

# Dynamic Testing Characterizes the Viscoelastic Properties of Vocal Fold Tissue

## The Challenge:

To Measure the High Frequency Performance of Healthy Vocal Fold Mucosa

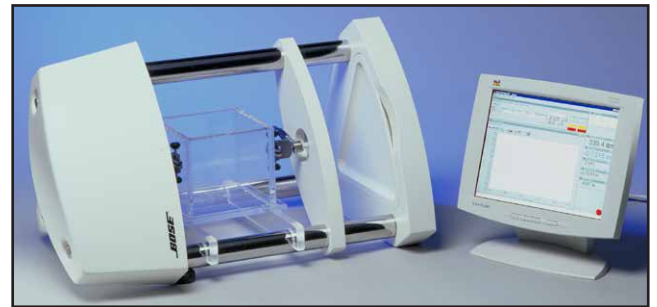
## Background

Mechanical properties of vocal-fold tissues are important in the study of acoustics and biomechanics of voice production. Biomedical engineering researchers at MIT and voice scientists in the Department of Audiology and Speech Sciences at Purdue are key participants in a new research initiative to understand the physical properties of vocal fold tissue and behavior of the vocal cord structure. Laryngeal trauma and cancer can seriously damage the mechanical and vibratory properties of the vocal folds. Such scarring and trauma reduces the natural elasticity of the vocal folds and can cause partial or complete loss of a person's vocal capability. The research teams are working long-term to develop a tissue-engineered implant that would restore functionality to damaged vocal folds.

## Meeting the Challenge

Published data on the dynamic properties of vocal fold tissue is rare and typically limited to frequencies of up to 15 Hz.<sup>1</sup> Dynamic Mechanical Analysis data in the frequency range of typical human speech (150-300 Hz) would be useful to fully evaluate the mechanical properties of native or replacement tissue. In addition, stress relaxation and strain creep data help determine the range of mechanical properties of vocal tissue.

Due to its combination of dynamic performance, precision and sensitivity for resolving force and displacement, the ElectroForce® 3200 test instrument was chosen to test healthy porcine and sheep vocal fold mucosa. The pig and sheep are common animal models of the human larynx. The ElectroForce 3200 test instrument is rated for cyclic testing up to 200 Hz. For this study, a custom 3200 instrument was used with a 450 Hz maximum test frequency.

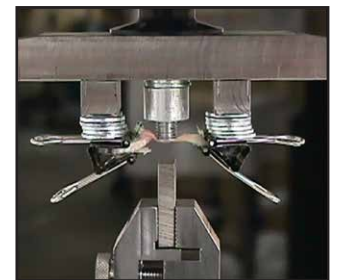


ElectroForce® 3200 test instrument in horizontal position with saline or incubator chamber

## Materials and Methods

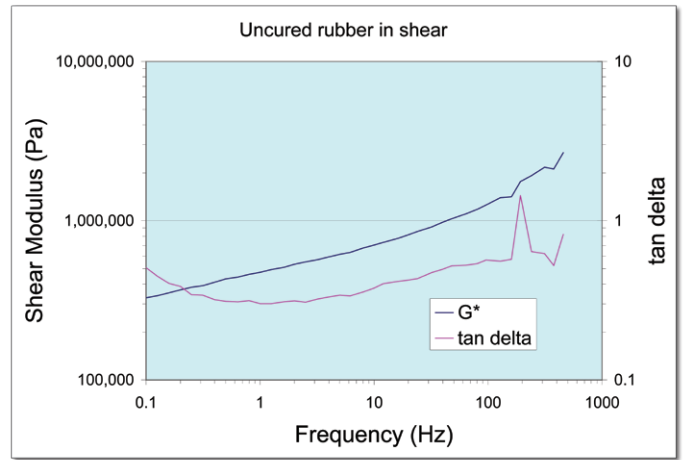
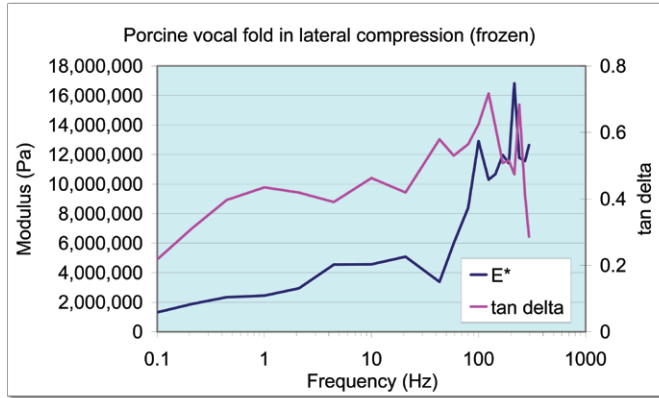
A complete porcine vocal cord with the cartilage attached was tested in lateral compression using a 3-point bend fixture adapted specifically for these dynamic mechanical tests. The section of the sample being tested was 5 mm long x 2.5 mm wide x 1 mm thick. Samples were held in the fixture under minimal tension. Care was taken to keep the sample hydrated during testing. The standard system LVDT was used for displacement measurement with a 45 N maximum capacity strain-gaged force transducer.

The following graphs represent typical data using this test method. Tan delta agrees very well with published data.<sup>1</sup> The compression modulus is higher than existing data in shear or tension primarily due to the pre-compressive stress (in excess of 2000 Pa) required to maintain contact during testing.

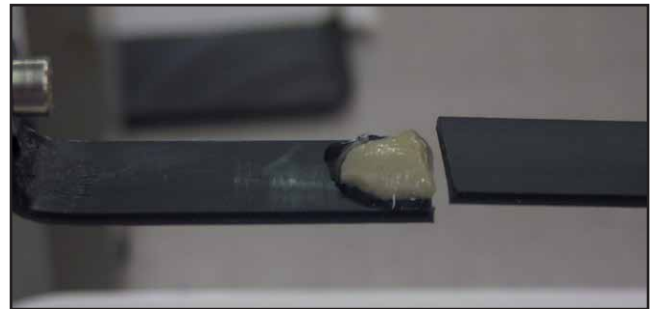
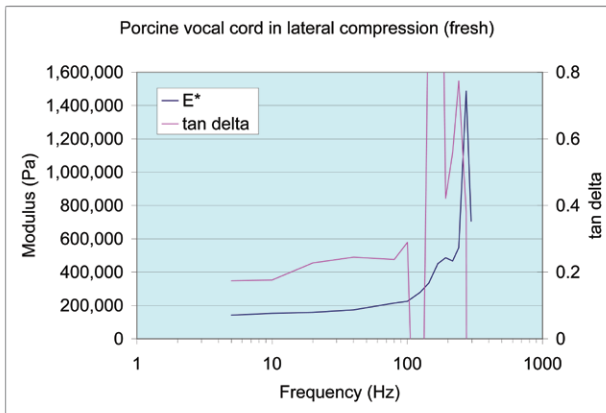


A porcine vocal cord sample in a custom 3-point bend fixture undergoing DMA tests

Refinement of the test methodology may permit a reduction in the pre-stress and, thus provide more representative modulus measurements. The first graph is from a sample that was frozen in air. The second graph is from a fresh unfrozen tissue that was well hydrated. There is an order of magnitude difference in modulus and the tan delta is also higher in the frozen specimen. There is considerable noise in the data above 100 Hz that further optimization of fixtures and transducers could reduce.



To test the soft tissue, a shear fixture was developed of lightweight rigid plastic material. The bottom reaction assembly was designed to allow vertical motion to preload the sample to create a frictional bond between the sample and the reaction fixtures. The amount of preload was not measured but is estimated to be 10-50 grams. A 50 gram strain gage force transducer was used to measure the small forces generated in shear.



Sheep vocal fold tissue sample



Single lap shear of tissue sample with fixture assembled

## Porcine Vocal Fold in Compression

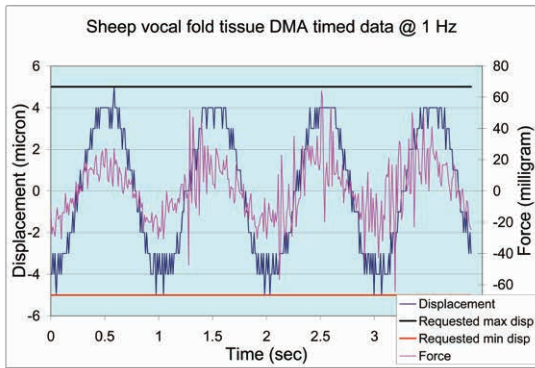
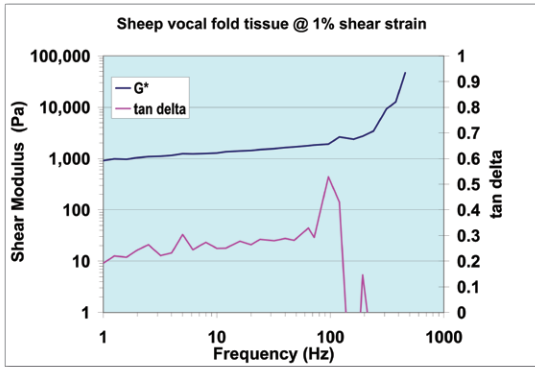
A different test set up was used to test the sheep vocal fold tissue in shear. The ElectroForce® 3200 test instrument was placed in a horizontal configuration and a single lap shear fixture was used to excite the sample. As proof of concept, an uncured rubber sample was tested using a piezoelectric force transducer, which has excellent high frequency performance. The following is an image of the shear fixture used for testing the elastomer and an example of typical data. Other than one out lying data point for tan delta, the data is reasonable out to 450 Hz.



Single lap shear of uncured rubber

The following test data is from a sheep vocal fold. The specimen is mounted so that it is loaded in shear. The sample size is approximately 10 mm x 7.7 mm x 1.2 mm and sheared in the direction of the 7.7 mm dimension. It shows very good complex shear modulus and tan delta data well past 100 Hz with an indication of a resonance at around 450 Hz.

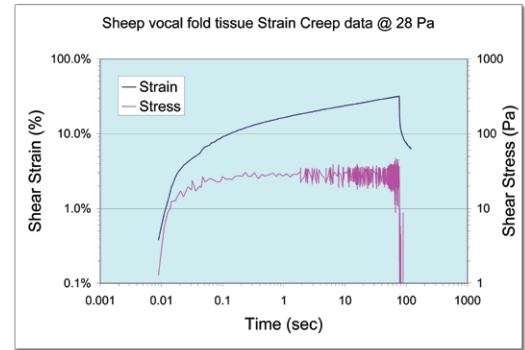
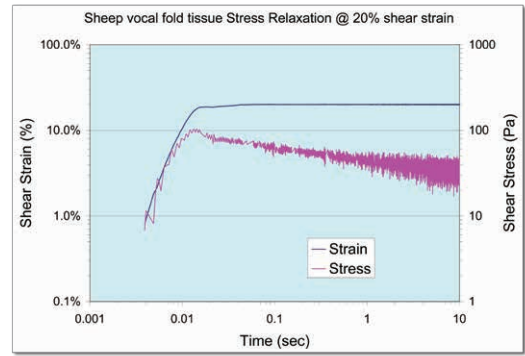
The measured modulus is many orders of magnitude lower than that measured from the compression tests and agrees well with published data taken at frequencies below 15 Hz using a torsional shear rheometer. The tan delta measured in all tests, including the torsional shear rheometer, agree very well with values in the 0.2 to 0.4 range for data falling outside resonance areas.



The timed data shows that the noise to signal ratio is relatively high. The use of a higher resolution piezoelectric force transducer and displacement transducer would be desirable to optimize the frequency response of the test system above 100 Hz. A shear fixture that has higher stiffness and the ability to measure and control the pre-compressive stress might also reduce the amount of resonance at high frequencies.

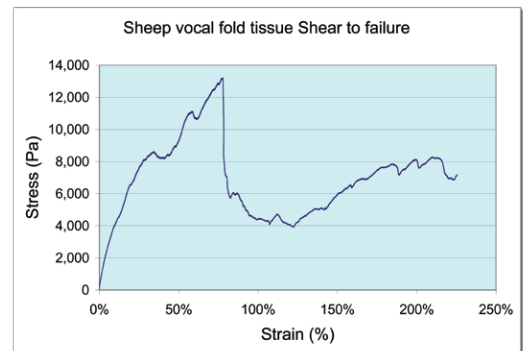
## Creep and Relaxation Data

Additional data was collected to evaluate the creep and relaxation properties of the tissue. Various levels of stress (for creep) and strain (for relaxation) were applied to the specimen and the changes in the relative force and displacement were measured. Of particular note is the ability to control small amplitude forces with the soft tissue sample at relatively high levels of displacement. 28 Pa stress represents 2 mN force and 30% strain represents 0.300 mm displacement. The following graphs are representative of the data collected during this segment of the evaluation.



## Shear Strain Ramp to Failure

As a final test, the sample used for the higher force creep testing was tested to failure at a rate of 0.1 mm per second (4% strain per second for a 2.5 mm thickness). Recorded forces were as high as 2 N at initial failure with bridging occurring after initial failure.



## Summary

This initial series of tests demonstrates that the custom ElectroForce® 3200 instrument with DMA software provides an excellent tool for determining the viscoelastic properties of soft tissues such as vocal folds. Although the dynamic testing of vocal fold tissue at high frequency by the MIT and Purdue teams is still in the early stages, the capabilities of the system will allow them to better determine the elasticity of human vocal folds and help pioneer the use of tissue engineering in vocal fold restoration.

(1) Chan, R. W., and Titze, I.R. (1999). "Viscoelastic shear properties of human vocal fold mucosa: Measurement methodology and empirical results" in J. Acoust. Soc. Am. 106, 2008-2021