Biaxial mechanical properties of human vocal fold cover under vocal fold elongation

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Abstract: Mechanical properties of the human vocal fold cover layer were experimentally investigated in uniaxial and biaxial tensile tests. The results showed a coupling effect between the stress conditions along the anterior-posterior and transverse directions, with vocal fold elongation increasing vocal fold stiffness along both directions, thus allowing more efficient control of the fundamental frequency of voice through vocal fold elongation. This study also shows that vocal folds were nearly isotropic at resting conditions, thus a tendency to vibrate with incomplete glottal closure, but became increasingly anisotropic with increasing vocal fold elongation.

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1. Introduction

The mechanical conditions within the human vocal folds play an important role in determining the fundamental frequency of vocal fold vibration and the resulting voice quality. As the vocal folds are postured for the production of different voice types, the mechanical conditions are also expected to vary (Hirano, 1974). A better understanding of the mechanical conditions of the vocal folds across different voice types would facilitate the development of biomaterials for vocal fold injection or tissue engineered vocal fold replacement with similar material properties. Computationally, a more quantitative characterization of the vocal fold mechanical properties would provide data for developing material constitutive models, linear or nonlinear, for use in computational models of phonation.

There have been many previous reports experimentally characterizing vocal fold mechanical properties (e.g., Hirano and Kakita, 1985; Alipour-Haghighi and Titze, 1991; Haji *et al.*, 1992; Chan and Titze, 1999; Alipour and Vigmostad, 2012; Kelleher *et al.*, 2013; Kazemirad *et al.*, 2014). While these studies have provided valuable contribution to our understanding of the vocal fold mechanical properties, most of these studies were performed in a uniaxial tensile test, different from the physiological conditions in which vocal folds are subject to tensions or constraints along multiple directions. Although elongating the vocal folds along the anterior-posterior (AP) direction increases vocal fold stiffness and tension along this direction, which is considered the primary means of regulating the fundamental frequency (F0) of voice (Titze, 1994; Zhang, 2016b), little is known about how vocal fold elongation affects the mechanical conditions in the transverse plane (the plane perpendicular to the AP direction), which may also contribute to F0 control. Previous studies have shown a cross-axis coupling effect such that AP elongation may also stiffen the vocal folds in the transverse plane (e.g., Yin and Zhang, 2013). With this extra vocal fold stiffening in the transverse plane, vocal fold elongation would be more effective in regulating the overall vocal fold stiffness and F0 of vocal fold vibration, compared to F0 control through varying the stiffness and tension along the AP direction alone. This cross-axis coupling effect of vocal fold elongation cannot be investigated in a uniaxial tensile test, as in most of the experimental studies so far.

Also, mechanical properties are often quantified around the resting vocal fold conditions, and it still remains unclear how the anisotropic mechanical conditions vary under different conditions of vocal fold posturing. For example, although there have been some recent studies toward quantifying stiffness anisotropy of the vocal folds (Hirano and Kakita, 1985; Kelleher *et al.*, 2013), these studies focused mainly on the stiffness anisotropy at the resting state of the vocal folds, due to limitations of uniaxial

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tests in evaluating biaxial mechanical properties at conditions of varying vocal fold elongation. Maintaining a degree of stiffness anisotropy has been shown to play an important role in achieving complete glottal closure during vocal fold vibration (Zhang, 2011; Xuan and Zhang, 2014).

The present study presents our first step toward quantification of the anisotropic mechanical properties of the vocal folds in a physiologically more realistic setting, and how they vary with vocal fold elongation. In this study, we focus on quantifying the mechanical properties of the cover layer of the vocal folds, which consists of the superficial and intermediate layers of the lamina propria and the epithelium. The cover layer directly interacts with the glottal airflow and is involved in vocal fold contact, and its mechanical properties are thus of particular importance. While the thyroarytenoid muscle, which forms the bulk of the vocal folds, also plays an important role in regulating vocal fold stiffness and tension and we expect similar passive material behavior as the cover layer, the thyroarytenoid muscle also affects vocal fold stiffness through active muscle contraction, measurement of which requires a different measurement protocol (Alipour-Haghighi *et al.*, 1989) and was thus not attempted in this study. We will show that the constraints along the AP ends of the vocal folds, induced by varying degrees of vocal fold elongation, have a large effect on the mechanical properties within the transverse plane as well as the degree of stiffness anisotropy of the vocal folds.

2. Method

Two cadaveric human larynges (L1 at age 20 and L2 at age 50, both male) were harvested at autopsy and frozen at −80 °C until use. After thawing, the entire membranous vocal folds were resected, with care to exclude the cartilaginous vocal process. The vocal fold cover layer (the epithelium and lamina propria) was dissected off the underlying muscle layer and used in the tensile tests. An approximately square-shaped segment of the cover layer, with axes along the AP and transverse direction (perpendicular to AP), was used in the experiments. The specimen was mounted to a Bose ElectroForce 4-motor biaxial testing system using silk sutures placed at each edge of the specimen so that the two axes of the biaxial testing system aligned approximately to the AP and transverse directions of the specimens (Fig. 1). The mounting was adjusted so that the sutures were neither under tension nor sagging noticeably. Suture marks were also placed in the center region of the testing specimen and monitored during testing using a high-speech camera (Phantom V711, Vision Research) mounted directly above the specimen.

Four experiments were performed for each specimen, two in a uniaxial setting and two in a biaxial setting [Figs. 1(c)–1(f)]. In the first two uniaxial experiments, the specimen was vibrated along either the AP [Fig. 1(c)] or transverse direction [Fig. 1(d)], with the other edges of the specimen unconstrained and free to move. The imposed vibration was a sinusoidal stretch at 1 Hz, from the resting position to a maximum stretch of 60% of the *in situ* length of the specimen along the testing direction. Results from these tests would provide data comparable to previous tensile tests. In the third experiment, the specimen was stretched to and held at six given degrees of elongation (0%–50% in step of 10%) along the AP direction [Fig. 1(e)], and for each of the six AP elongation conditions, the specimen was stretched sinusoidally at 1 Hz along the transverse direction, with a maximum stretch of 60%. Data from these biaxial tests quantify vocal fold mechanical properties along the transverse direction at different degrees of AP elongations, simulating conditions of different combinations of thyroarytenoid and cricothyroid muscle activations. In the fourth experiment [Fig. 1(f)], the specimen was held at the original length along the transverse direction while a sinusoidal vibration of 1 Hz was imposed along the AP direction. Data from this experiment allow evaluation of the AP stiffness when the vocal fold is subject to transverse constraint, as in *in vivo* vocal fold conditions.

Each test lasted 50 s, and was followed by a short period of rest (less than one minute) which was required to reprogram for the next experimental conditions, during which the specimen was kept hydrated by dripping phosphate-buffered saline on the specimen. Force relaxation was observed during the initial preparation stage, with the peak force of each cycle decayed with time. No further preconditioning was applied during the experiment. In the data reported below, force relaxation was generally small except for conditions of large vocal fold elongation (greater than 30%) in which the peak force decayed by about 15% over 50 cycles of vibration. Although this force relaxation may be further reduced by longer preconditioning, it was not pursued in this study considering that preconditioning is not often required or occurs in human

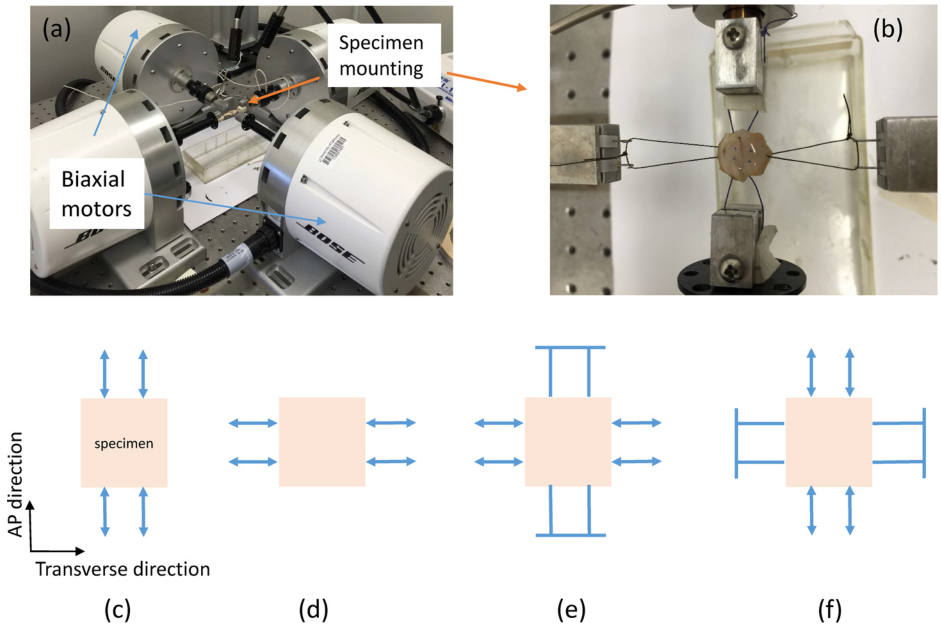

Fig. 1. (Color online) The experimental setup for the biaxial tests (a) and tissue mounting (b). (c)–(f) show the four experimental protocols used in this study, including two uniaxial settings and two biaxial settings.

voice communication. In other words, force relaxation may be a natural component of human phonation.

For each test, the displacements and forces along each axis were measured by an accelerometer included in the Bose ElectroForce system and a 250-g load cell (Honeywell, OH), respectively, at a sampling rate of 5000 Hz. The Bose ElectroForce system used a feedback control loop to achieve accurate displacement control. This introduced some high-frequency noise in the force data, which was filtered out by passing the force measurement through a low-pass filter with a cut-off frequency of 100 Hz (which is much higher than the 1 Hz of vibration of the experiment). The displacement and force data were then used to calculate the stress σ and strain ε using the initial dimensions of the specimens. To calculate vocal fold stiffness, the stress-strain data were averaged over loading and unloading and a tangent Young's modulus was calculated as the slope of the resulting curve (i.e., $d\sigma/d\varepsilon$) at different vocal fold deformations.

3. Results

3.1 Uniaxial tests: Material properties at resting state

Figure 2(a) shows the stress-strain curves from the first two experiments with uniaxial tests along the transverse direction (thick solid line) and the AP direction (thick dashed line), for larynx L2. Similar to previous experimental findings (e.g., Hirano and Kakita, 1985; Alipour and Vigmostad, 2012), the stress-strain curves for both uniaxial tests include a roughly linear range at small strains, in which the stress increased slowly with increasing strain, and a nonlinear range at large strains, in which the stress rapidly increased with increasing strain.

As in previous studies (Hirano and Kakita, 1985; Alipour and Vigmostad, 2012; Kelleher *et al.*, 2013), the vocal fold was significantly stiffer along the AP direction than the transverse direction. The derived tangent Young's moduli are shown in Fig. 2(b). For both larynges, the tangent Young's modulus was larger along the AP direction than the transverse direction. At the resting state (unstretched in either direction), the tangent Young's modulus for L1 was 3.73 and 1.17 kPa along the AP and transverse direction, respectively, with a stiffness anisotropy ratio of 3.2. For L2, the tangent Young's modulus was 1.05 and 0.60 kPa along the AP and transverse direction, respectively, with a ratio of 1.8. Note that because the AP and transverse uniaxial tests provided information about the mechanical properties along two different directions, estimation of the AP and transverse stiffnesses at one same vocal fold state was only possible for the resting unstretched vocal fold state. In other words, uniaxial tests do not allow estimation of the degree of stiffness anisotropy at conditions of varying degree of vocal fold elongation.

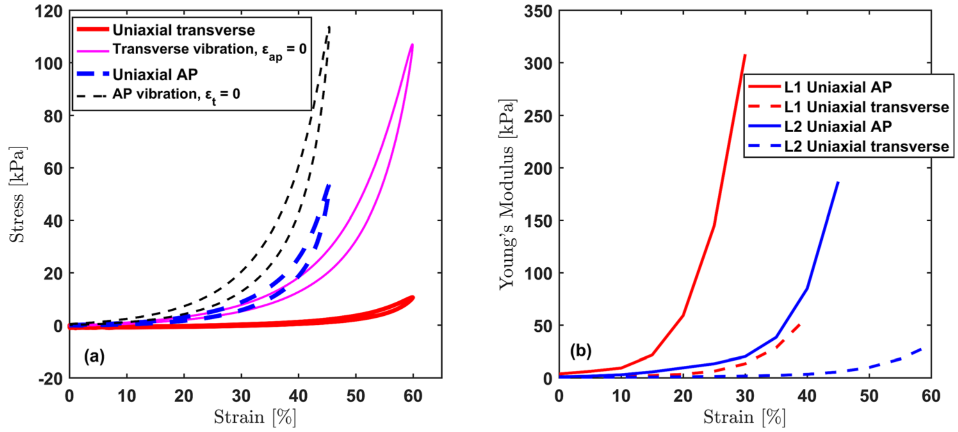

Fig. 2. (Color online) (a) The stress-strain curves obtained from uniaxial and biaxial tests for L2. (b) The derived tangent Young's moduli derived from the uniaxial tests for larynges L1 and L2.

3.2 Biaxial tests: Effect of vocal fold elongation

Figure 2(a) also shows data obtained in two biaxial tests, when the vocal fold specimen was vibrated along one direction while the specimen edges at the other direction, unlike being free in uniaxial test, were fixed at the initial length (thin solid and dashed lines). This fixed condition at the two edges induced a coupling effect between the transverse and AP directions, and increased the stress along the direction of vibration, compared to that in a uniaxial test. In other words, greater force was required in the biaxial tests to produce the same amount of vocal fold deformation as in a uniaxial test. More importantly, the slope of the stress-strain curve was larger in the biaxial setting, indicating an increase in vocal fold stiffness. Note that this coupling effect appears to be larger when the AP direction was constrained as compared to that when the transverse direction was constrained.

For biaxial experiments with the AP direction held at different degrees of elongation [Fig. 1(e)], the tangent Young's modulus along the transverse direction was extracted and plotted in Fig. 3 as a function of transverse strain, for different degrees of AP elongation of both larynges. The bottom half of Fig. 3 shows the same data but focuses in a smaller range of transverse strains which is more likely to occur during normal phonation. For both larynges, the coupling effect significantly increased the transverse Young's moduli. This magnitude of the coupling effect was larynx specific. For L1, increasing the AP elongation ε_{ap} from 0 to 0.33 led to the transverse Young's modulus at $\varepsilon_t = 0$ being almost doubled. For L2, the transverse Young's modulus at $\varepsilon_t = 0$ increased by almost four times as the AP elongation increased from 0 to 0.45. For both larynges, this increase gradually plateaued as the degree of AP elongation approached the nonlinear range (AP strain below about 0.2 for L1 and 0.3 for L2).

3.3 Stiffness anisotropy

Quantifying the degree of stiffness anisotropy requires data of the AP and transverse tangent Young's moduli measured around the same vocal fold state. In the present study, the two tangent Young's moduli data were available only for conditions of zero transverse strain (i.e., the specimen length along the transverse direction was kept constant at the initial value) at different degrees of AP elongation. For these conditions, an anisotropy ratio was calculated as the ratio between the AP tangent Young's modulus [measured from experiment 4 in Fig. 1(f)] and the transverse tangent Young's modulus [experiment 3 in Fig. 1(e)], and is shown in Fig. 4 as a function of AP elongation, for larynx L2 (solid line). Figure 4 shows that at zero AP elongation, the degree of stiffness anisotropy was about 1.5, indicating a nearly isotropic mechanical behavior. The anisotropy ratio increased significantly with increasing AP elongation, increased to 4.8 and 55 for an AP elongation of 18% and 45% AP elongation, respectively.

Unfortunately, the fourth experiment [Fig. 1(f)] was not performed for L1 so that the AP Young's modulus data for conditions of zero transverse strain were not available for L1. However, an estimation of the anisotropy ratio for L1 can be obtained as the ratio between the AP Young's modulus measured in a uniaxial test [i.e., with the transverse edges unconstrained; experiment 1 in Fig. 1(c)] and the transverse Young's modulus at zero transverse strain [experiment 3 in Fig. 1(e)]. Such estimations are shown in Fig. 4 for both larynges. Similar trends as to the directly

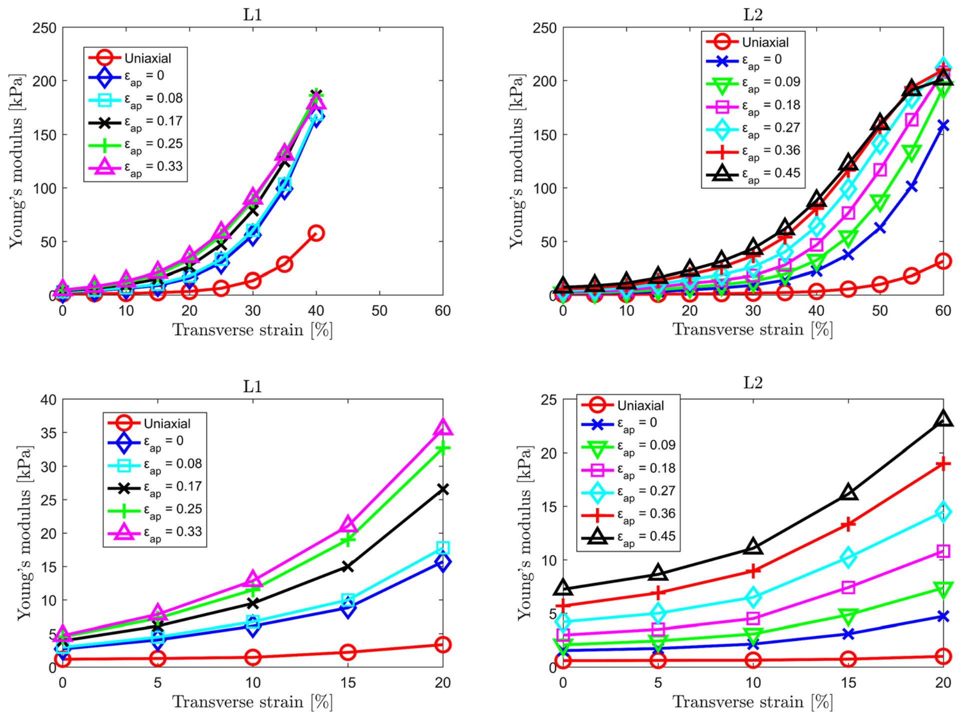

Fig. 3. (Color online) The transverse tangent Young's modulus as a function of the transverse strain for different conditions of AP elongation. The bottom panels show the same data but for a smaller range of transverse strain that is more likely to occur in normal phonation.

measured anisotropy ratio in L2 can be observed: the degree of stiffness anisotropy was generally low at resting states and increased with increasing AP elongation. Because the AP Young's modulus measured in a uniaxial test was expected to be smaller than that at a zero transverse strain (Fig. 2), this approximation would lead to an underestimation of the anisotropy ratio. Thus, the stiffness anisotropy was generally higher in L1 than in L2.

4. Discussion and conclusion

This study shows that in a physiologically more realistic condition with the vocal fold subject to constraints along both the AP and transverse directions, the mechanical stress within the vocal fold was higher than that measured in a uniaxial tensile test. In particular, the boundary constraints at the AP ends of the vocal folds can significantly increase both the stress and stiffness along the transverse direction (the transverse tangent Young's modulus increased by four times for L2). This coupling effect between the AP

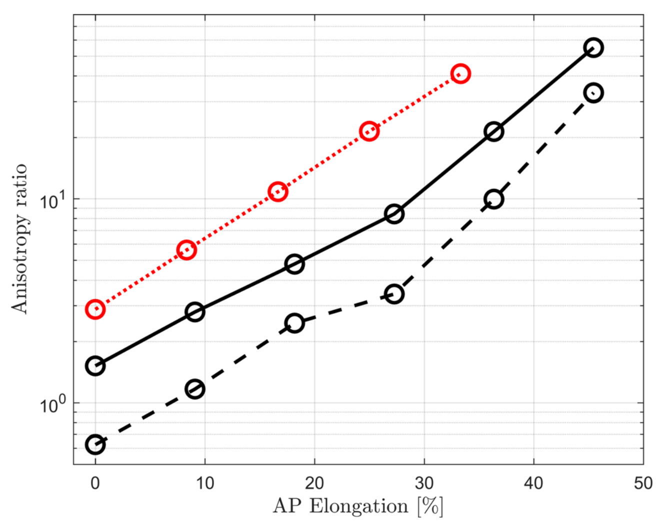

Fig. 4. (Color online) The stiffness anisotropy ratio, calculated as the ratio between the AP and transverse tangent Young's moduli, as a function of AP vocal fold elongation. Solid line: L2. The dotted and dashed line are an underestimation of the anisotropy ratio for L1 and L2, respectively.

and transverse directions, specifically the increase in the transverse stiffness accompanying vocal fold elongation, indicates that vocal fold elongation is more effective in F0 control than previously understood through regulation of vocal fold stiffness and tension along the AP direction alone, especially at low-pitch voice production. The effect of this cross-axis coupling on F0 control can be estimated by examining the effect of vocal fold elongation on vocal fold eigenfrequencies, which are important determinants of F0 (Zhang, 2016b). Using the data obtained for L2 and a vocal fold geometry similar to that used in Zhang (2016a), an eigenvalue analysis showed that a vocal fold elongation of 30%, which increased AP stiffness from 1.5 to 20 kPa and the transverse stiffness from 1.5 to 4.5 kPa, would increase the first *in vacuo* eigenfrequency of the vocal fold from 31 to 69 Hz, which is an extra 46% increase compared to an increase from 31 to 57 Hz if the accompanying increase in the transverse stiffness was not accounted for.

This study also shows that the degree of stiffness anisotropy was generally low at resting conditions and increased significantly with increasing vocal fold AP elongation. It is interesting to note that L2 was almost isotropic at the resting state whereas L1 was more anisotropic. This inherent difference in the stiffness anisotropy may explain the observation that in excised larynx experiments some vocal folds are able to vibrate with complete closure whereas others cannot (Isshiki, 1989). Our previous study shows that vocal fold models of nearly isotropic mechanical properties tend to vibrate with incomplete glottal closure (Xuan and Zhang, 2014). Thus, vocal folds with nearly isotropic mechanical properties, such as L2 in the present study, are likely to vibrate with incomplete glottal closure, whereas vocal folds with a higher degree of anisotropy, such as L1 in the present study, may be able to vibrate with better glottal closure.

The two larynges in this study exhibited large difference in their mechanical behaviors, with L1 having a smaller linear range and higher degree of stiffness anisotropy compared to L2 (Fig. 2 and Fig. 4). It may be tempting to attribute such differences to the age difference (age 20 for L1 vs age 50 for L2), among other factors. However, due to the small sample size of this study, this potential age effect would require further investigation in future studies.

Acknowledgments

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