the potential of A^* as a characteristic value to analyze the degree of anisotropy of the investigated material. It also provides evidence that the pipette aspiration technique should be suitable for investigating homogeneous anisotropic materials.

4. Discussion

In this work, we have presented an approach for fabricating transversely isotropic silicone specimens with varying degrees of fiber density for use in vocal fold modeling and demonstrated that the pipette aspiration technique can yield empirical data for characterizing these specimens. We measured the displacement profiles of several specimens with varying degrees of local anisotropy at different positions and compared the results using a parameter, A^* , to quantify the displacement profile axisymmetry. The results showed that the profile axisymmetry was strongly influenced by specimen strain and fiber density. A^* decreased with increasing strain and fiber density for the measurement above a single fiber and increased with increasing strain for the measurement between two fibers. The repeatability was also tested by measuring the displacement profiles of five different specimens at different strains. The calculated A^* values showed small standard deviations along a given fiber, although differences in A^* values between specimens were observed due to variation in fiber spacing, depth, and prestrain inherent in the manual fabrication process. As a result, the suitability of the measurement of the displacement profiles to analyze anisotropic material response has been demonstrated. Moreover, a simulation of the setup yielded profiles that were consistent with the measured profiles, i.e., both simulation and measurement results exhibited a local minimum for the pipette positioned directly above a single fiber and also yielded A^* values different from 1. The strengths, limitations and suggestions for future studies are given in the following sections.

4.1. Specimens

By means of embedded polyester fiber bundles in the top layer of a flexible silicone specimen, the stiffness in the fiber direction was increased, thereby causing locally transversely isotropic behavior. The anisotropy increased with strain of the specimen, whereas the material was approximately isotropic in the unstretched state. In human vocal folds the ligament also shows transverse isotropy under strain [57, 58]. This is thus a further step towards the development of a more realistic vocal fold model. However, in contrast to real tissue, the specimens investigated in this study were inhomogeneous and only locally anisotropic on a macroscopic level due to the bundles of fibers and the isotropic regions between adjacent fibers. In future tests this limitation could be overcome by using an even higher fiber density than that which was used here. Another possibility would be to unweave the fibers and distribute individual fibers uniformly over the sample width. Furthermore, the manual fabrication process could be improved to achieve more consistent specimens. One promising alternative might be the use of electrospinning, a method that uses electrical forces to draw nanometer-scaled fibers from a liquid polymer [59, 60]. If many such fibers could be aligned in a flexible silicone mixture, a more uniform fiber distribution and hence a more homogeneous material would result.

4.2. Experimental studies

The results demonstrated that analysis of the aspiration displacement profile gives information about the general material behavior. By introducing the A^* as a parameter for quantifying the degree of displacement profile axisymmetry, the amount of local anisotropy could be estimated. An increase in strain and fiber density both significantly influenced the material's A^* value. Furthermore, the measurement position also influenced the profile axisymmetry in such a way that A^* values greater than one correlated with the isotropic region between adjacent fibers, whereas values less than one were found in the region directly above one fiber. Moreover, the overall displacement was lower for the transversely isotropic specimens relative to that of an isotropic specimen.

Because displacement is directly related to material stiffness, it is possible that these measurements could be used to not only characterize the material response, but also to calculate specific mechanical material properties. Further studies dealing with the use of an inverse scheme [35] to estimate the Young's moduli of the investigated materials based on the displacement profile results are anticipated.

One of the advantages of the pipette aspiration technique is the ability to measure dynamic material response. To compare the current study with previously published methods for determining the dynamic material behavior of real and artificial vocal fold tissue, Table 6 summarizes the frequency ranges reported in several studies. The ergometer used by [26] could be used to measure the dynamic behavior up to about 10 Hz, whereas the torsional controlled strain rheometer presented in [61] enabled measurements up to about 50 Hz. With the simple-shear rheometer in [38], viscoelastic properties within the range of 1 Hz – 250 Hz were measured. Each of these studies required excised specimens, thus precluding *in vivo* application. The vibration transmission analyzer technique used in [33, 34] was demonstrated to yield the frequency-dependent material properties up to 400 Hz. However, this method could only be applied to the measurement of synthetic specimens with known geometry. The pipette aspiration setup presented here is expected to be capable of obtaining measurements within the frequency range of approximately 50 Hz to 700 Hz. This frequency range is limited by the pistonphone-electromechanical shaker combination. Because a certain force is needed to overcome the lobe's stiction, frequencies lower than 50 Hz could not be measured. The upper frequency is limited by the shaker capabilities. However, the physiological relevant frequency range of most human voicing (e.g., 78 Hz – 698 Hz [30]) falls within this measurement range. By comparing the maximum displacements and the A^* -values for different applied pressure frequencies, information on the frequency-dependent properties could be acquired.

The setup could be used to obtain measurements on real vocal fold tissue. Real vocal fold tissue data could be used to improve both computational and synthetic vocal fold models. The empirical methods presented here could thus serve as a basis for future studies. The data demonstrate the suitability of this technique to determine the anisotropic response of real and artificial vocal fold tissues over a range of frequencies.

4.3. Computational studies

A harmonic simulation of the experimental setup using the model presented in Sec. 2.4 yielded results similar to the experimental results in terms of displacement profile lines perpendicular to the fiber direction. Since the specimens investigated in this study were inhomogeneous and only locally anisotropic, the potential of the pipette aspiration technique to analyze homogeneous transversely isotropic material was further demonstrated by simulating the response of such a material. The results showed differing profile cross sections in orthogonal directions in a manner that is similar with the experimental results. Moreover, A^* can in general be used to characterize the investigated material and gives information regarding the degree of anisotropy. As a result, computational studies could study the way that the cross-sectional aspiration area, as well as the material parameters, affect the displacement profile, thus assisting in the determination of optimal configuration and parameters for analyzing anisotropic materials using the pipette aspiration technique.

5. Conclusion

Silicone specimens with embedded fiber bundles were fabricated to mimic the transversely isotropic behavior of the human ligament and move towards a more realistic vocal fold model. The pipette aspiration technique was applied to characterize the spatially-resolved material response of these specimens when subjected to an input frequency of 120 Hz. The

technique could be applied across a range of frequencies to determine the frequency-dependent response. The results showed deviations in profile axisymmetry in comparison to isotropic materials. The parameter for quantifying the profile axisymmetry was a useful characteristic value related to the material's local degree of anisotropy. Computational studies using models similar to the experimental specimens were in agreement with the experimental results and thus underscore the potential of this technique for both homogeneous and non-homogeneous transversely isotropic material characterization. Since this method enables non-destructive, spatially-resolved measurements as well as the analysis of the dynamic material behavior, it is a promising tool for use in the study of voice production, with the ultimate aim of improving the clinical care of the human voice.

Acknowledgments

The work was supported by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Grant No. FOR 894/2, "Strömungsphysikalische Grundlagen der menschlichen Stimmgebung" (i.e., fluid dynamical basics of human voice production) and by Grant Number R01DC009616 from the U.S. National Institute on Deafness and Other Communication Disorders (NIDCD). S.L. Thomson gratefully acknowledges support as a visiting professor from the University of Erlangen Graduate School in Advanced Optical Technologies (SAOT).

References

1. Lallana, EC.; Uy, MN. The Information Age. 2003.
2. Hirano, M. Clinical examination of Voice. Springer; 1981.
3. Gray SD, Hammond E, Hanson DF. Benign pathologic responses of the larynx. *Annals of Otology, Rhinology and Laryngology*. 1995; 104:13–18.
4. Hirano, M.; Kakita, Y. *Speech Science: Recent Advances*. College-Hill Press; 1985. Cover-body theory of vocal fold vibration; p. 1-46.chap
5. Finck, C.; Dejeune, L. *Handbook of Mammalian Vocalization*. Elsevier; 2010. Structure and oscillatory function of the vocal folds; p. 427-438.chap
6. Gray SD. Cellular physiology of the vocal folds. *Voice disorders and Phonosurgery I*. 2000; 33:679–697.
7. Hammond TH, Zhou R, Hammond EH, Pawlak A, Gray SD. The intermediate layer: A morphologic study of the elastin and hyaluronic acid constituents of normal human vocal folds. *Journal of Voice*. 1997; 11(1):59–66. [PubMed: 9075177]
8. Chan RW, Titze IR. Viscoelastic shear properties of human vocal fold mucosa: measurement methodology and empirical results. *Journal of the Acoustical Society of America*. 1999; 106:2008–2021. [PubMed: 10530024]
9. Döllinger M, Berry DA, Hüttner B, Bohr C. Assessment of local vocal fold deformation characteristics in an in vitro static tensile test. *Journal of the Acoustical Society of America*. 2011; 130:977–985. [PubMed: 21877810]
10. Hüttner B, Sutor A, Luegmair G, Rupitsch SJ, Lerch R, Döllinger M. Optical 3-d metric measurements of local vocal fold deformation characteristics in an in vitro setup. *IEEE Transactions on bio-medical engineering*. 2011; 58(10):2758–2766. [PubMed: 21427016]
11. Thomson SL, Mongeau L, Frankel SH. Aerodynamic transfer of energy to the vocal folds. *Journal of the Acoustical Society of America*. 2005; 118:1689–1700. [PubMed: 16240827]
12. Chen, LJ.; Zanartu, M.; Cook, D.; Mongeau, L. *Flow Induced Vibration*. 2008. Effects of acoustic loading on the self-oscillations of a synthetic model of the vocal folds.
13. Thomson SL, Mongeau L, Frankel FH. Physical and numerical flow-induced vocal fold model. *MAVEBA*. 2003:147–150.
14. Spencer M, Siegmund T, Mongeau L. Determination of superior surface strains and stresses, and vocal fold contact pressure in a synthetic larynx model using digital image correlation. *Journal of the Acoustical Society of America*. 2008; 123:1089–1103. [PubMed: 18247910]
15. Becker S, Kniesburges S, Müller S. Flow-structure-acoustic interaction in a human voice model. *Journal of the Acoustical Society of America*. 2009; 125:1351–1361. [PubMed: 19275292]

16. Sidlof P, Doare O, Cadot O, Chaigne A. Measurement of flow separation in a human vocal folds model. *Experiments in Fluids*. 2011; 51:123–136.
17. Zhang Z, Neubauer J, Berry DA. Influence of vocal fold stiffness and acoustic loading on flow-induced vibration of a single-layer vocal fold model. *Journal of Sound and Vibration*. 2009; 322:299–313. [PubMed: 20161071]
18. Drechsel, JS. Master's thesis. Brigham Young University; 2007. Characterization of synthetic, self-oscillating vocal fold models.
19. Riede T, Tokuda IT, Munger JB, Thomson SL. Mammalian laryngeal air sacs add variability to the vocal tract impedance: Physical and computational modeling. *Journal of the Acoustical Society of America*. 2008; 124:634–647. [PubMed: 18647005]
20. Pickup BA, Thomson SL. Influence of asymmetric stiffness on the structural and aerodynamic response of synthetic vocal fold models. *Journal of Biomechanics*. 2009; 42:2219–2225. [PubMed: 19664777]
21. Murray P, Thomson SL. Synthetic multi-layer, self-oscillating vocal fold model fabrication. *Journal of Visualized Experiments*. In press.
22. Shaw SM, Thomson SL, Dromey C, Smith S. Frequency response of synthetic vocal fold models with linear and nonlinear material properties. *Journal of Speech, Language and Hearing Research*. In press.
23. Titze, IR. *Principles of Voice Production*. Allyn & Bacon; 1994.
24. Solomon NP, Ramanathan P, Makashay MJ. Phonation threshold pressure across the pitch range: preliminary test of a model. *Journal of Voice*. 2007; 5:541–550. [PubMed: 16753281]
25. Alipour-Haghighi F, Titze IR. Viscoelastic modeling of canine vocalis muscle in relaxation. *Journal of the Acoustical Society of America*. 1985; 78:1939–1943. [PubMed: 4078169]
26. Alipour-Haghighi F, Titze IR. Elastic models of vocal fold tissues. *Journal of the Acoustical Society of America*. 1991; 90:1326–1331. [PubMed: 1939897]
27. Zhang K, Siegmund T, Chan RW. Modeling of the transient responses of the vocal fold lamina propria. *Journal of the Mechanical Behavior of Biomedical Materials*. 2009; 2:93–104. [PubMed: 19122858]
28. Kelleher JE, Zhang K, Siegmund T, Chan RW. Spatially varying properties of the vocal ligament contribute to its eigenfrequency response. *Journal of the Mechanical Behavior of Biomedical Materials*. 2010; 3:600–609. [PubMed: 20826366]
29. Kelleher JE, Siegmund T, Chan RW, Henslee EA. Optical measurements of vocal fold tensile properties: Implications for phonatory mechanics. *Journal of Biomechanics*. 2011; 44:1729–1734. [PubMed: 21497355]
30. Hollien H, Dew D, Philips P. Phonational frequency ranges of adults. *Journal of Speech, Language and Hearing Research*. 1971; 14:755–760.
31. Madigosky WM, Lee GF. Automated dynamic young's modulus and loss factor measurements. *Journal of the Acoustical Society of America*. 1979; 66:345–349.
32. Madigosky WM, Lee GF. Improved resonance technique for materials characterization. *Journal of the Acoustical Society of America*. 1983; 73:1374–1377.
33. Rupitsch SJ, Ilg J, Sutor A, Lerch R, Döllinger M. Simulation based estimation of dynamic mechanical properties for viscoelastic materials used for vocal fold models. *Journal of Sound and Vibration*. 2011; 330:4447–4459.
34. Ilg J, Rupitsch SJ, Sutor A, Lerch R. Determination of dynamic material properties of silicone rubber using one-point measurements and finite element simulations. *IEEE Transactions on Instrumentation and Measurement*. In press. 10.1109/TIM.2012.2203449
35. Rupitsch SJ, Lerch R. Inverse method to estimate material parameters for piezoceramic disc actuators. *Applied Physics A: Material Science & Proceedings*. 2009; 97:735–740.
36. Rupitsch SJ, Wolf F, Sutor A, Lerch R. Reliable modeling of piezoceramic materials utilized in sensors and actuators. *Acta Mechanica*. 2012:1–13. in press. 10.1007/s00707-012-0639-7
37. Titze IR, Klemuk SA, Gray S. Methodology for rheological testing of engineered biomaterials at low audio frequencies. *Journal of the Acoustical Society of America*. 2004; 115:392–401. [PubMed: 14759030]

38. Chan RW, Rodriguez ML. A simple-shear rheometer for linear viscoelastic characterization of vocal fold tissues at phonatory frequencies. *Journal of the Acoustical Society of America*. 2008; 124:1207–1219. [PubMed: 18681608]
39. Haji T, Mori K, Omori K, Isshiki N. Experimental studies on the viscoelasticity of the vocal fold. *Acta Otolaryngologica*. 1992; 112:151–159.
40. Chhetri DK, Zhang Z, Neubauer J. Measurement of young's modulus of vocal folds by indentation. *Journal of Voice*. 2011; 25:1–7. [PubMed: 20171829]
41. Pailler-Mattei C, Bec S, Zahouani H. In vivo measurements of the elastic mechanical properties of human skin by indentation tests. *Medical Engineering & Physics*. 2008; 30:599–606. [PubMed: 17869160]
42. Samur E, Sedef M, Basdogan C, Avtan L, Duzgun O. A robotic indenter for minimally invasive measurement and characterization of soft tissue response. *Medical Image Analysis*. 2007; 11:361–373. [PubMed: 17509927]
43. Nava A, Mazza E, Furrer M, Villiger P, Reinhart WH. In vivo mechanical characterization of human liver. *Medical Image Analysis*. 2008; 12:203–216. [PubMed: 18171633]
44. Long JL, Neubauer J, Zhang Z, Zuk P, Berke GS, Chhetri DK. Functional testing of a tissue-engineered vocal fold cover replacement. *Otolaryngology-Head and Neck Surgery*. 2010; 142:438–440.
45. Cox MAJ, Driessen NJB, Boerboom RA, Bouten CVC, Baaijens FP. Mechanical characterization of anisotropic planar biological soft tissues using finite indentation: Experimental feasibility. *Journal of Biomechanics*. 2008; 41:422–429. [PubMed: 17897653]
46. Aoki T, Ohashi T, Matsumoto T, Sato M. The pipette aspiration applied to the local stiffness measurement of soft tissues. *Annals of Biomedical Engineering*. 1997; 25:581–587. [PubMed: 9146811]
47. Matsumoto T, Abe H, Ohashi T, Kato Y, Sato M. Local elastic modulus of atherosclerotic lesions of rabbit thoracic aortas measured by pipette aspiration method. *Physiological Measurement*. 2002; 23:635–648. [PubMed: 12450265]
48. Zhao R, Wyss K, Simmons CA. Comparison of analytical and inverse finite element approaches to estimate cell viscoelastic properties by micropipette aspiration. *Journal of Biomechanics*. 2009; 42:2768–2773. [PubMed: 19765713]
49. Nava A, Mazza E, Kleinermann F, Avis NJ, McClure J. Determination of the mechanical properties of soft human tissues through aspiration experiments. *Lecture Notes in Computer Science*. 2003; 2878:222–229.
50. Henriksen JR, Ipsen JH. Measurement of membrane elasticity by micro-pipette aspiration. *The European Physical Journal E*. 2004; 14:149–167.
51. Ohashi T, Abe H, Matsumoto T, Sato M. Pipette aspiration technique for the measurement of nonlinear and anisotropic mechanical properties of blood vessels under biaxial stretch. *Journal of Biomechanics*. 2005; 38:2248–2256. [PubMed: 16154412]
52. Zhao R, Sider KL, Simmons CA. Measurement of layer-specific mechanical properties in multilayered biomaterials by micropipette aspiration. *Acta Biomaterialia*. 2011; 7:1220–1227. [PubMed: 21056128]
53. Zörner S, Kaltenbacher M, Lerch R, Sutor A, Döllinger M. Measurement of the elasticity modulus of soft tissues. *Journal of Biomechanics*. 2010; 43:1540–1545. [PubMed: 20189571]
54. Kaltenbacher, M. *Numerical Simulations of Mechatronic Sensors and Actuators*. Springer; 2007.
55. Pickup BA, Thomson SL. Flow-induced vibratory response of idealized versus magnetic resonance imaging based synthetic vocal fold models. *Journal of the Acoustical Society of America*. 2010; 128:EL124–EL129. [PubMed: 20815428]
56. Bower, AF. *Applied Mechanics of Solids*. Crc Pr Inc; 2009.
57. Hunter EJ, Titze IR. Refinements in modeling the passive properties of laryngeal soft tissue. *Journal of Applied Physiology*. 2007; 103(1):206–219. [PubMed: 17412782]
58. Cook DD, Nauman E, Mongeau L. Reducing the number of vocal fold mechanical tissue properties: Evaluation of the incompressibility and planar displacement assumptions. *Journal of the Acoustical Society of America*. 2008; 124(6):3888–3896. [PubMed: 19206814]

59. Doshi J, Reneker DH. Electrospinning process and applications of electrospun fibers. *Journal of Electrostatics*. 1995; 35:151–160.
60. Reneker DH, Chun I. Nanometre diameter fibres of polymer, produced by electrospinning. *Nanotechnology*. 1996; 7:216–223.
61. Chan R. Measurements of vocal fold tissue viscoelasticity: approaching the male phonatory frequency range. *Journal of the Acoustical Society of America*. 2004; 115(6):3161–3170. [PubMed: 15237840]

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1. Human larynx: (left) sagittal plane schematic showing the larynx and vocal tract, and (right) frontal plane close-up showing the vocal fold structure

Figure 2. Specimen preparation. Left: mold with fibers positioned using cutouts. Center: transversely isotropic silicone specimen and close-up of fiber bundle. Right: setup for stretching specimen

Figure 3.
Pipette aspiration setup

Figure 4.

Geometry of the finite element model based on the experimental setup: The fibers were modeled as long cylinders with 0.99 mm spacing and 0.4 mm beneath the surface. The pipette wall and the edges were fixed in all directions and a fluctuation pressure was applied to the aspiration area.

Figure 5. Comparison of the displacement profiles of (a) an isotropic specimen and (b) a locally anisotropic specimen. The contour plots (upper row) show that the profile of the locally anisotropic specimen is elliptically-shaped. The profile cross sections in orthogonal x - and y -directions (lower row) show a deviation from profile axisymmetry due to local anisotropy.

Figure 6.

Top view of the displacement profiles of (a) an isotropic specimen showing concentric circles about the centerpoint and at different positions on a locally anisotropic sample (above a single fiber (b) and between adjacent fibers (c)) that are somewhat elliptically-shaped due to a decrease in displacement at the fiber regions; the gray scale denotes displacement in μm .

Figure 7. Profile cross sections in x - and y -direction for the different positions demonstrating the deviation from profile axisymmetry for locally anisotropic specimens.

Figure 8. Profile cross sections perpendicular to the fiber direction for different fiber densities showing decreasing displacement with increasing fiber density.

(a) Above single fiber

(b) Between two fibers

Figure 9.

Comparison of the profile cross sections perpendicular to the fiber direction for different mixing ratios. The local minimum of the measurement above a single fiber (a) is more distinctive for the 1:1:4 mixture. The profile lines of the measurement between two fibers (b) shows increasing displacements due to the decreasing stiffness.

Figure 10.

Displacement profile perpendicular to the fiber as a function of strain (measurement above single fiber, $\rho_f = 0.72 \frac{\text{fibers}}{\text{mm}}$). Strain increases the effective stiffness near the fiber resulting in a more distinctive local minimum.

Figure 11.

A_{on}^* for specimens M1–M5 from the repeatability study. For each specimen, results acquired at three positions along a given fiber are shown for strain increments from 0% to 30% in steps of 10%.

Figure 12.

Microscopic views of specimen M4. The close-ups demonstrate the unequal fiber spacing in the unstretched case (a) and under strain (b) as well as varying fiber depths (c) due to the manual fabrication.

Figure 13.

The profile lines in (a) show the experimental result for the measurement on a fiber (specimen with a fiber density of $0.72 \frac{\text{fibers}}{\text{mm}}$). The profile lines in (b) and (c) show the numerical results. The profiles in (b) were obtained by modeling the fibers as long cylinders of a stiffer material. The profiles in (c) were obtained using the model with a homogeneous, transversely isotropic material.

Table 1

Mechanical material parameters of the transversely isotropic material tensor

Parameter	Value
E_{iso}	5.2 kPa
E_t	5.2 MPa
ν_{iso}	0.49
ν_i	0.4
G_t	1.73 MPa

Table 2

A^* value dependence on fiber density for two different positions for the transversely isotropic specimen:
higher fiber density yielded smaller A_{on}^* and larger A_{between}^*

ρ_f	A_{on}^*	A_{between}^*
0.24 $\frac{\text{fibers}}{\text{mm}}$	0.86	1.10
0.48 $\frac{\text{fibers}}{\text{mm}}$	0.84	1.24
0.72 $\frac{\text{fibers}}{\text{mm}}$	0.72	1.28
0.96 $\frac{\text{fibers}}{\text{mm}}$	0.71	1.38

Table 3

A^* value dependence on mixing ratio for two different positions for the transversely isotropic specimen: The more flexible the silicone mixture, the smaller A_{on}^* and the higher A_{between}^*

	A_{on}^*		A_{between}^*	
ρ_f	1:1:3	1:1:4	1:1:3	1:1:4
0.72 $\frac{\text{fibers}}{\text{mm}}$	0.72	0.62	1.28	1.35
0.96 $\frac{\text{fibers}}{\text{mm}}$	0.71	0.61	1.39	1.64

Table 4

A_{on}^* values for different strains (measurement above single fiber). Increasing strain results in higher deviations from profile axisymmetry.

Strain	A_{on}^*
0%	0.99
10%	0.87
20%	0.77
30%	0.72

Table 5

$\overline{A_{on}^*}$ values and standard deviations for different strains calculated from the A_{on}^* -measurements on five specimens with equal fiber density.

Strain	$\overline{A_{on}^*}$
0%	0.99 ± 0.02
10%	0.89 ± 0.03
20%	0.80 ± 0.07
30%	0.74 ± 0.05

Table 6

Comparison of several measurement techniques to determine the material properties of real and artificial vocal fold tissue with respect to the maximum frequency range.

Study	Type	Frequency range
Alipour-Haghighi and Titze (1991) [26]	Ergometer	0.1 Hz – 10 Hz
Chan (2004) [61]	Torsional rheometer	0.016 Hz – 50.33 Hz
Chan and Rodriguez (2008) [38]	Shear rheometer	1 Hz – 250 Hz
Rupitsch et al. (2011) [33] Ilg et al. (in press) [34]	Vibration transmission analyzer	10 Hz – 400 Hz
Current technique	Pipette aspiration	50 Hz – 700 Hz