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Review

Functional assessment of the ex vivo vocal folds through biomechanical testing: A review

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ABSTRACT

The human vocal folds are complex structures made up of distinct layers that vary in cellular and extracellular composition. The mechanical properties of vocal fold tissue are fundamental to the study of both the acoustics and biomechanics of voice production. To date, quantitative methods have been applied to characterize the vocal fold tissue in both normal and pathologic conditions. This review describes, summarizes, and discusses the most commonly employed methods for vocal fold biomechanical testing. Force–elongation, torsional parallel plate rheometry, simple-shear parallel plate rheometry, linear skin rheometry, and indentation are the most frequently employed biomechanical tests for vocal fold tissues and each provide material properties data that can be used to compare native tissue to diseased or treated tissue. Force–elongation testing is clinically useful, as it allows for functional unit testing, while rheometry provides physiologically relevant shear data, and nanoindentation permits micrometer scale testing across different areas of the vocal fold as well as whole organ testing. Thoughtful selection of the testing technique during experimental design to evaluate a hypothesis is critical to optimize biomechanical testing of vocal fold tissues.

Contents

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1. Introduction

As with synthetic materials, properties of biologic materials stem, in large part, from their mechanical characteristics. Understanding mechanical properties of biological materials allows for the creation of more accurate and useful biomechanical models. The human voice results from the interaction of numerous tissues, muscles, and nerves from the trachea to the oral cavity, of which the vocal folds (VF) play a central role (Fig. 1). Air passing between the apposed VFs generates vibration and subsequent vocalization. The vocal folds themselves consist of distinct layers, each with separate mechanical properties that allow for smooth, coordinated motion during vocalization to produce the precision required for speech and singing (Fig. 2). These layers are combined into the cover (epithelium and superficial lamina propria) and body (thyroarytenoid muscle) for analytical purposes, as the cover and body can move independently. These layers, moving independently under forces created by airflow through the glottis, create a wave-like flow of the VF cover that can be seen on videostroboscopy and is referred to as the mucosal wave. The propagation of the mucosal wave from the subglottis through the vocal folds is fundamental to the production of vocalization and relies on the varying properties of the vocalis muscle, ligament, lamina propria, and epithelium. The vocal folds are lengthened, shortened, raised, and lowered by the intrinsic laryngeal muscles to subtly modulate glottal airflow and create variations in timbre and pitch. Disruption of this delicate mechanical orientation from trauma, neoplasm, or inflammation can alter biomechanical tissue properties and voice.

The vast majority of the vocal fold cover is comprised of extracellular matrix [1]. The extracellular matrix proteins of the VF lamina propria consist of fibrous and interstitial proteins, in addition to lipids and carbohydrates. Many authors have posited that the fibrous proteins, collagen and elastin, dictate the tensile stress–strain characteristics of the vocal folds, whereas interstitial proteins such as proteoglycans and glycoproteins determine the viscoelastic shear properties by affecting fluid content and thickness of the lamina propria layers [1–3].

In historically-significant work, Hirano et al. described the superficial lamina propria as loosely organized with few collagen or elastin fibers, the intermediate layer with increased number of elastin fibers, and the deep layer with increased collagen fibers [4]. Gray et al. further elucidated that elastin was present throughout all three layers of the lamina propria, but elastin fibers are noted at an increased concentration within the vocal ligament [1]. Both elastin and collagen fibers are oriented longitudinally in the intermediate and deep layers of the lamina propria to ideally withstand the stress of the intrinsic laryngeal muscles [1,5].

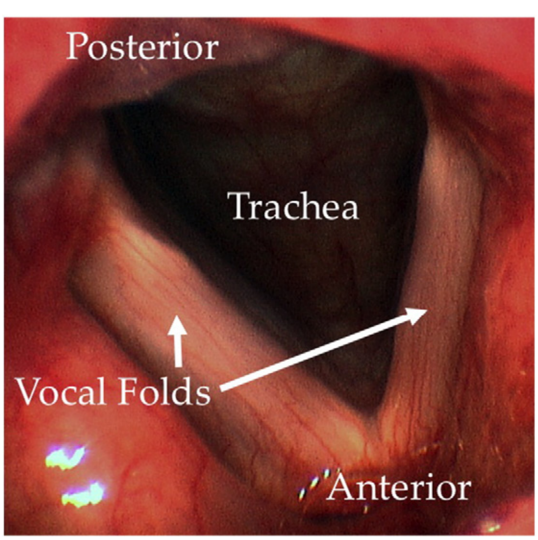

Fig. 1. Videolaryngoscopic view from above of fully abducted healthy vocal folds.

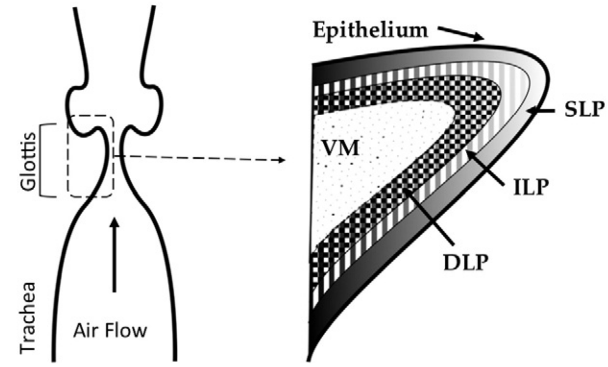

Fig. 2. Coronal sketch of the vocal tract from trachea to supraglottis (left). Cross-sectional view of the vocal folds (right) depicting the various layers that comprise the vocal fold. SLP = superficial lamina propria, ILP = intermediate lamina propria, DLP = deep lamina propria, VM = vocalis muscle.

The mechanical properties of VF are fundamental to the study of both the acoustics and biomechanics of voice production. Significant resources have been dedicated to quantifying the precise biomechanical properties of these tissues to design and develop improved synthetic and biologic materials, ultimately for clinical application. However, the specific techniques employed for quantification of the ex vivo biomechanical properties of the VFs are not standardized. This lack of standardization is problematic, as many laryngology and voice science laboratories are investigating novel, translational therapeutics to alter wound healing, develop regenerative models, or frank replacement of the lamina propria. Traditional histological outcomes do not adequately capture vocal fold dynamics, and more advanced assessment of the vocal folds is warranted. To provide a foundation for future investigation, the current literature on techniques and results of ex vivo laryngeal experimentation were reviewed.

2. Materials and methods

A database search to include Medline/Pubmed, Embase, and Web of Science was undertaken to compile relevant articles. Both a keyword search and MeSH analysis were employed to identify pertinent literature between January 1st, 1965 and November 30th, 2015. Keywords included vocal fold biomechanics, vocal fold and stress, strain, properties, in vivo testing, in vitro testing, ex vivo testing, elasticity, biomechanical testing, rheometry, indentation and nanonindentation, and mechanical testing. Relevant texts on biomechanical testing and materials science were searched for additional references for inclusion. Literature was then compiled and categorized for inclusion in the current manuscript.

3. Biomechanical testing overview

The biomechanical properties of VFs have been measured in vivo, in vitro, and ex vivo. In vivo techniques are currently impractical due to inherent access and anatomical limitations. Early work in the 1990s by Tran, Berke, and Tanaka attempted to obtain in-vivo data [6–9]. Tanaka and Hirano designed a small tube that attached to the operative channel of a fiber optic laryngoscope to measure stiffness via suction pressure applied to the VF [9]. Berke, Tran, and colleagues modified a force gauge to function through a laryngoscope and record in vivo vocal fold elasticity [6–8]. More recently, Hsiao and colleagues attempted to derive Young's modulus of the VF using color Doppler imaging, string vibration estimations, and known vocal fold properties [10]. To date, no simple, accurate mechanism exists to measure vocal fold biomechanics in vivo.