Characterization of the Vocal Fold Vertical Stiffness in a Canine Model

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Summary: Objectives/Hypothesis. Characterizing the vertical stiffness gradient that exists between the superior and inferior aspects of the medial surface of the vocal fold. Characterization of this stiffness gradient could elucidate the mechanism behind the divergent glottal shape observed during closing.

Study Design. Basic science.

Methods. Indentation testing of the folds was done in a canine model. Stress-strain curves are generated using a customized load-cell and the differential Young's modulus is calculated as a function of strain.

Results. Results from 11 larynges show that stress increases as a function of strain more rapidly in the inferior aspect of the fold. The calculations for local Young's modulus show that at high strain values, a stiffness gradient is formed between the superior and inferior aspects of the fold.

Conclusions. For small strain values, which are observed at low subglottal pressures, the stiffness of the tissue is similar in both the superior and inferior aspects of the vocal fold. Consequently, the lateral force that is applied by the glottal flow at both aspects results in almost identical displacements, yielding no divergence angle. Conversely, at higher strain values, which are measured in high subglottal pressure, the inferior aspect of the vocal fold is much stiffer than the superior edge; thus, any lateral force that is applied at both aspects will result in a much greater displacement of the superior edge, yielding a large divergence angle. The increased stiffness observed at the inferior edge could be due to the proximity of the conus elasticus.

Key Words: Vocal folds–Elasticity–Young's modulus.

INTRODUCTION

During vocal fold vibration, the glottis can take a convergent shape during opening (where the inferior aspect between the vocal folds is wider than the superior aspect) and a divergent shape during closing. This convergent-divergent transformation stems from the mucosal wave traveling in the inferior-superior direction, which was noted qualitatively using a full larynx model (Hirano[1]) and detailed in a hemilarynx model (Jiang and Titze[2]). In both models, the authors noted that during opening, when viewed from above, the inferior aspect of the folds was hidden by the superior aspect, which suggested that the glottis assumed a converging shape. Jiang and Titze were able to visualize the glottal dynamics from a coronal view, noting that during closing, the medial displacement of the inferior aspect of the fold preceded the displacement of the superior aspect, implying that the glottis assumes a diverging shape during closing. According to the myoelastic-aerodynamic theory, the convergent-divergent shape of the glottis is formed due to a phase delay in the inferior-superior direction of the mucosal wave velocity (Titze and Alipour[3]).

The convergent-divergent shape transformation of the glottis also affects the dynamics of the intraglottal flow. The intraglottal flow will attach (ie, follow the contour) to the converging walls of the glottis during opening but will separate (ie, not follow the contour) from the diverging walls during closing. The intraglottal flow separation during closing was demonstrated in computational, mechanical, and excised canine models (Shadle et al,[4] Pelorson,[5] Alipour and Titze,[6] Zhao et al,[7] and Kucinschi et al[8]), but the influence of intraglottal flow separation, if any, on the vibrations of the vocal folds is still being evaluated.

It is well known in the field of fluid mechanics that flow separation can significantly alter the pressure distribution in the flow (Anderson[9]). During phonation, the intraglottal pressure distribution determines the forces applied on the glottal wall by the intraglottal flow. The impact of the forces generated by flow separation in the glottis varies according to the models being used: The two-mass model of Ishizaka and Flanagan[10] did not consider flow separation to occur during closing, whereas the three-mass model of Story and Titze[11] and Pelorson et al[5] accounted for flow separation in the glottis during closing, but for simplification assumed that the intraglottal pressure downstream of the separation point was atmospheric. Other computational models (Zhao et al,[7] Zhang et al,[12] and Mihaescu et al[13]) predicted that intraglottal flow separation could lead to intraglottal vortices forming near the superior aspect of the folds. These flow separation vortices can produce pressure that is less than atmospheric (ie, negative pressure), thus applying an additional suction force on the superior aspect of the folds during closing. The magnitude of the negative pressure that can develop near the superior aspect of the fold is determined by the dynamic characteristics of the flow, which are influenced by the magnitude of the divergence angle of the folds (Figure 1).

Because the divergence angle may affect intraglottal pressures, it is important to understand mechanisms that create and increase the divergence angle. Our work, using particle image velocimetry to determine intraglottal geometry and velocity fields (Oren et al[14]), shows that at low subglottal pressure (ie,

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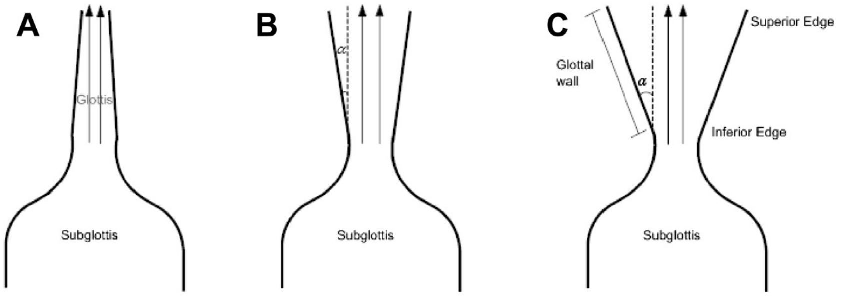

FIGURE 1. Schematics of the vocal folds vibration. (**A**) During opening, showing a convergent shape of the glottis. The intraglottal flow follows the contour of the wall. (**B**) During closing with a small divergence angle (α). The intraglottal flow may separate from the wall (**C**) during closing with a larger divergence angle. The intraglottal flow separates from the wall. Flow separation vortices may form in the glottis.

lung pressure), the divergence angle is small, and as the subglottal pressure increases, the divergence angle increases. The divergence angle will be increased by any factor that increases the phase difference between the upper and lower masses. It has been proposed in theoretical models that vocal tract inertance will increase this phase difference (Titze[15]). On the other hand, simulations done without a vocal tract show minimal phase difference (Story[16]). Our hypothesis for this behavior, and motivation for this study is that at low pressures, there is similar stiffness between the superior and inferior aspects of the fold; however, as subglottal pressure increases, the inferior aspect of the fold becomes stiffer than the superior aspect. This hypothesis attempts to address the question of how a phase delay can occur without a vocal tract.

The difficulty in accurate characterization of the elastic properties of the vocal folds stems from the inhomogeneous composition and viscoelastic characteristics of the tissue. The composition of the vocal fold tissue is often approximated by the two-layer body-cover model (Hirano[17]): The body, consisting of the deep layer of the lamina propria and the thyroarytenoid (TA) (vocalis) muscle, is largely responsible for maintaining muscular tone and accounts for the majority of the overall stiffness of the tissue (Titze and Alipour[3]). The cover, consisting of the epithelium and the superficial and intermediate layers of the lamina propria, is the locale most associated with mucosal wave propagation and has little mechanical integrity. The inhomogeneity of the body-cover layers also leads to an anisotropic response of the tissue.

The elastic properties of the vocal folds are most commonly studied in human or canine models. Canine vocal fold tissue is the preferred (animal) model because of the anatomical and acoustical similarities with human samples (Titze[18]). Additionally, testing in canines is performed immediately postmortem with minimal (or no) tissue decomposition, which reduces the variations in tissue properties that can stem from the decay process.

The data reported in the literature for the elastic properties of the vocal folds varies significantly depending on the specimen type and the testing method examined in the study (eg, the direction of loading). For example, Chan et al[19] used elongation technique to evaluate the longitudinal elastic properties of the tissue and showed that human males had higher levels of collagen in the tissue, which yielded a much higher Young's modulus (ie, stiffness) compared with females. Chan et al noted that the value for Young's modulus was a function of the strain in the tissue. At 40% strain level, they reported values of 1750/350 kPa for the ligament layer in human male/female, respectively, and 1000/480 kPa for the cover layer in male/female, respectively. These data can be compared with the data reported by Min et al[20] who used the same (elongation) technique but reported values of 600 kPa for the same (40%) strain level in the human ligament. Min et al did not show a gender-based difference like Chan et al, but their study was conducted on a smaller set of data (two males and two females compared with 12 males and eight females). Another study by Perlman and Durham[21] using human vocal fold tissue reported a longitudinal value of 400 kPa for the Young's modulus at 50% strain.

In the elongation technique, the vocal fold is normally mounted to the testing apparatus at its anterior/posterior ends and subject to a controlled tension. A drawback of this method is that it requires removal of the vocal fold from the larynx, eliminating the physiological prestress that is imparted on the fold by the surrounding tissue. The elongation technique then extracts the *global* longitudinal elastic properties. In comparison, the microindentation technique uses a solid indenter, which displaces the tissue a known distance and the resultant force is recorded. The microindentation technique neglects (local) tissue inhomogeneity that may exist in the tissue and allows for *local* elasticity variations to be resolved. The microindentation technique also allows the vocal fold to be kept intact in its normal physiological surroundings.

The microindentation technique was used by Chhteri et al[22] to evaluate the local elastic properties of human vocal folds at the mid-membranous plane. They noted that the stiffness of the tissue changes if the folds are removed from the larynx because of the elimination of the prestress condition. Chhetri et al showed that the inferior medial surface was stiffer than the superior medial surface. For a low strain value (vocal fold at rest), they measured a Young's modulus of 8.6 kPa at the mid-membranous plane. These data can be compared with the Young's modulus values reported by Berke and Smith[23] of 2.4 kPa (at low strain) and by Tran et al[24] who reported 7.6 kPa. Both these studies used microindentation at the mid-membranous plane.

Another measurement technique used to evaluate the elastic properties of the vocal folds is linear skin rheometry. The