Quantitative study of the effects of dehydration on the viscoelastic parameters in the vocal fold mucosa

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Abstract:

Objectives— The goal of this study is to quantify the viscoelastic parameters of vocal fold mucosa at varying dehydration levels.

Study Design and Methods— Healthy canine larynges were obtained post-mortem, and the samples were separated from the subglottal wall. The samples were dehydrated in a vacuum dryer. According to the total dehydration time per sample, dehydration levels were divided to four degrees 0%, 40%, 60%, 80%. The stepper was set to stretch the sample to a level of 35% strain at the same rate (0.5mm/s). Data collection was repeated 5 times under each dehydration condition. The compression resilience, $RC\% = S'/S * 100\%$, and the hysteresis area were measured according to the stress-strain curves. The varying properties of the samples under different dehydration levels were investigated by fitting the curves.

Results— The area of the hysteresis loops observed in the stress-strain curves increased exponentially with dehydration levels, while the $RC\%$ decreased linearly. For all curves, low-strain stages can be explained by Hooke's law ($\sigma = E_0 * \epsilon$). With increasing levels of dehydration, E_0 , was shown to increase while the linear range was shortened. High-strain stages resembled exponential rather than the linear curves. And the nonlinear stage of the curve became increasingly apparent in the stress-strain curves of increased dehydration levels.

Conclusions—The quantitative results in this study not only provide a numerical reference for future experimental measurements, but also can be used to verify the biphasic model in future studies.

Keywords

Vocal fold, Mucosa, Dehydration levels, Quantify, Viscoelastic parameters

I. INTRODUCTION

Normal phonation heavily depends on the complete functionality of the vocal organ. Anatomically, the vocal folds consist of a layered structure, with each layer exhibiting different mechanical properties¹⁻³. The mucosal hydration levels of the vocal fold have a significant impact on vocal function, and thus maintenance of hydration is a typical method of treatment and prevention of vocal fold dehydration^{4,5}. It has been proposed that the impact of dehydration on voice production is caused by the altered mechanical properties, such as mobility and deformability, of the vocal fold mucosa⁶. Factors contributing to vocal fold dehydration include inhalation of dry air or fever, alcohol or caffeine use, oral breathing and certain medications⁷. It has been hypothesized that vocal fold dehydration can cause the formation of vocal fold pathologies, such as polyps, nodules, and cysts, given that some common symptoms of laryngeal disorders, including hoarseness, increased vocal effort and worsening voice quality are caused by dehydration⁸. Therefore, studying vocal fold dehydration is not only important for understanding the mechanism of sound production, but is also an essential focus in clinical voice science.

In previous studies, Verdolini et al. and Chan et al.^{9,10} found that hydration levels are inversely related to phonation threshold pressure (PTP) as a physiological measure of phonatory effort. Jiang et al.⁶ also found that superficial dehydration of the vocal fold increases PTP and mean flow rate. The dehydration may be correlated with an increased difficulty to produce efficient levels of phonation. Therefore, quantifying the effects that dehydration has on the mechanical properties is crucial.

Chan and Tayama⁸ tested the hypothesis that vocal fold hydration affects voice production by mediating changes in vocal fold tissue rheology. They tested the change of elastic shear modulus,

dynamic viscosity and the damping ratio of the vocal fold mucosa under both dehydration and rehydration conditions. The results obtained confirmed their hypothesis by showing that the parameters listed above increased with dehydration and returned to normal levels after rehydration. In another study¹¹, viscoelasticity of the vocal fold was experimentally assessed through analyzing the stress-strain relationship in excised canine larynges. The results indicated that dehydrated vocal fold mucosa was associated with greater stiffness¹¹. However, the magnitude of increased stiffness when subjected to different dehydration levels had not been quantified.

Biphasic theory^{12,13} and the corresponding model involved introduce a new angle to investigate the effect of dehydration on the vocal folds. The vocal fold lamina propria is described as a biphasic material composed of a solid phase and an interstitial fluid phase. Zhang et al.¹² used the biphasic theory to predict the stress relaxation of the vocal fold lamina propria. The overall stress, as well as solid dynamics and fluid dynamics of vocal fold mucosa, during the stress relaxation process have been investigated separately. The interstitial fluid was found to be an important factor for the stress load support, particularly at the early stage of stress loading. Also, when the time becomes sufficiently large, the solid phase eventually supported the full stress. Furthermore, Hanson et al.¹⁴ and Philips et al.¹⁵ measured the volume and mass of both the solid and liquid components of canine vocal fold lamina propria tissue at varying dehydration levels. The liquid mass and volume fractions and liquid:solid mass and volume ratios were significantly different at each dehydration level, except for the liquid:solid volume ratios at 30% versus 50% dehydration¹⁴.

Alipour and Titze¹ divided the vocal fold into the mucosa layer (cover) and the muscular layer (body) to investigate their biomechanical properties separately. Stress relaxation of canine vocal fold

tissues (muscle and mucosa) was studied following these methods^{13,16-18}, and the results showed that the vocal fold body exhibits more of a nonlinear elastic response than the cover, and also that the period of time for vocal fold relaxation is longer in the body. Also, it was shown that the vocal fold cover exhibits tendencies closer to those of elastic materials. The stress-strain^{1,11,16-19} data of the body and cover tissue samples were obtained under sinusoidal displacement, and were fitted numerically by polynomial and exponential models. Therefore, the elastic properties of canine vocal fold tissue could be studied.

The objectives of the studies mentioned above were to better understand the biomechanics of the vocal folds and the effects that various levels of hydration have on the functionality of the vocal folds. However, the quantification of a relationship between hydration levels and the viscoelastic properties of the vocal folds has not been studied systematically. The application of the biphasic theory under different hydration levels is a novel approach to reach this goal.

The purpose of this study is to use stress-strain curves to quantify the effect of four dehydration levels on the viscoelastic properties of the vocal fold mucosa. It is hypothesized that the viscoelastic parameters will be altered across the various dehydration levels. These differences in the values of the parameters will give insight on the impact that dehydration has on phonation and non-periodic vocal fold vibrations. Also, the data could establish an objective dehydration and phonotrauma criteria that could be used for clinical treatment.

II. MATERIAL AND METHOD

A. Stress-strain experiment

The stress-strain experiments were completed to investigate how the stress-strain curves were altered due to increasing dehydration levels. The methods used in this paper resemble the study designs

utilized by Alipour et al.^{16,17} in which they built an elastic model of the vocal fold. The experimental setup is shown as Fig. 1. During the experiment, the stepper was set to stretch the sample to a level of 35% strain before releasing the sample at the same rate (0.5mm/s). For each of the various dehydration cycles, 5 trials were run in which data was collected.

B. Materials

B.1 Sample preparation

Ten canine larynges were harvested post mortem and inspected to ensure the absence of all irregularities, such as vocal nodules, polyps, and cysts. The excised larynges were then placed in a 0.9% saline solution to maintain hydration, and data were collected on them 12 to 36 hours after excision^{20,21}. The true vocal folds were separated from the subglottal wall. To retain the mucosa of the vocal fold, the arytenoid muscle inside was then dissected away using a scalpel. For both hemilarynges, a piece of cartilage was left at both ends of each sample for mounting and application of axial tension tests. All the samples were dissected as single layer, with a width of 3-4 mm and a depth of about 1 mm. After dissection, dimensions of each sample were measured while they were mounted on the test platform with no tensile load applied by a caliper with an accuracy of 0.02 mm. The average of 5 repeated measurements of each sample was used to reduce the error. The lengths of the mucosa samples ranged between 13.50 mm-14.50 mm.

B.2 Test apparatus

An OMEGA stress-strain gauge (DFG55-0.5) and a stepper machine (PMC100, Beijing TianRuiZhongHai precision Instrument Co., Ltd.) was used to record the changes in stress and control the displacement of the samples. The resolution of the gauge and the stepper was 0.5 mN and 0.1 mm

respectively. The gauge was linked to a computer, while the software (MESUR Lite, OMEGA) was set to record the data reported on the gauge at a frequency of 10 Hz. As shown in Figure 1, the stepper machine, on which the prepared mucosa sample was fixed by a suture, was set vertically during the test to avoiding the interference of gravity.

A vacuum dryer (XMTD-8222, Shanghai JingHong Laboratory Instrument Co.,Ltd.) was used to fulfill the dehydration procedure. The temperature of the dryer was set to 40 °C. The sample quality was measured by an electronic balance (YH-A3003, Shanghai YingHeng Weighting Co.,Ltd.) after every dehydration cycle.

C. The dehydration process

The temperature of the vacuum dryer was preheated to 40°C before inserting the samples. The samples were placed in the vacuum dryer initially for periods of 120s. When the sample was closer to complete dehydration, the periods were decreased to 60s to reduce error and so that the samples were not damaged. Complete dehydration was achieved when the weight of the sample did not change between two consecutive measurements, which were taken immediately after each period in the dryer. The total time taken to achieve complete dehydration for each sample was used to calculate the total time necessary to achieve the conditions of 0, 40, 60, and 80% dehydration for the corresponding sample. These times varied as a result of the different mass and water content of the vocal folds of each sample.

D. The Parameters of Analysis

D.1 Cross-sectional area of the samples

Due to the inability to consistently measure the cross-sectional area of the samples, the following function was used :

$$A=V_m/L \quad (1)$$

Where V_m is the mucosa volume and L is the reference length. Since values of strain are dependent upon reference length, it was very important to define and measure the length of vocal fold sample in a consistent manner to reduce errors.

Stress, σ , was calculated using the equation:

$$\sigma=F/A \quad (2)$$

Where F was the force-elongation recorded by the stress-strain gauge.

The mass of each sample, m_0 , was determined without the attached cartilages. Perlman²² measured the densities of canine vocal fold tissues and found that they were relatively constant across multiple larynges ($\rho_0 = 1.02 \text{ g/cm}^3$ for the cover), and thus the initial volume can be calculated by the equation:

$$V_0=m_0/\rho_0 \quad (3)$$

The cross sectional area was calculated as described in Eq. (1). This area could be determined using Hanson's^[9] method, from the measured sample mass (m_s), the original sample mass (m_0) and the measured density of vocal fold mucosa (ρ_s). The mucosa mass was initially measured with a piece of cartilage was left at both ends of samples. However, in a preliminary study it was found that the cartilage mass had little change while the mucosa reached the total dehydration, and therefore the cartilage was removed. The mass of the removed cartilage was measured and defined as m_c after total dehydration. Therefore, the mucosa mass (m_m) was assumed and calculated to be:

$$m_m = m_s - m_c \quad (4)$$

The liquid in the tissue was assumed to be in equilibrium with the saline, thus it was also assumed that the liquid density approached the saline density ($\rho_s = 1.004 \text{ g/ml}$)¹⁴. Therefore, the volume of the mucosa was calculated to be:

$$V_m = m_0 / \rho_0 - (m_0 - m_s) / \rho_s \quad (5)$$

D.2 Stress-strain curve analysis

The slope of the stress-strain curve represents the stiffness of the samples, while the hysteresis area mainly represents the viscosity of the samples. To evaluate viscoelasticity quantitatively, there are two approaches that have been used in previous studies. One approach is to calculate compression resilience ($RC\%$), which is a parameter for viscosity.

$$RC\% = S' / S * 100\% \quad (6)$$

In Eq. 6, S' represents the integral of unloading curve and S represents the integral of the loading curve. If the sample is more viscous, more internal energy is consumed as internal friction, indicating a lower $RC\%$.

Another approach is calculating the hysteresis area which is defined as the area of the closed loop formed by the loading and unloading curves. The change in viscosity is equal to the total hysteresis area.

Young's modulus, defined as the inclination of the stress-strain curve, is the measurement of mechanical stiffness. The varying properties of the curve under different dehydration levels can be investigated by fitting the curve. The low-strain stages behave linearly and the curves follow Hooke's

law. The function $y=a+bx$ was used to fit linear stage of the curves where the slope (b) represents E_0 .

High-strain stages appear to be exponential, rather than linear, and therefore the function $y=A*e^{(-x/t)}+y_0$ was used to fit the whole curve.

III RESULTS

The force versus elongation displacement results of one of the trials of subjecting 35% strain to the sample can be seen in Figure. 2.

The results show that the curves form closed, hysteresis loops as expected. The upper curves correspond to loading and the lower curves correspond to unloading. The viscosity of the vocal fold mucosa causes variation between loading and unloading curves. The areas of the loops increased with increasing dehydration levels. The hysteresis loops express the change of viscoelastic properties of the vocal fold mucosa. The loading and unloading curves are similar in initial trials. The increasing variation between the curves is introduced in highly dehydrated samples. The compression resilience ($RC\%$) and the hysteresis areas have been calculated and the results are shown in Table 1.

The stress-strain data from the 10 samples of vocal fold mucosa were averaged and can be visualized with their corresponding standard errors in Figure 3(a)(b).

The parameters of the fitted curves are shown in Table 2. And the Young's modulus was calculated by a linear regression between 5% and 14% strain values.

The properties of the release portion of the stress-strain curves varied from the stretch portions. The parameters of the fitted curves are shown in Table 3.

IV.DISCUSSION

In this study, the effects of dehydration on the viscoelastic parameters in the vocal fold mucosa were quantified. In consideration of the change of acceleration in the transition of loading to unloading, only the data beneath the level of 32% strain was used. The stress-strain curves shown in Fig. 2. indicate that the curves can be divided into two stages. The first stage is linear, the range of which spans under 10-20% strain, and second stage is nonlinear. The change of inclination between the curves shows that the linear parts of curve shorten with increasing levels of dehydration, and also that Young's modulus increased. This can explain why vocal fold dehydration can elicit disordered voice signals and introduce chaotic vibrations. The higher PTP required to drive vocal fold vibration during dehydration shortens the linear range, and causes the vocal fold stress to enter the nonlinear stage quicker. This became increasingly apparent when the samples were subjected to longer periods of dehydration. These results can also explain why chaotic vocal fold vibrations are likely to be present when the vocal fold cover is dehydrated.

The hysteresis loops express the change of viscoelastic properties of the vocal fold mucosa. The quantitative analysis shows that the change in hysteresis area during different dehydration levels displays exponential tendencies, revealing an exponential relationship between viscosity and dehydration levels.

Greater standard errors can be observed in high dehydration levels, which can be attributed to the entropy theory, as shown in Fig. 3. There is an exponential relationship between strain and stress, stress changed faster with the increase of strain. Compared to the results observed in the stretch phase, Fig. 3(a), the spans of the linear regions in the release phase were found to shorten with increasing dehydration levels. However, the slopes of the linear regions remained similar between the stretch and

release phase. The absolute values of the parameter t decreased with increasing dehydration levels. Also, under the same dehydration levels, the absolute values of the parameter t of the release curve were always less than that of the stretch curve.

In the previous study by Zhang et al.¹² focusing on the biphasic structure solid-fluid of the vocal fold mucosa, the fluid is found to support the majority of the stress at an early stage of stress relaxation. The presented results can verify the biphasic theory. Compared with the research of Hanson et al.¹⁴, the stress-strain results of this study may model vocal fold vibratory behavior and physiology more accurately.

As discussed above, the viscoelastic parameters were found to be important factors that affected the vibration of the vocal folds. As a result of these effects, the vocal fold vibration mode during phonation increased in complexity after the samples were dehydrated. Gaining insight on the mechanical parameters became an essential focus. The results illustrate laryngeal disorders caused by dehydration through mechanical and quantitative parameters which can provide objective data to assess laryngeal disorders caused by dehydration in clinical research.

V. CONCLUSIONS

Studying the effect of dehydration on the mechanical properties of the vocal fold cover is a fundamental step to deeply understand the complex variation of vibration modes. In this paper, we collected stress-strain measurements of ten vocal fold mucosa samples in different dehydration levels. The viscoelastic parameters were calculated by measuring and fitting the stress-strain curves. The results quantified the viscoelastic parameters, the linear parts of curve shortened with increasing dehydration levels, and Young's modulus increased as well. A larger Young's modulus was found to

increase PTP, while a shorter linear region caused nonlinear regions to be entered earlier. These results explain how vocal fold dehydration can elicit disordered voice signals and introduce chaotic vibrations, which are both factors in developing a vocal disorder. Also, these results provide a series of experimental data to verify the biphasic theory. Further study on the vocal fold pathologies caused by dehydration can use these quantified mechanical parameters to setup an objective assessment criteria.

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Table 1. Results of $RC\%$ and hysteresis areas in different dehydration levels

	0	40%	60%	80%
$RC\%$	83.5%	70.7%	59.4%	45.2%
hysteresis areas	6.5	15.2	30.5	105.2

Table 2. Parameters of stretch fit curve under different dehydration levels

Dehydration levels	Stage I: $y=a+bx$			Whole curve: $y=A * e^{(-x/t)} + y_0$	
	b	span	R^2	t	R^2
0	41374.50	17.5%	0.957	-0.104	0.999
40%	44846.05	16.4%	0.958	-0.095	0.999
60%	50198.98	15.5%	0.954	-0.093	0.999
80%	78157.41	14.3%	0.960	-0.091	0.998

Table 3. Parameters of release fit curve under different dehydration levels

Dehydration levels	Stage I: $y=a+bx$			Whole curve: $y=A * e^{(-x/t)} + y_0$	
	b	span	R^2	t	R^2

0	23589.36	18.9%	0.961	-0.070	0.999
40%	29810.85	17.4%	0.953	-0.060	0.999
60%	23198.20	16.7%	0.958	-0.055	0.999
80%	22833.13	15.8%	0.951	-0.048	0.999

FIGURE CAPTIONS

Figure 1. The experimental setup of stress-strain experiments

Figure 2. The stretch and release force-elongation displacement curves of vocal fold mucosa under different dehydration levels

Figure 3. The stress-strain graphs of the averaged data for each dehydration levels. The loading (a) and unloading(b) portions are plotted separately.

(a)

(b)