Young's Modulus of Canine Vocal Fold Cover Layers

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Summary: Objectives. The objective of this study was to measure the elastic modulus (Young's modulus) of canine vocal fold cover layers.

Study Design. Basic science study.

Methods. Cover layers from vocal folds of eight canine larynges were dissected. Cover layer samples from the mid-membranous, medial vocal fold surface area were used to measure material stiffness using a previously validated indentation method. Cover layers from two human larynges were also measured as control references. Superior and inferior medial cover layers were measured separately. A total of 15 superior medial surface and 17 inferior medial surface specimens from the canine and two and four specimens, respectively, from the human were tested.

Results. In the canine larynges, the mean Young's modulus of the superior medial surface was 4.2 kPa (range, 3.0–5.4 kPa; standard deviation [SD], 0.6 kPa) and of the inferior medial surface was 6.8 kPa (range, 5.4–8.5 kPa; SD, 0.8 kPa). Measurements on human cover samples were 5.0 kPa (range, 4.7–5.4 kPa; SD, 0.5 kPa) and 7.0 kPa (range, 6.7–7.3 kPa; SD, 0.3 kPa) for the superior medial and inferior medial surface, respectively. Human measurements were similar to the previously validated measurements. There was no difference between the stiffness measurements in the human and canine cover layer samples ($P > 0.05$).

Conclusions. The elastic stiffness (Young's modulus) of the canine and human vocal fold cover layers is similar. Findings support the use of canine larynx as an externally valid model to study voice production.

Key Words: Vocal fold–Laryngeal physiology–Young's modulus–Indentation.

INTRODUCTION

In vivo animal models have been used extensively to understand human laryngeal physiology.[1,2] One of the main advantages of animal models is the ability to perform invasive laryngeal experiments, which would not be ethically possible in humans.[3] The canine is the most commonly used *in vivo* model in laryngeal physiology research. The advantages of the canine larynx include excellent anatomic size match of laryngeal muscles and cartilaginous framework, as well as a nearly identical neuromuscular anatomy that is experimentally accessible for neuromuscular stimulation and measurement of phonatory parameters.[4] However, despite these similarities, the external validity of this model is frequently questioned as there are some differences between the larynges, such as a longer cartilaginous glottis in the canine larynx.[5] Thus, to extend the applicability of research findings from the canine model (or any other model) to human laryngeal physiology, it is important to ensure that the model larynx is as similar to the human larynx as possible.

Many previous reports have compared the gross morphology of the canine larynx and other mammalian species to the human larynx. Cox et al[6] quantified the geometric structure of the cricothyroid (CT) and thyroarytenoid (TA) muscles in human and canine larynges. They found that the basic gross function of the CT and TA muscles were the same, and although the canine CT muscle was slightly larger in cross section, the ratios of mass and cross-sectional areas of the CT and TA muscles between the two species were not significantly different. In considering this slight difference in muscle cross section, it should be remembered that in these studies, postmortem human larynges come from the elderly cohort (who have higher propensity for age-related vocal fold atrophy), whereas the canine larynx is harvested from young healthy animals. Jiang et al[7] compared laryngeal anatomy and function in pig, dog, deer, and human larynges. They found that although vocal fold length was similar in all the animals, the best anatomic match was between the human and the canine larynx, whereas those of the deer and pig were slightly longer. However, unlike the study by Cox et al, they found the CT muscles similar in all the animals. Kim et al[8] compared human, canine, and sheep laryngeal dimensions and found near perfect match between the human and canine larynges in terms of overall dimensions and arc of rotation of CT and cricoarytenoid joints. The ovine larynx was significantly different.

The acoustic output and phonatory aerodynamics of the larynx are dependent not only on the gross vocal fold neuromuscular anatomy, which sets up the glottic phonatory posture[9] but also on the specialized histopathology of the vocal fold cover layer, which facilitates self-sustained oscillation of the glottis.[10,11] The body-cover theory of phonation, which is the most contemporary paradigm for our understanding of voice production, states that the layered histology of the vocal fold can be divided biomechanically into the "body" layer, consisting of the TA muscle and the adjacent deep collagen fibers, and the "cover" layer consisting of the superficial and intermediate lamina propria layer and the vocal fold epithelium.[12] In this model, the fundamental frequency (F_0) of voice is primarily dependent on cover layer tension, which is controlled by muscular forces from the TA and CT muscles, if the amplitude of vocal fold motion is restricted to the cover. The cover layer elastic properties determine its response to the muscular forces

Accepted for publication December 4, 2013.

Financial Disclosure: This study was supported by grant no. RO1DC011300 from the National Institutes of Health.

Conflict of Interest: None.

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Journal of Voice, Vol. 28, No. 4, pp. 406-410

0892-1997/$36.00

http://dx.doi.org/10.1016/j.jvoice.2013.12.003

affecting its tension, and small variations in elastic properties of the cover layer can result in significantly altered phonation.[13] Thus, it is important that an ideal animal laryngeal model not only share similar overall muscular dimensions but also similar cover layer elastic properties.

Although there have been multiple reports comparing the gross morphology of human and canine laryngeal muscles (the body layer) and cartilaginous laryngeal framework, there is a relative paucity comparing the cover layer elastic properties between these species. The purpose of this study was to measure the elasticity of the canine vocal fold cover layer. Herein, we measure the Young's (elastic) modulus of the canine vocal fold cover layers using a previously validated indentation technique that was used to measure the modulus of human vocal fold cover layers.[14]

MATERIALS AND METHODS

The University of California, Los Angeles, Animal Research Committee and the Institutional Review Board approved the use of larynges for research. Eight canine larynges were obtained from humanely euthanized canines used for other approved research protocols. Each canine was of the mongrel breed aged between 1 and 2 years and weighed about 20–25 kg each. We also remeasured the Young's modulus of several human vocal fold cover layers to serve as internal control reference specimens. Two human larynges were harvested from autopsy cases within 48 hours postmortem. All larynges were kept quick-frozen after harvest in −80°C freezer until the day before the experiments, when they were thawed overnight in a refrigerator at 4°C.

On the experiment day, the fully thawed larynges were removed from the refrigerator and allowed to reach room temperature within closed plastic bags on a laboratory workbench for 2–3 hours. The larynges were bisected at the anterior and posterior commissures, exposing the vocal folds. Cover layers were then sharply dissected off from both vocal folds of each larynx using fine iris scissors and ×3.5 magnification from surgical loupes. The cover layer excision proceeded from the infraglottic vocal fold toward the superior medial vocal fold margin and then laterally toward the ventricle on the superior surface of the vocal fold. During the dissection, care was given not to remove any fibers of the TA muscle, which was left attached to the larynx.

The mid-membranous areas of the dissected cover layers were then separated into their three vocal fold surface parts: (1) superior surface, (2) superior medial surface (superior 3–4 mm of the medial vocal fold surface cover layer), and (3) inferior medial surface (inferior 3–4 mm of the medial vocal fold surface cover layer). Measurements of Young's modulus were made immediately after dissection. A detailed theoretical background and methodological considerations of the indentation technique for measurement of vocal fold elastic modulus was described previously and the same apparatus and methodology were used in this study.[14] Samples were placed on the indenter platform with the epithelium surface facing up toward the indenter and kept in contact but not submerged in 0.9% saline solution to keep moist and prevent desiccation (Figure 1). The indenter was mounted onto a force transducer (Shimpo DF-0.5R, 220 g load cell; Shimpo Instruments, Itasca, IL), which was mounted onto a motorized linear traverse (Model MA2506W1- S2.5-0; Velmex, Bloomfield, NY). The motorized linear traverse moved the cylindrical indenter into the sample in a direction perpendicular to the sample. Measurements were made with the 1-mm diameter indenter from the middle of each specimen. The voltage from the strain gauge of the force transducer was amplified by a factor of 100 and recorded with a PC-based AD board (UEI PowerDAQ, 16 bit resolution of 10 V input span, 2000 Hz sampling rate). Before beginning each measurement, the indenter was manually positioned as close as possible to the testing sample, without making contact. During measurements, the indenter was moved by the traverse in steps of 0.02 mm toward the testing sample (loading) and then moved back to its original position (unloading). After a wait time of 1.5 seconds after each traverse movement, the average of the force signal over 0.5 second was recorded as the indentation force (F) for the imposed indentation depth (h).

To maintain consistency of the procedural and measurement techniques with previous indentation measurements on the human larynx, human vocal fold covers served as positive controls. Young's moduli of human vocal fold covers were measured first to ensure that measurements were consistent with previously published data.[14] Once this was confirmed, measurements of the canine samples immediately followed. For each cover sample, Young's modulus was calculated from the slope of the initial portion of the unloading indentation cycle (dF/dh) based on the Hertzian model for a cylindrical contact as described previously.[14] The optimal indentation depth for measurements was about 0.08 mm. This depth was determined after performing numerous loading-unloading cycles and was the indentation depth with linear dF/dh relationship and optimal signal-to-noise ratio. An indentation depth close to 10% of the total sample thickness has been consistently

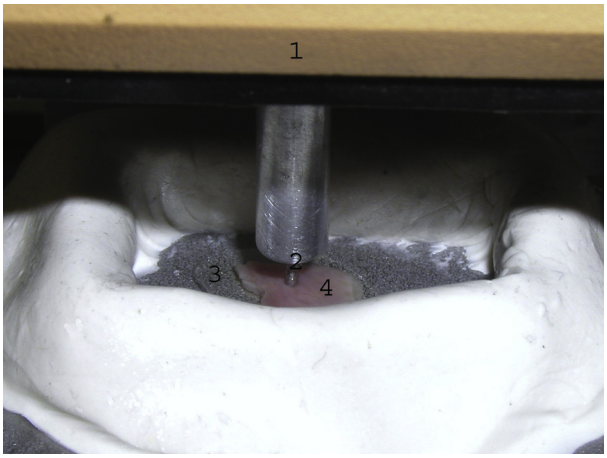

FIGURE 1. Indentation apparatus for measuring the elastic modulus of vocal fold cover layers, showing (1) force transducer, (2) indenter, (3) normal saline to keep specimen moist, and (4) vocal fold cover layer specimen.