CONTROL OF VOCAL FOLD COVER STIFFNESS BY LARYNGEAL MUSCLES

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ABSTRACT

Objectives: The objective of this study is 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 ex vivo human larynx and an in vivo canine larynx.

Methods: A modified linear skin rheometer adapted for laryngeal viscoelasticity measurement applied shear stress to the mid-membranous vocal fold medial surface via an attached suction probe. The measured probe displacement achieved at each level of laryngeal muscle contraction was 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 gradual tension of arytenoid adduction sutures and manual cricothyroid approximation, respectively. In the in vivo canine, graded current applied to the recurrent laryngeal nerves (RLN) the superior laryngeal nerves (SLN), respectively.

Results: Baseline shear modulus was calculated between 1076 to 1307 pascals. In the ex vivo human larynx, the shear modulus increased gradually to maximum of 1.6 times baseline value with graded arytenoid adduction and maximum of 3.7 times baseline value with manual 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: While both RLN and SLN stimulation increase cover stiffness, cricothyroid muscle activity results in the most dramatic increase.
INTRODUCTION

The ability to control the fundamental frequency (F0) of voice is critical to human communication, expression, and singing. Hirano (1974) laid the groundwork for understanding of F0 control when he introduced the “body-cover” theory of phonation. He proposed that the histology of the vocal fold lends itself to division into two distinct layers: 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 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. The cover layer has elastic properties necessary for the propagation of mucosal waves that 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 is primarily responsible for F0 control and the TA and the cricothyroid (CT) muscles change the stiffness of the cover layer by altering its length. Contraction of the CT muscle elongates and stiffens the cover layer, thus increasing F0, while activation of the TA muscle shortens the body layer while concurrently creating a slack in the cover layer, thus decreasing F0. This model provides 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 also allows for the theoretic possibility to obtain the same F0 at various combinations of TA and CT activation level. While other parameters such as subglottic pressure also affect F0, these
other factors are considered minor compared to the activity of the TA and CT muscle (Titze 1989).

The body-cover model has been further expanded upon using mathematical models where relative contributions of the TA and CT in F0 control have been assigned (Fujimura (1981), Titze (1988), and Lowell (2006)). Whereas computational models of F0 control have become more complex and sophisticated, in vivo data supporting these models is severely lacking. Current computational models are based on basic assumptions derived from measurements of the anatomic, histologic, acoustic, aerodynamic and biomechanical properties of the larynx, and consider the overall stiffness of the cover layer the most important factor in controlling F0. However, there is a paucity of in vivo measurements of vocal fold stiffness and there are no in vivo measurements of stiffness with concurrent laryngeal muscle activation. Such in vivo investigations have been hampered by lack of a reliable tensionometer to measure stiffness.

Study of vocal fold viscoelasticity has applications beyond the study of F0 control. A reliable 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 tissue engineering. This study is a preliminary report on the measurement of vocal fold viscoelasticity with laryngeal muscle activation. While the ultimate goal is a systematic and detailed measurements of in vivo vocal fold stiffness with isolated as well as combinations of TA and CT muscle activation levels, this study is a preliminary step testing the feasibility of
and obtaining reliable measurements using the LSR with various levels of intrinsic laryngeal muscle activation.

MATERIALS AND METHODS

Ex vivo larynx: An adult human larynx was harvested from an autopsy case less than 48 hours post-mortem and kept quick-frozen at -80°C until the day before the experiment. The larynx was then 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 processes 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 (Figure 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 tightened to simulate CT muscle action and the shear modulus measured at baseline (no suture tension), medium (CT approximation midway between baseline and maximal), and maximum tension (maximun 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 suprathyoid pharyngotomy and division of the pharynx circumferentially at this level. The larynx was 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, Minnesota). The nerves were stimulated at 80 Hz, 1.5 msec pulses, at approximately 0.06 mA increments.

The Linear Skin Rheomertor: Measurements of the vocal fold shear modulus were obtained using a modified Linear Skin Rheometer (LSR) [Matts 1998]. This device was originally developed to measure the biomechanical properties of the stratum corneum of skin, and was identified [Goodyer 2003] 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 (Goodyer 2007-3, 2006-2, Hess 2006, Licht 2007, Hertegaard 2006, 2004, Dailey 2007, Goodyer 2007-1) and the device concept has also been successfully adapted to measure human vocal folds in-vivo (Goodyer 2007-2, 2006-1).

The LSR (Figure 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 with a full-scale reading of 50 g. The force sensor can be attached to the tissue under test using a variety of special probes. For this study, a suction cannula probe with a right angle tip and 2 mm diameter was used and the left vocal fold was selected for measurement. The LSR with the probe was aligned at right angles to the longitudinal axis of the vocal fold such that there was no gap between the tip of the suction probe and the superior medial vocal fold epithelium, at which time 50mbar of suction was applied. The suction force was then released and the instrument zeroed prior to measurement. While some stress is applied to the vocal fold during this maneuver, this arrangement minimizes the effect and also maintains the probe in position. We found from experimentation with various probe designs that the suction probe was the most reliable at maintaining position after vocal fold stimulation. Also the LSR operates by applying a cyclical force and any DC offset due to initial loading is removed. The force sensor and suction attachment are gently cycled in a sinusoidal manner such that a cyclical shear force of one gram is applied to the vocal fold.

**Calculation of the Shear Modulus:** The LSR applies a known amount of force (one 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, or shear modulus, of the vocal fold. Using a simple shear model the geometry of this setup was defined as follows. The shear force (F) applied by the LSR is transmitted to the vocal fold cover over the area determined by the probe diameter (A). The displacement (P) is the resultant shear strain, which is tangential to the epithelial surface, and is measured by the LSR. The thickness of the lamina propria layer (H) is about 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, with manual cricothyroid approximation, the shear modulus increased from a baseline value (1307 Pa) to 3.7 times baseline value (4786 Pa) at maximal CT approximation (Figure 3). With gradual increase in the force of arytenoid adduction with graded increase in weights, the vocal fold shear modulus gradually and linearly increased from a baseline value (1076 Pa) to a maximum of 1.6 times baseline value (1723 Pa) at an adduction force of 60 grams, and thereafter remained relatively unchanged with increasing weight (Figure 4).

In the *in vivo* canine larynx, with graded neuromuscular stimulation of bilateral SLNs, the vocal fold shear modulus remained stable around the baseline values (1134 Pa) until stimulation level reached 0.23 mA. At 0.27 mA a hint of cricothyroid activity was noted. Shear modulus increased from 0.23 mA to 0.31 mA to a maximum of 2.5 times baseline value (2818 Pa), and thereafter remained stable or slightly decreased with further stimulation.
When graded neuromuscular stimulation was applied to the RLN, the vocal fold shear modulus remained stable around the baseline value (1077 Pa) until stimulation level 0.21 mA. At this point the onset of visible vocal fold motion was also appreciated. With further stimulation shear modulus increased to a maximum of 1.6 times baseline (1762 Pa) at 0.32 mA, and thereafter remained stable or slightly decreased with further increasing stimulation (Figure 6).

**DISCUSSION**

The results of this study indicate that while both RLN and SLN stimulation increase cover stiffness, cricothyroid muscle activity results in the most dramatic increase. In the human ex vivo larynx, CT approximation was applied manually and an almost four fold increase in shear modulus was achieved between baseline and maximal CT approximation. In the canine larynx, in vivo SLN stimulation could achieve only a 2.5 fold increase in the shear modulus despite maximal stimulation. The canine response is more physiologic because whereas with manual approximation it is physically possible to nearly completely appose the cricoid and the thyroid cartilages anteriorly this is not possible physiologically. The cricothyroid muscles insert at the edges of the thyroid and the cricoid cartilages and during muscular contraction the CT muscle will shorten but not to the degree that the cricoid and the thyroid cartilages would approximate completely.

Control of vocal fold stiffness via RLN stimulation appears more complex. Adductory muscles innervated by the RLN include the interarytenoid (IA), lateral cricoarytenoid (LCA), and the TA muscles. In the ex vivo human larynx, gradual increase in the force of arytenoid adduction lead to gradual increase in shear modulus to a maximum
increase of 1.6 times baseline value. This result is logical because during 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. However, no further lengthening can take place once the limits of cricoarytenoid joint rotation is reached and further force of adduction does not lead to additional increase in the shear modulus. Interestingly, the increase in shear modulus with RLN stimulation also reached a maximal value 1.6 times baseline values. The \textit{ex vivo} experiment would suggest that it would be possible to reach this increase in the shear modulus with arytenoid adduction (LCA action) alone. This could explain the excellent response to arytenoid adduction surgery for unilateral vocal fold paralysis (ref Chhetri). However, patients who undergo combined adduction and laryngeal reinnervation surgery for unilateral vocal fold paralysis do report an additional improvement three to six months after surgery, presumably when the laryngeal reinnervation kicks in. Therefore, what role does the TA play in modulating the cover stiffness? In a previous study from our laboratory, a gradual increase in F0 was seen with graded stimulation of the TA muscle in the present of constant LCA stimulation that maintained posterior glottic closure (Choi et al). While it seems reasonable to assume that both TA and LCA are contributing to cover stiffness their individual contributions are unknown. It is also entirely possible that the medial bulging of the vocal fold during TA activity affects phonation via mechanisms separate from viscoelastic changes of the cover layer, such as improving closure. Future studies measuring cover stiffness with isolated stimulation of adductor branches could delineate the role of each muscle.

The baseline shear modulus is similar in human and the canine larynx. The canine larynx is the closest match to the human larynx, both in its overall dimensions as well as
histopathologic characteristics (ref). The canine F0 (low 200 Hz range) is also similar to human larynx. Therefore, perhaps the similarity in the shear modulus between these larynges result from their overall common characteristics. It is possible that animals with higher F0 would have higher shear modulus.

**CONCLUSION**

Both RLN and SLN stimulation lead to increased viscoelasticity of the vocal fold. However, a more dramatic change in stiffness is seen with CT stimulation. Therefore, CT likely plays a more important role in F0 control.
REFERENCES

[Dailey 2007]

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[Licht 2007]  

[Matts 1998]  
Appendix

Derivation of Shear Modulus

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

1. $F = F_{\text{max}} \sin(t)$
2. $X = X_{\text{max}} \sin(t + \tau)$

Where
- $F =$ instantaneous force
- $F_{\text{max}} =$ the maximum force
- $t =$ time
- $X =$ instantaneous displacement
- $X_{\text{max}} =$ the maximum displacement
- $\tau =$ the phase shift in radians.

The DSR of the tissue is defined as $F_{\text{max}} / X_{\text{max}}$, 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 $F$ for $F_{\text{max}}$ and $X$ for $X_{\text{max}}$. DSR can then be used to estimate the shear modulus of the displaced vocal fold tissue using knowledge of the geometry of the test site, as follows:

The stress $\sigma$ is the applied force $F_{\text{max}}$ per unit area $A$ given by

2. $\sigma = F_{\text{max}} / A$

The resultant strain $\varepsilon$ is given by tangential displacement $X_{\text{max}}$ per material thickness $H$.

3. $\varepsilon = X_{\text{max}} / H$

Shear modulus $G$ is defined as stress per unit strain

4. $G = \sigma / \varepsilon$
5. $G = (F_{\text{max}} / X_{\text{max}}) \cdot (H / A)$

As DSR $= F_{\text{max}} / X_{\text{max}}$ then

6. $G = \text{DSR} \cdot H / A$

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. This model derives shear modulus from indentation data [Hayes 1972]. We first evaluated this correction methodology using data collected from 40 human hemilarynges at UKE [Goodyer 2006-2], 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 from 2 mm by 1.5 mm to 3.5 mm.
Figure 3

Shear Modulus - Human Vocal Fold

Cricothyroid Approximation

Figure 4

Shear Modulus - Human Vocal Fold

Arytenoid Adduction Force (grams)