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Research Paper

Determination of the elastic properties of rabbit vocal fold tissue using uniaxial tensile testing and a tailored finite element model

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ABSTRACT

The aim of the present study was to quantify the effects of the specimen shape on the accuracy of mechanical properties determined from a shape-specific model generation strategy. Digital images of five rabbit vocal folds (VFs) in their initial undeformed conditions were used to build corresponding specific solid models. The displacement field of the VFs under uniaxial tensile test was then measured over the visible portion of the surface using digital image correlation. A three-dimensional finite element model was built, using ABAQUS, for each solid model, while imposing measured boundary conditions. An inverse-problem method was used, assuming a homogeneous isotropic linear elastic constitutive model. Unknown elastic properties were identified iteratively through an error minimization technique between simulated and measured force-time data. The longitudinal elastic moduli of the five rabbit VFs were calculated and compared to values from a simple analytical method and those obtained by approximating the cross-section as elliptical. The use of shape-specific models significantly reduced the standard deviation of the Young's moduli of the tested specimens. However, a non-parametric statistical analysis test, i.e., the Friedman test, yielded no statistically significant differences between the shape-specific method and the elliptic cylindrical finite element model. Considering the required procedures to reconstruct the shape-specific finite element model for each tissue specimen, it might be expedient to use the simpler method when large numbers of tissue specimens are to be compared regarding their Young's moduli.

1. Introduction

The vocal folds, the primary organs of human phonation, are two lips of very soft tissue located on opposite sides of the larynx. Their elastic properties need to fall within a certain range for proper phonation. Accurate engineering methods and data are required to characterize vocal fold tissue. The rabbit animal model has often been used to investigate the vocal fold ultra-structural alterations in clinical problems such as wound healing and scarring (Dahlqvist et al., 2004;

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Hansen and Thibeault, 2006; Hertegård et al., 2006; Thibeault et al., 2002). The quantification of the rabbit vocal fold mechanical properties, however, presents experimental challenges because the tissue is very soft, and the specimens are very small. Shear rheometry and conventional uniaxial tensile testing have been used in previous studies to quantify the mechanical properties of rabbit vocal folds (Miri et al., 2014; Thibeault et al., 2002). The preparation of standard uniformly shaped test specimens for shear rheometry and conventional uniaxial testing of rabbit vocal folds is impractical because of their tortuous shapes and small dimensions. Thus, there is a need for robust methods that allow testing of specimens with arbitrary shapes.

In uniaxial tensile tests, strain is often estimated as the ratio between the plunger displacement and the gauge length of the specimen in-situ (Alipour and Titze, 1991; Riede et al., 2011). Stress is then estimated as the ratio between the measured force and the average cross-sectional area, assuming a cylindrical specimen shape. But local deformations around the anchor points may significantly bias the estimation of the stretch from the plunger displacement. Local deformation measurements yield more accurate results, as revealed and emphasized in recent studies on human vocal fold tissue. Kelleher et al. (2010) obtained local strain values by means of digital image correlation[1] (DIC). The Young's modulus obtained locally from the ratio of stress and local longitudinal strain was found to be significantly greater than that based on the plunger displacement (Kelleher et al., 2010). Small and soft tissues, such as rabbit vocal folds, often yield results with significant experimental uncertainties (Riede et al., 2011). Errors in the cross-sectional area and the length of the specimen are known to significantly affect the calculated Young's modulus (Cook and Mongeau, 2007).

In an earlier study, finite element analysis has been combined with inverse determination procedures to identify the Young's modulus of porcine vocal fold tissue (Bufi, 2011). The shape of the specimen was approximated as rectangular, which was believed to have caused errors in the results. Shape-specific finite element models have been recently used to estimate the hyper-elastic properties of the human sternocleido-mastoideus muscle from uniaxial tensile testing (Gras et al., 2012). The dependency of vocal fold tissue mechanical properties on the specimen geometrical uncertainties has not yet been systematically investigated. This motivated the present study on the use of uniaxial tensile testing and shape-specific finite element modeling to estimate rabbit vocal fold tissue mechanical properties. The tissue nonlinear, anisotropic, viscoelastic behavior was neglected at this time for the sake of simplicity. A linear isotropic elastic constitutive model was assumed to quantify the influence of tissue specimen shape on its mechanical properties. A shape-specific finite element model[2] (FEM) was created for each one of five rabbit vocal folds, and their elastic moduli were estimated using an inverse determination procedure. The accuracy of the proposed method was first investigated using three synthetic silicon rubber specimens with different shapes and sizes. Then, the method was applied for the actual tissue specimens, and the results were compared with those obtained from approximate methods.

[1]Digital image correlation: DIC.
[2]Finite element model: FEM.

2. Methods

2.1. Specimens

New Zealand rabbits ($n=5$, i.e., two female and three male rabbits) were provided by the animal facility of the department of surgery of the University of Wisconsin in Madison, with the help of Dr. Nicole Li and Professor Susan Thibeault. The larynges were excised and shipped to McGill University at a temperature below $-50\,^{\circ}$C. Following removal of extraneous muscle and shortening of the trachea, as shown in Fig. 1(b), a straight cut was made in the sagittal plane, to create two hemi-larynges (Miri et al., 2014). The right hemi larynx is shown in Fig. 1(c). The vocal folds, which are located between the arytenoid cartilage and the thyroid cartilage, were dissected out of the epiglottis area and the connecting tissues. Portions of the laryngeal wall and of the thyroid cartilage were kept at the anterior and posterior ends of the vocal folds to facilitate gripping the specimen within the testing machine, as shown by the dashed closed curve in Fig. 1(c). The small size of the tissue specimen precluded the removal of the epithelium, which was kept along with the lamina propria and the vocalis muscle.

Synthetic eco-flex 10 Platinum Cure silicon rubber specimens (Smooth-On, Easton, PA) were fabricated using a mixing ratio of 1:1:0.5 (i.e., Eco-flex with one part A, one part B, and 0.5 part silicon thinner) in the laboratory. Following air evacuation, the specimens were molded and cured at room temperature. The synthetic specimens were then trimmed into smooth arbitrary shapes, with no sharp notches or cuts. A typical silicon rubber specimen with arbitrary shape is shown in Fig. 2.

2.2. Experimental procedures

A 3D reconstruction software (iModeller 3D professional, UZR GmbH & Co KG, Hamburg, Germany) was used to construct a three-dimensional solid model of each specimen. This software was selected for its high quality mesh texture based on a previous 3D model reconstruction of the human skull (Abreu et al., 2007). A rotating platform was designed and built for the acquisition of the rabbit tissue specimen images, as shown in Fig. 3(a). The dimensions of the platform enclosure were $10.0\ \text{cm} \times 14.0\ \text{cm} \times 17.0\ \text{cm}$. A fiber-optic light source (Cole-Parmer Instrument Co., Montreal, QC) was used to minimize glare. Three synthetic silicon rubber specimens with different shapes and sizes were prepared, as detailed in Section 2.1. A white speckle pattern was applied on the surface of the synthetic specimens using an enamel spray. The specimens were mounted on the rotating platform in their initial undeformed condition using four black-silk sutures (3.0 metric). A total of 18 planar digital images were captured for each specimen following rotations in equal angular increments of 20°. The superficial layer of the excised rabbit vocal fold tissue is mostly avascular, and thus it was hard to distinguish the boundaries between the tissue and its