Mapping The Visco-Elastic Properties of the Vocal Fold

Eric Goodyer (1), Heather Gunter (2,3), Asa Masaki (3), and James B. Kobler (3,4)

(1) DeMontfort University, Leicester, UK, eg@dmu.ac.uk.; (2) Division of Engineering and Applied Science, Harvard University, Cambridge, MA.; (3) Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA.; (4) Harris Peyton Mosher Laryngological Research Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA, 02114, USA, James_Kobler@meei.harvard.edu

Abstract: The Linear Skin Rheometer (LSR), which measures skin visco-elasticity, was adapted for measurements of vocal fold properties. In excised larynges small patches of mucosa were driven sinusoidally at .3 Hz over 1-2 mm distances using a small probe. Forces on the order of one gram gave optimal measurements. Stiffness and viscosity values were derived from stress/strain data and using a simple shear model. The instrument was able to measure the visco-elasticity of the tissue in a repeatable manner and it could detect areas where the tissue was artificially stiffened. 2D maps of the viscous and elastic properties of the laryngeal mucosa were obtained showing local variations in elasticity and viscosity both parallel and perpendicular to the vocal fold edge.

Introduction: In phonosurgery there is a need for an instrument that can measure the material properties of the superficial lamina propria of the vocal fold, a layer that is critical for normal phonation, that is frequently damaged and that is a key target for novel vocal fold augmentation techniques. Such an instrument would ideally help the surgeon sense the extent and degree of tissue abnormality and provide objective measures before, during and after treatment.

The Linear Skin Rheometer

We undertook a pilot study to see if a commercially available rheological device, the Linear Skin Rheometer, or LSR, could be used to make measurements of vocal fold viscoelastic properties. An important feature of this instrument is the long lightweight tissue probe, which makes it suitable for potential use via a surgical laryngoscope. A series of bench tests of the LSR was conducted to address the following questions: (1) Is the LSR adequately sensitive to measure vocal fold mucosal material properties? (2) Can reproducible measurements be obtained? (3) Can differences in tissue properties be detected and correlated with different anatomical locations or tissue modifications?

The experiments to be described have been directed towards establishing the feasibility of using the LSR as a measurement tool rather than the gathering of normative data. The results suggest that the LSR has potential for providing clinically useful intraoperative assessment of vocal fold material properties.

Methods: The LSR is a precision mechatronic instrument that was designed to measure the visco-elastic properties of the stratum corneum of the skin [1]. Based upon an original concept developed by Hargens in the 1960's (The Gas Bearing Electrodynanometer or GBE) [2,3], the LSR uses modern micro-mechanical components to achieve force feedback control in real time, and precision position measurement.

LSR Measurement principles: When measuring the elastic and viscous properties of a material we are seeking to determine how far the material moves when a lateral force is applied to it. If we apply a sinusoidal force, then we expect to see a resultant displacement that also changes sinusoidally. The phase shift between the force and displacement curves is also of great interest. This technique has been used to measure skin elasticity, infer hydration levels [4] and assess skin ageing [5].

To make a measurement a probe is attached to the surface of the skin or larynx, and a sinusoidal force is then applied along its axis and thereby onto the tissue. Typically the peak force applied will be in the region of 3g for skin and 1g for the larynx. By simultaneously measuring the displacement caused by the force then a pair of readings is obtained as shown schematically in figure 1.

Three parameters can be obtained from the curves - *F Max* which is the peak force that is applied to the skin surface, *P Max* which is the peak displacement that occurs as a result of that force, and *T* which is the phase shift between the two signals. The elastic component of the skin is given simply by *F max / P max*, and is usually expressed in units of grams force per millimetre. The usual way of presenting this data is to plot force directly against displacement, in which case an ellipse will be formed, as the component parts are two sine waves with an identical period, but shifted in time. Such a picture, as taken from the LSR, is shown in figure 2. The phase shift is due to the viscous properties of the tissue. The LSR captures the force and displacement waveforms, and performs a linear regression on that data in order to determine the coefficients of the biodynamic equations for variation of force and displacement with time. (1) $F = FmaxSin(t)$ and (2) $P = PmaxSin(t+T)$, where F = instantaneous force, $Fmax$ = the maximum force, t = time over one cycle in radians, P = instantaneous displacement, $Pmax$ = the maximum displacement, and T = the phase shift in radians. To summarise, the slope of the ellipse along its major axis is the elastic parameter, and the area of the ellipse is the viscous parameter [6].

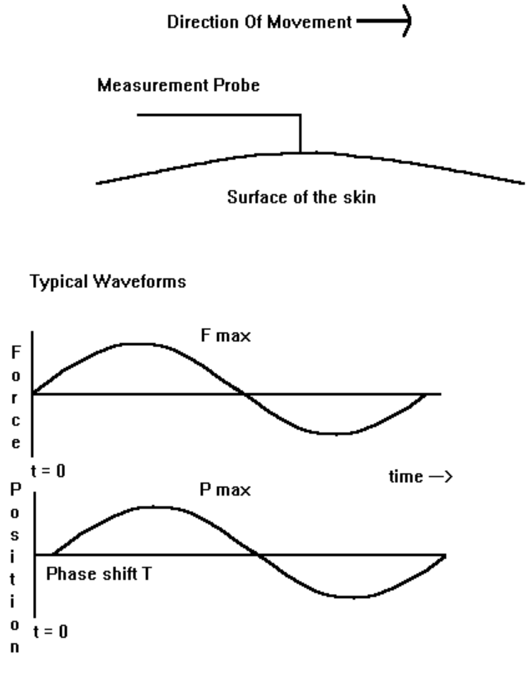
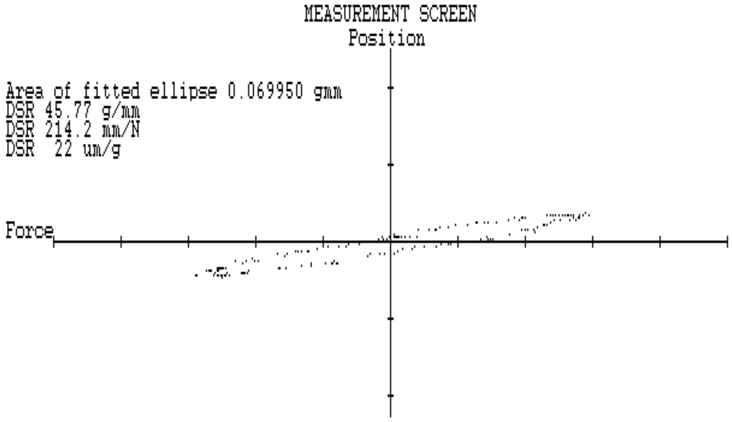


Figure 1 Typical Waveforms of Force and Displacement



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MEASUREMENT SCREEN

Area of fitted ellipse 0.069950 gmm
DSR 45.77 g/mm
DSR 214.2 mm/N
DSR  22 um/g
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Figure 2 Typical measurement screen

LSR device: The key to the design of the LSR is the means to apply a continuously varying, but controlled, force to the surface of the tissue. From the measuring head a light stiff probe protrudes, the far end of which is bent through a right angle and its tip is modified to attach to tissue. For laryngeal measurements we have used a variety of fine needle-tipped probes and also suction probes connected by flexible silicone tubing to a source of negative pressure. Inside the measuring head, the probe is attached to a load cell, which is moved along the probe axis by a motor-driven lead screw. An LVDT is used to monitor the position of the load cell and probe.

A PC is used to control the movement of the sensing head. Both force and position are continuously monitored at a rate of 1 kHz using a 12-bit ADC plug-in card. The motor is controlled with an analogue output signal also generated by the PC. The desired force/time cycle, which is normally a single sinusoid, is initially calculated from an equation, and then stored in memory as a table of values. The actual force applied to the probe is compared with the desired value in the table 1000 times per second. A feedback loop is used to control the motor, which moves the load cell in such a way as to minimise the discrepancy. The force applied thus follows closely the desired force/time cycle. The control loop uses an algorithm with proportional and integral terms, whose relative weighting can be varied.

The PC logs all the force and displacement readings over the complete measurement cycle, which is usually set to be 3 seconds thus generating 3000 pairs of points. This data is then used to generate the graphs that are shown above, and are analysed to determine the elastic and viscous parameters. Measurements of elasticity are expressed in terms of the Dynamic Spring Rate, in units of g/mm. The load cell used in the LSR is supplied by Maywood and has a full scale reading of 10g, with an overall accuracy of better then 0.02g. The LVDT is supplied by Solartron, type DF2.5, which has an accuracy of better than 4 microns. The motor is supplied by Maxon. Minor modifications were made to the LSR design to make it more suitable for measuring the visco-elastic properties of the larynx, but the underlying principles remain unchanged.

Bench tests on Laryngeal Specimens: Laryngeal specimens were prepared by hemisection, taking care to leave the vocal fold attachment to the thyroid cartilage at the anterior commisure region intact. The specimens were pinned to a wooden base attached to small XY/rotary machinist's fixture that allowed for accurate positioning and rotation. The specimens were kept moist with physiological saline and measurements were made at room temperature (roughly 20^{o}C). Most measurements were made using needle tipped probes. The most effective of several designs tested was made from a spring steel rod 1mm in diameter and 10 cm in length, which was bent to a right angle 5 mm from one end. A fine (000) insect pin was soldered to the short bent section so that it protruded 1.5 mm beyond the end of the rod. This needle was inserted into the tissue up to the rod, which controlled insertion depth to 1.5 mm. In some instances we used a suction-based probe made of lightweight aluminum tubing with an internal diameter of 1.4 mm.

Results:

Tests of repeatability: Six pig larynxes were measured at the centre of the vocal fold. Displacement axis was perpendicular to the length of the vocal fold. The table shows the standard deviations and means derived from 6 consecutive readings of the DSR taken from the same starting position.

Repeatability Tests	SD	Mean	SD/Mean
' middle' of sample 1	0.040591	0.691429	0.058706
' middle' of sample 2	0.058878	0.776667	0.075809
' middle' of sample 3	0.020659	0.69625	0.029672
' middle' of sample 4	0.036425	0.72125	0.050503
' middle' of sample 5	0.01169	0.488333	0.023939
' middle' of sample 6	0.005164	0.633333	0.008154

TABLE 1 – *Tests of repeatibility*

Variation in properties along length of vocal fold: The LSR was used to measure the DSR using a needle probe along the length of the vocal fold in a fresh pig vocal fold. The probe was placed perpendicular to the long axis of the vocal fold. Five readings were taken at each point, and the average plotted with respect to position of the measurement from the vocal process. The complete set of measurements is given in table 1. Stiffness was greatest near the vocal process and anterior commisure. The DSR over the vocal process was about 3-fold higher than over the membranous vocal fold

Position from Vocal Process (inches)	DSR 1	DSR 2	DSR 3	DSR 4	DSR 5
-0.07	2.81	2.89	2.98	2.84	2.97
0.05	0.88	0.9	0.9	0.86	0.88
0.1	0.69	0.68	0.69	0.69	0.71
0.2	1.28	1.29	1.26	1.25	1.25

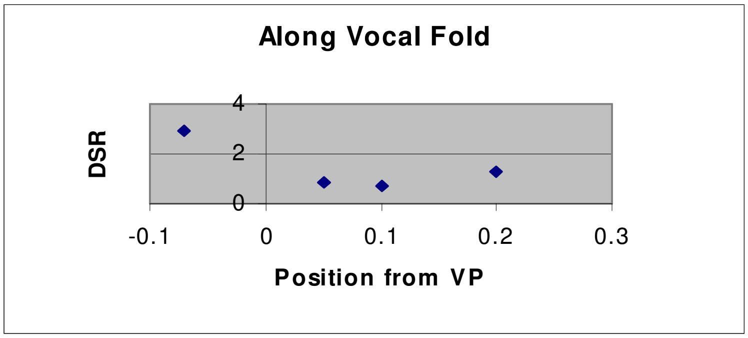


TABLE 2 – *Variation in DSR along length of vocal fold*

Effect of displacement direction relative to vocal fold axis: Readings were taken from the mid-membranous region of a pig vocal fold with the probe producing displacements at different angles relative to the long axis of the vocal fold. Five readings were taken at each angle, and the average DSR was plotted against that angle. The stiffness was least for displacements perpendicular to the long axis, and maximal for displacements along the long axis.

Angle	DSR 1	DSR 2	DSR 3	DSR 4	DSR 5
30	0.6	0.64	0.62	0.62	0.62
50	0.83	0.85	0.8	0.79	0.76
90	0.98	0.98	1.05	1.02	1.01
130	0.95	0.95	0.92	0.95	0.93
150	0.73	0.78	0.74	0.78	0.67
180	0.58	0.44	0.46	0.45	0.42

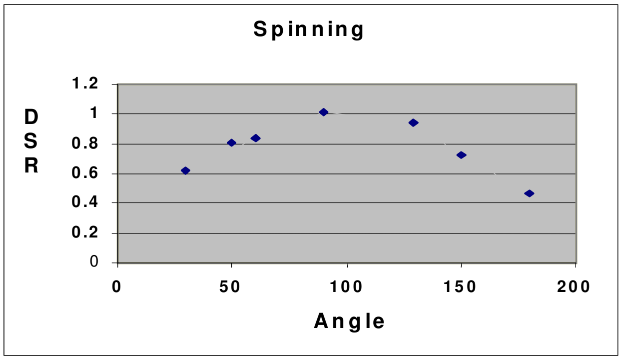
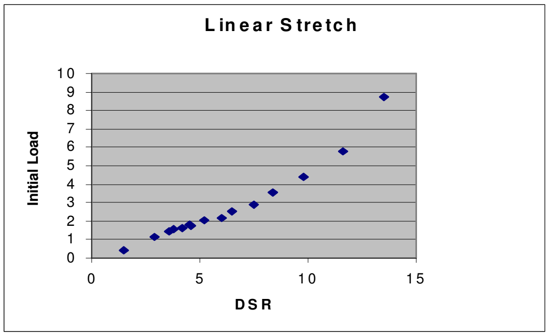


TABLE 3 – *Effect of displacement direction relative to vocal fold axis on DSR*

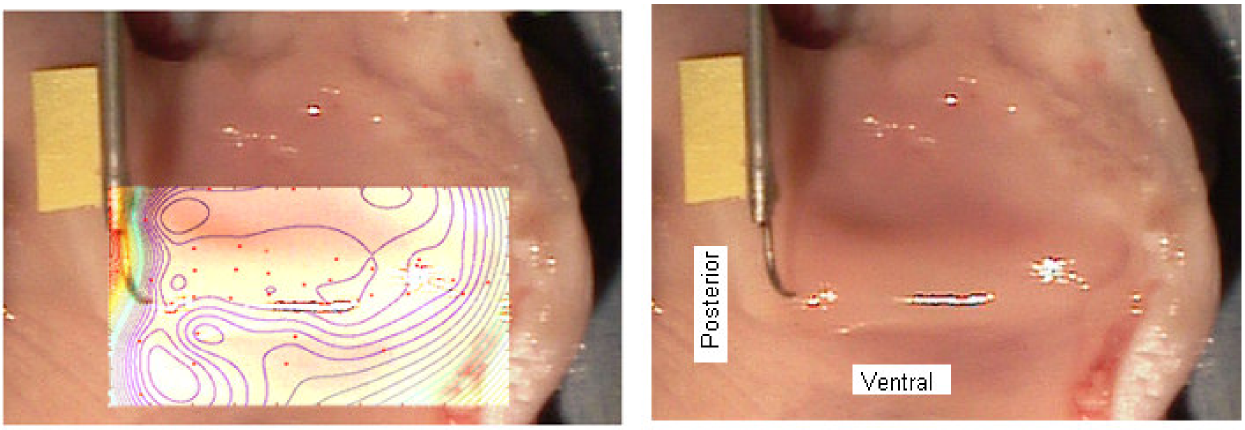
Measurements of excised lamina propria: In one calf larynx the lamina propria of the vocal fold was dissected out with attached vocal process and small attached piece of thyroid cartilage. The tissue was mounted such that it could be pre-tensioned and then measured. The tissue was pre-tensioned to a 'starting force', and a sinusoidal force of +- 1.5g was applied. The initial 'draw length' was also measured. The purpose of this trial was to determine if it would be possible to construct a classic stress/strain curve. The graph shown simply plots initial tension against measured DSR.



Draw length	0	0.05	0.065	0.075	0.085	0.095	0.095	0.11	0.125	0.155	0.17	0.185	0.2	0.215
Starting force	1.5	2.9	3.6	3.8	4.2	4.5	4.6	5.2	6	7.5	8.4	9.8	11.6	13.5
DSR	0.4	1.13	1.46	1.58	1.62	1.82	1.73	2.03	2.16	2.9	3.56	4.38	5.77	8.73

TABLE 4 – *Linear stress/strain data excised lamina propria*

Two-dimensional mapping of DSR: Using the XY table to position a sheep larynx, a systematic map of DSR at different locations on, above, and below the vocal fold was made. A reference digital photograph was taken at each point of measurement. Using this data, a 2D plot showing the variance of elasticity across the surface of the specimen was created by registering an isocontour 2D plot of elasticity (generated using Matlab software) to a lateral photographic view of the vocal fold. Contours reflect increments of .06 g/mm and range from .6 g/mm along vocal fold to 12 g/mm over arytenoids.



View of Vocal Fold with overlay of DSR contour map

View of Vocal Fold with LSR probe attachment

Images taken at Harvard, showing experimental setup of LSR with ex-vivo focal-fold, together with results obtained from research data.

FIGURE 3 *Visco-Elastic Isocontour Map*

Application of a simple model of shear viscoelasticity to derive viscosity estimates: The LSR derives viscous properties of a tissue by measuring the phase shift between the applied force and the resultant displacement. As we know the cycle time of the applied force, this measurement can be converted into the viscosity parameter for shear damping.

Assuming that the tissue can be characterised by shear stiffness *(k)* and shear damping *(B)* parameters that are in parallel, and that a sinusoidal shear force $(A*\sin(wt))$ is applied on the tissue surface, then the displacement of the surface *(x)* is $C*\sin(wt + z)$:

amplitude of force/amplitude of movement	$A/C = \mathrm{sqrt}(B^2w^2 + k^2)$	1.
Phase difference between force and movement	$z = \tan^{-1} Bw/k$	2.
Simplify (2) to obtain equation for B	$B = \tan(z)k/w$	3.
Simplify (1) to obtain equation for k	$(A/C)^2 = \tan(z)^2k^2 + k^2$	4.
Take out k^2	$(A/C)^2 = k^2(\tan(z)^2 + 1)$	5.
Apply trig. Rule $\sin^2 = 1/(\tan^2+1)$	$(A/C)^2 = k^2/\sin(z)^2$	6.
Solution for Shear Stiffness	$k = (A/C)\sin(z)$	7.
Replace k in (3)	$B = \tan(z)\,(A/C)\,\sin(z)/w$	8.
Apply trig. Rule $\tan(z)\sin(z)=\cos(z)$	$B = (A/C)\cos(z)/w$	9.

w is known since it is the applied frequency in Hz divided by 2*Pi. *A* is known since it is the applied amplitude of the force. *C* is known since it is the measured amplitude of the movement. *z* is the phase difference and is derived by a linear regression of the data obtained by the LSR with respect to time.

This basic relationship can be used to separate out the viscous and elastic components of the vocal fold biodynamics. The plot below shows the variation of these properties along the axis of the vocal fold. Shear damping units are given as gs/mm and the elastic units are given as g/mm.

Position	Elastic g/mm	Viscous gs/mm
2.54	0.686	0.460631
5.08	1.252	0.796346
-1.778	2.874	1.751961
1.27	0.8775	0.538705
0.71882	0.635	0.468439

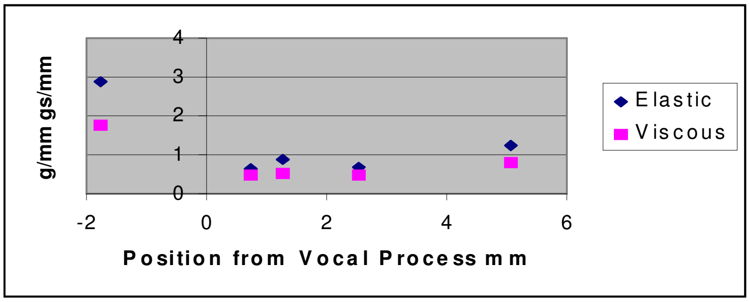
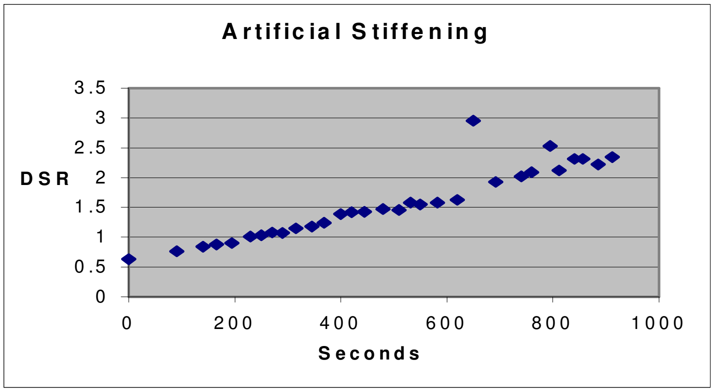


TABLE 5 – *Resolving out pure elastic and viscous components*

Artificial Stiffening:



A pig vocal fold was injected subepithelially with .1cc of 10% formaldehyde solution, and the DSR was measured over a period of 15 minutes. The graph shows change in DSR of the specimen as the tissue was artificially stiffened.

FIGURE 4 *Change in Stiffness over Time*

Effect of Differing Layers: The DSR of different layers of a calf larynx were measured in a direction parallel to the vocal fold. This was achieved by sequentially removing layers, and retaking the measurement at the same position. These initial results demonstrate how the layers contribute to overall tension development as the vocal fold is stretched.

A. Typical epithelium measurements.
B. A small island of epithelium about 2 x 3 mm which indicates the relative contribution of the epithelium versus the underlying amorphous layer.
C. Epithelium removed.
D. Amorphous layer removed
E. Directly in the muscle.

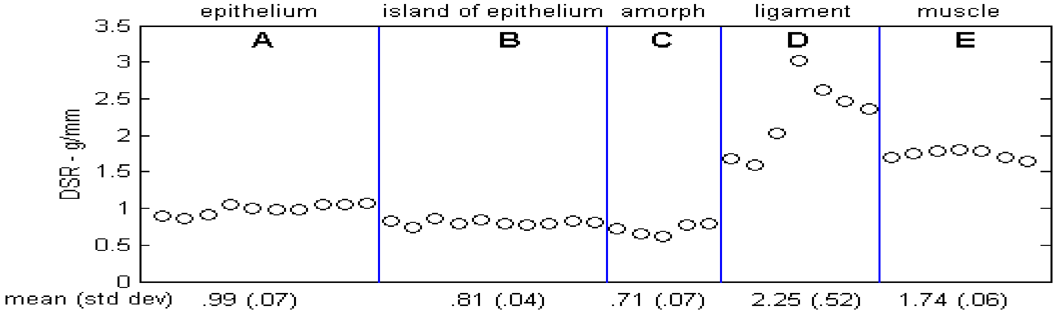


FIGURE 5 *Variation of DSR with Vocal Fold Layers*

Discussion: Over the last decade there has been a surge of interest in vocal fold material properties and a widening appreciation of the relevance of such data to better understanding the anatomy, pathology, aging, modeling and repair of the vocal folds.

For excised specimens, parallel plate rheometry has been become a standard for determining the shear viscoelasticity parameters of tissue from animals, cadavers or surgical specimens, of candidate augmentation materials and of animal vocal folds previously implanted with augmentation materials [10-17, 21, 29]. Complimentary stress/strain studies on excised vocal fold layers have provided data on longitudinal viscoelastic properties essential for understanding vibratory behaviour and contributions of the different layers to vibration as a whole [7,22,23,24].

Measurements on intact vocal folds has been technically difficult, but is clearly essential for many clinical applications. In vivo measurement could potentially help identify abnormal regions, provide feedback during augmentation surgery and aid in the objective assessment of surgical procedures designed to manipulate vocal fold material properties. Methods that have been applied to intact vocal folds include indentation with a probe attached to a servo motor-controlled force sensor [18,19], lateral displacement of the vocal fold with a trans-oral calibrated lever [8,9,27], and medial aspiration of the mucosa with a calibrated suction catheter [25]. The latter two methods have been tested on human subjects under general anesthesia.

In contrast to the *in vitro* rheological methods, the LSR is not capable of determining absolute values for viscoelasticity parameters on a unit area or volume basis because the exact volume of tissue that is deformed cannot be determined. Data obtained so far is comparable to previous intact larynx methods. Like the indentation approach and in contrast to the transoral lever, there is good spatial resolution. Relative measurements are potentially very useful in many clinical scenarios if abnormal tissue can be identified or changes resulting from a treatment can be documented. The results demonstrated that this method has the ability to make sensitive and repeatable punctate measurements that may allow for mapping areas of pathology, and for side-by-side comparison of a normal with an abnormal vocal fold. It is also well suited for testing the properties of the clinically important superficial lamina propria because it can be attached non-invasively to the epithelium with a suction cannula and gently wiggle the vocal fold cover. This is similar to the way surgeons intuitively test vocal fold properties by palpating the tissue with small surgical instruments.

Future Directions: While the LSR was able to reliably measure the most pliable tissues of the larynx, it was originally designed for measurements of skin on the dorsum of the hand, which is about 5 times more stiff than the vocal fold. Some adaptations to the hardware and software would improve performance of the feedback control system for vocal fold tissue. Following these improvements further in vitro testing of human and animal vocal folds will be pursued to obtain normative data under well-controlled conditions.

We have found that the probe of the LSR can be adapted to work through a surgical laryngoscope, but the arrangement is somewhat cumbersome. Our current technical review has indicated that the application of Surface Acoustic Wave (SAW) technology offers the potential to further miniaturize the LSR to the point where it is convenient for routine clinical use in the operating room.

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