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Research Paper

Indentation of poroviscoelastic vocal fold tissue using an atomic force microscope☆

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ABSTRACT

The elastic properties of the vocal folds (VFs) vary as a function of depth relative to the epithelial surface. The poroelastic anisotropic properties of porcine VFs, at various depths, were measured using atomic force microscopy (AFM)-based indentation. The minimum tip diameter to effectively capture the local properties was found to be 25 μm, based on nonlinear laser scanning microscopy data and image analysis. The effects of AFM tip dimensions and AFM cantilever stiffness were systematically investigated. The indentation tests were performed along the sagittal and coronal planes for an evaluation of the VF anisotropy. Hertzian contact theory was used along with the governing equations of linear poroelasticity to calculate the diffusivity coefficient of the tissue from AFM indentation creep testing. The permeability coefficient of the porcine VF was found to be $1.80 \pm 0.32 \times 10^{-15}$ m^4/N s.

1. Introduction

Mechanical elastic properties are important to ensure the functionality of remodeled engineered tissue constructs. The design of biomimetic injectable biomaterials requires information about the specific elastic properties of the host tissue (Heris et al., 2012). Changes in elastic properties associated with pathology can be best captured if characterized at the microscale (Athanasiou et al., 2000; Loparic et al., 2010). Indentation at different length scales has been frequently used for the mechanical characterization of materials. Indentation methods are well suited to soft biological material and can be used to map the mechanical properties of inhomogeneous biological tissues. Indentation at the microscale requires only a very small sample volume, on the order of 100 μL, which is especially helpful in studies using small animal models, such as rats.

The indentation of very soft tissue with elastic moduli on the order of kiloPascals requires high resolution measurements in terms of force and displacement. The indentation depth for a thin layer of such tissue should not exceed 10% of the sample thickness (Butt et al., 2005). Beyond this region,

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the tissue material may exceed linear elasticity because soft tissues undergo large deformations, and indentation results may be affected by substrate properties. Indentation forces depend on the indentation depth, probe size and elastic modulus of the sample. An atomic force microscope (AFM) with colloidal probes can overcome the resolution limitations of conventional indenters (Stolz et al., 2004). It can be used to image the topographical features of a small tissue sample and measure local elastic properties through indentation at different length scales depending on the probe size.

The vocal fold (VF) is inhomogeneous, anisotropic, and fluid-saturated porous. The VFs are located at the top of the trachea and under the epiglottis (Fig. 1a). They are stretched horizontally across the larynx along the anterior–posterior direction (Fig. 1b). To evaluate the inhomogeneity of the VFs, the tissue sample should be sliced to probe at different depths. This technique was recently applied to ex-vivo brain tissue to assess the elasticity of gray and white brain matter (Kaster et al., 2011). To investigate the mechanical anisotropy, indentation was performed for bone samples sectioned in planes along different orientations relative to the material axes, used for hard tissues such as bone (Fan et al., 2002; Swadener et al., 2001). In another approach, structural data was used to estimate the anisotropic properties of osteon lamellae through the implicit calculation of material constants fitted to measured indentation data (Reisinger et al., 2011). These techniques have not yet been adapted for the characterization of soft tissues, including the VFs. Conventional testing methods along with a transversely isotropic model were used to determine the anisotropic mechanical properties of porcine VFs (Miri et al., 2012b). Pipette aspiration method is another technique for characterization of the local, anisotropic properties of the VFs (Weiß et al., 2012).

Poroelastic models treat the tissue as a porous structure with deformable solid and penetrating fluid phases (Galli and Oyen, 2009). Upon mechanical loading, the solid phase undergoes normal and shear stresses while the fluid phase experiences hydrodynamic pressure. In a numerical study, a continuous poroelastic linear model of VF was developed to simulate the liquid flow in the tissue and study the fluid dynamics during VF oscillations (Tao et al., 2009). No empirical data was presented on the permeability of VF tissue. The

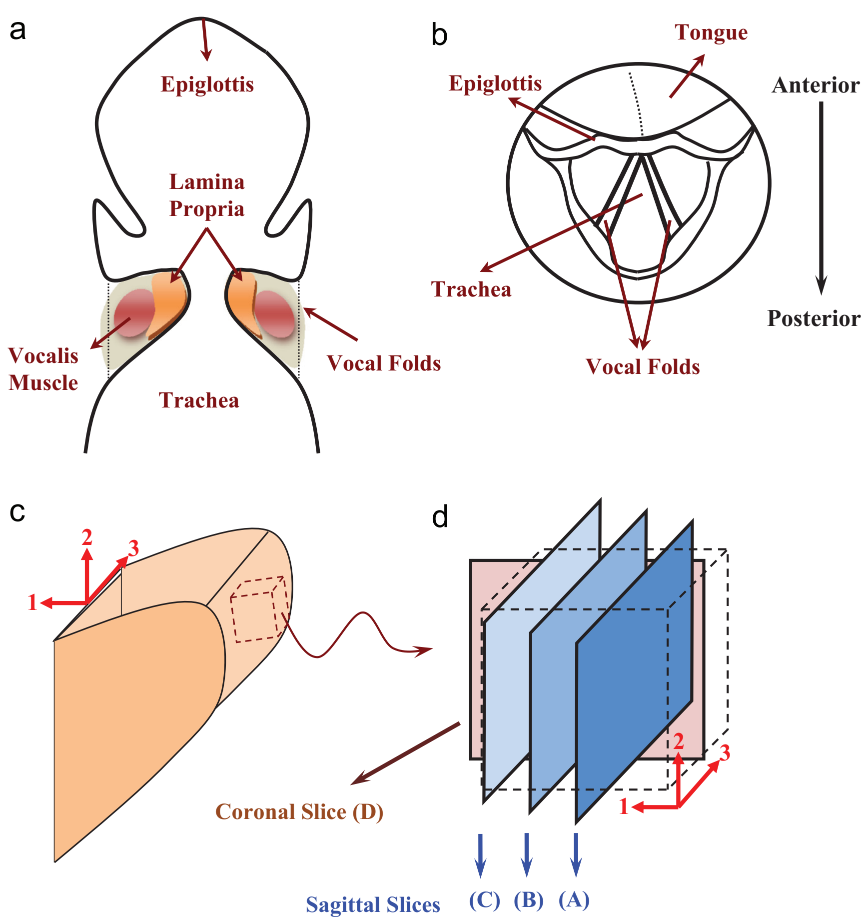

Fig. 1 – (a) Coronal view of larynx. (b) Transverse view of Larynx. (c) Sketch of one vocal fold and material coordinate system. A small cubical portion of the tissue is shown by the dashed lines. The location was around one third of the span near the thyroid cartilage, where the impact stress is believed to be the greatest. (d) Cubic tissue sample was then sectioned in three sagittal slices shown in blue (A, B, C), and one coronal slice (D) shown in pink. A is the superficial layer, closer to the surface of the vocal folds. B is the intermediate layer and C is the deep layer of the vocal folds. Direction 3 is the anterior–posterior direction. Direction 2 is the inferior-superior direction and direction 1 is the medial-lateral direction. The sagittal planes are denoted as 1-planes (perpendicular to direction 1) and coronal planes as 3-planes (perpendicular to direction 3).
(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)