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Could Spatial Heterogeneity in Human Vocal Fold Elastic Properties Improve the Quality of Phonation?

JORDAN E. KELLEHER,[1] THOMAS SIEGMUND,[1] and ROGER W. CHAN[2,3]

[1]School of Mechanical Engineering, Purdue University, 585 Purdue Mall, West Lafayette, IN 47907, USA; [2]Otolaryngology–Head and Neck Surgery, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA; and [3]Biomedical Engineering, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA

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Abstract—The physical mechanisms leading to the acoustic and perceptual qualities of voice are not well understood. This study examines the spatial distribution of biomechanical properties in human vocal folds and explores the consequences of these properties on phonation. Vocal fold lamina propria specimens isolated from nine excised human male larynges were tested in uniaxial tension (six from non-smokers, three from smokers). An optical method was employed to determine the local stretch, from which the elastic modulus of three segments in the anterior-posterior direction was calculated. Several specimens exhibited a significant heterogeneity in the modulus with the middle segment stiffer than the other segments. It was concluded that such modulus gradients are stronger in specimens from non-smokers than smokers. To understand the functional implications of a modulus gradient, the first eigenmode of vibration was calculated with a finite element model. With a modulus gradient, the vocal fold's eigenmode deflection was spread along the anterior-posterior length, whereas for a homogeneous modulus distribution, the deflection was more focused around the mid-coronal plane. Consequently, the strong modulus gradient may enable more complete glottal closure, which is important for normal phonation, while a more homogeneous modulus may be responsible for poor glottal closure and a perceived ''breathy'' voice.

Keywords—Larynx, Smoking, Glottal area, Elastic modulus, Functional gradient, Biomechanics.

INTRODUCTION

The glottis is defined as the space between the true vocal folds, and the glottal area is the area lying in the transverse plane between the vocal folds. Determination of the glottal area is crucial to analyze the voice.[6] It has implications to the glottal airflow, the conditions of contact between the vocal folds and hence contact stresses, which all contribute to acoustic characteristics of the voice as well as perceptual voice qualities.[51] Photoglottography,[5] video stroboscopy,[31] and kymography[50] have been common techniques for estimating the glottal area. More recently, advancements in high-speed endoscopic techniques have enhanced the accuracy and capabilities of measuring the glottal area.[47] The myoelastic-aerodynamic theory of voice production[56] connects tissue elastic properties to phonation events *via* the vibration characteristics of the vocal folds as excited by the glottal airflow. It is therefore appropriate to establish a potentially more direct link between the glottal area and the biomechanical properties of the vocal folds.

Spatially varying properties of the vocal folds have been investigated by several others in the past. Haji *et al.*[28] examined the transverse force–deflection response at the anterior commissure, mid-membranous vocal fold, and vocal process locations on canine vocal folds, and reported that the mid-membranous region was more compliant, though no quantitative analysis was performed. Similarly, the local transverse stiffness was measured in static tension *via* sutures and was found to generally increase from posterior to anterior.[18] Others have found a gradation of shear stiffness in porcine larynges increasing from the vocal fold inferiorly to the trachea[23] and change in local shear stiffness in the anterior-posterior direction.[32] However, spatial variations have not been examined for the longitudinal tensile elastic modulus in human vocal folds, except for one case study.[37] In this recent case study on the biomechanical properties of a vocal

Address correspondence to Thomas Siegmund, School of Mechanical Engineering, Purdue University, 585 Purdue Mall, West Lafayette, IN 47907, USA. Electronic mail: siegmund@purdue.edu

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ligament specimen it was found that the tissue at the mid-coronal plane was approximately ten times stiffer than the tissue located at the anterior and posterior (i.e., macula flavae) regions when subjected to tensile stretch. We hypothesize that such a gradient indeed exists in the elastic properties of both the vocal fold cover and the vocal ligament, and that the elastic heterogeneity would be related to phonation. As smoking has been documented to potentially cause destructive consequences to biological tissues,[27] we also explore if smoking could affect the biomechanical properties of vocal fold tissue, potentially altering the modulus gradient. We further conjecture that such gradients, or the absence of gradients, may result in changes to the vibratory characteristics of the vocal folds leading to acoustic, aerodynamic, and perceptual changes in voice.

The inclusion of tissue specimens from subjects with a history of smoking is motivated by previous findings that smoking affects phonation. Several past studies have addressed such effects from a clinical perspective.[2,3,17] According to the Centers for Disease Control and Prevention, approximately 20% of U.S. adults smoke cigarettes as of 2009.[11] It has clearly been documented that smoking affects health.[20] Pertaining to the larynx, smoking is known to be a primary factor of laryngeal cancer,[8,10] Reinke's edema,[41] thickening of the vocal fold epithelium,[33] atypical cellular changes in the epithelium,[1,45] inflammation in vocal fold fibroblasts,[7] and possibly even causes neurological defects.[22] Acoustically, the fundamental frequency of vocal fold vibration F_0 during phonation has been shown to be lower for smokers than for non-smokers,[49] even if one has been smoking for less than 10 years.[22] Smoking has been shown to also affect the biomechanics of certain tissues, such as vascular tissues.[19] Smoking raises the blood pressure which significantly reduces the arterial distensibility thereby causing an increased stiffness. However, the effect of smoking on the biomechanics of laryngeal tissues should not necessarily be compared with that of vascular tissues due to the different physiological conditions. The studies on laryngeal tissues cited above[1,7,8,10,33,41,45] have indeed shown changes in the tissue structure, but these studies do not provide information on the tissue mechanical properties. How changes in biomechanical properties could translate to acoustical and aerodynamic changes observed in vocal disorders associated with smoking is thus not well understood.

MATERIALS AND METHODS

Experimental

Tissue specimens were isolated from the excised larynges of nine human cadaveric subjects, see Table 1. Six male non-smoker subjects, as well as three male smoker subjects were included, all of whom were Caucasian. For each subject, one vocal ligament specimen and the contralateral vocal fold cover specimen were tested with a tensile cyclic stretch-release paradigm. The sample preparation and testing protocols were approved by the Institutional Review Board of University of Texas Southwestern Medical Center. Following the protocol of Chan *et al.*,[13] the cover and ligament specimens were dissected with instruments for phonomicrosurgery, separated from the underlying vocalis muscle and immediately placed in phosphate buffered saline (PBS). 3-0 nylon sutures were inserted through the center of a section of the arytenoid cartilage and the center of a section of the thyroid cartilage, both naturally attached to the vocal ligament or vocal fold cover specimen. The suture inserted through one cartilage section was connected to the actuator (lever arm) of a servo-controlled lever system,[1] while the suture in the other cartilage section was connected to the support.

Figure 1 depicts the experimental setup. The lever system was under displacement feedback control, and was connected to a function generator and an oscilloscope to monitor the displacement input. A small amount of pre-load (less than 0.023 N) was maintained before the tensile test began to keep the sutures taut and the specimen aligned vertically. The tensile force response of the specimen was detected by the servo-control lever system with a resolution of 0.3 mN, digitized at 500 samples/s and output for further analysis. The applied displacement was sinusoidal at 1 Hz with an amplitude of 3.0–8.0 mm, depending upon the initial resting vocal fold length L_0. The applied displacement was set such that each tissue specimen was stretched to approximately 130–140% of its initial length. The uniaxial tensile test was conducted for 180 cycles, similar to previous studies on vocal fold elasticity.[13,36]

A monochrome CCD camera[2] (pixel size of 9.9×9.9 μm, maximum frame rate of 75 fps) together with a macro-lens was used to capture images continuously during the experiment. A spirit level was used to ensure that the optical path of the camera was perpendicular to the specimen axis. A traceable speckle pattern was applied to the surface of the tissue specimen by applying black ink dots with a fine tipped paint brush, or by using black enamel based spray paint. This procedure resulted in a covering of the tissue specimen surface with a discontinuous speckle pattern such that specimen stiffness and moisture ingress were not disturbed.[36] During the experiments, specimens

[1]Aurora Scientific Model 300B-LR, Aurora, Ontario, Canada.

[2]Allied Vision Technologies, Stingray F-033B.

TABLE 1. Details on the tissue specimens for this study (L_0 = initial specimen length, D_0 = initial specimen cross-sectional diameter).

Subject	Age	Postmortem hours	Specimen	L_0 (mm)	D_0 (mm)
A	68	20	Ligament	14.3	3.6
			Cover	15.5	2.0
B	68	32	Ligament	21.2	3.4
			Cover	13.8	2.5
C	87	43	Ligament	15.1	3.5
			Cover	15.2	2.9
D	45	18	Ligament	17.12	2.8
			Cover	18.95	2.1
E	64	19	Ligament	14.77	2.9
			Cover	12.82	1.6
F	57	44	Ligament	14.58	3.3
			Cover	12.12	1.4
G (smoker)	70	11	Ligament	14.9	6.1
			Cover	14.0	3.9
H (smoker)	78	48	Ligament	11.2	2.8
			Cover	15.9	1.7
I (smoker)	86	41	Ligament	15.0	2.4
			Cover	15.7	1.7
Average	69	31	–	15.1	2.7

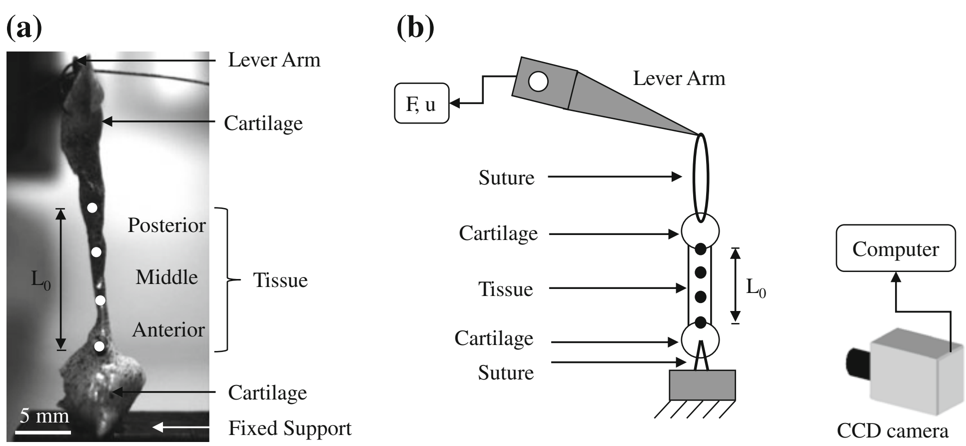

FIGURE 1. The experimental setup for the cyclic tensile stretch test. (a) the vocal fold cover from a non-smoker (subject E). The four points that were optically tracked are shown as white circles. (b) Schematic drawing of the setup.

were kept in air to avoid optical distortions related to the use of a glass chamber with physiological solution, as well as dissolution of the ink or spray paint. Instead, specimens were hydrated periodically by dripping PBS onto the tissue.

Optical measurements of tensile deformation have been shown to be crucial for vocal fold tissues.[36] Thus, displacements of four points on the specimen surface at equidistant longitudinal locations were obtained from image sequences *via* digital image correlation functions of the Image Processing Toolbox™ of Matlab® (version 7.10), which have a resolution of 0.1 pixels. This resulted in the specimen being divided into three longitudinal segments—anterior, middle, and posterior segments—of equal length ($L_0/3$). The stretch λ of each segment can then be computed by

$$\lambda_{\text{anterior}} = 1 + \frac{\Delta L_{\text{anterior}}}{L_0/3},\ \lambda_{\text{middle}} = 1 + \frac{\Delta L_{\text{middle}}}{L_0/3},$$
$$\lambda_{\text{posterior}} = 1 + \frac{\Delta L_{\text{posterior}}}{L_0/3} \tag{1}$$

where ΔL is the elongation of the corresponding segment as determined from the displacement data at the four measurement points. The medio-lateral strains were not of interest for this particular study and thus were not measured. Distance measurements were calibrated by taking an image of an object of known dimensions to establish a pixel-to-mm ratio. The specimen diameter of each segment was optically measured at five equidistant locations, and the cross-sectional area was calculated assuming the tissue specimen to be of circular cross-section. A second

CCD camera, orthogonal to the first, confirmed the circular cross-sections to be a reasonable assumption. Then the five areas measurements were averaged to estimate the cross-sectional area $A_{0,i}$ of each segment (where $i =$ anterior, middle, or posterior). The cross-sectional diameter of the overall specimen (all three segments) D_0 is given in Table 1. Finally, the nominal stress $\sigma = F/A_{0,i}$ of each segment was determined from the force output from the lever arm F and the segment's cross-section area $A_{0,i}$ in the undeformed state. The elastic modulus of each segment was estimated as the slope of the initial linear regime of the loading portion of the stress–stretch curve.

Analysis Model

For the analysis, the tissue specimens were approximated as cylinders with length $\bar{L}_0$, and unit cross section. The finite element method and a linear perturbation method were used for the computation of the eigenmode shapes by solving.

$$\left(-\omega^2 \mathbf{M}^{pq} + \mathbf{K}^{pq}\right)\phi^q = 0 \tag{2}$$

where $\mathbf{M}$ is the mass matrix, $\mathbf{K}$ is the stiffness matrix, p and q are the degrees of freedom, ω is the eigenfrequency, and ϕ is the corresponding eigenvector (eigenmode of vibration). Based on the eigenmodes, the glottal area values and closure conditions can be obtained.

In order to arrive at a concise analysis model, several model abstraction steps were considered. To arrive at a unique geometry, the model considered an average length $\bar{L}_0 = 15.1$ mm based on data of all tested vocal fold tissue specimens (Table 1). The model assumed a constant cross-section in order to negate the effects of subject-specific tissue geometry, enabling us to only focus on the effects due to the spatially varying elastic properties. Quantitative analysis of high-speed endoscopic imaging revealed that healthy subjects exhibited a much higher degree of symmetry in vocal fold vibrations than dysphonic subjects.[40] Thus, the two vocal folds were considered to be symmetric with respect to the mid-sagittal plane. Consequently, only one vocal fold would need to be considered in the numerical analysis. In order to obtain a basic model, the vocal fold was considered to possess a length and elastic moduli of the combined means of the non-smokers and smokers derived from the vocal ligament and vocal fold cover specimens.

The model considered isotropic, linear elasticity, as an approximation for small-amplitude oscillation. From the experiments, elastic moduli data were available as three discrete data values. A bell-shaped curve consisting of the sum of two Gaussian distributions[37] was fitted to the average elastic modulus of the three segments. The spatial distribution of the elastic modulus E along the anterior-posterior coordinate $x_{\mathrm{a-p}}$ is

$$E(x_{\mathrm{a-p}}) = a_1 \exp\left(-\frac{x_{\mathrm{a-p}} - b_1}{c_1}\right)^2 + a_2 \exp\left(-\frac{x_{\mathrm{a-p}} - b_2}{c_2}\right)^2. \tag{3}$$

One set of the parameters of this function was computed for all vocal fold specimens. Eleven equally-spaced points along $x_{\mathrm{a-p}}$ were selected and moduli data at these locations were used as input into the finite element model. A model with a homogeneous elastic modulus was also created in order to elicit the effects of a spatially varying modulus. The homogenous elastic modulus $\bar{E}$ was taken to be the average of the Gaussian distribution, thus

$$\bar{E} = \frac{1}{\bar{L}_0} \int_0^{\bar{L}_0} E(x_{\mathrm{a-p}})\, dx_{\mathrm{a-p}} \tag{4}$$

Furthermore, a Poisson's ratio of 0.45 was considered since studies have shown that soft tissue has some compressibility at high deformation rates.[46]

57,825 first order, reduced integration, eight-node brick elements (C3D8R) were used to discretize the geometry using a sweeping technique. The characteristic element size was 0.182 mm with 76 elements along the length of the vocal fold's surface. Computations were conducted using the commercially available finite element software package Abaqus (version 6.11). The nodes representing the interface to the anterior commissure and the vocal process (i.e., top and bottom surfaces of the cylinder) of the model were constrained in all degrees of freedom. Also, all nodes were constrained such that motion occurred only in the plane spanned by the anterior-posterior and medial–lateral direction. The shape of the first eigenmode of vibration was obtained as normalized values of displacements $\hat{u}_{\mathrm{m-l}}$ of points located along the medial surface of the cylinder. The finite element model is appropriate for this study because obtaining closed-form analytical solutions of the mode shapes of a beam with a spatially varying elastic modulus is not trivial.

For computation of glottal areas A_{g} the anterior and posterior vocal fold ends were assumed to be separated by pre-phonatory anterior and posterior glottal gaps $d_{\mathrm{a}} = 0.5$ mm and $d_{\mathrm{p}} = 1.0$ mm, respectively, such that the glottis can be described by a physiologically relevant trapezoidal shape.[55] The displacements of the vocal folds along the length of the vocal fold were computed from the normalized deflections predicted by the finite element model as $U_{\mathrm{m-l}} = \eta \cdot \hat{u}_{\mathrm{m-l}}$. The amplitude factor η was obtained by the condition that contact between the vocal folds was incipient in the closed configuration. The area of the glottis A_g can be calculated as

$$A_{\mathrm{g}} = \frac{1}{2}\left(d_{\mathrm{a}} + d_{\mathrm{p}}\right)\bar{L}_0 \cos\alpha \pm 2\int_0^{\bar{L}_0} \eta \cdot \hat{u}_{\mathrm{m-1}}(x_{\mathrm{a-p}})dx_{\mathrm{a-p}} \tag{5}$$

where $\alpha = \sin^{-1}\left[\left(d_{\mathrm{p}} - d_{\mathrm{a}}\right)/(2\bar{L}_0)\right]$ and $\pm$ is for the open and closed states, respectively. The integral calculation (area under the eigenmode shape) was conducted by the trapezoidal rule.

RESULTS

Experimental

All vocal fold tissue specimens exhibited a time dependent response with the stress at maximum applied stretch declining continuously with the number of applied load cycles N so that no steady state response was reached, as in other studies.[14] Thus, the tissue elastic moduli were obtained from a representative stretch cycle such that transient effects associated with tissue preconditioning were minimized. In particular, data are presented for the 55th loading cycle of the protocol. From cycles $N = 1$ to $N = 180$, the average peak stress decayed by 28%. The detailed analysis of the tissue specimens was conducted for cycle $N = 55$ where the peak stress had experienced on average 74% of the total decay. As an example, the stress-stretch response of the anterior, middle, and posterior segments of the 55th cycle for the vocal ligament of subject G is shown in Fig. 2. Further analysis focuses on the small stretch region.

The values for the elastic moduli of all subjects are given in Table 2. For all subjects, the elastic modulus of the middle segment was generally larger than that of the anterior or posterior segments. Hypothesis testing of the data was conducted to determine if the heterogeneity in the elastic modulus was statistically significant ($p < 0.05$). The mean ratios of the elastic modulus of the middle segment to anterior segment and middle segment to posterior segment were the test statistics. The null hypothesis was that the mean middle-to-anterior and the mean middle-to-posterior modulus ratios were equal to one—signifying that no spatial gradient exists and the tissue is homogeneous. The alternative hypothesis was that the average modulus ratios were greater than one—signifying that the middle segment is significantly stiffer than the anterior and/or posterior segments. The hypothesis tests were performed using a one-tailed Student's t-distribution for three cases: $n = 9$ for all subjects, $n = 6$ for the non-smokers, and $n = 3$ for the smokers.

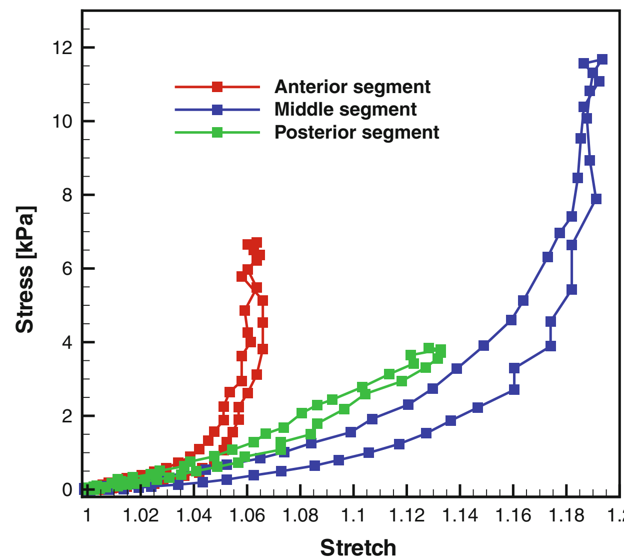

FIGURE 2. Typical stress–stretch curves of the anterior, middle, and posterior segments of a vocal fold tissue sample. The 55th load cycle for the vocal ligament from subject G.

TABLE 2. The elastic moduli of the anterior, middle, and posterior segments for all subjects.

		Elastic modulus (kPa)		
Subject	Specimen	Anterior	Middle	Posterior
A	Ligament	130	116	24
	Cover	36	644	24
B	Ligament	151	117	30
	Cover	36	364	65
C	Ligament	219	881	60
	Cover	145	5129	167
D	Ligament	11	41	28
	Cover	65	226	32
E	Ligament	23	39	14
	Cover	126	637	197
F	Ligament	8	21	17
	Cover	64	626	284
G (smoker)	Ligament	19	13	18
	Cover	196	291	19
H (smoker)	Ligament	13	20	28
	Cover	72	149	49
I (smoker)	Ligament	3	7	10
	Cover	21	62	20
Average	–	74	521	60

The results on modulus ratios are displayed in Fig. 3 with the error bars representing one standard deviation. For all subjects, the middle-to-anterior modulus ratio was significantly greater than one for the vocal fold cover ($p = 0.021$) and vocal ligament ($p = 0.022$), and the middle-to-posterior modulus ratio was significant only for the vocal fold cover ($p = 0.014$).

Upon examining the non-smokers and smokers separately, several interesting observations could be made. For non-smokers, it was found that the stretch

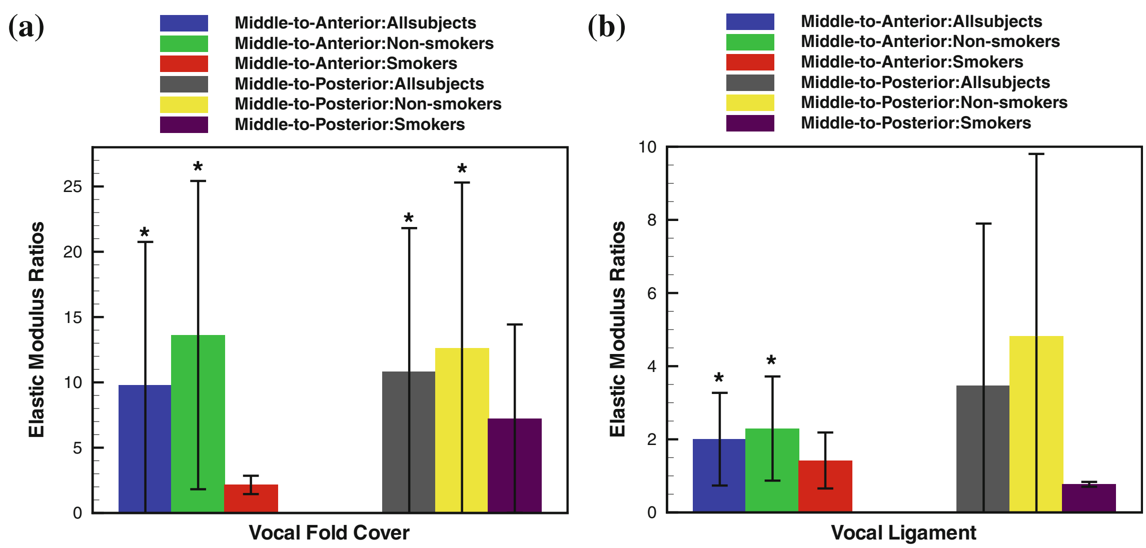

FIGURE 3. The ratio between elastic moduli of the middle, anterior, and posterior vocal fold segments for all subjects (n = 9), non-smokers (n = 6), and smokers (n = 3) for (a) vocal fold cover and (b) vocal ligament specimens. *denotes that the ratio is greater than one with statistical significance ($p < 0.05$).

of the middle segment was generally lower than those of the anterior and posterior segments in both vocal fold cover and vocal ligament specimens. This finding corroborates the results of our previous case study,[37] but was not found to be true for vocal fold tissues from the smokers. Consequently, for non-smokers the elastic moduli of the middle segments were much larger than the moduli of the anterior or posterior segments, yielding a strong heterogeneity/gradient. The elastic moduli of the three segments in the smokers were more homogeneous and a modulus gradient is not nearly as strong as in the non-smokers.

For the vocal fold cover from non-smokers, it was found that the middle-to-anterior and middle-to-posterior ratios of elastic moduli were significantly greater than one, with $p = 0.024$ and $p = 0.038$, respectively. Also, for the vocal ligament from non-smokers, the middle-to-anterior modulus ratio was significantly greater than one ($p = 0.038$), and the middle-to-posterior modulus ratio was nearly significant ($p = 0.060$). The hypothesis testing on the elastic moduli of vocal fold tissues from smokers did not yield statistical significance for the middle-to-anterior or the middle-to-posterior modulus ratios, for neither the vocal fold cover nor the vocal ligament specimens. Thus, we concluded that the elastic modulus gradient was largely absent in the smokers, unlike that of the non-smokers. The anterior-to-posterior elastic modulus ratio was tested against unity following the same procedure as described above. For both the non-smokers and the smokers no significant differences were found, i.e., there was no evidence of a spatial gradient in elastic modulus between the anterior and the posterior segments. Finally, a one-way analysis of variance (ANOVA) was conducted to compare the mean elastic modulus ratios between groups (between cover and ligament or between non-smokers and smokers). Eight individual ANOVA were performed: (1) middle-to-anterior ratio in covers between non-smokers and smokers, (2) middle-to-anterior ratio in ligaments between non-smokers and smokers, (3) middle-to-posterior ratio in covers between non-smokers and smokers, (4) middle-to-posterior ratio in ligaments between non-smokers and smokers, (5) middle-to-anterior ratio in non-smokers between covers and ligaments, (6) middle-to-anterior ratio in smokers between covers and ligaments, (7) middle-to-posterior ratio in non-smokers between covers and ligaments, and (8) middle-to-posterior ratio in smokers between covers and ligaments. Results of ANOVA revealed statistical significance only for the case of the middle-to-anterior ratio in non-smokers between the cover and ligament specimens ($p = 0.042$).

The vocal fold cover and the vocal ligament specimens for the non-smokers demonstrated substantial heterogeneity in tissue elasticity, with the average elastic modulus of the middle segment being approximately 8 times higher than that of the anterior segment, and approximately 9 times higher than that of the posterior segment. However, for the tissue specimens from smokers, the degree of heterogeneity in the elastic modulus is lesser since the average elastic modulus of the middle segment was only 1.8 and 4.0 times higher than those of the anterior and the posterior segments, respectively. How this difference in the spatial heterogeneity of tissue properties (i.e., graded vs. homogeneous elastic modulus) may affect vocal fold vibration characteristics was examined next.

Eigenmodes and Glottal Area

The set of parameters from Eq. (3) describing the elastic modulus spatial distribution computed for all vocal fold specimens was $a_1 = 558.6$ kPa, $b_1 = 7.53$ mm, $c_1 = 2.66$ mm, $a_2 = 1.05 \times 10^{15}$ kPa, $b_2 = -2.65 \times 10^4$ mm, $c_2 = 4.77 \times 10^3$ mm, depicted as normalized values in Fig. 4. Additionally, the amplitude factor η, which ensures that contact was initiated between the two vocal folds in the closed configuration, was first met in the model for the graded elastic modulus at $\eta = 0.353$. The total amplitude of vibration ($2\eta = 0.706$ mm) is relatively small compared to typical vibration amplitudes but is within the physiological range.[52] Results of the computations using the finite element model (Fig. 4) are displayed in Fig. 5. Figures 5a and 5b are included to show the physiologically relevant vocal fold motion while 5c and 5d are to provide a detailed view of the differences in the shape of the first eigenmodes of vibration for comparisons of graded vs. homogeneous elastic modulus spatial distribution. Since the modulus at the anterior and posterior segments in the graded case was approximately 8 times smaller than that at the mid-coronal section, the bending resistance of such vocal fold tissue is locally low. This is reflected in the finding that the deflection exhibits a more plateau-like shape. On the other hand, for the constant elastic modulus case, the shape of the first eigenmode follows the parabolic shape typically exhibited by homogeneous beam-like structures. Thereby, the deflection is more concentrated towards the mid-point (mid-coronal plane) of the vocal fold.

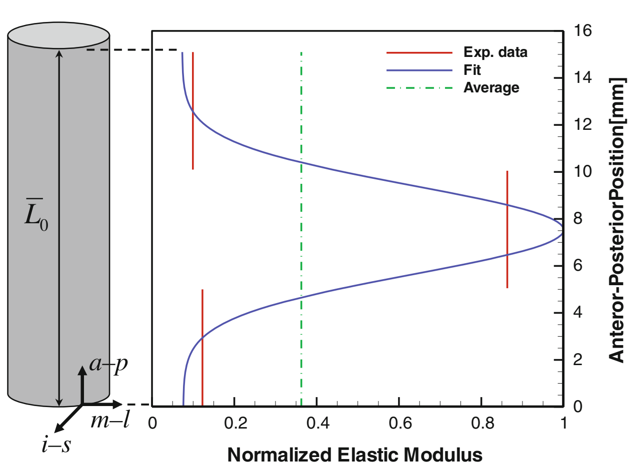

FIGURE 4. The elastic modulus of the three vocal fold segments for all specimens is shown along with a fitted Gaussian curve and the average modulus for the homogeneous case. The geometry of the finite element model and the coordinate system is displayed. a–p is the anterior–posterior, m–l is the medial–lateral, and i–s is the inferior-superior direction, respectively.

Based on the predicted mode shapes, the glottal areas in the open and the closed states were calculated. The predicted broad or plateau-like shape (i.e., the deflection is more evenly distributed across the length of the vocal fold) of the eigenmode for the graded case led to a smaller glottal area in the closed configuration and a larger glottal area in the open configuration. On the other hand, the predicted narrower mode shape (i.e., the deflection is more concentrated at the mid-point of the vocal fold) for the homogeneous case indicated a larger glottal area in the closed configuration and a smaller glottal area in the open configuration. Specific values of glottal area are provided in Table 3. In addition, the ratio of the glottal areas of the open and closed states was calculated and defined as the *open-to-closed (OC) index*. The OC index computed for the graded case was nearly 1.5 times greater than that computed for the homogeneous case. This finding suggests that a vocal fold with a more heterogeneous distribution of the elastic modulus in the anterior-posterior direction could show more contrast in vocal fold deflection between the closed phase and the open phase during vocal fold vibration, a factor important for normal phonation.[24] Finally, the choice of Poisson's ratio had very little influence on the predicted mode shape, hence the glottal areas. Changing Poisson's ratio to 0.4 or 0.49999 caused the OC index of the graded and homogeneous cases to change by approximately 2%.

DISCUSSION

For six of the nine subjects tested (i.e., the non-smokers), the elastic modulus of the vocal fold lamina propria was found to be on average 8 and 9 times greater in the middle of the vocal fold than in the anterior and posterior regions, respectively. However, for three of the nine subjects tested (i.e., the smokers), the average elastic modulus of the middle segment was only 1.8 and four times greater than the anterior and posterior regions, respectively. The large spatial variation in the elastic modulus caused the deflection to be more broadly distributed along the length of the vocal fold than for the case of a homogeneous tissue. In an elastically homogeneous tissue, the deformation was more uniform leading to a narrower eigenmode. The results of the present biomechanical simulation model indicate differences in the open and closed phases of phonation between graded and non-graded vocal folds.

For the subjects who were smokers, the experimental results imply that smoking could affect the biomechanical properties of vocal fold tissues and lead

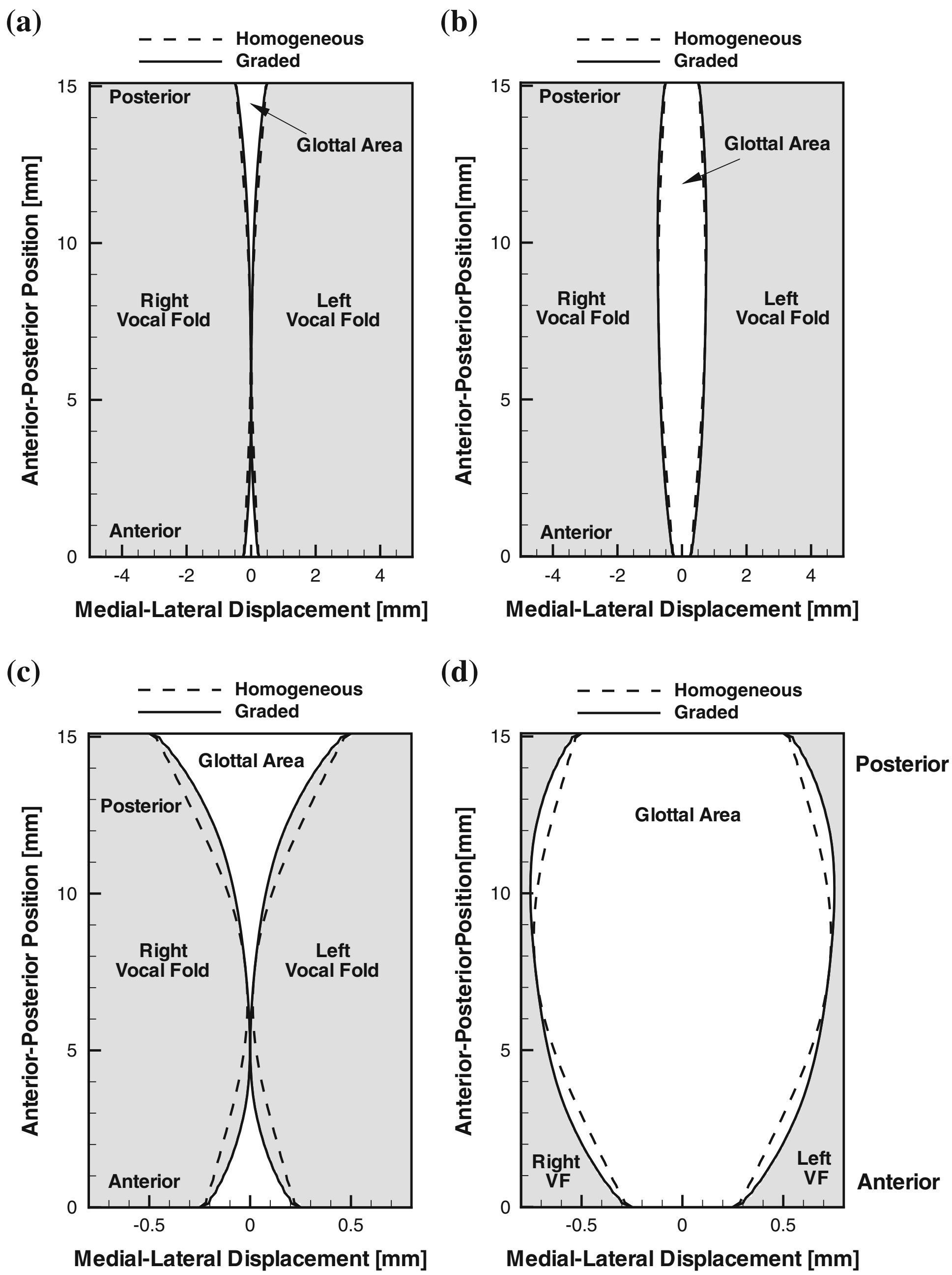

FIGURE 5. A superior view of the glottis when the vocal folds are in the (a), (c) closed configuration, and (b), (d) open configuration. The abscissa of (c) and (d) has been scaled by a factor of 5 from the images (a) and (b) to show the differences in the deflection of the graded and homogeneous cases. The vocal fold configurations are determined from the first eigenmode shapes of the finite element model accounting for the spatial variations of elastic moduli as determined experimentally and for the average of the graded modulus (i.e., homogeneous case).

to a reduced elastic heterogeneity in the tissue. It can be deduced that as the histological architecture of the vocal folds might be compromised by smoking,[33,41] any tissue microstructural changes could be reflected in the tissue elasticity as well. The altered vocal fold histology of the smokers could change the longitudinal heterogeneity of the elastic modulus, if the present findings could be corroborated by further studies involving more subjects. Indeed, the present findings should be considered as preliminary. Tissue biomechanical properties depend upon a host of subject-specific characteristics, such as gender, age, genetic makeup, external stimuli, etc. Age-related effects on the histology have been well documented in vocal fold tissues. It has been documented that arrangement of collagen fibers are less organized in geriatric vocal folds.[26] However, in related studies, there was shown to be no significant differences in the collagen fiber

TABLE 3. The predicted values of glottal area in the closed and open configurations, and the open-to-closed index for the graded and homogeneous elastic modulus cases.

	Glottal area (mm^2)		
	Closed	Open	Open-to-closed index
Graded	3.27	19.37	5.92
Homogeneous	4.45	18.20	4.09

content between adult (age 22–54 years) and geriatric (age 65–82 years) vocal folds,[29] and that most age-dependent changes of vocal fold biomechanical properties could occur below age 60.[57] Since all of the subjects (except subject D) of the present investigation are in the geriatric category, age-related effects may not be a significant confounding factor. The subjects analyzed were selected based upon their biographical similarities, i.e., the fact that they were all males, Caucasian, and of comparable age. It is not our goal by any means to extrapolate our results to the overall population of smokers. Additional subjects and data are definitely required to provide a more compelling conclusion.

Sufficient glottal closure is important to normal voice production because it facilitates self-sustained vocal fold oscillation. From an aerodynamic perspective, phonation threshold pressure, or the minimum subglottal pressure required to initiate and sustain vocal fold oscillation, is directly related to the prephonatory glottal width.[52] That is, the larger the glottal gap, the more energy it takes to achieve sustained phonation. Incomplete glottal closure can also lead to turbulent airflow. This turbulence causes pressure fluctuations in the vocal output and is perceived as a ''breathy'' voice.[54] Various measures of turbulence have been proposed to quantify the breathiness of the voice signal, and to correlate to the degree of closure of the vocal folds.[9,42,44] Alternatively, the OC index may be another valid measure of quantifying breathiness, with a smaller value suggesting less contrast in the glottal area of the open and the closed configurations. With advancements in the accuracy of high-speed endoscopic imaging,[47] the OC index could be quantified clinically and correlated to a subject's voice quality. The voice of smokers has commonly been characterized as breathy, among other perceptual voice qualities that are typical of pathological phonation. Studies have shown that hoarseness, which can be characterized as a voice that sounds breathy, raspy, strained, or harsh, was more common among smokers than non-smokers,[21] and that the mean airflow rate of passive smokers (i.e., non-smokers who are exposed to environmental tobacco smoke) was higher than that of non-smokers.[38] Also, the mean glottal gap during the closed phase of phonation was found to be larger in female smokers than non-smokers.[4] The present results suggest that insufficient glottal closure may exist in vocal folds with a more homogeneous elastic modulus distribution. It is interesting to note that the vocal folds excised from smokers in this study demonstrated a more homogeneous elastic modulus distribution than the non-smoker specimens. This could then lead to a more breathy voice which has been clinically documented in the studies above. However, it must also be noted that breathiness can be due to age-related effects, as indicated in previous studies showing increased occurrence of glottal gaps[34] and higher noise-to-harmonic ratios, which correlates with breathiness[25] in geriatric men. Yet age alone may not be able to fully account for these effects, as it has been stated that ''the age-related change in glottal closure in men is not particularly dramatic.''[39]

There were several potential limitations in this study. First, the tensile experiment on the specimens was conducted in air at room temperature. We do recognize that most previous studies on vocal fold tissue properties have been conducted at 37 °C, presumably to simulate the physiological temperature environment of the human larynx *in vivo*. However, it has also been suggested in the literature that the actual tissue temperature in the vocal folds during phonation may be somewhat lower than the systemic body temperature of 37 °C, due to convective and evaporative cooling effects of the laryngeal airflow on the vibrating vocal folds.[12,16] Second, the tissue specimens were not tested in a physiological fluid as most previous vocal fold biomechanical studies. Vocal fold hydration is composed of systemic and superficial components.[48] Since the tissue was excised, hydration was maintained by submerging the tissue in PBS and by periodically dripping PBS during the testing. Dehydration studies typically submerge the tissue in a hypertonic solution for 30 min or greater to examine the effects of dehydration (e.g., increased stiffness).[15,43] Superficial hydration is largely determined by the airflow around the vocal folds and humidity of the ambient environment.[30] The experiments were conducted in a laboratory environment in Dallas, Texas in June where the relative humidity is typically 50–75%, which is much higher than the desiccated air (0% humidity) used in Hemler *et al.*[30] Given this level of humidity, the periodic dripping of PBS onto the tissue, and the relatively short duration (less than 10 min) in which the tissue was not submerged in PBS, any dehydration effects were likely to be minimal. Third, this investigation focuses on the vocal fold eigenmodal response in only the small stretch regime of the stress-stretch curve. Under normal speaking conditions, vocal fold length changes in the anterior-posterior direction are within the linear portion of the stress-stretch response (i.e., less than 10% elongation).[35] It is mainly during situations involving significant vocal pitch changes (such as during singing) where there is substantial vocal fold longitudinal stretch, which would induce nonlinear effects. Additionally, the various mechanical stresses encountered during normal phonation have been predicted to be relatively minor[53]; hence, the strains would likely be small. Therefore, the analysis of the small stretch (linear) regime of the stress-stretch curve is a legitimate first approximation for describing many situations of normal phonation. The eigenmode analysis could be evaluated at higher levels of axial stretch by linearizing the material properties at that stretch level (i.e., using the tangent elastic modulus). The behavior of the eigenmodes and glottal areas at higher axial loads was not considered in this investigation, and should be addressed in further studies.

In summary, the results of the current study suggested that heterogeneity in spatial distribution of the elastic modulus along the length of the vocal fold could enable more complete glottal closure, resulting in improved phonation. On the other hand, a relatively homogeneous modulus distribution could lead to poor glottal closure and a more breathy voice quality. While such phonatory differences could also be correlated to the effects of smoking, further studies with a larger number of subjects and knowledge of the subject's lifestyle and health history are necessary to establish a more definitive link.

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CONFLICT OF INTEREST

No conflict of interest exists which would have inappropriately influenced this work.

REFERENCES

[1]Auerbach, O., E. C. Hammond, and L. Garfinkel. Histologic changes in the larynx in relation to smoking habits. *Cancer* 25:92–104, 1970.

[2]Awan, S. N. Automatic estimation of vocal harmonics-to-noise ratio using cepstral analysis. In: Investigations in Clinical Phonetics and Linguistics, edited by F. Windsor, M. L. Kelly, and N. Hewlett. Mahwah, NJ: Lawrence Erlbaum Associates, 2002, pp. 449–458.

[3]Awan, S. N. The effect of smoking on the dysphonia severity index in females. *Folia Phoniatr. Logop*. 63:65–71, 2011.

[4]Awan, S. N., and D. L. Morrow. Videostroboscopic characteristics of young adult female smokers vs. nonsmokers. *J. Voice* 21:211–223, 2007.

[5]Baer, T., A. Löfqvist, and N. S. McGarr. Laryngeal vibrations: a comparison between high-speed filming and glottographic techniques. *J. Acoust. Soc. Am*. 73:1304–1308, 1983.

[6]Bielamowicz, S., R. Kapoor, J. Schwartz, and S. V. Stager. Relationship among glottal area, static supraglottic compression, and laryngeal function studies in unilateral vocal fold paresis and paralysis. *J. Voice* 18:138–145, 2004.

[7]Branski, R. C., H. Zhou, D. H. Kraus, and M. Sivasankar. The effects of cigarette smoke condensate on vocal fold transepithelial resistance and inflammatory signaling in vocal fold fibroblasts. *Laryngoscope* 121:601–605, 2011.

[8]Bridger, G. P., and P. Reay-Young. Laryngeal cancer and smoking. *Med. J. Austm*. 2:293–294, 1976.

[9]Castillo-Guerra, E., and A. Ruiz. Automatic modeling of acoustic perception of breathiness in pathological voices. *IEEE Trans. Biomed. Eng*. 56:932–940, 2009.

[10]Cattaruzza, M. S., P. Maisonneuve, and P. Boyle. Epidemiology of laryngeal cancer. *Eur. J. Cancer* 32:293–305, 1996.

[11]Centers for Disease Control and Prevention (U.S.). Vital signs: current cigarette smoking among adults aged ≥18 years—United States, 2009. *MMWR Morb. Mortal Wkly. Rep*. 59:1135–1140, 2010.

[12]Chan, R. W., M. Fu, and N. Tirunagari. Elasticity of the human false vocal fold. *Ann. Otol. Rhinol. Laryngol*. 115:370–381, 2006.

[13]Chan, R. W., M. Fu, L. Young, and N. Tirunagari. Relative contributions of collagen and elastin to elasticity of the vocal fold under tension. *Ann. Biomed. Eng*. 35:1471–1483, 2007.

[14]Chan, R. W., T. Siegmund, and K. Zhang. Biomechanics of fundamental frequency regulation: constitutive modeling of the vocal fold lamina propria. *Logop. Phoniatr. Voco*. 34:181–189, 2009.

[15]Chan, R. W., and N. Tayama. Biomechanical effects of hydration in vocal fold tissues. *Otolaryngol. Head Neck Surg*. 126:528–537, 2002.

[16]Cooper, D. S., and I. R. Titze. Generation and dissipation of heat in vocal fold tissue. *J. Speech Hear. Res*. 28:207–215, 1985.

[17]Damborenea Tajada, J., R. Fernández Liesa, E. Llorente Arenas, M. J. Naya Gálvez, P. Rueda Gormedino, C. Marín Garrido, and A. Ortiz García. The effect of tobacco consumption on acoustic voice analysis (Spanish). *Acta Otorrinolaringol. Esp*. 50:448–452, 1999.

[18]Döllinger, M., D. A. Berry, B. Hüttner, and C. Bohr. Assessment of local vocal fold deformation characteristics in an in vitro static tensile test. *J. Acoust. Soc. Am*. 130: 977–985, 2011.

[19]Failla, M., A. Grappiolo, S. Carugo, I. Calchera, C. Giannattasio, and G. Mancia. Effects of cigarette smoking on carotid and radial artery distensibility. *J. Hypertens*. 15:1659–1664, 1997.

[20]Fielding, J. E. Smoking: health effects and control. *N. Engl. J. Med*. 313:491–498, 1985.

[21]Figueiredo, D. C., P. R. F. Souza, M. I. R. Gonçalves, and N. G. Biase. Auditory perceptual, acoustic, computerized and laryngological analysis of young smokers' and non-smokers' voice. *Rev. Bras. Otorrinolaringol.* 69:791–799, 2003.

[22]Gonzalez, J., and A. Carpi. Early effects of smoking on the voice: a multidimensional study. *Med. Sci. Monit.* 10:649–656, 2004.

[23]Goodyer, E., M. Gunderson, and S. H. Dailey. Gradation of stiffness of the mucosa inferior to the vocal fold. *J. Voice* 24:359–362, 2010.

[24]Gordon, M., and P. Ladefoged. Phonation types: a cross-linguistic overview. *J. Phonetics* 29:383–406, 2001.

[25]Gorham-Rowan, M. M., and J. Laures-Gore. Acoustic-perceptual correlates of voice quality in elderly men and women. *J. Commun. Disord.* 39:171–184, 2006.

[26]Gray, S. D., I. R. Titze, F. Alipour, and T. H. Hammond. Biomechanical and histological observations of vocal fold fibrous proteins. *Ann. Otol. Rhinol. Laryngol.* 109:77–85, 2000.

[27]Guo, X., M. J. Oldham, M. T. Kleinman, R. F. Phalen, and G. S. Kassab. Effect of cigarette smoking on nitric oxide, structural, and mechanical properties of mouse arteries. *Am. J. Physiol. Heart Circ. Physiol.* 291:H2354–H2361, 2006.

[28]Haji, T., K. Mori, K. Omori, and N. Isshiki. Mechanical properties of the vocal fold Stress-strain studies. *Acta Otolaryngol. (Stockh.)* 112:559–565, 1992.

[29]Hammond, T. H., S. D. Gray, and J. E. Butler. Age- and gender-related collagen distribution in human vocal folds. *Ann. Otol. Rhinol. Laryngol.* 109:913–920, 2000.

[30]Hemler, R. J. B., G. H. Wieneke, J. Lebacq, and P. H. Dejonckere. Laryngeal mucosa elasticity and viscosity in high and low relative air humidity. *Eur. Arch. Otorhinolaryngol.* 258:125–129, 2001.

[31]Hertegård, S., and J. Gauffin. Glottal area and vibratory patterns studied with simultaneous stroboscopy, flow glottography, and electroglottography. *J. Speech Hear. Res.* 38:85–100, 1995.

[32]Hess, M. M., F. Muller, J. B. Kobler, S. M. Zeitels, and E. N. Goodyer. Measurements of vocal fold elasticity using the linear skin rheometer. *Folia Phoniatr. Logop.* 58:207–216, 2006.

[33]Hirabayashi, H., K. Koshii, K. Uno, H. Ohgaki, Y. Nakasone, T. Fujisawa, N. Shono, T. Hinohara, and K. Hirabayashi. Laryngeal epithelial changes on effects of smoking and drinking. *Auris Nasus Larynx* 17:105–114, 1990.

[34]Honjo, I., and N. Isshiki. Laryngoscopic and voice characteristics of aged persons. *Arch. Otolaryngol.* 106:149–150, 1980.

[35]Hoppe, U., F. Rosanowski, M. Döllinger, J. Lohscheller, M. Schuster, and U. Eysholdt. Glissando: laryngeal motorics and acoustics. *J. Voice* 17:370–376, 2003.

[36]Kelleher, J. E., T. Siegmund, R. W. Chan, and E. A. Henslee. Optical measurements of vocal fold tensile properties: implications for phonatory mechanics. *J. Biomech.* 44:1729–1734, 2011.

[37]Kelleher, J. E., K. Zhang, T. Siegmund, and R. W. Chan. Spatially varying properties of the vocal ligament contribute to its eigenfrequency response. *J. Mech. Behav. Biomed. Mater.* 3:600–609, 2010.

[38]Lee, L., J. C. Stemple, D. Geiger, and R. Goldwasser. Effects of environmental tobacco smoke on objective measures of voice production. *Laryngoscope* 109:1531–1534, 1999.

[39]Linville, S. E. Source characteristics of aged voice assessed from long-term average spectra. *J. Voice* 16:472–479, 2002.

[40]Lohscheller, J., U. Eysholdt, H. Toy, and M. Döllinger. Phonovibrography: mapping high-speed movies of vocal fold vibrations into 2-D diagrams for visualizing and analyzing the underlying laryngeal dynamics. *IEEE Trans. Med. Imaging* 27:300–309, 2008.

[41]Marcotullio, D., G. Magliulo, and T. Pezone. Reinke's edema and risk factors: clinical and histopathologic aspects. *Am. J. Otolaryngol.* 23:81–84, 2002.

[42]Mehta, D. D., and R. E. Hillman. Voice assessment: updates on perceptual, acoustic, aerodynamic, and endoscopic imaging methods. *Curr. Opin. Otolaryngo.* 16:211–215, 2008.

[43]Miri, A. K., F. Barthelat, and L. Mongeau. Effects of dehydration on the viscoelastic properties of vocal folds in large deformations. *J. Voice*, 2012. http://dx.doi.org/10.1016/j.jvoice.2011.09.003.

[44]Mori, K., S. M. Blaugrund, and J. D. Yu. The turbulent noise ratio: an estimation of noise power of the breathy voice using PARCOR analysis. *Laryngoscope* 104:153–158, 1994.

[45]Müller, K.-M., and B. R. Krohn. Smoking habits and their relationship to precancerous lesions of the larynx. *J. Cancer Res. Clin. Oncol.* 96:211–217, 1980.

[46]Saraf, H., K. T. Ramesh, A. M. Lennon, A. C. Merkle, and J. C. Roberts. Mechanical properties of soft human tissue under dynamic loading. *J. Biomech.* 40:1960–1967, 2007.

[47]Schuberth, S., U. Hoppe, M. Döllinger, J. Lohscheller, and U. Eysholdt. High-precision measurement of the vocal fold length and vibratory amplitudes. *Laryngoscope* 112:1043–1049, 2002.

[48]Sivasankar, M., and C. Leydon. The role of hydration in vocal fold physiology. *Curr. Opin. Otolaryngol. Head Neck Surg.* 18:171–175, 2010.

[49]Sorensen, D., and Y. Horii. Cigarette smoking and voice fundamental frequency. *J. Commun. Disord.* 15:135–144, 1982.

[50]Švec, J., and H. Schutte. Videokymography: high-speed line scanning of vocal fold vibration. *J. Voice* 10:201–205, 1996.

[51]Titze, I. R. Parameterization of the glottal area, glottal flow, and vocal fold contact area. *J. Acoust. Soc. Am.* 75:570–580, 1984.

[52]Titze, I. R. The physics of small-amplitude oscillation of the vocal folds. *J. Acoust. Soc. Am.* 83:1536–1552, 1988.

[53]Titze, I. R. Mechanical stress in phonation. *J. Voice* 8:99–105, 1994.

[54]Titze, I. R. Principles of Voice Production, 2nd ed. Denver: National Center for Voice and Speech, 320 pp, 2000.

[55]Titze, I. R. The Myoelastic Aerodynamic Theory of Phonation. Iowa City: National Center for Voice and Speech, 240 pp, 2006.

[56]van den Berg, J. Myoelastic-aerodynamic theory of voice production. *J. Speech Hear. Res.* 1:227–244, 1958.

[57]Zhang, K., T. Siegmund, and R. W. Chan. A constitutive model of the human vocal fold cover for fundamental frequency regulation. *J. Acoust. Soc. Am.* 119:1050–1062, 2006.