5pSC3. Non-invasive in vivo measurement of the mechanical properties of human vocal fold tissue

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A non-invasive method was developed and examined to obtain the mechanical properties of human vocal fold tissue in vivo via measurements of the mucosal wave propagation speed during phonation. Images of four human subjects’ vocal folds were captured from the high speed imaging (HSI) and magnetic resonance imaging (MRI) experiments at different phonation pitches, the frequency of the vocal folds vibrations, in the range from 110 to 440 Hz. The MRI images were used to obtain the dimensions of the subjects' vocal folds in the high-speed images. The mucosal wave propagation speed was determined for each subject at different pitches using an automatic image processing algorithm. The shear modulus of the vocal fold mucosa in the transverse direction was then calculated from a surface (Rayleigh) wave propagation dispersion equation using the measured wave speeds. It was shown that the mucosal wave propagation speed and the shear modulus of the vocal fold tissue were generally greater at higher pitches. The results were in good agreement with those from other studies obtained via in vitro measurements, thereby supporting the validity of the proposed measurement method. This method offers the potential for in vivo clinical assessments.
INTRODUCTION

Human voice is produced by airflow expelled from the lungs through the airway and modulated by the self-sustained oscillation of the vocal folds, two membranes located within the trachea at the level of Adam’s apple. The oscillation involves mainly medial and lateral wavy motion of the vocal fold mucosa, commonly referred to as the mucosal wave. The generation and propagation of the mucosal wave depends on the shear viscoelastic mechanical properties of the mucosa. Therefore, a knowledge of the mechanical properties of vocal fold tissue is needed for the development of injectable biomaterials used for vocal fold augmentation and repair (Caton et al., 2007; Heris et al., 2012), for the diagnostic of scarred tissue (Thibeault et al., 2002; Rousseau et al., 2004), and for the assessment of surgical procedures used in vocal fold treatment (Thibeault et al., 2011).

In vitro measurements of the viscoelastic shear properties of human vocal fold tissue have been mostly performed using parallel plate or linear skin rheometry (Chan and Titze, 1999; Goodyer et al., 2003; Chan, 2004; Hess et al., 2006; Chan and Rodriguez, 2008; Dailey et al., 2009; Goodyer et al., 2009). For example Chan and Rodriguez (2008) designed a custom-built, controlled-strain, linear, simple-shear rheometer for the measurement of the viscoelastic properties at frequencies up to 250 Hz. These existing methods are inaccurate for frequencies greater than at most 250 Hz, and cannot be used for in vivo measurements.

In vivo measurements of the vocal fold elastic modulus have been performed (Berke, 1992; Berke and Smith, 1992; Tran et al., 1993; Goodyer et al., 2006; Goodyer et al., 2007a; Goodyer et al., 2007b). Berke and Tran et al. (1992; 1993) constructed an apparatus, which worked based on direct lateral force and displacement measurements, to obtain the vocal folds elastic modulus in vivo. This invasive measurement technique had a limited range of application due to its cumbersome apparatus. Goodyer et al. (2006; 2007a; 2007b) developed a custom built instrument to measure the vocal folds elastic properties in vivo. This measurement technique is invasive, and is performed in a quasi-static manner; i.e., the frequency-dependent properties of human vocal fold tissue were not obtained at different phonation frequencies.

The mucosal wave propagation has been assessed clinically by different measurement techniques such as electrography, photoglottography, ultrasound and visualization techniques including videokymography, stroboscopy and high speed imaging (Boessenecker et al., 2007; George et al., 2008; Voigt et al., 2010; Krausert et al., 2011). Krausert et al. (2011) investigated the advantages and disadvantages of each technique. George et al. (2008) studied the generation and propagation of the mucosal waves. They used a laser-triangulation endoscope as well as high-speed cameras to capture the human vocal folds profile during vibration in vivo. Voigt et al. (2010) presented a detection algorithm for mucosal wave propagation using HDSI. No attempt was made, however, to obtain the wave propagation speed.

In the present study, a novel method for non-invasive in vivo measurement of the shear modulus of the human vocal fold mucosa was developed. High-speed and MRI images of the vibrating vocal folds of four human subjects were obtained at different pitches within the fundamental range between 110 to 440 Hz. The pixel size of each set of high-speed images was determined by overlapping images of specific anatomical features obtained from HSDI and MRI. An automatic image processing algorithm was developed to detect and track the mucosal wave propagating on the superior surface of the vocal folds, through which the speed of mucosal wave propagations at different pitches were obtained for different subjects. An inverse surface (Rayleigh) wave propagation problem was solved to calculate the shear modulus of the subjects’ vocal folds mucosa in the transverse direction from the measured wave speeds. The dependency of the mucosal wave speed and the shear modulus on pitch was investigated. The results were found to be in good agreement with those obtained by Chan and Rodriguez (2008) in vitro, and thus support the validity of the proposed in vivo measurement method for clinical applications.

METHODS

The review board of the Research Ethics and Compliance (IRB) of McGill University, Montreal Neurological Institute and Hospital Research Ethics Board (REB) approved the protocol and consent procedure for the HSDI and MRI experiments, respectively. Four non-smoker adults, three men and one woman, aged between 22 to 29, participated in this study. The subjects were healthy with no current or past medical history, and no voice disorders.
No subject was a trained voice user or had any background in clinical voice practice. Each subject participated in the HSDI and MRI experiments during two different sessions.

**Image Processing Algorithm**

Several methods have been suggested for vocal fold edge detection in the literature (Yan *et al.*, 2006; Lohscheller *et al.*, 2007; Zhang *et al.*, 2010). A threshold-based edge detection algorithm was used in the present study is. High-speed images were imported in Matlab® for image processing. The glottal midline was defined interactively by selecting and connecting the anterior and posterior commissures points in the first image with the maximum glottal gap (Figure 1 (a)). The same line was considered as the glottal midline for subsequent images in each set of data, because of the high imaging rate and the negligible movement of the larynx within the short imaging time interval. The midline center region of the vocal folds along the anterior-posterior direction was determined as the region of interest and the images were rotated to make the glottal midline vertical as shown in Figure 1 (b). The intensity of each pixel in the high-speed digital images, \( I(x, y) \), determined at each point. To demarcate the vocal fold edges, the cropped high-speed digital images were converted to binary ones and the perimeter of the binary images was identified as shown in Figure 1 (c) and Figure 1 (d), respectively. The conversion threshold was obtained from the first local minimum of the image histogram (Wittenberg *et al.*, 1995; Zhang *et al.*, 2010). The locations where the intensity became unity in the binary images represented the vocal fold edge location, \( VF(x, y) \). The obtained vocal fold edge overlapped on the gray scale image is shown in Figure 1 (e).

![Glottal Midline](image-a)  
![Region of Interest](image-b)  
![Magnified Region of Interest](image-c)  
![Binary Image](image-d)  
![Perimeter of Binary Image](image-e)  

**FIGURE 1.** Image processing algorithm: (a) pre-processed high-speed image, region of interest and non-vertical glottal midline; (b) magnified region of interest with vertical glottal midline; (c) binary image; (d) perimeter of the binary image, where boundaries represent the vocal fold edge; (e) the search window for mucosal wave detection, obtained vocal fold edge is overlapped on the gray scale image; (f) obtained mucosal wave overlapped on the gray scale image.

It was observed that the image intensity changed significantly in the vicinity of the vocal fold edge and the mucosal wave peak. To illustrate this, the gradient of the intensity along the arrow line in Figure 1 (e) is shown in Figure 2. The two main extrema represent the location of the right and left vocal fold edges. A local extremum is also present on the left fold, at the location of the mucosal wave peak amplitude. To detect the mucosal wave, a search window was defined near the vocal fold edge as shown in Figure 1 (e). The search window moved in the lateral direction with a velocity equal to the average edge velocity, \( \bar{V}_{edg} = \Delta VF/N \), in which \( \Delta VF \) is the maximum edge displacement in the opening phase, and \( N \) is the number of frames in which the maximum displacement occurs. The width of the search windows was selected to be equal to \( \Delta VF \) pixels. The gradient of intensity in the search window was calculated and the location of mucosal wave peak, \( MW(x, y) \), was obtained. The mucosal wave peak is shown overlapped with the gray scale image in Figure 1 (f).
The mucosal wave propagation speed, $c_m$, for each row in the image was calculated from:

$$c_m = \Delta MV \cdot SR \cdot \frac{L_m}{L_H},$$  

where $\Delta MV$ (pixel/frame) is the change in the location of the mucosal wave in the lateral direction, $SR$ is the frame rate of imaging (4000 frame/sec), $L_m$ is the known length of vocal folds in the MRI image and $L_H$ (pixel) is the length in the high-speed images.

### Wave Propagation Model

It was shown that the movement of the medial and superior surfaces of vocal folds, featuring the mucosal wave propagation, are respectively elliptical and circular in general (Boessenecker et al., 2007). This suggests that, mucosal waves include motion in both the longitudinal and transverse directions (Figure 3), with respect to the propagation direction. This behavior is similar to that of Rayleigh waves; i.e., a type of surface waves which propagates near stress-free surfaces. Therefore, the well-known of Rayleigh wave propagation model was employed to calculate the shear modulus of the vocal fold mucosa from the measured mucosal wave speeds, assuming ideal Rayleigh waves. The Rayleigh wave dispersion equation for a macroscopically homogeneous and isotropic medium is as follows:

$$\left(2 - \xi^2 \right)^4 - 16 \left(1 - \xi^2 \right) \left(1 - \frac{\xi^2}{\kappa^2} \right) = 0,$$  

where

$$\kappa^2 = \left(\frac{c_s}{c_c} \right)^2 = \left(\frac{k_s}{k_c} \right)^2 = \frac{\lambda + 2\mu}{\mu} = \frac{2 - 2\nu}{1 - 2\nu}, \quad \xi = \frac{k_s}{k_R},$$

and $\lambda(\omega)$ and $\mu(\omega)$ are frequency-dependent Lame functions. The Poisson’s ratio of the vocal fold tissue, $\nu$, was assumed to be 0.495. The constants $k_R$, $k_c$ and $k_s$ are the Rayleigh, compressional and shear wavenumbers, respectively, known as (Graff, 1975)

$$k_R = \frac{\omega}{c_R},$$
\[ k_r = \frac{\omega}{c_r} = \frac{\omega}{\sqrt{(\lambda + 2\mu)/\rho}}, \quad (4b) \]
\[ k_s = \frac{\omega}{c_s} = \frac{\omega}{\sqrt{\mu/\rho}}, \quad (4c) \]
in which \( \omega \) is the angular frequency, and \( c_r \), \( c_c \) and \( c_s \) are Rayleigh, compressional and shear wave speeds, respectively.

The mucosal wave propagation speed at different pitches was obtained from the image processing procedure for each of the subjects, \( c_m \), and treated as the Rayleigh wave propagation speed, \( c_R \). Then, the shear wavenumber, \( k_s \), and consequently the shear wave speed, \( c_s \), were obtained from Eqs. (2) and (3) in terms of the Rayleigh wavenumber, \( k_R \). Finally, the shear modulus of the vocal fold mucosa was calculated from \( \mu = \frac{\omega^2 \rho}{k_s^2} \). The vocal fold mucosa density, \( \rho \), was assumed to be 1060 and 1075 kg/m\(^3\) for male and female subjects, respectively (Titze, 1994).

The shear modulus obtained from such surface wave propagation method is representative over the depth of wave penetration. The mucosal wave propagates on the surface of vocal folds mainly within the mucosa during phonation. Therefore, the obtained shear modulus is believed to be that of the vocal fold mucosa.

**RESULTS AND DISCUSSION**

Data were obtained for different phonation pitches in the range from 110 to 260 Hz and 220 to 440 Hz for male and female subjects, respectively. The mean value and standard deviation of the measured data obtained from each subject at each pitch were calculated. The standard deviation values of the measured mucosal wave speed and shear modulus were less than 6 and 12 percent of the corresponding mean values, respectively. Thus, only the mean values are shown.
The shear modulus of human vocal fold mucosa in the transverse direction at fundamental frequencies from 110 to 440 Hz is shown in Figures 4. The same trends as those of mucosal wave propagation speed were observed as expected. The frequency-dependent behavior of mechanical properties of human vocal fold tissue is influenced by two main parameters, namely the inherent viscoelastic properties of the tissue and the longitudinal tension.

The vocal fold tissue includes a large concentration of fibers such as elastin and collagen within interstitial fluid (Titze, 1994), and thus feature a highly viscoelastic mechanical behavior. The viscoelastic behavior of such fibrous material is dependent on its molecular entanglement network, i.e., the coupled configurational motion of neighboring molecules. The magnitude of the tissue shear modulus depends on the configurational rearrangements that occur within the time period of one dynamic loading cycle. At low frequencies, the fibers have sufficient time to adjust themselves with the applied deformation by moving and sliding alongside each other. Therefore, the tissue stiffness is relatively small. At higher frequencies, only minor adjustment can take place within the period of deformation, and thus the tissue is stiffer. A longitudinal tension is required for the self oscillation of vocal folds. This tension is applied by the thyroarytenoid muscle and sustained by collagenous fibers of the ligament, which consists of the intermediate and deep layers of the lamina propria (Titze, 1994). The global (effective) stiffness of the tissue is directly related to vocal fold tension (Titze, 1994). Greater tensions are applied to the vocal folds at higher pitches, which results in greater stiffnesses.

As shown in Figure 4, considerable differences were observed between the obtained shear modulus values from different subjects’ vocal folds. The rate of increase of shear modulus values with pitch was also different for different subjects. The extension of the vocal fold length varied with pitch. For example the length of the vocal folds of Subject 1 at 260 Hz was 45 percent greater than that at 110 Hz. For a relative elongation of 45 %, the increase in shear modulus values was 225 % between the corresponding pitches. For Subject 3, for which the rate of increase of the shear modulus was the least, the values for the relative elongation of vocal folds and the increase in shear modulus were 19 % and only 13 %, respectively.

![FIGURE 4. The shear modulus of human vocal fold mucosa at different phonation pitches from 110 to 440 Hz. ○: Subject 1; □: Subject 2; ◇: Subject 3; △: Subject 4; - - - - - : regression for Subject 1; - - - : regression for Subject 2; --- - : regression for Subject 3; -- -- -- : regression for Subject 4. (Regressions are to guide the eye) The mean value and standard deviations of the shear modulus of three subjects’ vocal fold mucosa, Subjects 1 to 3, were compared with those obtained by (Chan and Rodriguez, 2008) from in vitro measurements in Figure 5.](image-url)
FIGURE 5. The means and standard deviations (upper error bars) of the shear modulus of human vocal fold mucosa versus frequency in the range from 100 to 260 Hz. ●: the mean value of three male subjects from in vivo measurements (present study); ■: the mean value of seven specimens obtained from in vitro measurements (Chan and Rodriguez, 2008).

As seen in this figure, the results fall within the same range while those from in vivo measurements were generally greater than in vitro ones. Chan and Rodriguez (2008), used a parallel rheometer to measure the shear modulus of the human vocal fold cover with no extension (tension). The method used in the present study, however, yields data during phonation, while the vocal folds underwent large extensions. This allowed the effect of vocal fold tension to be taken into account.

CONCLUSIONS

A novel non-invasive method was developed for the in vivo measurements of the shear modulus of human vocal fold tissue during phonation. Four subjects underwent high speed digital and magnetic resonance imaging tests at different pitches, for fundamental frequencies in the range from 110 to 440 Hz. The images obtained from MRI were used to determine pixel size in those from HSDI. Using the high-speed images and an automatic image processing algorithm, the mucosal wave propagation speed was determined for different human subjects at different phonation pitches. The shear modulus of the subjects’ vocal fold mucosa was then obtained using a surface (Rayleigh) wave propagation model and the measured wave speeds.

The results were compared to those of previous study from in vitro measurements and found to be in good agreement. The obtained shear modulus increased with pitch. This was speculated to be because of the inherent viscoelastic properties of the tissue and the increased longitudinal tension applied to vocal folds at higher pitches. The magnitude of the obtained shear modulus and the rate of increase with pitch were considerably different for different subjects. This emphasizes the need for in vivo clinical assessments of any specific patients’ vocal folds for diagnostic purposes or the evaluation of vocal fold treatment procedures.

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REFERENCES

Graff, K. F. (1975). Wave Motion in Elastic Solids (Ohio State University Press, Columbus, Chapters 5, 6).