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Quantification of Porcine Vocal Fold Geometry

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Abstract

Objective—Quantify porcine vocal fold medial surface geometry and three-dimensional geometric distortion induced by freezing the larynx, especially in the region of the vocal folds.

Study Design—Basic science

Methods—Five porcine larynges were imaged in a microCT scanner, frozen, and rescanned. Segmentations and three-dimensional reconstructions were used to quantify and characterize geometric features. Comparisons were made with geometry data previously obtained using canine and human vocal folds as well as geometries of selected synthetic vocal fold models.

Results—Freezing induced an overall expansion of approximately 5% in the transverse plane and comparable levels of non-uniform distortion in sagittal and coronal planes. The medial surface of the porcine vocal folds was found to compare reasonably well with other geometries, although the compared geometries exhibited a notable discrepancy with one set of published human female vocal fold geometry.

Conclusion—Porcine vocal folds are qualitatively geometrically similar to data available for canine and human vocal folds, as well as commonly used models. Freezing of tissue in the larynx causes distortion of around 5%. The data can provide direction in estimating uncertainty due to bulk distortion of tissue caused by freezing, as well as quantitative geometric data that can be directly used in developing vocal fold models.

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Keywords

porcine vocal folds; vocal fold medial surface geometry; tissue distortion; histological processing; vocal fold modeling

INTRODUCTION

In terms of the underlying physics of voice production, the manner in which the human vocal folds respond to flow through the respiratory airway is primarily governed by the spatial distribution of tissue layers of differing stiffness. Thus two of the most important characteristics of the vocal folds, from a mechanical point of view, are geometry and material properties. A few specific examples include the following: First, geometry is needed to describe mass distribution, which in turn plays an essential role in vocal fold vibratory characteristics. Second, the shapes of the vocal folds' medial surfaces define the shape of the glottal airway, directly influencing glottal air flow. Finally, studies of vocal fold tissue material properties depend on geometric details for interpretation since the spatial distribution of the stiffness of the various tissue layers is fundamentally a geometric description.

Several studies have highlighted and characterized connections between vocal fold geometry and voicing. For example, it has been shown that surgical geometric alterations can cause significant change in voice quality,^{1, 2} that pre-phonatory glottal shape influences vocal register and vibration,³ and that mathematical^{4, 5} and computational^{6, 7} models are highly dependent on initial and boundary conditions (the latter of which, in particular, are directly tied to model geometries). In addition, an understanding of vocal fold geometry is essential for developing and studying anatomically-accurate synthetic vocal fold replicas that exhibit realistic vibratory responses and that thus have the potential to advance voice production research.⁸⁻¹⁰

Considering these factors, it is apparent that accurate geometrical descriptions of the human vocal folds are needed in order to develop a more complete understanding of the physics of voice production, in addition to the clear surgical and clinical implications. Indeed, knowledge of both the geometry and material properties of vocal fold tissue is important for accurate model creation. A significant amount of research has been done to quantify the material properties of the various tissue layers¹¹⁻¹⁸ and has been complemented by a somewhat smaller, though important body of work involving a variety of methods to obtain vocal fold geometric data, including that which is summarized below.

Casting techniques have been used to create detailed three-dimensional (3D) models of the airway in the region of the vocal folds. Berry et al.¹⁹ measured the medial surface geometry of canine larynges using a lost wax technique, and Sidlof et al.²⁰ provided quantitative descriptions of profiles along the medial surface for excised female human larynges using a plaster casting technique. These studies have provided important quantitative information about the geometry of the laryngeal lumen, although more data are needed to more completely quantify airway geometry for a broader population. The casting technique is

limited to providing information about the shape of the medial surface and is not able to be used to quantify geometry of internal vocal fold tissue layers.

Medical imaging modalities, such as X-ray, magnetic resonance (MR), and computed tomography (CT), have yielded vocal fold dimension and shape data, but have not yet been fully exploited to provide complete 3D geometrical descriptions. Agarwal et al.²¹ used laminagraphic tracings of the larynx to obtain dimensions of the vocal folds in one plane. Bakhshaei et al.²² described the use of CT scans to create a generic standard model of the laryngeal framework and models of the airway, but no geometric descriptions were included (e.g., providing equations for surface profiles or making 3D computer models available). Others have used CT imaging to study conditions such as unilateral vocal fold immobility,²³ paralysis,²⁴ or post-surgical airflow characteristics,²⁵ but without full 3D geometric descriptions, instead providing basic geometric measures such as glottal area²⁵ or vocal fold length.²³ Pickup and Thomson¹⁰ created an MRI-based model of the vocal folds, and provided an equation for the medial surface; however, this was only for one patient and no subglottic or supraglottic data were included. In general, CT imaging is well-suited for quantifying geometry of the airway, cartilage, or bulk tissue, but it is not useful for distinguishing between soft tissue layers. MR has potential for imaging soft tissue layers, but it has not yet been widely utilized for imaging vocal fold layer structure because of challenges with spatial resolution and tissue distinction.

Processing histological sections can yield information regarding the geometry of the various vocal fold layers, but the technique is limited to two dimensions and often induces significant tissue deformation. This approach appears to be the best option for generating layer geometric descriptions, but more in-depth knowledge of the geometrical artifacts induced by histologic processing is required in order to rely on geometric data obtained by such methods. Traditional histologic sample processing induces high levels of deformation. Various researchers have shown that resection²⁶ and fixation in formalin²⁷ create significant artifacts in tissue geometry. Johnson et al.²⁶ measured the change in linear dimensions from in situ to post resection, fixation in 10% formalin, and slide preparation for tissue from canine labiobuccal mucosal margins and tongue. They reported a change in linear dimensions of 20 to 40% for tissue resection, 8 to 10% change after fixation, and a further shrinkage of 2 to 10% during slide preparation. Kimura et al.²⁷ compared the geometry in the mid-coronal plane of formalin-fixed and unfixed canine larynges and found that significant shrinkage was caused by the formalin fixation and histological processing. The most shrinkage was observed in the depth (medial-lateral direction) of the vocal fold body, with around 42% shrinkage induced by fixation in formalin and less than 17% induced from histological treatment. The depth of the vocal fold cover exhibited less shrinkage than the body, with less than 20% shrinkage from fixation and less than 20% shrinkage in cover from histological processing. Less shrinkage, on the order of 9% to 24% for fixation, was observed in the thickness (inferior-superior direction), demonstrating the influence of direction on shrinkage.

In order to avoid the excessive shrinkage caused by fixation, Eckel and Sittel²⁸ snap froze human larynges in liquid nitrogen and performed a morphometric analysis of the entire larynx based on horizontal sections. However, since freezing vocal fold tissue still induces

some expansion, accurate quantification of the expansion induced by freezing the tissue would enable better approximation of actual vocal fold dimensions. Tayama et al.²⁹ began this process by measuring the linear shrinkage that occurred in coronal sections of canine larynges. They snap froze the larynges and then measured vocal fold depth and thickness as the larynges thawed in saline. They found that freezing the tissue induced small changes in vocal fold length (<2%), depth (<5%), and thickness (5 to 10%), and that thawed larynges returned to pre-freezing dimensions.

The first purpose of this paper is to build on the work of Tayama²⁹ by quantifying the expansion of the tissue in three dimensions, providing needed insight into multi-dimensional effect of freezing on airway and vocal fold tissue geometry. It is intended as an intermediate step towards a more complete quantitative description of the geometry of the vocal folds, which would entail a three-dimensional description of all of the tissue layers. To obtain information regarding the internal tissue distribution found in the vocal folds, either medical imaging capable of differentiating between the layers or information obtained from histological processing is necessary. However, medical imaging has not yet been demonstrated for vocal fold tissue layer distinction. Though histological processing enables very high spatial resolution, since it induces deformation, quantification of the deformation is necessary to obtain accurate geometry from histology. Therefore, in this paper the three-dimensional-deformation induced by freezing tissue is quantified, which is one step towards quantifying the overall deformation that results from histological processing.

Animal modeling, particularly excised larynx studies, has been an important fixture in voice production research. Though canine vocal folds have been the primary animal model for the study of phonation, in some aspects of voice production, other animals may more closely model human vocal folds. Porcine vocal folds exhibit both similarities and differences with respect to human vocal folds. Some differences include the vibration of the superior folds in pigs³⁰, the incline of the porcine vocal folds in the inferior direction (anterior aspect is more inferior than posterior), as well as differences in phonation threshold pressure, subglottal pressure, sound pressure level, and Young's modulus.³¹ Further, it has been observed that porcine vocal folds lack a mucosal wave and intraglottal phase differences during vibration, limiting the utility of the excised porcine larynx in fluid-structure interaction studies.³² On the other hand, research has shown that for some situations porcine vocal folds serve as a suitable model, especially those focusing on variations in pitch.³⁰⁻³³ Jiang et al.³³ observed that in the thickness and structure of the vocal fold cover, overall vocal fold stiffness, fundamental frequency, and range of phonation, the pig most closely resembles that of human compared with dog and deer. Alipour et al.³¹ found that the frequency range and non-linear stress-strain patterns of pigs most closely resembled those of human when compared with dog, sheep, and cow. Garrett et al.³² found that though the pig only has a two-layer lamina propria, in contrast with the three-layer lamina propria of humans, the composition of the superficial and deep layers are almost identical between the pig and human. Alipour et al.³¹ hypothesized that this similarity is what contributes to the large dynamic range in both species. In addition, the availability of pig larynges is significantly more convenient than canine vocal folds.

Notably, given the advantages of using porcine vocal folds as a model, quantitative descriptions of porcine vocal fold geometry beyond length, depth, and thickness have not been reported. In this paper additional aspects of porcine vocal fold geometry are quantified and compared to the canine vocal fold data of Berry et al.¹⁹, the human data of Sidlof et al.²⁰, and other geometries that have been used for vocal fold modeling purposes. Therefore, the second purpose of this work is to quantify geometric aspects of porcine vocal folds relative to human vocal folds.

METHODS

Five fresh porcine larynges (Fig. 1) were obtained from a local abattoir. Less than six hours from procurement, all five larynges were placed in specimen bags and imaged with a Siemens Inveon MicroPET/CT scanner using Inveon Acquisition Workplace (IAW) software for data acquisition at the University of Wisconsin-Madison. The folds were in the resting position and were placed with the posterior larynx flat on the imaging bed of the scanner. After scanning, the larynges were then frozen with isopentane cooled over liquid nitrogen in order to prevent cracking and then rescanned. Voxel size for all scans was 0.1029 mm along each side, with the interrogation region containing 992 voxels in each dimension.

The scans were then analyzed using two different methods. First, medial surface profiles were extracted from the scans in order to compare porcine vocal fold geometry with other commonly used models. Second, tissue and airway volumes were analyzed to quantify the effects of freezing the tissue on the geometry. These are described in detail below.

Medial Surface Profile

The laryngeal airway was segmented using ITK-SNAP³⁴ for both scans (room temperature and frozen). A sagittal section of the larynx and a segmented airway is shown in Figure 2. The volumes were imported into a computer-aided design (CAD) software package (NX, Siemens PLM Software, and ProEngineer Wildfire 3.0) and a profile of the medial surface of the vocal folds was obtained by defining a reference plane perpendicular to the sagittal plane and perpendicular to the folds, placed halfway between the anterior commissure and the vocal process, as shown in Figure 3. Note the porcine vocal folds are inclined inferiorly at about a 40° angle to the horizontal, as described by Alipour and Jaiswal³⁰ and as can be seen in Figure 2, so this reference plane differs somewhat from the human coronal plane that is typically used to characterize human vocal fold medial surface profiles. The intersection data of the CAD geometry and the reference plane was exported and converted into a series of points in a plane using custom MATLAB codes, yielding medial surface profiles such as shown in Figure 3. Because the superior folds vibrate in pigs³⁰, superior fold geometry was also reported.

Tissue and Airway Volume

The microCT scans were resampled in Analyze 8.1 (AnalyzeDirect, Inc.) such that the voxel size was 0.218 mm along each side. The room temperature and frozen volumes were manually registered to each other in Analyze using the Analyze 3-D Voxel Registration Tool. Since the tissue expanded unevenly with freezing, the manual registration proceeded such

that there was an even amount of overlap across the larynx in all three directions as illustrated in Figure 4. The registered volumes were then exported as a series of transverse plane images. In MATLAB, the tissue was segmented from the surrounding air by thresholding each image and the area of the tissue in each cross section was then calculated.

RESULTS AND DISCUSSION

Medial Surface Profile

The medial surface profile for each larynx was obtained and analyzed. Figure 5 contains the left and right sides of the profiles superimposed on each other for each of the five larynges at room temperature. The horizontal axis corresponds to the sagittal plane, from inferior to superior. The vertical axis denotes medial to lateral sense. A degree of natural asymmetry on the order of 10 to 30% between the left and right sides is manifest.

In order to account for size variations between samples, profiles from each larynx were normalized relative to a characteristic height (the average distance from the most superior point to the middle of the superior folds) and a characteristic width (the average distance from the sagittal plane to the edge of the true vocal fold), and are plotted in Figure 6a for the room temperature larynges and Figure 7a for the frozen larynges. The average of the profiles with one standard deviation above and below the mean for the room temperature and frozen larynges, respectively, is shown in Figures 6b and 7b. The normalized, average profile from the room temperature larynx could be used in vocal fold modeling. Since the shape is not able to be readily described by an equation, the data will be made available in the online data repository www.larynxdata.org.

The averaged profiles from the room temperature and frozen scans are shown together in Figure 8. No consistent variation in either the medial-lateral or inferior-superior direction is evident, other than the fact that the room temperature inferior folds were not all fully adducted in the mid-membranous plane. However, it is clear from the standard deviation plots in Figures 6 and 7 that distortion was introduced by freezing the tissue. Tayama et al.²⁹ measured canine vocal fold geometry in the mid-membranous coronal plane at various stages of thawing after quick freezing. They showed that the frozen tissue expands slightly, around 5%, though with thawing then returns to within 1 to 2% of pre-frozen dimensions. This is comparable to the level of distortion seen in the present study.

The medial surface profiles from one of the room temperature larynges at locations other than the mid-membranous section are shown in Figure 9; profiles correspond to planes offset 2 mm anterior and posterior to the mid-membranous profile, as denoted in Figure 9a. Slight variation of the porcine vocal folds can be seen, but in general the shape of the vocal folds is similar in the anterior-posterior direction. Even though the vocal folds are inclined inferiorly, there is little variation in the direction of the length of the folds. The ability to neglect variation in the third dimension significantly simplifies modeling the shape of the vocal folds for computational and experimental studies.

The averaged profile obtained from the porcine folds at room temperature is compared with data obtained by other researchers and two models in Figure 10. Berry et al.¹⁹ provided an

equation for the surface profile of canine larynges, and the profile shown here corresponds to the profile midway between the anterior and posterior sides of the surface. Sidlof et al.²⁰ reported data for two female human larynges, and the data shown here is the mid-membranous coronal section from their specimen labeled Larynx 8 when it was positioned for phonation at 148 Hz. The Agarwal et al.²¹ data is for human false fold geometry obtained from coronal laryngeal laminagrams near the midmembranous location of the vocal fold length; the combined mean data for non-singers is shown. The shape of two models is also included: the commonly used M5 model from Scherer et al.³⁵ and a recent variation of the M5 model, used by Murray and Thomson⁸, the primary variation being a larger inferior glottal radius. This latter model is referred to as the EPI model (due to the inclusion of an epithelial layer in the model).

All models and vocal fold data are qualitatively similar, although some significant quantitative differences existed. The porcine mid-membranous profile from the present study is similar to other commonly used models of human vocal folds and the EPI profile is remarkably similar to the canine data of Berry et al.¹⁹ Differences can be seen in the inlet and exit radii and the slopes of the inferior regions. The shape of the superior porcine fold is relatively similar to that of the human false folds, as reported by Agarwal et al.²¹, though it should be noted that in the pig, the superior folds also oscillate, whereas the false folds generally do not oscillate significantly during normal human phonation.²¹ The data of Sidlof et al.²⁰ exhibited notable differences compared with that of the others, possibly because the larynx was from a female and/or due to the folds having been postured for phonation prior to casting. More data are clearly needed in order to accurately quantify vocal fold geometry, including the vast individual variations that are known to occur.

Alipour and Jaiswal³⁰ found that the superior fold also oscillates in pigs. Removal of the superior folds to make them more geometrically similar to excised human vocal folds should be done with caution since doing so changes a fundamental aspect of in vivo porcine vocal fold vibration. Alternatively, leaving the false folds in place and allowing them to vibrate creates a different situation from that of either in vivo (false folds in place) or excised (false folds frequently removed) human vocal folds. No model perfectly resembles human vocal folds, but this significant difference should be considered when working with porcine larynges.

Tissue and Airway Volume

The changes in tissue and airway area in all three dimensions due to freezing are here reported and discussed. Plots of area at each transverse cross section along the inferior-superior direction for the room temperature and frozen larynges are shown in Figure 11. In this plane the tissue generally seems to expand when frozen.

The percent difference in area between the room temperature and frozen larynges at each cross section for each of the five larynges is shown in Figure 12. The difference is on the order of 5%, but none of the larynges exhibit a uniform level of expansion at each cross section.

The tissue volume was calculated by summing all of the areas from all of the cross sections, and the changes in volume between the room temperature and frozen larynges were

calculated; see Table 1. All changes are positive, meaning that the tissue expanded, as would be expected. The average change in volume for all the larynges was 5%, but as shown in Figure 12, the expansion is not uniform throughout the volume. Therefore, one cannot predict a change of 5% in any given dimension, even if it lies in the transverse plane.

The area of each airway is shown in Figure 13. Note that the areas of the secretory laryngeal gland ducts or other sinus ducts were not considered. The distortion in the geometry caused by freezing is much less uniform—in some places, the airway constricted, due to inward displacement of the tissue, while in other places the airway was enlarged.

Figures 14 and 15 show the area of the tissue at cross sections in the sagittal and coronal plane, respectively, along with the area of the airway. No consistent changes in area between room temperature and frozen specimens are observable. The tissue expansion in the transverse plane is accompanied by non-uniform distortions in the other areas. Given the wide variety of tissue types present in the larynx, including cartilage, muscle, and soft tissue, each of which would likely tend to expand differently when frozen, such variation is not surprising. It is therefore evident that no generalizable description of the expected direction of distortion in a single coronal cross section can be given, which is unfortunate given that a single coronal cross section of the vocal folds provides the most detail regarding the distribution of the internal layers of the vocal fold tissue. However, the present data do provide some idea of the degree of distortion that could be expected. The data in Table 1 suggest that freezing can be expected to cause distortion on the order of five percent.

CONCLUSION

Despite the fact that the geometry plays such an important role in phonation, relatively little quantitative information is available regarding the shape of the vocal folds, particularly that of the internal layers. This is due to the challenge of obtaining such information—the difficulty associated with obtaining sufficient spatial resolution in medical imaging and the distortion associated with histological processing. The present work addresses one part of the challenge of obtaining accurate geometrical information from histological processing by quantifying the overall three-dimensional effect of freezing, one step in a common method of fixation shown to have less distortion, on tissue geometry.

By comparing the medial surfaces at the mid-membranous section of the vocal folds, it is shown that freezing does cause a geometrical distortion of the tissue, but not in a consistent manner. Overall, the tissue experiences about a 5% change in volume in the entire larynx, but this does not appear to cause consistent or uniform shrinkage or expansion in any one direction in the region of the vocal folds.

The present work also quantifies the geometry of the medial surface of porcine vocal folds, and compares it with human and canine vocal folds and various synthetic model geometric data. The shape is similar to the other comparisons, though the porcine folds appear to be slightly thicker. It is difficult to compare exactly since the human data was from females; thus more human geometric data is needed for a more general comparison. Other obvious differences between pig and human vocal folds exist, such as the underlying tissue structure

and the 40 degree downward angle of the porcine folds, but otherwise it is evident that the geometry of porcine vocal folds is relatively similar to that of canine and human vocal folds.

As model development and geometrical quantification of the vocal folds becomes more sophisticated and extends into three dimensions, description of the vocal folds with drawings and simple splines becomes quite limiting. Therefore, to facilitate access by other researchers, all data described in this paper will be made available at www.larynxdata.org, including the original microCT scans and the curve describing the medial surface of the porcine vocal folds.

Though traditional medical imaging does not yet allow the differentiation of tissue layers, the technology is continually improving and the future development of such capabilities seems likely. The method outlined in this paper to go from medical images to a 2D profile describing the geometry could be applied to obtain descriptions of the different tissue layers on excised human larynges, and even to in vivo humans, once this level of detail becomes technologically feasible. One obvious limitation of histological processing is that the excised tissue cannot be postured for voicing in precisely the same way as that which occurs in vivo. Even when the vocal folds are adducted via sutures or other means for phonation, the nerves controlling muscles activation are not stimulated, thus geometry obtained in this manner is still only a limited representation of in vivo conditions. The ability to posture the folds for speech is a major advantage of in vivo medical imaging and will certainly be used in the future to obtain more accurate geometrical models of the vocal folds. However, until that time, the methods described in this paper can contribute to an overall estimate of the accuracy of future geometries derived from histological section processing.

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(a)

(b)

FIGURE 1.
Photographs of porcine Larynges 1 (a) and 2 (b).

FIGURE 2.
Sagittal view of porcine larynx (left) and computer rendering of segmented porcine laryngeal airway (right).

FIGURE 3.
Medial surface profile (left) and airway CAD model rendering (right).

FIGURE 4.

Manual registration of Larynx 1 at room temperature (left column) to Larynx 1 when frozen (right column). The center column includes the overlap in yellow, the frozen tissue in green, and the room temperature tissue in red. The three rows are the three orthogonal views of the larynx that roughly correspond to the transverse, coronal, and sagittal planes. The yellow, red, and green lines show the locations of the coronal, sagittal, and transverse planes, respectively.

FIGURE 5. Left and right medial surface profiles of Larynges 1 through 5 (a through e, respectively) at room temperature.

FIGURE 6.

(a) Average of left and right medial surface profiles of the five room temperature larynges, normalized. (b) Mean profile (solid line) of all five larynges, including left and right sides, and envelope corresponding to plus and minus one standard deviation (dotted line).

FIGURE 7.

(a) Average of left and right medial surface profiles of the five frozen larynges, normalized.

(b) Mean profile (solid line) of all five larynges, including left and right sides, and envelope corresponding to plus and minus one standard deviation (dotted line).

FIGURE 8.
Mean frozen (dashed line) and room temperature (solid line) profiles

FIGURE 9.

(a) Location of planes anterior and posterior to the mid-membranous plane. (b) Profiles from three planes: 2 mm anterior (dashed lines), mid-membranous (solid lines), 2 mm posterior (dotted lines).

FIGURE 10.

Medial surface profile from present study along with profiles from human and canine data as well as with models from other studies.

FIGURE 11.

Area of the tissue in the transverse plane for Larynges 1 through 5 (a through e), including room temperature (solid lines) and frozen (dashed lines). The horizontal and vertical scales of plots a through d are the same as those denoted by the axis labels in plot e.

FIGURE 12.
Normalized difference between room temperature and frozen larynges.

FIGURE 13.

Cross-sectional area of the airway in the transverse plane for Larynges 1 through 5 (a through e) when the tissue was at room temperature (solid lines) and frozen (dashed lines).

FIGURE 14.

Tissue (larger) and airway (smaller) areas (e.g., as illustrated in plot e) in the sagittal plane for Larynges 1 through 5 (a through e), including room temperature (solid lines) and frozen (dashed lines).

FIGURE 15.

Tissue (larger) and airway (smaller) areas in the coronal plane for Larynges 1 through 5 (a through e), including room temperature (solid lines) and frozen (dashed lines).

TABLE 1

Percent change in volume between room temperature and frozen larynges

Larynx	Percent Change in Volume
1	4.6
2	5.1
3	4.4
4	5.4
5	3.0
Average	4.5