Summary: Objective. This study mapped the variation in tissue elasticity of the subglottic mucosa, applied these data to provide initial models of the likely deformation of the mucosa during the myoelastic cycle, and hypothesized as to the impact on the process of phonation.

Study Design. Six donor human larynges were dissected along the sagittal plane to expose the vocal folds and subglottic mucosa. A linear skin rheometer was used to apply a controlled shear force, and the resultant displacement was measured. These data provided a measure of the stress/strain characteristics of the tissue at each anatomic point. A series of measurements were taken at 2-mm interval inferior of the vocal folds, and the change in elasticity was determined.

Results. It was found that the elasticity of the mucosa in the subglottic region increased linearly with distance from the vocal folds in all 12 samples. A simple deformation model indicated that under low pressure conditions the subglottic mucosa will deform to form a cone, which could result in a higher velocity, thus amplifying the low pressure effect resulting from the Venturi principle, and could assist in maintaining laminar flow.

Conclusions. This study indicated that the deformation of the subglottic mucosa could play a significant role in the delivery of a low pressure airflow over the vocal folds. A large scale study will now be undertaken to secure more data to evaluate this hypothesis, and using computational fluid dynamics based on actual three-dimensional structure obtained from computed tomography scans the aerodynamics of this region will be investigated.

Key Words: Vocal folds–Phonation–Biomechanics–Tissue deformation–Aerodynamics.

INTRODUCTION

The principles underlying the ability to phonate are now well established. The purpose of this study was to focus on the tissue structures in the subglottic region that are less well studied. Previous studies identified that the stiffness of subglottic mucosa in pig and canine larynges increased with distance inferior from the vocal folds. The objective of this study was to determine if human tissue exhibited the same variation. To achieve this, the team deployed a linear skin rheometer (LSR), as used in comparable studies by other teams who were examining the elastic properties of vocal fold tissue.

Our reason for examining this region is that although the subglottic region is rarely studied due to the difficulty in visualization, some studies have indicated that it may play a role in phonation, and that scarring in this region could affect the quality of voice. Sundberg identified that there are mechanoreceptors in this region that are used to sense the transfer of energy from the subglottal airflow into the vocal folds in advance of the mucosal wave. Smith examined the change in pitch in 14 female patients (mean age: 53) following a cricotracheal resection. In all cases, the mean fundamental frequency fell significantly, typically by 21 Hz. Both studies indicate that the subglottal region does play a role in phonation, and this study was devised to gain a better understanding of how that region functions during phonation, and to see if the biomechanics could explain these reported impacts.

Having obtained the results, which indicated that the mucosa does exhibit a gradation in stiffness, the manner in which this structure will deform during the low pressure phase of the myoelastic cycle was modeled using a simple deformation model. The impact of the deformed structure is considered with respect to how it would affect the aerodynamics of the airflow in this region. This simplistic analysis indicated that the end result is that the flow rate would be increased, possibly magnifying the extent of vocal fold closure, and could assist laminar flow.

MATERIALS AND METHODS

Six excised larynges were used for this study, three male and three female. All were obtained in accordance with approved ethical principles. They were obtained from mortuary cases, and then deep-frozen before gradual thawing and use; all were stored for many months before use. The larynges were hemisected to reveal the vocal folds and subglottic mucosa, providing the study with 12 sample hemi-larynges. These samples were mounted on a three-axis micrometer-controlled table. An LSR adapted for use with soft tissues was deployed for this study. The LSR has been deployed in a range of previous studies that examined the variation in vocal fold biomechanics with respect to anatomic position and direction of applied stress. The current experimental setup has been enhanced as a result of the experiences gained from deploying the LSR in these earlier studies, as shown in Figure 1. A glass cannula with a 90° bend was used to apply a tangential force to the epithelium, and was attached using a suction pressure of 50 mbar. The LSR delivered a controlled cyclical force of ±1 g to the attachment point and the displacement was logged. From these data, a regression algorithm provides a best fit to the pair of sine waves, which provide a measure of the stress/strain characteristics in terms of grams force per millimeter displacement. Although it is possible to convert these..
readings into estimates of shear modulus using established methods based on the geometry of the experimental setup, we are only interested in the change in elastic properties. Therefore, the results are presented in g/mm unit, and the graphical representations are normalized with respect to the stiffness of the vocal folds in order to present the change of stiffness of the mucosa with respect to vocal fold stiffness.

Ten readings were taken at each sample point and were averaged to obtain a series of point-specific readings with respect to anatomic context, being the distance inferior from the vocal folds.

The calculations carried out made use of the following established formulae.

The change in pressure arising from laminar flow through a constriction is given in Equation 1. This was used to calculate the drop in air pressure in the region near the vocal folds.

$$\Delta P = \rho / (V_1^2 - V_2^2)$$  \hspace{1cm} (1)

where $\Delta P$ is the change in pressure within the constricted flow region; $\rho$ is the density of air; $V_1$ is the airflow velocity before the constriction; and $V_2$ is the airflow velocity within the constriction.

Young modulus for linear extension is given in Equation 2 and was used to model the mucosal deformation.

$$l = F / (L \times A \times Y)$$  \hspace{1cm} (2)

where $l$ is linear extension; $F$ is the uplift force; $L$ is the initial length of the material; $A$ is the surface over which the uplift force is applied; and $Y$ is Young modulus.

To develop the next stage of the study, which is modeling the aerodynamics of this region, a computed tomography (CT) scan of a human larynx was converted to provide a three-dimensional (3D) model based on the actual anatomic structure for use with a computational flow dynamics (CFD) program. CT images of the excised larynges have been used to create 3D reconstructions with the help of an open-source medical imaging software, 3D Slicer (http://www.sci.utah.edu/cibc-software/seg3d.html; The Center for Integrative Biomedical Computing, University of Utah). This model will be used to determine the changes in the aerodynamics that arise when the subglottic mucosa is deformed under low pressure in the next stage of this research program.

**RESULTS**

Table 1 shows the raw results. The keys for the donor column are the following: M for male, F for female, age of donor, L for left, and R for right side. The first column is the measurement

| Donor Male (M) or Female (F), Age, Left (L) or Right (R) | Distance Inferior From the Vocal Folds | Quality Indices |
|--------------------------------------------------------|---------------------------------------|-----------------|
|                                                        | 2 mm | 4 mm | 6 mm | 8 mm | 10 mm | 12 mm | Coefficient of Variance (%) | Correlation Coefficient Left Versus Right |
| F, 63, L                                                | 0.44 | 0.52 | 0.53 | 0.63 | 0.59 | 0.65 | 0.72 | 14 | 0.86 |
| F, 63, R                                                | 0.36 | 0.42 | 0.45 | 0.51 | 0.56 | 0.65 | 0.55 | 15 | 0.96 |
| F, 74, L                                                | 0.44 | 0.52 | 0.49 | 0.53 | 0.53 | 0.62 | 0.64 | 9  | 0.97 |
| F, 74, R                                                | 0.33 | 0.42 | 0.46 | 0.48 | 0.49 | 0.58 | 0.63 | 6  | 0.78 |
| F, 67, L                                                | 0.15 | 0.17 | 0.19 | 0.22 | 0.26 | 0.28 | 0.28 | 4  | 0.97 |
| F, 67, R                                                | 0.13 | 0.17 | 0.19 | 0.2 | 0.24 | 0.27 | 0.24 | 6  | 0.78 |
| M, 57, L                                                | 0.57 | 0.53 | 0.64 | 0.68 | 0.7 | 0.69 | 0.65 | 13 | 0.97 |
| M, 57, R                                                | 0.34 | 0.39 | 0.47 | 0.45 | 0.57 | 0.6 | 0.6 | 11 | 0.71 |
| M, 85, L                                                | 0.37 | 0.5 | 0.58 | 0.72 | 0.74 | 0.65 | 0.62 | 14 | 0.96 |
| M, 85, R                                                | 0.37 | 0.48 | 0.51 | 0.55 | 0.56 | 0.66 | 0.69 | 14 | 0.96 |
| M, 68, L                                                | 0.32 | 0.41 | 0.44 | 0.45 | 0.48 | 0.53 | 0.62 | 14 | 0.96 |
| M, 68, R                                                | 0.35 | 0.4 | 0.45 | 0.44 | 0.51 | 0.49 | 0.57 | 14 | 0.96 |

Units are g/mm.
taken at the mid-membranous location on the vocal folds. Thereafter, readings are taken at 2-mm intervals inferior from the vocal folds along the midline.

To provide information on the quality of data, two statistical parameters are provided. For each set of readings taken from each point, the coefficient of variance is given. In addition, the data derived from the left side of each hemi-larynx are correlated with the data obtained from the right side to provide a correlation coefficient (CC).

Figure 2 presents the same data as a set of 12 line graphs. The final visual presentations in Figures 2 and 3 present the normalized and mean normalized data from all 12 hemi-larynges, presented as change in mucosal tissue stiffness with respect to distance inferior of the vocal folds.

The key finding was that the stiffness of the mucosa increases as the measurements are taken more inferior from the vocal folds. This can be seen in Figure 2, in which all the lines show an upward trend of increasing stiffness with respect to distance from the vocal folds. Figure 3 normalizes each line with respect to the initial stiffness measured at the vocal folds, and this shows more clearly that all the samples exhibited the same phenomena of increasing stiffness in the inferior direction. Figure 4 takes the mean of the normalized results to which the trend line indicates that stiffness increased by approximately 0.058 g/mm for a displacement of 1 mm.

**DISCUSSION**

**Variation in mucosal stiffness**

This preliminary study indicated that there is a variation in the elastic properties of the subglottic mucosa, with tissue stiffness increasing as the measurements were taken in a more inferior direction. During the myoelastic cycle, there are variations in subglottal pressure that will cause a variable deformation in this region, which will result in a cone, in effect a gradual narrowing of the air path as the airflow moves superior toward the vocal folds. What is now required is for an aerodynamic analysis to be carried out to determine if this deformation has an impact on the nature of the airflow, as a gradual narrowing of a tube can reduce turbulence, increase velocity, and support more laminar flow.

Table 1 and Figure 2 present all the data obtained, and it can be seen that the stiffness of the mucosa increases with respect to the distance inferior of the vocal folds. Figure 4 used all the data, including the few rogue data points, to determine the mean variation with respect to the vocal fold stiffness, and this shows an almost linear relationship of 12% increase in stiffness for each millimeter in the inferior direction.

The significance of this study arises when consideration is given to the deformation characteristics of the mucosa during the low pressure phase of the myoelastic cycle, which would exhibit an uplift similar to the vocal folds, but with a much reduced magnitude.
Uplift arises from the Venturi effect such that when an airflow passes through a constriction, there is a pressure drop that arises from the increased velocity of the air through that restriction. This occurs because conservation of mass requires an increase in velocity through the constriction to maintain the same magnitude of air mass flowing through the constricted pipe. Conservation of energy requires a compensation for the increase in kinetic energy that arises from the increase in velocity, and this manifests itself as a drop in air pressure.

Using Equation 1, it is possible to calculate the pressure drop at the vocal folds arising from a continuous flow of air from the trachea starting with the mean flow rate during normal phonation.

Mean airflow rate is given by Holmberg et al as 185 mL/s for men and 139 mL/s for women. Terasawa et al give a figure of 155 mL/s for men and 153 mL/s for women for “comfortable phonation.” Yoshioka et al report 112 mL/s for men and 89 mL/s for women for “easy sustained phonation.” For this model, 151 mL/s was used for male larynges and 127 mL/s for female larynges as the mean airflow rate.

The internal diameter of the trachea is taken as 19.63 mm for men and 16.26 mm for women using the extensive study by Breatnach et al, which examined the dimensions of 693 tracheas. The values used here were for samples aged 20–69 years, with the sagittal and coronal values averaged.

The length of the true vocal folds is given by Su et al as 14.6 mm for men and 11.2 mm for women, based on 165 samples of photographs taken during laryngeal surgery. Titze gives a range of 17.5–25 mm for male vocal folds and 12.5–17.5 mm for female vocal folds. Cho et al give the results of 17 mm for men and 14.2 mm for women during the “comfort phonation phase,” measured using ultrasonics. The mean of these figures, being 17.61 mm and 13.47 mm, respectively, for male and female larynges was used to give the length of the vocal folds before closure. Given the density of air is 1.225 kg/m³, it is possible to compute change in air pressure with respect to the vocal fold gap. This is given in Figure 4, and a pressure drop of around 1000 Pa was computed. This is in the same order of magnitude as the work published by Oren, who measured the air pressure in hemisected canine larynges, and produced graphs showing the change in air pressures superior and inferior to the vocal folds with respect to the measured air pressure from the trachea. Oren’s figures from three canine samples (converted to Pascal) show changes of around 1500, 2500, and 3000 Pa superior of the vocal folds, and around 800, 1000, and 1400 Pa inferior of the vocal folds. Even though the change in inferior pressure is lower than that which has been measured superior, any change in pressure will result in the mucosa being deformed to be a cone, and our figure of 1000 Pa as used in the following calculations aligns with the actual measurement for change in air pressure given by Oren. Figure 4 presents a graph that shows the predicted pressure drop over the vocal folds with respect to the amount of closure; as can be seen, as the width of the gap decreases, the air pressure change rises to around 1000 Pa.

Moving to the final synthesis of this discussion, the results presented in Figures 4 and 5 were used to model the deformation of the subglottic mucosa during closure. The extent of the uplift in the mucosa was determined by applying the simple linear extension model given by Equation 2.

Close to the vocal fold edge, the uplift pressure is similar; therefore, the key differentiating factor is the tissue thickness. The availability of data for the subglottic region is scarce in the literature, but one useful source is the study by Kutta et al, in which the team analyzed the structure of the subglottic region, and concluded that it is divided into three or four layers composed of collagenous and elastic fibres in which seromucous glands are embedded. Of interest to this model are the figures provided, which Kutta’s team obtained from scanning electronic microscope images of sections of the subglottal region. The high-resolution image gives a tissue depth of 860 μm for the top three layers, the fourth being disregarded as it is defined as “loose connective tissues.” This is, however, a simplification of the anatomy revealed by these images, which will be taken forward by our next study. The vocal folds are between 3- and 5-mm thick. Using 4 mm as the mean, the linear deformation of the subglottic mucosa will be about 22% (860 of 4000) of that achieved by the vocal folds for regions with a similar Young modulus.

The team has now derived figures for the typical thickness of the vocal folds and subglottic mucosa, the change in stiffness of that tissue with respect to distance inferior of the vocal folds, and the change in uplift pressure during the myoelastic cycle. Using all these data, we present a simple model of the deformation of the subglottic region during the myoelastic cycle.

The main finding of this study, as can be seen in Figures 5 and 6, is that the mucosa exhibits a conical deformation characteristic that will constrict the airflow inferior of the vocal folds. This constriction increases with vocal fold closure, which will result in an increase in flow velocity in this region. As flow velocity increases more gradually over a longer distance (i.e., lower acceleration), the kinetic energy increase will also be lower with respect to change in flow path distance, and this will reduce the occurrence of turbulent flow.
In developing our next phase of study, we will take account of the findings by Oren et al., who presented results that show that flow separation from divergent glottal walls during closure gives rise to vortices that create negative pressure near the superior aspects of the vocal folds. Oren’s later work concluded that a convergent shape of the subglottis can produce a significant reduction in turbulence, and the cone deformation that we are predicting may support that process. We will investigate if the cone deformation impacts on both vortices and the potential reduction in turbulence.

Future work
Our hypothesis arising from these findings is that the deformation of the subglottic mucosa will reduce the occurrence of vortices and enhance laminar flow. As yet, we do not have sufficient data to prove that claim, and the aerodynamic modeling may in fact disprove it. Either way, this study needs to be carried out to determine if we are right or wrong. To test this hypothesis, we will extend this research as follows. First, we will embark on a large-scale study of more larynges to enhance the above data, with substantially more samples obtained with a finer pitch; this will present us with a “map of the variation of tissue elasticity” in this region to be used in the deformation model. Second, we will construct a representative 3D model of the subglottal region using CT scans and analyze the aerodynamics of that structure using a CFD analysis tool. A sample image of a 3D reconstruct obtained from CT scans using open-source medical imaging software—Seg3D2—developed by the NIH Center for Integrative Biomedical Computing at the University of Utah Scientific Computing and Imaging (SCI) Institute—is shown in Figure 5. The CT scan of the subjects’ larynges was based on 240 images, with an increment of 1 mm. Automatic image segmentation algorithm built in Seg3D2 software was used to convert the CT scan images to develop computer-aided design format, such as STL files. These volume data will be used to generate high-fidelity coupled CFD model to take account of both the true geometry of the subglottic region and the deformation of the subglottic mucosa. The research findings from the fluid structure CFD modeling of human larynges will be reported in a subsequent journal publication (Figure 7).

Quality of data
Two indices of quality were determined to support our data. One is the average coefficient of variance of all the sets of 10 readings taken from each location on hemi-larynx, defined as normalized standard deviation with respect to mean. This represents the spread of the original datasets, with 0% meaning that all samples are identical. Our aim was to achieve 10% or better, so the range of 4%–15% is acceptable but will be improved upon by taking a larger number of samples at each measurement point for the wider study.

The second measure of quality is a determination of the CC between the left and right hand sides of each larynx, where a CC of 1 means that there is a perfect match. There is no standard as to what level of coefficient represents acceptable data, and our aim was to achieve 0.8 or better. The range of 0.71–0.97 is also acceptable, but will be improved upon in the following study.

CONCLUSION
These results indicated that the stiffness of the subglottic mucosa increases linearly with distance inferior of the vocal folds. The deformation of the mucosa results in a conic constriction inferior of the vocal folds, causing an increase in airflow velocity and a tendency to support laminar flow.

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REFERENCES
1. Titze IR. The myoelastic aerodynamic theory of phonation. National Center for Voice and Speech, 2006. ISBN 9780874141566.
2. Goodyer E, Gunderson M, Dailey SH. Gradation of stiffness of the mucosa inferior to the vocal fold. J Voice. 2010;24:359–362.
3. Oren L, Dembinski D, Gutmark E, et al. Characterization of the vocal fold vertical stiffness in a canine model. J Voice. 2014;28:297–304.
4. Dailey SH, Tateya I, Montequin D, et al. Viscoelastic measurements of vocal folds using the linear skin rheometer. *J Voice*. 2009;23:143–150.
5. Hess MM, Mueller F, Kobler JB, et al. Measurements of vocal fold elasticity using the linear skin rheometer. *Folia Phoniatr Logop*. 2006;58:207–216.
6. Sundberg J, Iwasson J, Billström AH. Significance of mechanoreceptors in the subglottal mucosa for subglottal pressure control in singers. *J Voice*. 1995;9:20–26.
7. Smith ME, Roy N, Stoddard K, et al. How does cricotracheal resection affect the female voice? *Ann Oto Rhino Laryngology*. 2008;117:85–89.
8. Holmberg E, Hillman R, Perkell J. Glottal airflow and transglottal air pressure measurements for male and female speakers in soft, normal, and loud voice. *J Acoust Soc Am*. 1988;84:511–529.
9. Terasawa R, Hibi SR, Hirano M. Mean airflow rates during phonation over a comfortable duration and maximum sustained phonation results from 60 normal adult subjects. *Folia Phoniatr*. 1987;39:87–89.
10. Yoshioka H, Sawashima M, Hirose H, et al. Clinical evaluation of air usage during phonation. *Jpn J Logoped Phoniatr*. 1977;18:87–93.
11. Breatnach E, Abbott GC, Fraser RG. Dimensions of the normal human trachea. *AJR Am J Roentgenol*. 1984;142:903–906.
12. Su MC, Yeh TH, Tan CT, et al. Measurement of adult vocal fold length. *J Laryngol Otol*. 2002;116:447–449.
13. Titze IR. *Principles of Voice Production*. Englewood Cliffs, NJ: Prentice Hall; 1994. ISBN 978-0-13-717893-3.
14. Cho W, Hong J, Park H. Real-time ultrasonographic assessment of true vocal fold length in professional singers. *J Voice*. 2012;26:819.e1–819.e6.
15. Oren L, Gutmark E, Khosla S. Intraglottal velocity and pressure measurements in a hemilarynx model. *J Acoust Soc Am*. 2015;137:935–943.
16. Kutta H, Steven P, Paulsen F. Anatomical definition of the subglottic region. *Cells Tissues Organs*. 2006;184:205–214.
17. Hahn MS, Teply BA, Stevens MM, et al. Collagen composite hydrogels for vocal fold lamina propria restoration. *Biomaterials*. 2006;27:1104–1109.
18. Besnard D, Harlow FH, Johnson NL, et al. Instabilities and turbulence. *Los Alamos Sci Spec Issue*. 1987:145–184.
19. Oren L, Khosla S, Murugappan S, et al. Role of subglottal shape in turbulence reduction. *Ann Otol Rhinol Laryngol*. 2009;118:232–240.
20. Oren L, Khosla S, Gutmark E. Intraglottal pressure distribution computed from empirical velocity data in canine larynx. *J Biomech*. 2014;47:1287–1293.