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Control of Vocal Fold Cover Stiffness by Laryngeal Muscles: A Preliminary Study

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Objectives: To perform preliminary measurements of the shear modulus of the vocal fold cover layer during intrinsic laryngeal muscle contraction.

Study Design: Shear modulus was measured in an in vivo canine larynx and an ex vivo human larynx.

Methods: Shear stress was applied to the transverse axis of the vocal fold using a modified linear skin rheometer (LSR) via an attached suction probe. The probe displacement in response to the applied force was measured at various levels of laryngeal muscle contraction. The force-displacement data were used to derive the shear modulus using a simple shear model. In the ex vivo human larynx, lateral cricoarytenoid (LCA) muscle and cricothyroid (CT) muscle activity was simulated with arytenoid adduction and cricothyroid approximation sutures, respectively. In the in vivo canine, adductor muscle and CT muscle contraction was induced with graded stimulation of the recurrent laryngeal nerve (RLN) and the superior laryngeal nerves (SLN), respectively.

Results: Baseline shear modulus was between 1,076 and 1,307 Pascals. In the ex vivo human larynx, the shear modulus increased gradually to a maximum of 1.6 times baseline value with graded arytenoid adduction and 3.7 times baseline value with cricothyroid approximation. In the in vivo larynx, the shear modulus increased to a maximum of 1.6 times baseline value with RLN stimulation and 2.5 times baseline value with SLN stimulation.

Conclusions: Findings are in agreement with the cover-body model in that cricothyroid muscle activity generates a greater change in cover stiffness than laryngeal adductors. The role of the individual laryngeal adductors (thyroarytenoid [TA] vs. LCA) in control of vocal fold cover stiffness remains to be further elucidated.

Key Words: Shear modulus, vocal fold, viscoelasticity, LSR, larynx.

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INTRODUCTION

The ability to control the fundamental frequency (F0) of voice is critical to human communication, expression, and singing. Hirano[1] laid the groundwork for understanding of F0 control when he introduced the "body-cover" theory of phonation. He proposed that the vocal fold can be mechanically divided into two distinct layers based upon its histology: the "body" layer consisting of the thyroarytenoid (TA) muscle and the adjacent deep collagen fibers, and the "cover" layer consisting of the superficial lamina propria and the epithelium. The body layer is the "active" layer with inherent contractile properties as it is able to shorten with neuromuscular stimulation while the cover layer is the "passive" layer whose tension is affected by the actions of the intrinsic laryngeal muscles. However, the cover layer has special elastic properties, imparted by its lamina propria layer, necessary for the propagation of mucosal waves, which is ultimately responsible for the quality of the generated sound.

The body-cover model of phonation facilitated an explanation of F0 control based on tension or stiffness of the vocal fold. In this model, cover layer stiffness primarily determines the F0, and the TA and the cricothyroid (CT) muscles control F0 by determining the stiffness of the cover layer by altering its length. Thus, contraction of the CT muscle elongates and stiffens the cover layer, increasing F0, while contraction of the TA muscle shortens the body layer by creating a slack in the cover layer, decreasing F0. This model provided antagonistic roles for TA and CT muscles, and laid the groundwork for F0 control based on variable levels of TA and CT muscle contraction. Interestingly, this model also allowed for the theoretic possibility to obtain the same F0 at various combinations of TA and CT activation levels. While other parameters such as subglottic pressure have also been demonstrated to affect F0, these other factors are considered minor compared with the activity of the TA and CT muscle.[2]

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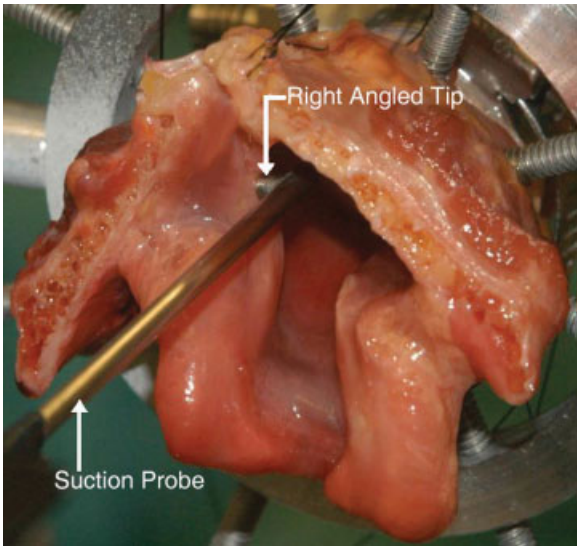

Fig. 1. Ex vivo larynx setup for measurement of shear modulus. The suction probe is attached to the left vocal fold.

It should be noted that the body-cover model was based on histologic and endoscopic observations of the larynx and not on actual measurements of cover stiffness during intrinsic laryngeal muscle contraction. However, this model was widely accepted, and mathematical models were subsequently developed by various authors to assign relative contributions of the TA and CT in F0 control, particularly in regards to explaining findings during laryngeal electromyographic studies that increased TA activity could lead to both increase *and* decrease in F0.[2–4] While computational models are based on assumptions derived from measurements of the anatomic, histologic, acoustic, aerodynamic, and biomechanical properties of the larynx and consider the cover stiffness the most important parameter controlling F0, there have been no in vivo measurements of cover stiffness during intrinsic laryngeal muscle activation. Such in vivo investigations have been hampered by lack of a reliable tensionometer to measure vocal fold stiffness. Whereas computational models of F0 control have become more complex and sophisticated, in vivo data supporting these models are lacking.

Study of vocal fold viscoelasticity has applications beyond the study of F0 control. A reliable and quantitative method of measuring vocal fold pliability is necessary to understand vocal fold changes induced by diseases such as vocal fold edema, scar, and neoplasm. A reliable methodology is also necessary to objectively assess the results of vocal fold treatments such as laryngeal reinnervation, lamina propria replacement therapy, and vocal fold replacement with tissue engineering. This study is a preliminary report on the measurement of vocal fold viscoelasticity during laryngeal muscle activation. With the ultimate goal of a systematic measurement of vocal fold cover stiffness in vivo during individual and combinations of intrinsic muscle activation, herein we report preliminary results evaluating the feasibility of reliably obtaining such measurements.

MATERIALS AND METHODS

Ex Vivo Larynx

An adult human larynx was harvested from an autopsy case less than 48 hours postmortem and kept quick-frozen at −80°C until the day before the experiment. The larynx was removed from deep freeze and allowed to thaw overnight at −4°C in the refrigerator, then kept soaked in isotonic saline in the morning of the experiment until it was thawed soft. The supraglottic structures, including the epiglottis and the false vocal cords, were excised. Arytenoid adduction sutures (3-0 nylon) were then placed through the left muscular process and brought out through the anterior inferior thyroid lamina to adduct the vocal fold, thus simulating lateral cricoarytenoid (LCA) muscle contraction. A 3-0 nylon suture was placed circumferentially through the anterior cricoid and the anterior inferior border of the thyroid cartilages for manual cricothyroid approximation, thus simulating CT muscle contraction. The larynx was then mounted horizontally on a custom-designed laryngeal holder for experimental measurements (Fig. 1). Increasing weights in 10-gram increments were placed on the adduction sutures using a pulley mechanism to simulate increasing vocal fold adduction. The cricothyroid approximation suture was manually tightened to simulate CT muscle action and the shear modulus measured at baseline (no suture tension), medium (CT approximation midway between baseline and maximum), and maximum tension (maximum CT approximation possible with tightening of the CT approximation sutures). The larynx was periodically sprayed with saline to keep the surface moist.

In Vivo Canine Model

A mongrel canine (25 kg) was used. The animal study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act. Our institutional Animal Research Committee approved the research protocol. After anesthesia was induced with intravenous thiopental, the animal was orally intubated and placed under halothane general anesthesia.

A vertical midline skin incision was then made on the anterior neck to widely expose the larynx and the trachea. Bilateral recurrent laryngeal nerves (RLNs) and superior laryngeal nerves (SLNs) were isolated. A low tracheotomy was performed for intra-operative ventilation, and the oral endotracheal tube was removed. The larynx was exteriorized into the neck by first performing a suprahyoid pharyngotomy and then

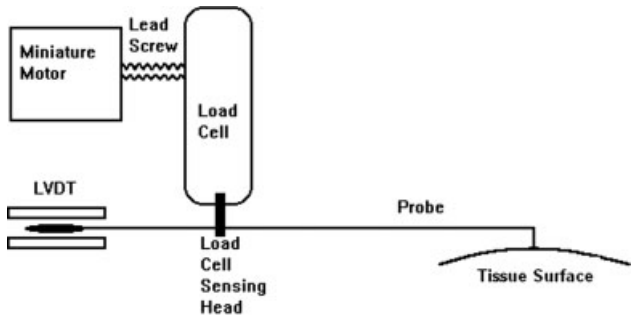

Fig. 2. Schematic of the linear skin rheometer (LSR) for measurement of vocal fold shear modulus. (LVDT = linear voltage differential transformer, a device that measures small linear displacements.)

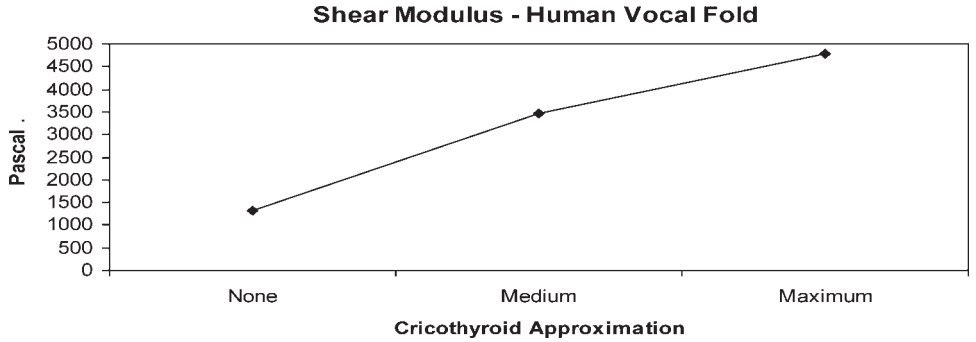

Fig. 3. Shear modulus of the vocal fold (ex vivo human larynx) with manual cricothyroid approximation.

by dividing the pharynx circumferentially at this level. This allowed the larynx to be slightly lifted off the neck and fixed in placed using a custom-designed laryngeal holder. This exposure allowed placement of the LSR probe on the vocal fold externally and unhindered by oral and pharyngeal structures. Custom-designed monopolar electrodes with silicone insulation were applied to the isolated nerves bilaterally. The electrodes were attached to a constant current nerve stimulator (WR Medical Electronics Co., Model 2SLH, St. Paul, MN). The nerves were stimulated at 80 Hz, 1.5 msec pulses, at approximately 0.06 mA increments. Although stimulation of RLN causes contraction of both adductors and abductor, the overall effect is vocal fold adduction.

The Linear Skin Rheometer

Measurements of the vocal fold shear modulus were obtained using a modified linear skin rheometer (LSR).[5] This device was originally developed to measure the biomechanical properties of the stratum corneum of skin and was identified[6] as a potential method to quantify the viscoelastic properties of the human vocal fold and to quantify the effectiveness of tissue augmentation therapy. This device has been used to measure vocal fold viscoelasticity in a variety of reports,[7–13] and the device has also been successfully adapted to measure vocal fold viscoelasticity in human in vivo.[14,15]

The LSR (Fig. 2) is a programmable tensionometer capable of measuring displacement to a resolution of 4 μm using a linear variable displacement transducer (LVDT) and force to a resolution of 20 mg using a built-to-order force sensor (load cell) with a full-scale reading of 50 gm. The force sensor can be attached to the tissue under test using a variety of special probes. For this study, a 2-mm-diameter suction cannula probe with a right-angle tip was used, and the left vocal fold was selected for measurements. The LSR was positioned so that the probe was perpendicular to the longitudinal (anterior-posterior) axis of the vocal fold, and the right-angled tip of the probe was aligned with the medial vocal fold surface at the mid-membranous vocal fold level such that there was no gap between the tip of the suction probe and the vocal fold epithelium. Fifty millibars of suction was then applied to attach the probe tip to the vocal fold surface. The active suction force was then released, but the tip remained attached due to the vacuum force created within the suction cannula (Fig. 1). While some stress is applied to the vocal fold during this maneuver, the effect is minimal and previous experimentation with various probe designs suggested that the suction probe was the most reliable at maintaining position during vocal fold stimulation.[10] Also, the LSR is electronically zeroed after the vacuum is released and also just prior to application of the shear force. The LSR then applies a sinusoidal shear force of 1 gram on the transverse direction of the medial vocal fold. In other words, the applied force mimics a sine wave as it is increased from 0 to 1 gram to 0 as the probe is displaced inferiorly on the medial vocal fold surface, then force is applied similarly in the reverse direction and the probe is displaced in the superior direction. Five measurements were performed for each experimental parameter.

Calculation of the Shear Modulus

The LSR applies a known amount of force (1 gram in this study) and measures the displacement achieved, and this force/displacement data is used to derive the "dynamic spring rate" (DSR). In mechanical engineering, the term DSR defines the amount that a spring changes in length when a unit of force is applied to it. It is not a time-dependant term. By applying knowledge of the probe geometry, it is possible to estimate the stiffness (shear modulus) of the vocal fold. Using a simple shear model (modulus [G] = stress/strain), the geometry of this setup was defined as follows. The shear stress is the shear force (F) applied by the LSR transmitted to the vocal fold cover over the

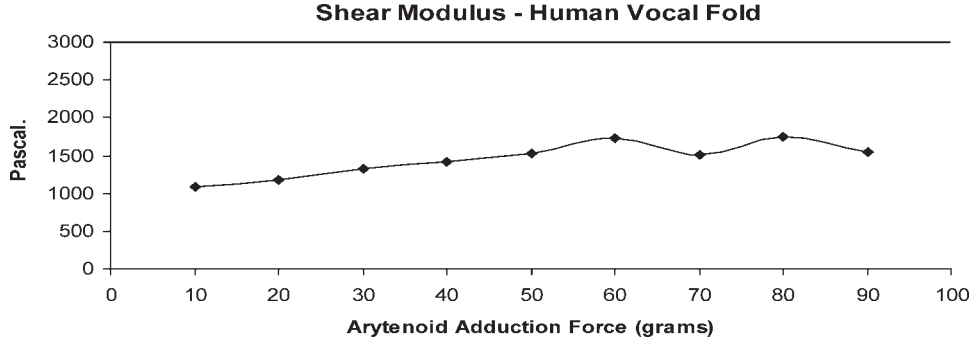

Fig. 4. Shear modulus of the vocal fold (ex vivo human larynx) with graded increase in the force of arytenoid adduction.

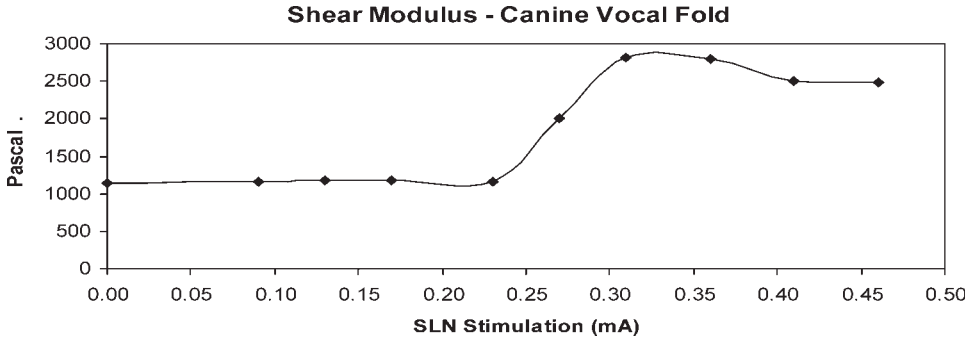

Fig. 5. Shear modulus of the vocal fold (in vivo canine larynx) with graded bilateral superior laryngeal nerve (SLN) stimulation.

area determined by the probe diameter (A). The shear strain is the resultant displacement (X), which is tangential to the epithelial surface and is measured by the LSR, over the thickness of the lamina propria layer (H). The lamina propria layer thickness (H) was assigned a value of 1 mm for the human larynx and 2 mm for the canine larynx. Thus, an estimate for the shear modulus (G) is derived as further elaborated in the appendix.

RESULTS

In the ex vivo human larynx, the shear modulus increased from 1,307 Pascals at rest to 4,786 Pascals (3.7 times baseline value) at maximal CT approximation (Fig. 3). With gradual increase in the force of arytenoid adduction, the shear modulus gradually increased from 1,076 Pascals at rest to a maximum of 1,723 Pascals (1.6 times baseline value) at an adduction force of 60 grams, and thereafter remained relatively unchanged with increasing adductory force (Fig. 4).

In the in vivo canine larynx, with graded neuromuscular stimulation of bilateral SLNs, the vocal fold shear modulus remained stable around the baseline stiffness of 1,134 Pascals at zero stimulation until the stimulation level reached 0.23 mA, at which point a hint of cricothyroid activity was noted. Cricothyroid approximation commenced with further stimulation, and the shear modulus increased to a maximum of 2,818 Pascals (2.5 times baseline value) at a stimulation level of 0.31 mA, and thereafter stiffness remained stable or slightly decreased with further stimulation (Fig. 5). Similarly, with graded neuromuscular stimulation applied to the RLN, the shear modulus remained stable around the baseline value of 1,077 Pascals at zero stimulation until the stimulation level reached 0.21 mA, at which point mild twitching of the vocal fold was observed. Vocal fold adduction commenced with further stimulation, and the shear modulus increased to a maximum of 1,762 Pascals (1.6 times baseline value) at 0.32 mA, and thereafter remained stable or slightly decreased with further stimulation (Fig. 6).

DISCUSSION

The body-cover theory assigns the CT muscles the primary role and the TA muscles the secondary role in F0 control.[3] The results of this study are consistent with this theory in that contraction of CT muscles leads to a greater increase in stiffness than the adductory laryngeal muscles and therefore the range of F0 control by CT muscles would be expected to be greater. SLN stimulation in the canine larynx achieved a two-and-a-half-fold increase in the shear modulus at maximal stimulation compared with baseline stiffness. CT approximation applied manually in the human larynx achieved an almost four-fold increase in shear modulus compared with baseline stiffness. The greater increase in stiffness achieved with the manual technique can be explained by the fact that with manual approximation it is possible to apply enough force to nearly completely appose the cricoid and the thyroid cartilages, whereas this cannot occur physiologically in vivo.

An unexpected finding was that simulated LCA activation by arytenoid adduction ex vivo resulted in a stiffness change that was similar in degree to that

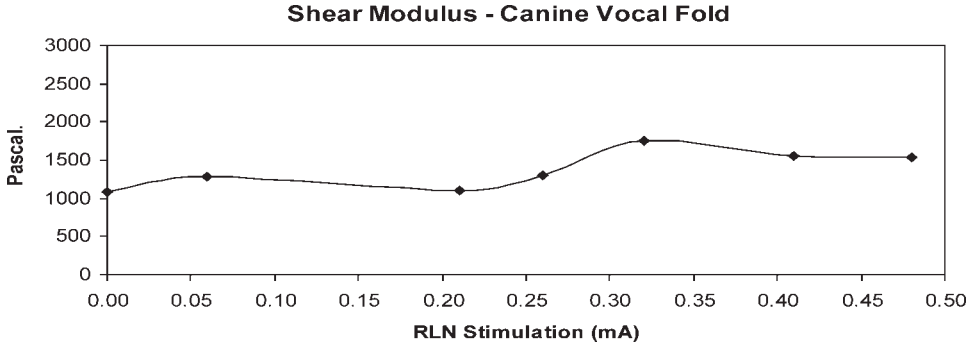

Fig. 6. Shear modulus of the vocal fold (in vivo canine larynx) with graded ipsilateral recurrent laryngeal nerve (RLN) stimulation.

achieved in vivo with RLN stimulation. An increase of 1.6 times baseline value of stiffness was seen in both instances. The stiffness change upon RLN stimulation would be attributable to activation of both LCA and TA muscles. Therefore, further in vivo studies testing these muscles individually are needed to assess the stiffness changes attributable to each muscle. Current theories of F0 control have not considered a role for the LCA muscle in control of cover stiffness. The ex vivo result appears physiologically consistent because during LCA contraction (simulated arytenoid adduction) the vocal process rotates medially and posteriorly thus adducting and lengthening the vocal fold, which would account for the increase in shear modulus. Once the limits of the cricoarytenoid joint rotation are reached, no further vocal fold lengthening can take place despite further arytenoid adduction force, and therefore additional increase in the shear modulus was not seen beyond 60 grams adductory force.

Arytenoid adduction and laryngeal reinnervation are established treatment modalities for unilateral vocal fold paralysis. The ex vivo results suggest that the generally excellent voice outcome after arytenoid adduction surgery for vocal fold paralysis may occur not only through improved closure but better stiffness match with the normal vocal fold as well.[16] However, patients who undergo laryngeal reinnervation in addition to arytenoid adduction for unilateral vocal fold paralysis also report an additional improvement in vocal quality three to six months after surgery, presumably when the laryngeal reinnervation kicks in.[16] Therefore, increase in body (TA) stiffness also appears to play an important role in modulating voice quality. While it would be reasonable to assume that both TA and LCA are contributing to cover stiffness, the nature of their individual contributions are unknown.

Previous in vivo canine studies in our laboratory showed that at high levels of CT stimulation, increasing TA activity *decreased* F0, presumably by slackening the cover layer, which is consistent with the classic cover-body theory.[17] However, in the absence of CT activity and while glottic closure was maintained by isolated stimulation of the LCA muscles, increasing TA activity resulted in gradual *increase* in F0. TA muscles may affect F0 in the latter setting not only via changes in the stiffness of the cover layer but also by improving closure, facilitating entrainment of the vocal folds, and controlling the effective depth of vibration as proposed by Titze.[3] Therefore, further in vivo studies are also needed to examine the mechanisms of F0 control by the TA muscle. Studies are also needed to evaluate and correlate the acoustic and aerodynamic effects of intrinsic laryngeal muscle activity with cover stiffness, and experimental strategies to measure the effective depth of vibration need to be developed.

CONCLUSIONS

Both RLN and SLN stimulation lead to increased stiffness of the vocal fold. However, a more dramatic change in stiffness is seen with SLN stimulation. Therefore, CT appears to play a greater role in control of F0 range. The role of the individual laryngeal adductors in changing cover stiffness needs further study, as both TA and LCA muscles appear to contribute to cover stiffness.

APPENDIX

Mathematical Model for Derivation of Shear Modulus Using the Linear Skin Rheometer (LSR)

A sinusoidal force (F) is applied to the material under test and the resultant displacement (X) is logged.

$$F = F_{max}\mathrm{Sin}(t) \tag{1}$$

$$X = X_{max}\mathrm{Sin}(t + \tau) \tag{2}$$

Where

F = instantaneous force;

F_{max} = the maximum force;

t = time;

X = instantaneous displacement;

X_{max} = the maximum displacement;

τ = the phase shift in radians.

The dynamic spring rate (DSR) of tissue is a measure of its elasticity and is the ratio of the peak force to peak displacement (F max / X max) when a sinusoidally varying force is applied to the material, causing it to deform, and is expressed in g/mm. As we are not using the time-dependant information associated with the sinusoidal nature of the applied force, we can substitute F_{max} for F and X_{max} for X. DSR can then be used to estimate the shear modulus of the displaced vocal fold tissue using knowledge of the geometry of the vocal fold, as follows:

The stress σ is the applied force F_{max} per unit area A given by

$$\sigma = F_{max}/A \tag{3}$$

The resultant strain ϵ is given by tangential displacement X_{max} per material thickness H.

$$\varepsilon = X_{max}/H \tag{4}$$

Shear modulus G is defined as stress per unit strain

$$G = \sigma/\varepsilon \tag{5}$$

$$G = (F_{max}/G_{max})^{*}\ (H/A) \tag{6}$$

As DSR = F_{max} / X_{max} then

$$G = DSR^{*}\ H/A \tag{7}$$

It is important to note that this simple shear model does not make any allowance for the attached tissue,

which is also subjected to shear stresses due to displacement of the tissue directly underneath and surrounding the suction attachment. This effect drops off rapidly as the force transmitted through a solid is inversely related to distance. However, a rigorous mathematical solution to describe the elastic processes involved has not been published. In the absence of a mathematical solution for the shear modulus of tissue attached to other tissue, we incorporated a simple correction derived experimentally based on a widely accepted mathematical model developed by W. C. Hayes.[18] This model derives shear modulus from indentation data. This correction methodology has been evaluated using data collected from 40 human hemilarynges,[9] which were tested using both the Hayes indentation method and the LSR. The data sets from the two methods correlated well when the surface area of attachment used to analyse the LSR data was increased by 0.75 mm in all dimensions. Based on these results, we employed a comparable correction to the data in this study by increasing the diameter of the area of attachment (A) from 2 mm by 1.5 mm to 3.5 mm.

BIBLIOGRAPHY

1. Hirano M. Morphological structure of the vocal cord as a vibrator and its variations. *Folia Phoniatr (Basel)* 1974; 26:89–94.
2. Fujimura O. Body-cover theory of the vocal fold and its phonetic implications. In: Stevens KN, Hirano M, eds. *Vocal Fold Physiology*. Tokyo: University of Tokyo Press; 1981: 271–288.
3. Titze IR, Jiang J, Drucker DG. Preliminaries to the body-cover theory of pitch control. *J Voice* 1988;1:314–319.
4. Lowell SY, Story BH. Simulated effects of cricothyroid and thyroarytenoid muscle activation on adult-male vocal fold vibration. *J Acoust Soc Am* 2006;120:386–397.
5. Matts PJ, Goodyer EN. A new instrument to measure the mechanical properties of the human stratum corneum. *J Cosmet Sci* 1988;49:321–323.
6. Goodyer EN, Gunter H, Masaki A, Kobler J. Mapping the visco-elastic properties of the vocal fold. 6th International Conference in Advances in Quantitative Laryngology, April 3–4, 2003, Hamburg, Germany.
7. Hertegård S, Dahlqvist A, Goodyer E. Viscoelastic measurements after vocal fold scarring in rabbits—short-term results after hyaluronan injection. *Acta Otolaryngol* 2006;126:758–763.
8. Hess M, Muller F, Kobler J, Zeitels S, Goodyer EN. Measurements of vocal fold elasticity using the linear skin rheometer. *Folia Phoniatr Logop* 58:207–216.
9. Goodyer EN, Hemmerich S, Mueller F, Licht K, Hess M. Characterization of the elasticity of the human vocal fold using electromechanical measurement techniques. In: 7th International Conference Advances in Quantitative Laryngology, Voice and Speech Research, Gronningen, October 2006.
10. Goodyer E, Welham NV, Choi S, Yamashita M, Dailey SH. The shear modulus of the human vocal fold in a transverse direction. *J Voice* 2008, Jan 19. doi:10.1016/j.voice.2007.09.006.
11. Licht K, Goodyer EN, Müller F, Kobler JB, Hess M. The anisotropic nature of the vocal fold. Pan European Voice Conference, Groningen, August 2007.
12. Goodyer EN, Hemmerich S, Müller F, Kobler JB, Hess M. The shear modulus of the human vocal fold, preliminary results from 20 larynxes. *Eur Arch Otorhinolaryngol* 2007;264:45–50.
13. Dailey SH, Tateya I, Montequin D, Welham N, Goodyer E. Viscoelastic measurements of vocal folds using the linear skin rheometer. *J Voice* 2007, May 5. doi:10.1016/j.voice.2007.01.002.
14. Goodyer EN, Muller F, Bramer B, Chauhan D, Hess M. In vivo measurement of the elastic properties of the human vocal fold. *Eur Arch Otorhinolaryngol* 2006;263: 445–462.
15. Goodyer EN, Müller F, Licht K, Hess M. In Vivo measurement of the shear modulus of the human vocal fold - interim results from 8 patients. *Eur Arch Otorhinolaryngol* 2007;264(6):631–635.
16. Chhetri DK, Gerratt BR, Kreiman J, Berke GS. Combined arytenoid adduction and laryngeal reinnervation in the treatment of vocal fold paralysis. *Laryngoscope* 1999;109: 1928–1936.
17. Kurita S, Kazuto N, Hirano M. A comparative study of the layer structure of the vocal fold. In: Bless DM, Abbs JH, eds. *Vocal Fold Physiology: Contemporary Research and Clinical Issues*. San Diego, CA: College Hill Press; 1981: 3–21.
18. Hayes WC, Keer LM, Herrmann G, Mockros LF. A mathematical analysis for indentation tests of articular cartilage. *J Biomech* 1972;5:541–551.