FUNCTIONAL DEFINITIONS OF VOCAL FOLD GEOMETRY FOR LARYNGEAL BIOMECHANICAL MODELING

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Precise geometric data on vocal fold dimensions are necessary for defining the vocal fold boundaries with respect to the laryngeal framework in physiological and biomechanical models of the larynx (eg, finite-element models). In the mid-membranous coronal section, vocal fold depth can be defined as the horizontal distance from the vocal fold medial surface to the thyroid cartilage, whereas vocal fold thickness can be defined as the vertical distance from the inferior border of the thyroarytenoid muscle to the vocal fold superior surface. Traditionally, such geometric data have been obtained from measurements made on histologic tissue sections. Unfortunately, it is very difficult to obtain reliable data by this method, unless the effects of sample preparation on vocal fold geometry are quantified. Significant tissue deformations are often induced by histologic processes such as fixation and dehydration, sometimes producing shrinkages as large as 30%. In this study, reliable geometric data of the canine vocal fold were obtained by the alternative method of quick-freezing for sample preparation, using liquid nitrogen. Coronal sections of quick-frozen larynges were thawed gradually in saline solution. Images of the mid-membranous coronal sections at various thawing stages were captured by a digital camera. Measurements of operationally defined vocal fold dimensions (depth and thickness) useful for biomechanical modeling were made with a graphics software package. The results showed that geometric changes of the vocal fold induced by freezing are likely reversed by thawing, such that the measurements made on thawed larynges are reliable approximations of the actual vocal fold dimensions.

KEY WORDS — biomechanical modeling, quick-freezing, thickness, vocal fold depth, vocal fold geometry.

INTRODUCTION

In the study of voice production, excised larynges from humans and animals have often been used. The canine larynx has frequently been used because of its relative ease of acquisition and its similarities in size and in basic anatomy with the human larynx. For instance, active contractile properties and passive stress-strain characteristics of the canine intrinsic laryngeal muscles have been measured previously[1-3] (also Tayama et al, unpublished data). These data are important for the physiological and biomechanical modeling of voice production. Geometric data on the larynx are also important information for biomechanical modeling, including the 3-dimensional orientations of the intrinsic laryngeal musculature,[4] as well as the dimensions of the laryngeal cartilage framework[5] and the vocal fold. The mechanics of phonation are critically dependent on the geometry of the vocal folds. For example, it has been shown that phonation threshold pressure, the minimum subglottal pressure required to initiate and sustain vocal fold oscillation, is dependent on the thickness of the vocal fold and the shape (convergence or divergence) of the glottis.[6] Differences in vocal fold geometry between subjects of different age and gender, and between humans and other species, are also valuable information for studies of their differences in voice production.

Vocal fold structure is often discussed on the basis of the body-cover theory.[7] Previous studies have reported geometric data on the cover, which is the major vibratory portion of the vocal fold.[8-10] From histologic tissue sections, Hirano et al[11,12] reported that the "thickness" of the vocal fold cover was 1.0 to 1.5 mm in the human larynx. Kurita et al[13] measured the "thickness" of the vocal fold mucosal layer in humans and in different animals, including dogs. They reported that it was around 3 mm in dogs. From grossly dissected canine vocal folds, Perlman and Durham[14] reported the "thickness" of the cover to be

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A previous version of this paper was presented at the 138th meeting of the Acoustical Society of America, Columbus, Ohio, November 1-5, 1999.

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TABLE 1. SUBJECT INFORMATION

Canine Subject	*Sex*	*Weight (kg)*
C1	M	20
C2	M	20
C3	M	20
C4	M	27
C5	M	22
C6	M	30
C7	F	25
C8	M	27
C9	F	23
C10	M	20
C11	F	19
C12	F	20
C13	F	21
C14	M	23
C15	F	25

around 2.5 mm in male dogs and 2.0 mm in female dogs.

In the above studies, the "thickness" of the vocal fold measured was not well defined geometrically, and the "depth" of the vocal fold was not measured. Therefore, these data are not optimal for the purpose of biomechanical modeling, which requires precisely defined measurements positioned in a coordinate system with respect to the laryngeal cartilage framework. For example, the vocal fold "thickness" in those studies was only measured at 1 point, and it was measured arbitrarily (in an oblique direction[11-13]), rather than in a well-defined coordinate system. A larger number of data points are needed to express more realistically the geometric contour of the vocal fold, but not so many data points that they would compromise the computational efficiency of the biomechanical models.

There are also methodological problems associated with the measurements of vocal fold geometry in the above studies. The vocal folds are made of soft tissues. In many studies, geometric measurements have been made on the mid-membranous coronal section of the vocal fold prepared histologically. Unfortunately, geometric deformations of different types of soft tissues are commonly observed during histologic sample preparation processes such as formalin fixation and dehydration. For example, shrinkages of human oral tissues as large as 30% have been reported.[15] The above reports on vocal fold dimensions did not consider these possible tissue shrinkages.

To minimize tissue deformation, Eckel and Sittel[16] measured the geometry of the human larynx in horizontal tissue sections using the alternative method of quick-freezing for sample preparation, instead of using histologic methods. Quick-freezing with liquid nitrogen has been shown to induce only minimal changes in the dynamic mechanical properties of vocal fold tissues.[17] It has also been commonly used for tissue sample preparation in electron microscopy[18] and in tissue culture.[19] Even with quick-freezing, however, there could still be some tissue deformation (eg, about 6% expansion in human head and neck muscles[20]). Still, quick-freezing is probably the best technique for the preservation of soft tissues, and causes only minimal changes in geometry. Although frozen tissues would expand in volume, the deformation is considerably less than that caused by histologic preparation methods.[16,18] The rate of deformation is different in different types of tissues with different processing methods. If we could quantify how much the vocal fold deforms during quick-freezing and across different stages of thawing, the actual dimensions of the vocal fold in vitro could be approximated.

This study attempted to operationally define the "depth" and "thickness" of the vocal fold with respect to the laryngeal cartilage framework, in order to efficiently represent the vocal fold geometry in biomechanical models of the larynx (eg, finite-element models[21]). We attempted to obtain reliable geometric data on the canine vocal folds by comparing frozen and thawed larynges. Canine tissues were used because they could be obtained immediately after death, while it was practically not possible to obtain fresh-enough human larynges. It was important to use fresh tissues so that the stages of rigor mortis would not induce significant tissue deformation before the vocal fold geometry could be preserved by quick-freezing. The geometric data on the canine vocal fold obtained in the present study are useful for functionally defining the vocal fold boundaries with respect to the laryngeal framework in biomechanical models of the larynx.

METHOD

Subjects. Fifteen canine larynges were excised within 10 minutes of death after cardiovascular experimentation and euthanasia of the animals, in accordance with the Institutional Animal Care and Use Committee of the University of Iowa. All specimens were obtained from adult mongrel dogs without evidence of laryngeal trauma or head and neck diseases (see Table 1 for subject information). No animals were sacrificed specifically for the present investigation.

Sample Preparation. For each larynx, soft tissues superior to the true vocal folds were removed, including the epiglottis, aryepiglottic folds, and false vo-

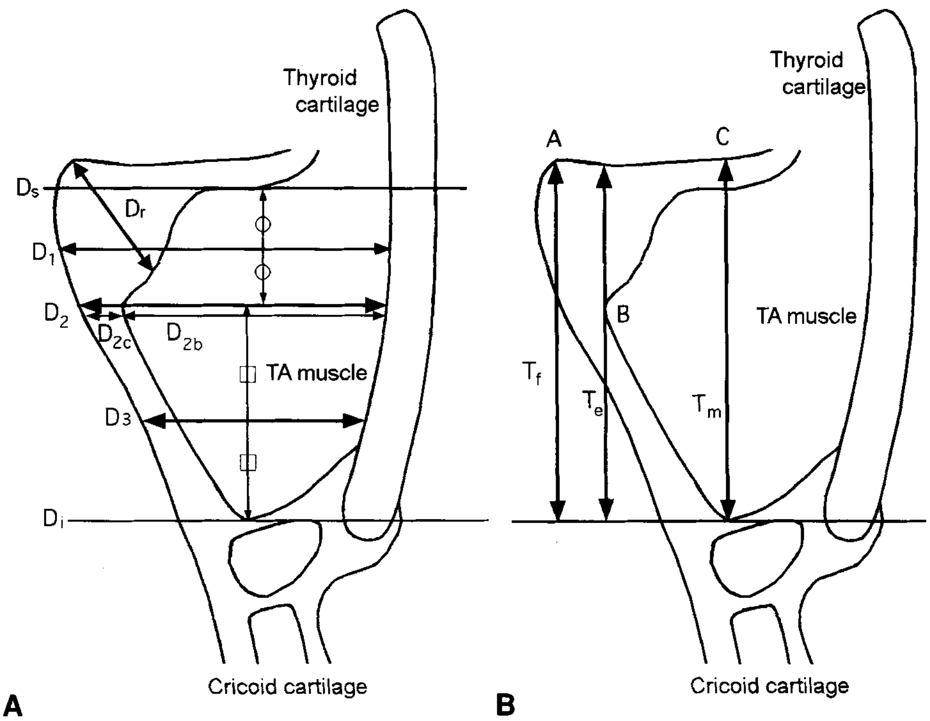

Fig 1. Schematic illustrations of operational definitions of **A**) vocal fold depth and **B**) vocal fold thickness in mid-membranous coronal section. See text for definitions.

cal folds. In order to measure vocal fold length, we made marks on the anterior commissure, the mid-membranous point, and the vocal process of each vocal fold with black India ink before freezing. The larynx was quickly frozen by chilling in liquid nitrogen (boiling point, −196°C). It was mounted by securing the trachea over a piece of polyvinyl chloride tube with an O-clamp around the first and second tracheal rings. Other clamps were also used to hold the thyroid and cricoid cartilages in order to securely fix the larynx. The vocal fold length of 10 larynges (C1 to C10), defined as the distance between the anterior commissure and the vocal process, was measured by a digital caliper before and after freezing. It was also measured after thawing for 5 of the 10 larynges (C1 to C5). In order to measure vocal fold

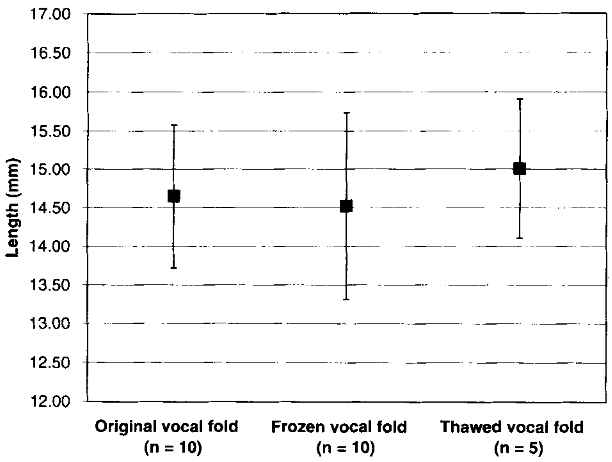

Fig 2. Vocal fold length changes with quick-freezing and thawing (mean length ± SD).

depth and thickness, 5 larynges (C11 to C15) were cut at the mid-membranous point in the coronal plane while they were still frozen, by means of a custom-built microtome. Thawing of the larynges was carried out in saline solution at room temperature (20°C). Images of the larynges in the mid-membranous coronal section were captured at the following times for subsequent geometric measurements: 1) during the frozen stage and 2) every 30 minutes during thawing, for up to 3 hours.

Image Acquisition. Scaled images of the vocal fold in the mid-membranous coronal section were captured with a digital camera (Ricoh, DC-3Z) with a spatial resolution of 640 × 480 pixels. The length resolution was 0.052 to 0.056 mm. The distortion of the digital images was less than 2% with the camera set at the condensed area mode. Image data in JPEG format were transferred to a computer, on which measurements of vocal fold depth and thickness were made with a graphic software package (Adobe Photoshop).

Definitions of Geometric Measurements. In the mid-membranous coronal section, the depth of the vocal fold (D) was defined as the distance from the medial surface of the vocal fold to the thyroid cartilage on a horizontal line, as shown schematically in Fig 1A. As this distance changes along the superior-inferior direction, it was defined at 5 different levels (Ds, Di, D1, D2, and D3) that can be clearly and consistently identified across larynges. Ds was defined at the level of the superior border of the thyroarytenoid (TA) muscle, while Di was defined at the level of the inferior border of the TA muscle. D_2 was defined at the level at which the medial edge of the TA muscle suddenly changes its curvature, such that the mucosal layer of the vocal fold (cover) becomes increasingly thicker superior to this level. D_1 was arbitrarily defined at a level equidistant to both D_s and D_2, whereas D_3 was defined at a level equidistant to both D_2 and D_i. In addition, each depth was also divided into 2 portions, one being the depth of the mucosal layer (the cover, D_c) and the other being the depth of the muscular layer (the body, D_b). Such definitions provide an efficient and functional representation of the vocal fold contour for biomechanical modeling. For comparison with previous studies, a reference "depth," D_r, is also shown in Fig 1A, which was defined in an arbitrarily oblique direction similar to the method of Hirano et al[11,12] and Kurita et al.[13]

Vocal fold thickness was defined as the distance from the inferior border of the TA muscle to the vocal fold superior surface on a vertical line (Fig 1B). It was defined at 3 specific points (A, B, and C) that can be consistently identified across larynges, in order to capture the change of the vocal fold contour

TABLE 2. CHANGES OF VOCAL FOLD LENGTH AS RESULT OF QUICK-FREEZING AND THAWING

Subject	*Original Vocal Fold*	*Frozen Vocal Fold*	*Thawed Vocal Fold (at 2 h)*
C1	14.74	14.57	15.06
C2	13.42	12.91	13.48
C3	14.65	16.38	15.54
C4	15.24	15.62	15.21
C5	15.41	14.87	15.76
C6	16.32	15.54	MD
C7	14.67	13.35	MD
C8	13.54	12.79	MD
C9	13.57	14.23	MD
C10	14.93	15.00	MD
Mean	14.65	14.53	15.01
SD	0.93	1.21	0.90

Data are in millimeters. MD — missing data.

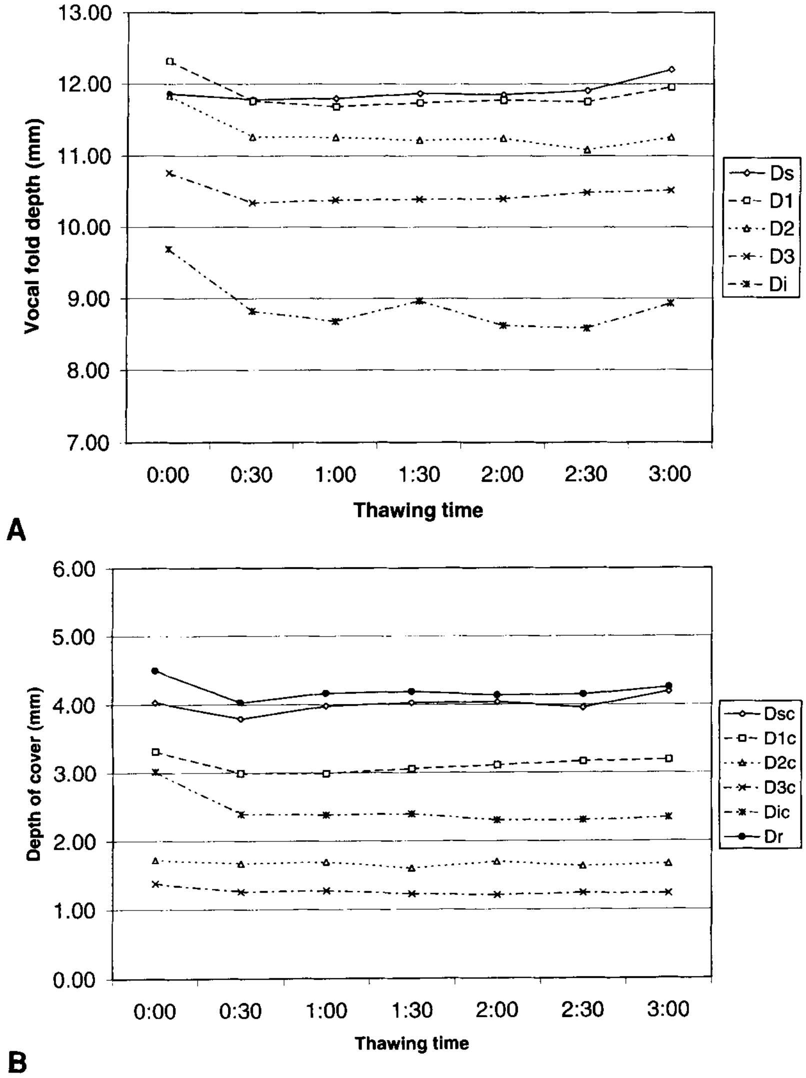

Fig 3. Mean vocal fold measurements as function of thawing time (n = 5 in each Figure). See text for definitions. **A)** Total depth. **B)** Depth of cover. (Continued on next page.)

along the medial-lateral direction: Tf was defined at the vocal fold free edge (point A: the "free-edge thickness"); Te was defined at the medial border of the TA muscle (point B: the "effective thickness"), at the level at which it suddenly changes its curvature (as in the definition of D2); and Tm was defined at the mucosal surface vertically above the inferior border of the TA muscle (point C: the "mucosal-muscle thickness").

These definitions of vocal fold depth and thickness emphasize the physiological and functional importance of the TA muscle in the control of vocal fold geometry, as it has been demonstrated that contraction of the TA muscle tends to bulge out the inferior portion of the vocal fold medially, creating a deeper and thicker vocal fold with a more rectangular glottal shape.[22] Furthermore, many of the definitions are based on the level at which the medial border of the TA muscle suddenly changes its curvature (point B in Fig 1B), which has previously been identified to be the point of mucosal wave upheaval.[23] Thus, the vocal fold depth D2 and the effective thickness Te, defined directly with respect to this point, should effectively capture the dimensions of the vibratory portion of the vocal fold. Data based on these definitions of vocal fold depth and thickness provide an efficient and functional representation of the vocal fold boundaries with respect to the laryngeal framework for biomechanical modeling applications.

RESULTS AND DISCUSSION

Vocal Fold Length. Figure 2 shows the changes of vocal fold length associated with the quick-freezing and thawing processes. Length measurements were obtained for 10 subjects (C1 to C10) before freezing (original in situ length) and after freezing (frozen length). Data were also obtained after 2 hours of thawing for 5 of the 10 subjects (C1 to C5). Because coronal sections were prepared for the other 5 subjects (C6 to C10) while the larynges were still frozen, length measurements made after thawing were not available for them. The average length of the vocal folds for the 10 subjects (C1 to C10) was 14.65 mm before freezing and 14.53 mm after freezing (ap-

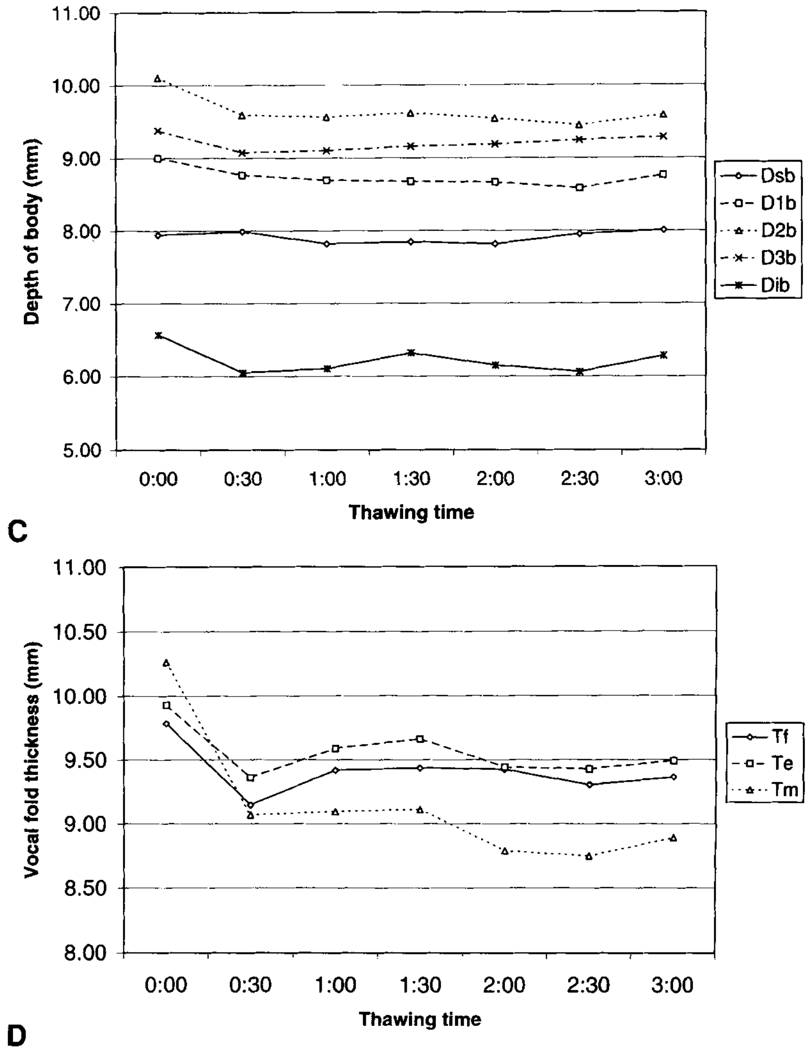

Fig 3, continued. **C)** Depth of body. **D)** Thickness.

TABLE 3. CHANGES OF TOTAL VOCAL FOLD DEPTH ACROSS FROZEN AND THAWING STAGES

			Thawing Time					
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
D_1	C11	11.33	11.18	11.11	11.33	11.22	11.40	11.42
	C12	10.97	9.99	10.14	10.67	10.21	10.31	11.11
	C13	12.15	12.21	11.68	11.82	12.07	12.11	12.00
	C14	13.10	12.38	12.50	12.18	12.03	11.78	11.85
	C15	14.05	13.06	13.02	12.70	13.36	13.17	13.40
	Mean	12.32	11.76	11.69	11.74	11.78	11.75	11.96
	SD	1.27	1.20	1.14	0.78	1.16	1.04	0.88
D_2	C11	11.01	10.54	10.48	10.71	10.59	10.67	10.72
	C12	10.72	10.26	10.57	10.84	10.44	10.46	11.05
	C13	11.80	11.56	10.92	10.86	10.94	10.96	10.87
	C14	12.51	11.70	12.07	11.71	11.52	11.01	11.22
	C15	13.11	12.27	12.27	11.98	12.72	12.32	12.43
	Mean	11.83	11.27	11.26	11.22	11.24	11.08	11.26
	SD	1.00	0.84	0.85	0.58	0.93	0.72	0.68
D_3	C11	10.36	9.48	9.48	9.56	9.40	9.77	9.75
	C12	10.36	9.88	9.99	10.24	9.91	9.77	10.44
	C13	10.76	10.97	10.92	11.09	11.58	11.94	11.19
	C14	10.95	10.75	10.60	10.52	10.15	10.22	10.17
	C15	11.38	10.62	10.91	10.55	10.94	10.74	11.03
	Mean	10.76	10.34	10.38	10.39	10.40	10.49	10.52
	SD	0.43	0.63	0.63	0.56	0.86	0.90	0.60
D_i	C11	8.98	7.98	7.58	7.79	7.48	7.48	7.68
	C12	10.40	9.67	9.78	10.14	9.76	9.69	10.18
	C13	MD	MD	MD	MD	MD	MD	MD
	C14	MD	MD	MD	MD	MD	MD	MD
	C15	MD	MD	MD	MD	MD	MD	MD
	Mean	9.69	8.82	8.68	8.97	8.62	8.58	8.93
	SD	1.00	1.19	1.55	1.66	1.61	1.56	1.77
D_s	C11	11.82	11.35	11.59	11.48	11.39	11.71	11.95
	C12	10.40	9.35	9.25	9.79	9.66	9.59	10.28
	C13	11.96	12.58	12.16	12.20	12.24	12.73	12.88
	C14	13.29	12.63	12.82	12.80	12.57	12.16	12.34
	C15	MD	13.00	13.19	13.08	13.40	13.35	13.55
	Mean	11.86	11.78	11.80	11.87	11.85	11.91	12.20
	SD	1.18	1.49	1.55	1.31	1.42	1.44	1.23

Data are in millimeters. See text for various definitions of total depth D. MD — missing data.

proximately a 1% decrease). For the 5 subjects in which measurements were also made after 2 hours of thawing (C1 to C5), the average vocal fold length was 14.69 mm before freezing, 14.87 mm after freezing (approximately a 1% increase), and 15.01 mm after thawing (another 1% increase). Table 2 shows the individual data for vocal fold length.

These results suggested that the magnitude of 1-dimensional changes in vocal fold tissue geometry as a result of quick-freezing and thawing was minimal (ranging from −1% to 2%) and was probably within the limits of measurement errors. It seemed that the method of quick-freezing, which has been commonly used for tissue sample preparation in electron microscopy and in tissue culture, indeed resulted in considerably less tissue deformation than that induced by histologic techniques of sample preparation (as much as 30% shrinkage).[15]

Vocal Fold Depth. Figure 3A-C shows the changes of vocal fold depth as a function of freezing and thawing, in terms of the different definitions of vocal fold depth illustrated in Fig 1A. Figure 3A shows the total depth D (body plus cover), which varied from approximately 8.6 to 11.8 mm along the superior-inferior direction across the thawing stages. The average depth D_s was 11.86 mm in the frozen stage and was 11.78 to 12.20 mm across the thawing stages (99% to 103% of the frozen dimensions). The average D_1 was 12.32 mm in the frozen stage and was 11.69 to 11.96 mm across the thawing stages (95%

TABLE 4. CHANGES OF VOCAL FOLD DEPTH OF COVER ACROSS FROZEN AND THAWING STAGES

			Thawing Time					
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
D_{1c}	C11	3.38	3.10	3.11	3.06	3.00	3.04	3.15
	C12	3.27	2.29	2.24	2.55	2.36	2.59	2.66
	C13	3.34	3.53	3.54	3.61	3.66	3.78	3.68
	C14	2.41	2.16	2.16	2.17	2.42	2.48	2.45
	C15	4.17	3.89	3.91	3.90	4.12	3.95	4.02
	Mean	3.31	2.99	2.99	3.06	3.11	3.17	3.19
	SD	0.63	0.76	0.78	0.72	0.77	0.67	0.66
D_{2c}	C11	2.03	2.18	2.05	2.08	2.15	2.15	2.13
	C12	1.61	1.64	1.82	1.67	1.91	1.72	1.88
	C13	1.45	1.27	1.10	1.07	1.04	1.09	1.13
	C14	1.24	1.10	1.24	1.03	1.06	0.90	0.89
	C15	2.31	2.16	2.28	2.17	2.35	2.32	2.32
	Mean	1.73	1.67	1.70	1.60	1.70	1.63	1.67
	SD	0.44	0.50	0.51	0.54	0.61	0.63	0.63
D_{3c}	C11	1.39	1.50	1.48	1.40	1.29	1.35	1.27
	C12	1.66	1.49	1.65	1.73	1.72	1.76	1.83
	C13	0.88	0.85	0.71	0.78	0.81	0.81	0.81
	C14	1.21	1.00	1.07	0.88	0.85	0.95	0.94
	C15	1.76	1.46	1.46	1.35	1.38	1.33	1.30
	Mean	1.38	1.26	1.28	1.23	1.21	1.24	1.23
	SD	0.36	0.31	0.38	0.39	0.39	0.37	0.40
D_{ic}	C11	3.04	2.62	2.15	2.34	2.24	2.15	2.34
	C12	3.20	2.92	3.00	2.96	2.70	2.90	2.96
	C13	2.57	1.82	1.96	2.03	1.80	1.58	1.56
	C14	3.36	2.62	2.65	2.69	2.74	2.83	2.82
	C15	2.93	2.00	2.17	1.98	2.05	2.07	2.03
	Mean	3.02	2.40	2.39	2.40	2.31	2.30	2.34
	SD	0.30	0.47	0.43	0.42	0.41	0.56	0.57
D_{sc}	C11	4.54	4.55	4.85	4.88	4.60	4.39	4.58
	C12	1.86	1.55	1.50	1.67	1.81	1.72	1.83
	C13	5.15	5.08	5.29	5.39	5.25	5.51	5.63
	C14	4.12	3.78	3.79	4.00	4.20	3.83	4.56
	C15	4.50	4.00	4.46	4.19	4.31	4.33	4.36
	Mean	4.04	3.79	3.98	4.03	4.04	3.96	4.19
	SD	1.27	1.35	1.49	1.43	1.31	1.39	1.41
D_r	C11	4.09	3.86	4.24	4.24	3.84	4.07	4.26
	C12	3.52	2.80	2.75	2.72	2.91	2.79	2.89
	C13	5.76	4.91	5.32	5.31	5.27	5.25	5.36
	C14	3.90	4.08	4.06	4.13	4.13	4.05	4.00
	C15	5.23	4.49	4.46	4.55	4.53	4.59	4.76
	Mean	4.50	4.03	4.17	4.19	4.14	4.15	4.26
	SD	0.95	0.79	0.93	0.94	0.87	0.91	0.92

Data are in millimeters. See text for various definitions of depth of cover D_c.

to 97% of the frozen dimensions). The average D_2 was 11.83 mm in the frozen stage and was 11.08 to 11.27 mm across the thawing stages (94% to 95% of the frozen dimensions). For D_3, the average was 10.76 mm in the frozen stage and was 10.34 to 10.52 mm across the thawing stages (96% to 98% of the frozen dimensions). For D_i, the average vocal fold depth was 9.69 mm in the frozen stage and was 8.58 to 8.97 mm across the thawing stages (89% to 93% of the frozen dimensions). In summary, it can be seen that the average vocal fold depth in the frozen stage was generally larger than that during thawing, which was almost steady over the entire course of thawing (for 3 hours). These data suggested that some tissue expansion (in most cases less than 5%) was likely induced by the liquid nitrogen quick-freezing process, and was effectively reversed by the thawing process.

TABLE 5. CHANGES OF VOCAL FOLD DEPTH OF BODY ACROSS FROZEN AND THAWING STAGES

	Subject	Frozen	Thawing Time					
			30 min	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
D1b	C11	7.95	8.09	8.00	8.26	8.22	8.36	8.27
	C12	7.70	7.70	7.90	8.12	7.84	7.72	8.45
	C13	8.80	8.68	8.13	8.22	8.42	8.33	8.32
	C14	10.69	10.23	10.34	10.00	9.62	9.31	9.39
	C15	9.87	9.16	9.11	8.80	9.24	9.22	9.38
	Mean	9.00	8.77	8.70	8.68	8.67	8.59	8.76
	SD	1.27	0.99	1.04	0.79	0.74	0.67	0.57
D2b	C11	8.98	8.36	8.44	8.63	8.43	8.52	8.59
	C12	9.11	8.61	8.75	9.16	8.54	8.74	9.17
	C13	10.35	10.29	9.82	9.79	9.90	9.87	9.73
	C14	11.27	10.60	10.83	10.68	10.47	10.11	10.33
	C15	10.81	10.10	9.99	9.81	10.37	10.00	10.11
	Mean	10.10	9.59	9.56	9.62	9.54	9.45	9.59
	SD	1.02	1.03	0.97	0.77	0.99	0.76	0.71
D3b	C11	8.97	7.98	8.00	8.16	8.12	8.42	8.48
	C12	8.70	8.39	8.34	8.51	8.19	8.01	8.61
	C13	9.88	10.12	10.21	10.31	10.77	11.13	10.38
	C14	9.74	9.75	9.52	9.64	9.30	9.26	9.23
	C15	9.62	9.16	9.45	9.19	9.56	9.41	9.73
	Mean	9.38	9.08	9.10	9.16	9.19	9.25	9.29
	SD	0.52	0.90	0.91	0.86	1.10	1.20	0.79
Dib	C11	5.94	5.36	5.43	5.45	5.24	5.34	5.34
	C12	7.20	6.75	6.78	7.18	7.06	6.79	7.22
	C13	MD	MD	MD	MD	MD	MD	MD
	C14	MD	MD	MD	MD	MD	MD	MD
	C15	MD	MD	MD	MD	MD	MD	MD
	Mean	6.57	6.05	6.11	6.32	6.15	6.06	6.28
	SD	0.89	0.98	0.95	1.23	1.29	1.03	1.33
Dsb	C11	7.27	6.80	6.73	6.60	6.78	7.32	7.36
	C12	8.54	7.80	7.75	8.12	7.86	7.87	8.45
	C13	6.80	7.49	6.87	6.81	6.99	7.22	7.25
	C14	9.17	8.86	9.03	8.80	8.36	8.32	7.77
	C15	MD	9.00	8.73	8.89	9.09	9.02	9.19
	Mean	7.94	7.99	7.82	7.84	7.82	7.95	8.01
	SD	1.10	0.93	1.05	1.08	0.96	0.74	0.81

Data are in millimeters. See text for various definitions of depth of body Db. MD — missing data.

Figure 3B shows the various definitions of the depth of the cover (Dc), which varied from approximately 1.2 to 4.0 mm along the superior-inferior direction. The smallest depth of the cover was observed for D3c (around 1.2 mm), while the largest depth was observed for Dsc (around 4.0 mm, not including the reference depth Dr). The obliquely defined cover depth (Dr) reported by Hirano et al[11,12] and Kurita et al[13] was around 3.0 mm in dogs, whereas in our measurements it was around 4.2 mm. Figure 3C shows the various definitions of the depth of the body (Db), which varied from approximately 6.0 to 9.6 mm along the superior-inferior direction. Dib was the smallest, because it did not include a significant muscle portion, while D2b was the largest, because it involved the deepest TA muscle. Most of the data for the cover depth (Dc) and the body depth (Db) changed with quick-freezing and thawing in a way very similar to that of the total vocal fold depth (D), as evidenced by the similar shapes of the curves in Fig 3A-C; ie, the average depth in the frozen stage was generally larger, but it became smaller and remained very stable across the thawing stages. Tables 3-5 show the individual data for vocal fold depth.

Vocal Fold Thickness. Figure 3D shows the changes of vocal fold thickness across the frozen and thawing stages, in terms of the various definitions of vocal fold thickness (T) illustrated in Fig 1B. The thickness varied from approximately 8.8 to 9.6 mm along the medial-lateral direction across the thawing stages. The average free-edge thickness (Tf) was 9.78 mm in frozen larynges and was 9.15 to 9.44 mm across the thawing stages (94% to 96% of the frozen dimen-

TABLE 6. CHANGES OF VOCAL FOLD THICKNESS ACROSS FROZEN AND THAWING STAGES

			Thawing Time					
	Subject	*Frozen*	*30 min*	*1 h*	*1 h 30 min*	*2 h*	*2 h 30 min*	*3 h*
Tf	C11	8.88	7.92	8.75	8.74	8.39	8.46	8.59
	C12	8.07	8.03	8.07	8.02	8.23	8.06	8.41
	C13	11.23	10.42	10.86	11.07	10.99	10.85	10.59
	C14	10.40	10.48	10.40	10.56	10.53	10.41	10.33
	C15	10.35	8.89	9.02	8.80	8.99	8.73	8.90
	Mean	9.78	9.15	9.42	9.44	9.43	9.30	9.36
	SD	1.28	1.25	1.17	1.31	1.26	1.24	1.02
Te	C11	9.09	8.13	8.90	8.99	8.34	8.52	8.76
	C12	7.86	8.18	8.24	8.33	8.29	8.06	8.41
	C13	11.49	10.76	11.07	11.25	10.83	11.06	10.93
	C14	10.55	10.64	10.61	10.77	10.57	10.62	10.37
	C15	10.66	9.10	9.13	8.99	9.18	8.87	9.00
	Mean	9.93	9.36	9.59	9.66	9.44	9.43	9.49
	SD	1.44	1.28	1.20	1.27	1.21	1.33	1.10
Tm	C11	9.41	7.92	8.23	8.57	7.69	7.78	8.38
	C12	9.48	8.83	8.66	8.33	7.69	7.68	7.89
	C13	11.49	10.48	10.53	10.69	10.49	10.53	10.33
	C14	9.98	9.07	8.99	9.12	9.05	9.00	8.96
	C15	10.97	9.06	9.08	8.85	9.03	8.77	8.90
	Mean	10.26	9.07	9.10	9.11	8.79	8.75	8.89
	SD	0.93	0.92	0.87	0.93	1.17	1.15	0.92

Data are in millimeters. See text for various definitions of thickness T.

sions). The average effective thickness (Te) was 9.93 mm in the frozen stage and was 9.36 to 9.66 mm during thawing (94% to 97% of the frozen dimensions). The average mucosal-muscle thickness (Tm) was 10.26 mm in the frozen stage and was 8.75 to 9.11 mm across the thawing stages (85% to 89% of the frozen dimensions). Similar to the data on vocal fold depth (Fig 3A-C), it can be seen that the average vocal fold thickness was generally larger while the tissues were still frozen, but became smaller and remained relatively stable over the course of thawing (for 3 hours). Again, these data suggested that the quick-freezing process likely induced some vocal fold tissue expansion (in most cases, within 5% to 10%), but such expansion was basically reversed by thawing. Table 6 shows the individual data for vocal fold thickness.

In summary, the findings of the present study support the hypothesis that there are only minimal 3-dimensional changes in vocal fold tissue geometry as a result of quick-freezing and thawing, in terms of vocal fold length (<2%), depth (<5%), and thickness (5% to 10%). These magnitudes of tissue geometric deformation are considerably smaller than those induced by histologic techniques of tissue sample preparation (as much as 30%).[15] Hence, it seems that the method of quick-freezing, which has already been commonly used for tissue sample preparation in electron microscopy and in tissue culture, is probably the better technique for the preservation and estimation of the in situ geometry of the vocal fold and other soft tissues in vitro. Figure 4 shows such an estimation of the reconstructed in situ vocal fold geometry based on the data of vocal fold depth and thickness averaged over the thawing process, as data from the thawing stages were good approximations of the prefreezing geometry.

Physiologically, the geometry of the vocal fold is heavily influenced by the activity of the TA muscle. Vocal folds with no or minimal TA muscle contraction tend to be thinner and have a more convergent glottal shape, while those with significant TA muscle

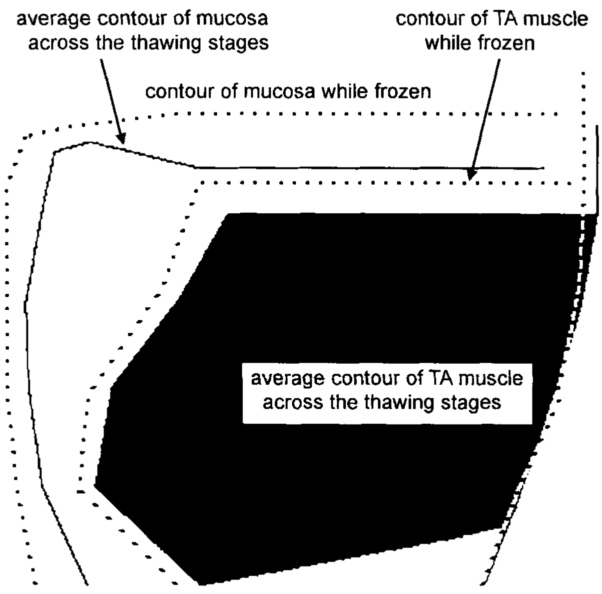

Fig 4. Reconstruction of vocal fold contour across frozen and thawing stages.

activity tend to bulge out medially along the inferior vocal fold border, creating a deeper and thicker vibrating structure with a more rectangular glottal shape.[22] These two vocal fold configurations are believed to be associated with the falsetto voice (vocal register) and the chest voice (or modal register), respectively.[22] Consequently, one major limitation of the present study was that geometric data were obtained from excised canine larynges, which obviously did not have any TA muscle activity. Nonetheless, the present data are representative of the resting vocal fold geometry, which are useful for establishing the baseline geometric configuration of laryngeal biomechanical models. Further studies are needed to quantify dynamic changes of vocal fold geometry as a function of TA muscle activity.

CONCLUSION

Data on vocal fold geometry are important information for the biomechanical modeling of voice production. In this study, we operationally defined the depth and the thickness of the vocal fold with respect to the laryngeal framework and the TA muscle, so as to efficiently represent the vocal fold geometry in laryngeal biomechanical models. In order to minimize the significant tissue deformation typically associated with histologic techniques, we used the alternative method of quick-freezing for preparing mid-membranous coronal sections of canine vocal folds. Measurements of the vocal fold dimensions were made on quick-frozen and thawing canine tissues with digital photography. The results are useful for defining the vocal fold boundaries with respect to the laryngeal framework in biomechanical models of the larynx, especially for continuum-mechanical models (eg, finite-element models[21]) that are based on the physiological properties (active contractile properties) of the canine laryngeal muscles[1-3] (also Tayama et al, unpublished data).

REFERENCES

1. Alipour F, Titze IR, Perlman AL. Tetanic contraction in vocal fold muscle. J Speech Hear Res 1989;32:226-31.

2. Alipour F, Titze IR. Elastic models of vocal fold tissues. J Acoust Soc Am 1991;90:1326-31.

3. Alipour F, Titze IR. Active and passive characteristics of the canine cricothyroid muscles. J Voice 1999;13:1-10.

4. Mineck CW, Tayama N, Chan R, Titze IR. Three-dimensional anatomic characterization of the canine laryngeal abductor and adductor musculature. Ann Otol Rhinol Laryngol 2000;109:505-13.

5. Tayama N, Chan RW, Kaga K, Titze IR. Geometric characterization of the laryngeal cartilage framework for the purpose of biomechanical modeling. Ann Otol Rhinol Laryngol 2000;110:1154-61.

6. Chan RW, Titze IR, Titze MR. Further studies of phonation threshold pressure in a physical model of the vocal fold mucosa. J Acoust Soc Am 1997;101:3722-7.

7. Hirano M. Phonosurgery: basic and clinical investigations. Otologia (Fukuoka) 1975;21:239-440.

8. Baer T. Investigation of phonation using excised larynges [Dissertation]. Cambridge, Mass: Massachusetts Institute of Technology, 1975.

9. Saito S, Fukuda H, Kitahara S, et al. Pellet tracking in the vocal fold while phonating. Experimental study using canine larynges with muscle activity. In: Titze IR, Scherer RC, eds. Vocal fold physiology. Denver, Colo: Denver Center for the Performing Arts, 1985:169-82.

10. Fukuda H, Kawasaki Y, Kawaida M, et al. Physiological properties and wave motion of the vocal fold membrane viewed from different directions. In: Gauffin J, Hammarberg B, eds. Vocal fold physiology: acoustic, perceptual, and physiological aspects of voice mechanisms. San Diego, Calif: Singular Publishing Group, 1991:7-14.

11. Hirano M, Kurita S, Nakashima T. The structure of the vocal folds. In: Stevens KN, Hirano M, eds. Vocal fold physiology. Tokyo, Japan: University of Tokyo Press, 1981:33-43.

12. Hirano M, Kurita S, Nakashima T. Growth, development and aging of human vocal fold. In: Bless DM, Abbs JH, eds. Vocal fold physiology. San Diego, Calif: College-Hill Press, 1983:22-43.

13. Kurita S, Nagata K, Hirano M. A comparative study of the layer structure of the vocal fold. In: Bless DM, Abbs JH, eds. Vocal fold physiology. San Diego, Calif: College-Hill Press, 1983:3-21.

14. Perlman AL, Durham PL. In vitro studies of vocal fold mucosa during isometric condition. In: Baer T, Sasaki C, Harris K, eds. Laryngeal function in phonation and respiration. Boston, Mass: College-Hill Press, 1987:291-303.

15. Johnson RE, Sigman JD, Funk GF, Robinson RA, Hoffman HT. Quantification of surgical margin shrinkage in the oral cavity. Head Neck 1997;19:281-6.

16. Eckel HE, Sittel C. Morphometry of the larynx in horizontal sections. Am J Otolaryngol 1995;16:40-8.

17. Chan RW. Shear properties of vocal fold mucosal tissues and their effect on vocal fold oscillation [Dissertation]. Iowa City, Iowa: The University of Iowa, 1998.

18. Bozzola JJ, Russell LD. Electron microscopy: principles and techniques for biologists. Boston, Mass: Jones and Bartlett Publishers, 1992.

19. Freshney RI. Culture of animal cells. 3rd ed. New York, NY: Wiley-Liss, 1994.

20. Pech P, Bergstrom K, Rauschning W, Haughton VM. Attenuation values, volume changes and artifacts in tissue due to freezing. Acta Radiol 1987;28:779-82.

21. Alipour F, Berry DA, Titze IR. A finite-element model of vocal-fold vibration. J Acoust Soc Am 2000;108:3003-12.

22. Titze IR. Principles of voice production. Englewood Cliffs, NJ: Prentice Hall, 1994:252-78.

23. Kurokawa H. High speed cinematographic analysis of subglottal mucosal vibration during experimentally induced phonation in excised larynges [in Japanese]. J Otolaryngol Jpn 1992;95:1151-63.