Tensile Properties of Human Vocal Folds from Smokers versus Non-Smokers

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SUMMARY

Uniaxial tensile tests were performed on the human vocal fold lamina propria of three healthy (i.e. non-smokers) and two smokers cadaveric subjects. For each subject, one vocal ligament specimen and the contralateral vocal fold cover specimen were obtained. A non-contact optical method was employed to determine the spatially varying tissue deformation. Results revealed that the tensile deformation was very heterogeneous. The data provided evidence to support the claim that for *non-smoker* specimens the stretch of the mid-point region is less than the average of the anterior and posterior regions of the vocal ligament (p=0.0017) and vocal fold cover (p=0.0558). Since the mid-point position has the smallest cross-sectional area, one would suspect this region to deform the most, yet this was precisely the opposite of what occurred for the non-smoker specimens. However, for the smokers, it was found (though not statistically significant) that the maximum stretch occurred at the mid-point position for one vocal ligament and one vocal fold cover specimen. We discuss potential histomechanical explanations for such heterogeneous deformation behavior and the differences in the healthy and smoker specimens.

INTRODUCTION

Phonation is the result of the vocal folds vibrating as air is passed up from the lungs and through the larynx; hence, phonation is critically determined by the biomechanical properties of the vocal folds. In a previous study, it was discovered that there was a gradient in the mechanical properties of a healthy vocal ligament specimen [1]. However, that study was limited in subject range and the load states considered. While there exists knowledge on the biomechanical properties of healthy vocal fold tissue [2-3], it is unknown how properties change due to disease or lifestyle choices. The present study seeks to understand how smoking may affect the deformation of vocal fold tissues and the consequences as they pertain to vibration. We hypothesize that the local deformation behavior of non-smokers may be different from that of smokers which would reveal deeper insight into the mechanisms underlying phonation.

METHODS

Tissue specimens isolated from the excised larynges of three male non-smokers and two smokers (one male, one female) human cadaveric subjects were considered. For each subject, one vocal ligament specimen and the contralateral vocal fold cover specimen were tested. Following the protocol of Chan et al. [2], the cover and ligament specimens were carefully dissected with instruments for phonomicrosurgery, separated from the underlying muscle and immediately placed in phosphate buffered saline (PBS). 3-0 nylon sutures were inserted through the center of the arytenoid cartilage and the center of the thyroid cartilage both naturally attached to the vocal ligament or cover specimens. The suture inserted through the thyroid cartilage section was connected to the actuator of a servo-controlled lever system, while the suture inserted through the arytenoid cartilage section was connected to a stationary support. A 1 Hz sinusoidal displacement was applied to the specimen for 300 cycles. A monochrome CCD camera (pixel size 9.9 x 9.9 µm, maximum frame rate 75 fps) together with a macro lens was used to capture images during tensile deformation. Traceable ink markers were applied by initially applying a spray-on cosmetic foundation to the moist tissue. Then, a fine tipped paint brush was used to place black ink markers onto the tissue specimen. Displacements of selected ink markers were obtained from image sequences via the Image Processing Toolbox™ of MATLAB®.

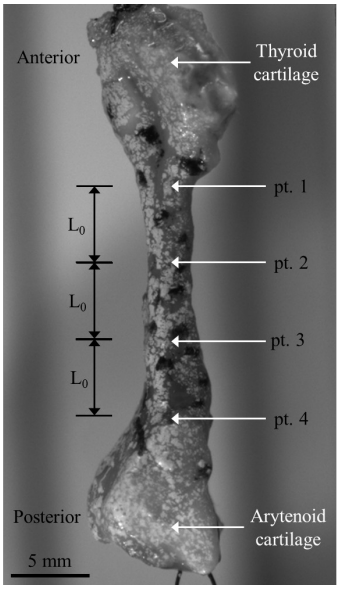

Figure 1: An undeformed image of a vocal ligament specimen from a non-smoker. The markers shown delineate the anterior, center, and posterior segments.

Four markers were chosen in the anterior-posterior (i.e. longitudinal) direction, so that each specimen was divided into three equidistant segments (Figure 1). The stretch λ_i of each segment ($i = 1,2,3$) could then be calculated as $\lambda_i = 1 + \delta_i / L_{i,0}$, where δ_i is the deformation of the segment and $L_{0,i}$ is its initial, undeformed length. Once the stretches were computed, the average of the anterior and posterior segments was compared to that of the center segment. Hypothesis testing of paired data with a t-test was conducted to determine if there was a statistically significant difference in the stretch of the center segment versus the average of the anterior and posterior segments. A typical specimen and the definition of the geometric properties are given in Figure 1.

RESULTS AND DISCUSSION

The 55th stretch cycle was analyzed because it provided good image sequences for all specimens. For non-smokers, the center segment of the specimens did deform less than the average of the anterior and posterior segments, with statistical significance (p=0.0017) for the vocal ligament specimens and near statistical significance (p=0.0558) for the vocal fold cover specimens (see Table 1). These findings confirm the earlier reported results on healthy subjects [1].

Interestingly, the opposite trend was discovered among the subjects who were smokers. The center segment from one vocal ligament and one vocal fold cover specimen from the smokers actually deformed more than the other segments. Even though this discovery is not statistically significant due to the small sample size and high variance, the finding may shed light onto the mechanics of diseased vocal fold tissues. Histology is clearly linked to the biomechanical response of the tissue through local collagen fiber architecture, volume fraction and collagen fiber properties, see [4] for an example on cardiovascular tissue. Thus, we speculate that smoking can affect these parameters and cause deviations from the histological architecture of healthy subjects. This in turn will lead to changes in the deformation and vibration characteristics and subsequently changes in voice. However, further microscopy studies must be performed to either validate or reject this hypothesis.

CONCLUSIONS

Uniaxial tensile tests of vocal fold tissue specimens from three non-smokers and two smokers were conducted. The spatial distribution of stretch was found to be very heterogeneous. For the healthy specimens, the smallest stretch often occurred in the center, where the cross-sectional area is the smallest. Among the smokers, the center deformed the most for one ligament and one cover specimen. These results improve our understanding on the mechanics of non-smoker versus smoker vocal fold tissues. Currently, we are unable to explain why the fundamental frequency of smokers is generally lower than non-smokers. However, there is potentially evidence of histomechanical differences which would affect the vibration characteristics and hence phonation. Consequently, the vocal folds appear to possess functionally graded material properties. The function of the graded properties may be to improve the structural bending and/or shear of the vocal folds. However, the graded properties may also be attributed to age-related effects, thus being an outcome of phonation rather than a requirement. The exact function of the graded properties is the subject of future research.

ACKNOWLEDGEMENTS

The authors are grateful to the National Institutes of Health (NIDCD grant R01 DC006101) for funding this investigation and to the National Science Foundation for supporting J.E. Kelleher in the form of a graduate research fellowship.

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Table 1: Average stretch values for the center segment and anterior and posterior segments and their corresponding p value indicating whether the spatial variation is statistically significant. Note: p value < 0.05 is statistically significant.

	Vocal Ligament					Vocal Fold Cover				
	Mean λ of segments					Mean λ of segments				
Subjects	**center**	**anterior & posterior**	***SD* of difference**	***p* value**	**Signifi-cant?**	**center**	**anterior & posterior**	***SD* of difference**	***p* value**	**Signifi-cant?**
Non-smokers	1.0476	1.0856	0.0039	0.0017	Yes	1.0642	1.1132	0.0310	0.0558	No
Smokers	1.1381	1.0943	0.1238	0.3525	No	1.0865	1.0759	0.0461	0.4004	No