Abstract  In earlier work we showed that low relative humidity (RH) of inhaled air causes acoustic voice parameters such as jitter and shimmer to deteriorate. Other authors have shown negative effects on vocal efficiency. To explain these changes in the mechanical properties of the vocal folds, the effects of changes in RH of the air passing over microdissected mucosa of sheep larynges were studied. The dissected surface of the tissue specimen just touched Ringer solution and air of varying RH was blown over the specimen. The mucosa specimen was subjected to sinusoidal oscillations of length (strain) and the resulting force (stress) was measured. The gain and phase angle between the imposed strain and resulting stress were measured, and elasticity and viscosity were calculated. Two different air conditions were tested: air with high RH (100%) vs air with low RH (0%). Viscosity and stiffness increased significantly in both ambient conditions ($P < 0.01$). Dry dehydrating air resulted in a stiffer and more viscous cover than humid air ($P < 0.001$). These changes in mechanical characteristics may contribute to the effects on voice parameters described in earlier work.

Keywords  Vocal fold · Mucosa · Hydration · Humidity · Biomechanics

Introduction

Several authors have shown an effect of hydration status of the vocal folds on voice quality and efficiency [1, 3, 4, 10, 11, 12]. We reported in previous work that short-term inhalation of dry air increases voice perturbation measures [4]. In a study on healthy subjects, Verdolini et al. [10, 12] reported an effect of dehydration on parameters of vocal efficiency. In another study on subjects with benign vocal fold lesions, the same authors found that after exposure of the vocal folds to hydrating conditions voice measures improved and vocal nodules and polyps tended to regress [11]. There has been subsequent discussion of whether it was the hydration status in the superficial vocal fold layers (cover) or in the underlying tissues (body) that resulted in these effects [4].

The vibratory pattern of vocal fold oscillations depends on many factors. In a fixed configuration, the pattern is mainly determined by mass, tension, internal friction or tissue viscosity, and the condition of the mucus layer on the surface of the vocal folds. In the current context, the latter two factors are the most relevant since these may be influenced by the relative humidity (RH) of inhaled air.

Viscosity depends on several things, including the amount of water present in the tissues, as reported by Finkelhor et al. [1]. These authors found a decrease in the oscillation threshold pressure with increasing hydration levels of the vocal fold tissues. This confirms the relation of viscous damping and subglottal pressure needed for sustained oscillations, as predicted theoretically by the inequality derived by Titze and Talkin [9]: $P_s \geq \frac{(k_i/T)(Bc)/2w}{1}$, where $P_s$ is subglottal pressure, $k_i$ is transglottal pressure coefficient, $T$ is vocal fold thickness, $B$ is the viscous damping coefficient of the vocal folds, $c$ is the mucosal wave surface velocity, and $w$ is the prephonatory glottal width. This inequality shows that if viscous damping increases (increased viscosity), a higher subglottal pressure is needed for phonation. Consequently, vocal efficiency will decrease.

The vocal folds are believed to behave as a two-mass oscillator consisting of a body (vocalis muscle and deep layers of the lamina propria) and a cover (mucosa and superficial layer of the lamina propria) [6]. Changes in hydration level in either mass will lead to changes in viscosity, resulting in different viscoelastic properties which in
Materials and methods

Mucosa

Healthy human laryngeal mucosa is very sparse: excised material is in principle always diseased; otherwise, surgery would be inappropriate. We chose to use sheep laryngeal mucosa, as in a comparative histological study Kurita et al. [7] showed that the vocal fold dimensions (i.e. length and mucosa thickness) of sheep are similar to those of humans. Moreover, they showed that there is a fairly distinct boundary between the superficial and the deep layer of the lamina propria. This makes it comparatively easy to dissect the superficial layers for use in studies of vocal fold cover.

As early as possible (at the most 1 h) after the sheep had been sacrificed (for other purposes), the larynx was taken out and transported to the laboratory in saline solution at about 20 °C. The larynx was divided into two hemilarynges. At specific points, the mucosa was marked with methylene blue and the distance between these marks was measured. The vocal fold mucosa was microdissected and adequately hydrated with Ringer solution. Using the marks on the mucosa, it was stretched to its original size and fixed with pins. The outlines of the specimen were drawn on the microdissected mucosa with methylene blue. Then the size was measured (around 3–4 mm long and 1 mm wide) and the specimen was cut accordingly. A T-clip cut out of aluminium foil was pressed around each end of the specimen for later mounting on the experimental set-up.

Strain–stress device

The mucosa specimen was mounted in a device and submerged in Ringer solution. At the beginning of an experiment the specimen was raised so that it just touched on the solution’s surface. A heating device at the bottom of the chamber kept the solution at 32 °C. The temperature was measured at the beginning and end of each experiment. A feedback mechanism controlled the temperature. Air of varying RH could be blown over the specimen. This controlled setting mimicked a physiological situation: the mucosa was well hydrated from its deep surface while being exposed to air of variable RH passing over its superficial surface.

The specimen was attached by the T-clips to two small vertical pins and stretched to its original length. One of the pins (the vibrating pin) was connected to a vibrator similar to a loudspeaker coil. The position and movements of the vibrating pin were precisely controlled by a position feedback system with a function generator as input. With this pin, a sinusoidal oscillation was induced, resulting in a sinusoidal strain on the specimen. The other pin (force pin) was attached to a force transducer (see Fig. 1 for a schematic drawing of the set-up). After low-pass filtering (1 kHz), the measured force and position were digitized (sampling rate 4 kHz) and stored in a personal computer. For a detailed description of the experimental set-up, see elsewhere in this issue [5].

Analysis

The recorded position and force signals were subjected to spectral analysis by fast Fourier transformation. The gain [ratio between