Quantification of Porcine Vocal Fold Geometry

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Summary: Objective. The aim of this study was to 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. The medial surface geometries of five excised porcine larynges were quantified and reported.

Methods. Five porcine larynges were imaged in a micro-CT 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 nonuniform 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.

Conclusions. 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.

Key Words: 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 airflow. Finally, studies of vocal fold tissue material properties depend on geometric details for interpretation because 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 changes in voice quality,[1,2] that prephonatory 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 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 layers[11–18] and has been complemented by a somewhat smaller, although important body of work involving a variety of methods to obtain vocal fold geometric data, including that which is summarized in the following.

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

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obtain dimensions of the vocal folds in one plane. Bakhshaee 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 (eg, 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 postsurgical airflow characteristics,[25] but without full 3D geometric descriptions, instead providing basic geometric measures such as glottal area[25] or vocal fold length.[23] Pickup and Thomson[10] 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 used 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 to rely on geometric data obtained by such methods. Traditional histologic sample processing induces high levels of deformation. Various researchers have shown that resection[26] and fixation in formalin[27] create significant artifacts in tissue geometry. Johnson et al[26] measured the change in linear dimensions from in situ to postresection, fixation in 10% formalin, and slide preparation for tissue from canine labiobuccal mucosal margins and tongue. They reported a change in linear dimensions of 20–40% for tissue resection, 8–10% change after fixation, and a further shrinkage of 2–10% during slide preparation. Kimura et al[27] compared the geometry in the midcoronal plane of formalin-fixed and formalin-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 9–24% for fixation, was observed in the thickness (inferior-superior direction), demonstrating the influence of direction on shrinkage.

To avoid the excessive shrinkage caused by fixation, Eckel and Sittel[28] snap froze human larynges in liquid nitrogen and performed a morphometric analysis of the entire larynx on the basis of horizontal sections. However, because 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 (5–10%), and that thawed larynges returned to prefreezing dimensions.

The first purpose of this article was to build on the work of Tayama et al[29] by quantifying the expansion of the tissue in three dimensions, providing needed insight into multidimensional effect of freezing on airway and vocal fold tissue geometry. It is intended as an intermediate step toward a more complete quantitative description of the geometry of the vocal folds, which would entail a three-dimensional description of all 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. Although histological processing enables very high spatial resolution because it induces deformation, quantification of the deformation is necessary to obtain accurate geometry from histology. Therefore, in this article, the three-dimensional deformation induced by freezing tissue is quantified, which is one step toward quantifying the overall deformation that results from histological processing.

Animal modeling, particularly excised larynx studies, has been an important fixture in voice production research. Although 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 pigs,[30] 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] Furthermore, 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 nonlinear stress-strain patterns of pigs most closely resembled those of human when compared with dog, sheep, and cow. Garrett et al[32] found that although 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 is 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.