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Vocal fold elasticity in the pig, sheep and cow larynges

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Abstract

Elastic characteristics of the pig, sheep and cow vocal folds were investigated through a series of *in vitro* experiments. Sample strips of the vocal fold tissue were dissected from pig, sheep and cow vocal folds and mounted inside a saline-filled ergometer chamber that was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Sinusoidal elongation was applied on the samples to obtain the passive force measurements. Force and elongation data from the samples were recorded electronically with a dual-servo system (ergometer). Stress-Strain data were compared to characterize the interspecies differences in the elastic properties of vocal folds. Pig vocal folds exhibited the most nonlinear stress-strain relationship, indicating the presence of a high level of collagen fibers. Cow vocal folds had the highest Young's modulus, but the tissue displayed a nearly linear stress-strain profile. Previous studies of phonation in these three species have indicated that pig larynges have the highest range of phonation frequencies, making them a good candidate for animal studies. The current study provides quantitative data for the elastic properties of the oscillating laryngeal tissue in these species and indicates that nonlinear behavior of these tissues may lead to wider oscillation ranges.

Keywords

Force-elongation; Stress-strain; Pig larynx; Young's modulus

INTRODUCTION

Excised larynx models have been used frequently to study the aerodynamics and acoustics of phonation. Depending on the distinctive geometrical and mechanical properties of the vocal folds in each animal model, a variety of oscillating conditions were observed. Besides the widely used canine larynx, the ease of availability of pig, sheep, and cow larynges makes them potential candidates for excised larynx studies. Although data regarding geometrical measurements and anatomical comparisons among larynges of different species is available in the literature¹⁻², study of inter-species tissue elasticity has been limited to the canine model.

Kurita et al¹ compared the vocal fold anatomy of five pig, dog, sheep and human excised larynges and observed that these animal species all had a two-layered lamina propria

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structure unlike the three-layered structure in human. In the human vocal folds, the density of the collagen fibers (and consequently stiffness) progressively increased in the direction of the vocalis muscle. The density of elastic fibers was greatest in the intermediate layer of lamina propria and gradually decreased in the superficial and deep layers. A similar slope in stiffness was observed in pig larynges and to some extent in sheep vocal folds. In contrast, the canine larynx had the greatest density of collagen and elastic fibers in the superficial layer with a less dense and more pliable deep layer of lamina propria. The arrangement of fibers in all the species was in the direction of the longitudinal axis of the vocal folds.

Elastic properties of canine vocal fold viable tissues (muscle and mucosa) have been measured and modeled by Alipour and Titze³. They showed that the elastic behavior for vocal fold tissues was linear at low strains and exhibited an exponential profile at higher strain values. They reported average low strain Young's modulus of 21 kPa for the canine vocal fold body (muscle) and a value of 42 kPa for its cover. While the elastic properties of other laryngeal muscles that were measured and reported in a similar manner⁴⁻⁵ are useful in the phonatory and posturing models, it is the elastic property of the cover that has the most influence on the vocal fold oscillation frequency due to its proximity to the airflow and Bernoulli pressure that produces mucosal wave. It is also subjected to impact from tissue on the opposite side with risk of possible damage such as tissue scarring, polyps and nodules. Most of the surgical treatments performed on the larynx are directed towards this layer for restoration of the mucosal wave.

Unlike the canine tissue, the elastic properties of human vocal folds have not been measured as extensively from fresh tissue samples using comparable force-elongation tests. There have been some studies that reported tissue stiffness and spring constant from the resilience of the tissue to external excitation. For example, by applying a 1-mm diameter probe at various locations on the excised human vocal folds and measuring the force and displacements, Haji et al⁶ estimated the degree of stiffness for human true and false vocal folds from the inclination of force-displacement curve. They also defined a parameter of stiffness from the area of force-displacement hysteresis curve. Based on these techniques, they reported that stiffness is lower in the middle of membranous portion and higher at the vocal process and anterior commissure. They also reported that false vocal folds have the lowest stiffness than other portions of laryngeal structure.

However, there are very few studies that have reported the measured elastic properties of human tissue samples. Mechanical properties of human vocal folds have also been reported by Min et al⁷ and Chan and Titze⁸. Chan and Titze⁸ measured the complex shear modulus of human vocal fold mucosa within 18-20 hours postmortem, using a rheometer. The tissue samples were placed between two circular disks and the oscillatory motion of one disk measured the complex shear modulus of the tissue sample. They reported that at low frequency, the shear modulus was about 10-100 kPa for males and 3-20 kPa for female vocal folds. Similar to the study by Alipour & Titze³, the elastic properties of human vocal ligament were measured by Min et al.⁷ using force-elongation experiments on samples acquired from surgical patients and cadaveric tissue. They reported an average low-strain Young's modulus of 33.1 ± 10.4 kPa (n=8) for the human vocal ligament with a steep increase at higher strain values.

Alipour et al.⁹ compared the acoustic properties of phonation from excised pig, sheep and cow larynges. They found a wide range in the inter-species phonation characteristics, in which the fundamental frequency range was highest in the pig larynx and lowest in the cow. The greater range observed in the pig was attributed to the presence of two oscillating vocal folds (superior and inferior folds), which are not present in the other animals studied.

Because of the high oscillation range for the pig larynx (100 to 300 Hz), the pig was suggested to be a good model for studying phonation.

In a similar study by Alipour and Jaiswal¹⁰, subglottal pressure, fundamental frequency, and glottal airflow resistance were compared between pig, sheep and cow larynges. A nonlinear relationship between subglottal pressure and airflow was observed in the three species, while a linear relationship had been previously demonstrated in excised canine larynges¹¹. Glottal airflow resistance was found to be similar amongst the three animals, and differences in maximum frequency ranges were attributed to stiffness variations and morphological differences between species.

The variations in the range of phonation frequencies amongst these animal models can be attributed to differences in vocal folds length, morphology, and biomechanical properties of the vocal folds based on their species-specific vocalization needs¹². The choice and description of an appropriate model requires further understanding and comparison of these biomechanical differences. Thus, it is important to do a comparative study between the animal models to further determine their suitability for excised larynx research. The purpose of the current study was to examine the elastic properties of the oscillating laryngeal tissue (cover) in these animal models, namely, pig, sheep and cow larynges, and to compare the results with canine data. Since pig larynx ventricle extends behind its supraglottal wall and can make it oscillate at low frequency⁹, elastic property measurement of its supraglottal wall was included to explain the oscillatory behavior.

METHODOLOGY

The experimental protocol used was similar to an earlier study by Alipour and Titze³. Tissue samples were prepared from the oscillating portions of the laryngeal tissue of pig, sheep and cow excised larynges. Before dissection, the average *in situ* length of the each sample was measured with a set of calipers. For the sheep larynx, one sample was made from each vocal fold. The larger dimensions of the cow larynx allowed two samples from each side of the vocal fold. In the pig, at least one sample was made from each superior fold (SVF), inferior fold (IVF) and from the supraglottal wall (GW). The details of sample preparation are as follows:

Pig Sample preparation

The frozen porcine larynx was slow thawed at room temperature. Epiglottis and strap muscle tissues were excised out and discarded. The larynx was placed horizontally on a dissection tray and a midline longitudinal section was made in the posterior surface. The midline section extended from the superior margins of the arytenoid cartilage to the tracheal rings. A similar section was made anteriorly through the thyroid midline to divide it into two hemilarynges. Care was taken to maintain the vocal fold edges and cartilaginous connections intact. The superior and inferior folds were identified along with the vocal process of the arytenoid cartilage, the attachment point for the folds. An effort was made to prepare three samples from each hemilarynx, one from the inferior fold, one from the superior and one from the supraglottal wall (Figure 1A). For the purpose of elastic measurements, thin strips were prepared from each vocal fold. The cartilaginous attachment points of the folds were identified and marked with permanent ink. The *in situ* lengths were measured and averaged for the superior and inferior folds (the inferior fold had a slightly curved edge so the length of its superior and inferior sides was measured separately and averaged). A deep incision was made in the thyroid lamina around the anterior attachment point of the superior fold to carve out the cartilaginous ends of the sample. A transverse incision was similarly made through the portion of the arytenoid cartilage apposing the superior vocal fold. Once the cartilaginous ends were marked and excised, two lateral incisions were made on either side

of the sample to extricate it from the surrounding tissue. The superficial layer was excised by gently teasing it out in the anterior-posterior direction from the underlying connective membrane (that separated the superficial layer from the muscle fibers). The superior folds were fairly well defined and usually their entire width above the ventricle was used for the sample preparation (Figure 1A). Any extraneous muscle fibers attached to the sample were carefully removed. A silk suture (#2.0 metric) attached to a curved needle was threaded through the cartilage ends of each tissue strip. The resulting sample consisted of the superficial layer of vocal fold tissue bordered by cartilage wedges with sutures running through them. The supraglottal wall samples were made as a 3-mm strip parallel to the superior edge and about 10 mm above it.

Sheep Sample Preparation

The sample preparation was similar to the pig larynx except for the lack of ventricle and hard to detect edges in the younger species (Figure 1B). A 3-mm anterior-posterior strip of tissue sample was incised from the middle of the vocal fold (between approximate edges) from each hemilarynx, bordered by thin wedges of thyroid and arytenoid cartilages at the anterior and posterior ends respectively. The softer arytenoid cartilage of the sheep larynges made it easier to cut, but required more careful needle insertion for suture attachment to avoid a tear.

Cow Sample Preparation

The greater width of the bovine vocal folds (up to 10 mm) allowed for preparation of two samples from each side (Figure 1C). The protocol for sample preparation was otherwise similar to the sheep larynx. First, an anterior-posterior incision is made in the middle of the vocal fold (between approximate edges) from each hemilarynx. Next two strips of tissue with average width of 3 mm were selected and incised to make two separate samples, bordered by pieces of thyroid and arytenoid cartilages at each end. In most samples, the cartilaginous end (near the thyroid notch) was stiffer and at times calcified. In such a case, an electrical saw was used to make the incisions at the edges with care so as to not damage the vocal folds.

Data Collection and Analysis

To obtain the passive properties of these tissues, samples were stretched and released by applying a 1-Hz sinusoidal signal to the ergometer as described by Alipour and Titze³. Samples were subjected to about 40% elongation in this manner for about 20 seconds. The displacement of the ergometer arm and the force exerted by the tissue was measured electronically with a Dual Servo ergometer (Cambridge Technology). The ergometer had a force resolution of 0.0005 N, displacement accuracy of 0.02 mm, and a rise time of 6 milliseconds. The analog signals of displacement and force of the ergometer were sent to an A/D converter (DI-410, DATAQ Instruments). The two signals were displayed and recorded on the computer at 2000 sample/sec with WINDAQ software (DATAQ Instruments). The force and displacement signals were calibrated and converted to stress and strain using the sample's cross-sectional area and length. The average cross-sectional area (A_0) was obtained from the sample mass (m) (without cartilages), *in situ* length (L_0) and density (ρ) at the end of the experiment according to

$$A_0 = \frac{m}{\rho L_0} \quad (1)$$

For the mass measurement, the attached cartilage ends were dissected out and the sample was dabbed with tissue paper to remove any extra moisture. The density value of canine

vocal fold cover tissue was measured previously by Perlman¹³ and reported to be 1.02 g/cm^3 that was used for the cover tissues in this study.

RESULTS

Anatomical Observations

On visual examination of the porcine vocal folds, the superior edge appeared ligamentous with a narrow, well-defined band like appearance (Figure 1A). The superior vocal fold (SVF) had a prominent cartilaginous process extending into it (which was determined by digital palpation). The inferior vocal fold (IVF) in contrast was wider and less defined. The tissue primarily consisted of a thin superficial layer with an underlying bulk of muscle fibers. The superficial layer appeared thicker at the attachment ends. The porcine arytenoids were larger than their human or canine counterparts and probably fused with the corniculate cartilage on their superior edge, extending the vertical size. A well-defined ventricle was present between the superior and inferior folds that extended from the anterior commissure up to the arytenoids. The ventricle was deeper at the posterior end and was found to extend laterally into the walls of the glottis. The anterior-posterior attachment points for the two folds were marked for making length measurements.

Kurita et al¹ described the inferior fold of the porcine larynx as the true vocal fold and the superior fold as the ventricular folds. The experimental evidence from airflow driven oscillation of excised porcine larynges⁹, suggest that both vocal folds vibrate during phonation (primarily in unison but out of phase) and probably differ in their morphology. It is debatable assigning the labels of 'true' and 'false' vocal folds on the basis of function for porcine larynges. Thus, the nomenclature 'superior' and 'inferior' folds were used in this study. For the superior fold, the length extended from the thyroid lamina (anterior commissure, thyroid notch) to the terminating point of the cartilaginous vocal process. The inferior edge extended anteriorly from the thyroid lamina attachment point to the base of the arytenoid cartilage posteriorly. The average length of the porcine vocal fold was $28 \pm 2.9 \text{ mm}$ for the superior samples, $28 \pm 2 \text{ mm}$ for the inferior samples, and $32 \pm 7.9 \text{ mm}$ for the wall samples. The inferior fold has been described as having two layers with an indistinct boundary between them¹. The superficial layer has fewer elastic and collagen fibers with the collagen fibers becoming more abundant in the deep layer as the muscle is approached. This concurs with our observation of a thinner superficial layer.

Figure 2 represents a typical sample of recorded data with displacement of the ergometer arm or sample elongation in the top panel and resultant force generated in the bottom panel. The applied elongation signal remained at constant amplitude of 10 mm for this sample while the amplitude of the generated force showed a decline with time, indicating tissue relaxation. To remove the sample slackness, some minor initial force was applied that shows as an offset in the bottom graph.

When the generated force signal was plotted against the elongation displacement, a hysteresis loop was obtained with the upper leg of the curve representing stretch part and lower leg representing the release of the sample (Figure 3). Each loop in the figure denotes successive cycles of elongation and release. The force generated as a result of the stretching of the sample was higher than that during its release, depicting a hysteretic property observed in most soft tissues¹⁴⁻¹⁵. Over successive cycles of stretching/releasing, the tissue is considered preconditioned, where the force-displacement curves become less variable. Even in this state, hysteresis is observed, demonstrating the viscoelastic properties of the vocal fold tissue. The stress values for each sample were obtained by averaging the force generated during stretch legs of the last 2-3 cycles and normalizing it to the cross-sectional area of the sample. To obtain the stress-strain relationship for the sample, the normalized

stress was plotted against sample strain (elongation normalized to *in vitro* length of the sample).

Figure 4 depicts the stress-strain data for 6 samples of the pig superior vocal fold (SVF) with the average values denoted by solid line. The Young's moduli range between 13.8 to 26.1 kPa with mean values of 19.2 ± 4.2 kPa ($n = 6$). The Young's modulus was calculated by a linear regression between 5% and 15% strain values. The variability in the values could be partly attributed to the presence of cartilaginous extension of the vocal process in some of the tissue samples.

The stress-strain curves for six samples of pig inferior vocal folds (IVF), and four samples of pig superior glottal wall (SGW) were averaged in a similar fashion and it was found that the pig inferior vocal fold had a mean Young's modulus of 16.3 ± 2.9 kPa ($n=6$). The pig superior glottal wall had a mean Young's modulus of 10.2 ± 2.1 kPa ($n=4$). The pig IVF samples (Figure 5) showed lesser variability in their stress-strain curves than that for SVF data with greater nonlinearity at higher strain values than was observed in the SVF samples.

Unlike the pig vocal fold samples, sheep vocal fold samples were mostly non-uniform in length and cross-sectional area, with the average length ranging from 15 to 20 mm. The Young's modulus ranged between 6.7 to 19.1 kPa with a mean value of 11.7 ± 4.6 kPa ($n=6$). Sheep vocal fold samples showed less nonlinearity during force-elongation experiments than pig tissue samples though the variability in data is also greater than that of the pig samples (Figure 6). The low range of Young's modulus and lower nonlinearity may have been responsible for the lower ranges of oscillation frequency in sheep larynges as reported by Alipour & Jaiswal⁹.

The cow vocal fold samples were longer in dimension and also stiffer during force elongation. Their sample length ranged between 24 to 30 mm. Their Young's modulus ranged between 18.1 to 44.4 kPa with a mean value of 29.9 ± 8.2 kPa ($n=9$). They showed very linear stress-strain curves, similar to the sheep samples (Figure 7). An examination of the force-elongation hysteresis loop (not shown here) demonstrated a much smaller area between stretch and release indicating minimal viscoelastic behavior. This linearity and lack of viscosity may have been responsible for the lack of pressure-frequency sensitivity in the cow larynges as reported by Alipour & Jaiswal⁹.

DISCUSSION

A comparative chart shown in Figure 8 demonstrates the difference between Young's modulus across species. The canine data is from the Alipour and Titze³ database which is included in this chart. The canine vocal fold cover has the highest Young's modulus with the values descending in the order: canine, cow, pig and sheep respectively. One major difference between these samples is that the canine vocal fold cover samples were viable, but the vocal fold covers from pig, sheep and cow were not; this lack of viability could have potentially decreased the viscosity and elasticity of these tissue samples. However, larynges with similar tissue conditions have been successfully oscillated on the excised larynx bench, indicating their mechanical properties were preserved to some degrees till a few hours after death.

Most soft tissues, including the vocal folds, contain two primary load-bearing proteins responsible for providing elasticity (elastin) and strength (collagen)¹⁶. Collagen is a wavy, fibrous protein and in the vocal folds is arranged in a complex 3-D network. During stretching of the tissue, the wavy collagen becomes taut initially, and the tension in the tissue increases as additional collagen fibers are recruited.

The stress-strain curves shown in Figures 4-6 demonstrate the nonlinear elastic behavior of the soft tissue, especially as elongation increases. The nonlinearity is likely a result of the collagen content in the tissue, where collagen recruitment occurs gradually as the sample is stretched^{15, 17}. For small strains (<0.15), the stress-strain relationship can be assumed linear, with the slope of the curve depicting a low Young's modulus value for the sample. In this low-strain, linear region of the curve, it is reasonable to assume that elastin is the primary load-bearing component within the tissue. Upon further displacement, initially unstretched collagen fibers are recruited and begin to bear load. In this way, Young's modulus for the elastin content in the tissue¹⁵ is provided by the linear region of the graph.

The variability of data in Figure 4 may be related to the variability in elastin and collagen content of the tissue. Several groups have shown that both age and gender affect the elastin and collagen content, which in turn would influence the material properties of the tissue¹⁷⁻¹⁸. As can be observed in the stress-strain curves, the linear strain region appears to vary between samples. Beyond the linear region, the strength and density of primarily the collagen fibers will affect the stress-strain relationship in the non-linear section.

Studies of histological and phonatory properties of different species indicated relations between phonation and structure. This includes a comparison of phonation and structure between pig, dog, deer, and human¹⁹, the collagen content of dog, monkey, and pig larynges²⁰, the collagen and elastin content of dog, ferret, and pig larynges²¹, and comparison to those of human. Overall, on examining collagen and elastin content and distribution within the larynx tissue¹⁹⁻²¹, it was found that pig larynges are most similar in terms of histological properties to human.

Although histological studies of sheep larynges vocal folds have not been performed extensively, the highly linear stress-strain relationship, coupled with the lower Young's modulus, indicate lower collagen content and more elastin presence in the cover tissue. Kurita et al.¹ had reported that the superficial layer of the lamina propria had abundant elastic and collagenous fibers with their density increasing in deep layer. This variation in the density of fibrous elements may contribute to some of the variability in the elastic properties.

As shown in Figure 7 for cow vocal folds, a high Young's modulus but minimal nonlinearity and viscoelasticity potentially indicate that collagen is present in high levels in the vocal folds, but that the fibers are relatively taut even at low strain, such that collagen fiber recruitment is minimal during elongation. Histological examination of the tissue would potentially confirm this hypothesis.

The greater nonlinearity in the pig vocal folds may be the reason that the pig larynges have a wide range of oscillation frequency as reported by Alipour and Jaiswal⁹. Use of a student t-test indicated that the difference between Young's modulus of SVF and IVF is not significant ($p=0.174$), but between SGW and IVF or SVF is significant ($p=0.003$). Biomechanically, the structure above the ventricle in the pig larynx is more comparable to the true vocal folds, hence, the term *superior vocal folds* is used. This similarity was also reported by Alipour & Jaiswal⁹ from their observation of oscillations of these two folds in the pig excised larynges. A higher Young's modulus indicates a higher density of collagen that contributes to the tensile strength of the tissue. Histological analyses of these layers within the pig larynx could possibly provide clearer explanation for the differences in material properties between the different regions.

Although, the dog vocal fold cover is twice as stiff as the pig larynx, at high strains the pig vocal fold exhibits much higher stiffness, similar to the human ligament which absorbs most of the longitudinal tension and allows the superficial layer to be lax and oscillate better³.

The great phonatory range of pig larynx may be also due to the possibility of an existence of ligament layer in the pig superior vocal folds that needs further examination and study.

CONCLUSIONS

The study provides a bio-mechanical comparison of the vibrating tissue samples across four different species. The results suggest that the physiological differences in the linearity and ranges of oscillation of excised larynges reported in a previous study⁹ are reflective of the tissue composition and mechanics. Histological examination of the vibrating tissue across these species would aid in further parsing out the effect of makeup and distribution of its fibrous composition on the differences in the tissue material properties between species. Future work should measure not only the mechanical properties with the tissue in viable condition, but extend these measurement to a biaxial stress-strain data that provide a complete set of elastic properties including transverse Young's modulus, shear modulus and Poisson's ratio, at least for the pig vocal folds which have been shown to be a good model of phonation.

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REFERENCES

1. Kurita, S.; Nagata, K.; Hirano, H. A comparative study of the layer structure of the vocal fold. In: Bless, DM.; Abbs, JH., editors. *Vocal Fold Physiology: Contemporary research and clinical issues*. College Hill Press; San Diego, CA: 1983. p. 3-21.
2. Kim MJ, Hunter E, Titze IR. Comparison of human and ovine laryngeal dimensions. *Annals of Otology, Rhinology and Laryngology* 2004;113:60–68.
3. Alipour F, Titze IR. Elastic models of vocal fold tissues. *J Acoust Soc Am* 1991;90(3):1326–1331. [PubMed: 1939897]
4. Alipour F, Titze I. Active and passive characteristics of the canine cricothyroid muscles. *Journal of Voice* 1999;13(1):1–10. [PubMed: 10223670]
5. Alipour F, Titze IR, Hunter EJ, Tayama N. Active and passive properties of laryngeal adductor/abductor muscles. *J Voice* 2005;19(3):350–359. [PubMed: 16102663]
6. Haji T, Mori K, Omori K, Isshiki N. Mechanical-Properties of the Vocal Fold - Stress-Strain Studies. *Acta Oto-Laryngologica* 1992;112(3):559–565. [PubMed: 1441998]
7. Min YB, Titze IR, Alipour F. Stress-Strain Response of the Human Vocal Ligament. *Annals of Otology Rhinology and Laryngology* 1995;104(7):563–569.
8. Chan RW, Titze IR. Viscoelastic shear properties of human vocal fold mucosa: measurement methodology and empirical results. *J Acoust Soc Am* 1999;106(4 Pt 1):2008–2021. [PubMed: 10530024]
9. Alipour F, Jaiswal S. Phonatory characteristics of excised pig, sheep, and cow larynges. *J Acoust Soc Am* 2008;123(6):4572–4581. [PubMed: 18537405]
10. Alipour F, Jaiswal S. Glottal airflow resistance in excised pig, sheep and cow larynges. *Journal of Voice* 2009;23(1):40–50. [PubMed: 18023324]
11. Alipour F, Scherer RC, Finnegan E. Pressure-flow relationships during phonation as a function of adduction. *Journal of Voice* 1997;11(2):187–194. [PubMed: 9181542]
12. Riede T, Lingle S, Hunter EJ, Titze IR. Cervids with different vocal behavior demonstrate different viscoelastic properties of their vocal folds. *J Morphol.* 2009 in press.
13. Perlman, AL. A technique for measuring the elastic properties of vocal fold tissue., The University of Iowa; Iowa City, Iowa: 1985. unpublished doctoral dissertation

14. Holzapfel, GA.; Ogden, RW., editors. Biomechanics of soft tissue in cardiovascular system. Springer Wien; New York: 2004. CISM Course & lecture No 441- International Centre for Mechanical Sciences
15. Humphrey, JD. Cardiovascular solid mechanics- cell, tissues and organs. Springer Wien; New York: 2002.
16. Gray SD. Cellular physiology of the vocal folds. *Otolaryngol Clin North Am* 2000;33(4):679–698. [PubMed: 10918654]
17. Sato, Kiminori; Minoru, Hirano; Tadashi, Nakashima. Age-related changes of collagenous fibers in the human vocal fold mucosa. *Annals of Otology, Rhinology & Laryngology* 2002;111(1):15–20.
18. Hahn MS, Kobler JB, Zeitels SM, Langer R. Quantitative and comparative studies of the vocal fold extracellular matrix II: collagen. *Ann Otol Rhinol Laryngol* 2006;115(3):225–232. [PubMed: 16572613]
19. Jiang JJ, Raviv JR, Hanson DG. Comparison of the phonation related structures among pig, dog, white-tailed deer, and human larynges. *Annals of Otology, Rhinology and Laryngology* 2001;110:1120–1125.
20. Garrett CG, Coleman JR, Reinisch L. Comparative histology and vibration of the vocal folds: implications for experimental studies in microlaryngeal surgery. *Laryngoscope* 2000;110(5 Pt 1): 814–824. [PubMed: 10807360]
21. Hahn MS, Kobler JB, Starcher BC, Zeitels SM, Langer R. Quantitative and comparative studies of the vocal fold extracellular matrix. I: Elastic fibers and hyaluronic acid. *Ann Otol Rhinol Laryngol* 2006;115(2):156–164. [PubMed: 16514800]

Figure 1A.

Dissected view of Pig hemilarynx with major components marked as superior vocal fold (SVF), inferior vocal fold (IVF), subglottal wall, supraglottal wall, arytenoid, thyroid cartilage (TC), and cricoid cartilage (CC).

Figure 1B.

Dissected view of sheep hemilarynx with vocal fold, subglottal wall, supraglottal wall, arytenoid, thyroid cartilage (TC), and cricoid cartilage (CC) marked.

Figure 1C. Dissected view of cow hemilarynx cut with vocal fold, subglottal wall, supraglottal wall, arytenoid, thyroid cartilage (TC), and cricoid cartilage (CC) marked.

Figure 2.

Typical force and elongation signals from a pig vocal fold sample. The top panel shows the sample elongation due to the sinusoidal stretch at 1-Hz. The bottom panel shows the force response of the sample measured with ergometer.

Figure 3. Force-elongation hysteresis loops of the same data as Figure 2. Samples are stretched in the upper loop and released in the lower loop.

Figure 4.
Stress-strain relations of 6 samples of pig superior vocal fold.

Figure 5.
Stress-strain relations of 6 samples of pig inferior vocal fold.

Figure 6.
Stress-strain relations of 4 samples of sheep vocal fold.

Figure 7.
Stress-strain relations of 3 samples of cow vocal fold.

Figure 8. Average Young's modulus comparison of vocal fold samples from different species. Each bar represents a single sample with its average on its error bar.