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Measurements of Vocal Fold Elasticity Using the Linear Skin Rheometer

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Key Words
Vocal fold elasticity · Linear skin rheometer · Dynamic spring rate

Abstract

Objective: The linear skin rheometer (LSR), which measures skin visco-elasticity, was adapted for measurements of vocal fold properties. A series of studies was performed on animal and human excised larynges to determine if the LSR technique can be applied to the vocal fold. ***Methods:*** In excised larynges, small patches of mucosa were driven sinusoidally at 0.3 Hz over distances of 1–2 mm using a small probe. Forces in the order of 1 g gave optimal measurements. Stiffness and viscosity values were derived from stress/strain data. ***Results:*** 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. Two-dimensional maps of the mechanical properties of the laryngeal mucosa were obtained showing local variations in elasticity both parallel and perpendicular to the vocal fold edge. Initial studies were undertaken using animal tissue; more recently, the LSR has been successfully used to obtain similar data from human tissue. ***Conclusion:*** The LSR was been demonstrated to be capable of measuring the elastic properties of the vocal fold in a repeatable and reliable manner. Further studies will now be undertaken to obtain data from a larger sample of human tissue.

Introduction

In phonosurgery there is a need for an instrument that can measure the pliability of vocal fold mucosa (epithelium and superficial lamina propria). The superficial lamina propria, which is the primary oscillator, is critical for normal phonation and is frequently damaged [1]. Therefore, it is a key target for novel vocal fold augmenta-

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tion substances and techniques to restore mucosal pliability. Consequently, it would be valuable to have an instrument that would assist the surgeon with assessing mucosal stiffness/pliability, and which could provide objective measures before, prior to, during and after treatment. Detailed understanding of the biomechanics of the vocal fold can only be achieved if we are able to measure its elastic properties in situ.

The Linear Skin Rheometer

The linear skin rheometer (LSR) [2, 3] is a precision electromechanical measuring instrument that was specifically designed to measure the visco-elastic properties of the stratum corneum. This technique was successfully adapted during a yearlong programme [4] to take visco-elastic measurements from vocal fold tissue. Of particular value is the use of a variable length probe, typically 300 mm long, that allows the user to probe along structures and within cylindrical shapes. This probe is attached to the tissue under test using a needle that is mounted at right angles to the primary rod axis. The rod is capable of rotating through a full circle, allowing the needle to be inserted at any angle into the tissue. It is this technique that allowed us to take measurements from within an intact larynx in a perpendicular direction with respect to the vocal fold.

The needle probe is inserted into the vocal fold epithelium such that the direction of motion is normal to the line that can be drawn between the vocal process and anterior commissure. Thus, the direction of the measurement is in the same axis as the motion generated by the mucosal wave generated during phonation. The force applied is sinusoidal, with a peak amplitude of 1 g, applied at a rate of 0.3 Hz. The resultant force/displacement data forms an ellipse; this is due to the displacement lagging behind the applied force due to the viscous properties of the tissue.

In this summary only the dynamic spring rate (DSR) data are presented, which is defined as the peak force/peak displacement. The greater the DSR, the stiffer the material under test. For all DSR measurements, the unit is grams per millimetre.

Instrument Hardware and Design

A schematic drawing is shown in figure 1. A force-controlled miniature direct-current servo (Maxon 23-12, 0.5 W rating, supplied in the UK by Trident Engineering), gearing and lead screw drive the LSR probe. The linear variable differential transformer has a unit of linearity of 0.3% (15 μm) and a sensitivity of 0.01% (0.5 μm) (Solartron type DF2.5, Schlumberger Industries). The force exerted on the probe is measured directly by a calibrated load beam (Minigram Beam Load Cell, type MBH50, rated 50 g, supplied in the UK by RDP Electronics) with an overall accuracy of $<$20 mg. The load beam is mounted vertically within the instrument casing. The needle probe is attached to the surface of the tissue under test, thus creating a shear force.

The needle was formed by tapering the tip of a 1-mm diameter stainless-steel rod over a distance of about 5 mm. The insertion depth was approximately 0.5 mm. There was no histological evaluation of tissue damage but neither was there any obvious damage or tearing of the tissue. The small displacements of typically 1 mm,

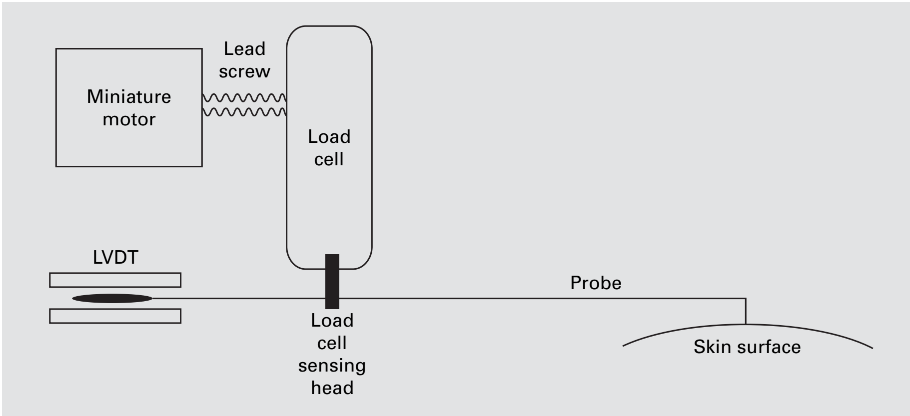

Fig. 1. The LSR sensor head. LVDT = Linear variable differential transformer.

and maximum force of 1 g, are within the range of the normal motion of the tissue during phonation.

All components fit into one casing measuring 20.0 × 14.8 × 6.9 cm, and the whole unit weighs 1.7 kg. The probe housing itself is a light-weight machined Perspex chuck mounted on a low-friction swivel assembly allowing 360-degree movement. This is protected from damage during routine usage by a metal collar. The chuck contains wire grips to allow the wire probes to be inserted or withdrawn by a simple, firm push or pull. A single lead connects the unit to a PC via a 25-pin D-type connector. Power for the LSR unit is taken from the PC via the connector.

Instrument Control

An IBM (or compatible) PC is used to control the movement of the probe and to log force and displacement data. Both force and displacement are monitored continuously at a rate of 1 kHz using a 12-bit ADC plug-in card (National Instruments ATMIO16). 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 calculated initially 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 1,000 times a second. A feedback loop is used to control the motor, which moves the load cell in such a way as to minimize any discrepancy. The force applied thus follows the desired force/time cycle extremely closely. The control loop uses an algorithm with proportional and integral terms, whose weightings can be varied.

The PC logs all the force and displacement values over a complete measurement cycle, which is usually set at 0.33 Hz, thus generating 3,000 pairs of points over a 3-second cycle. Two waveform plots are then obtained, as seen in figures 2 and 3.

Three parameters may be obtained from these curves:

F_{max}, the peak force that is applied to the skin surface;

P_{max}, the peak displacement occurring as a result of that force;

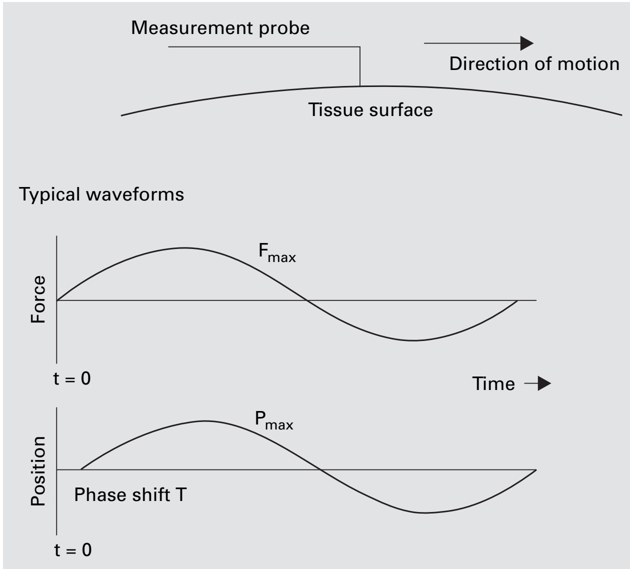

Fig. 2. Typical waveforms of force and displacement.

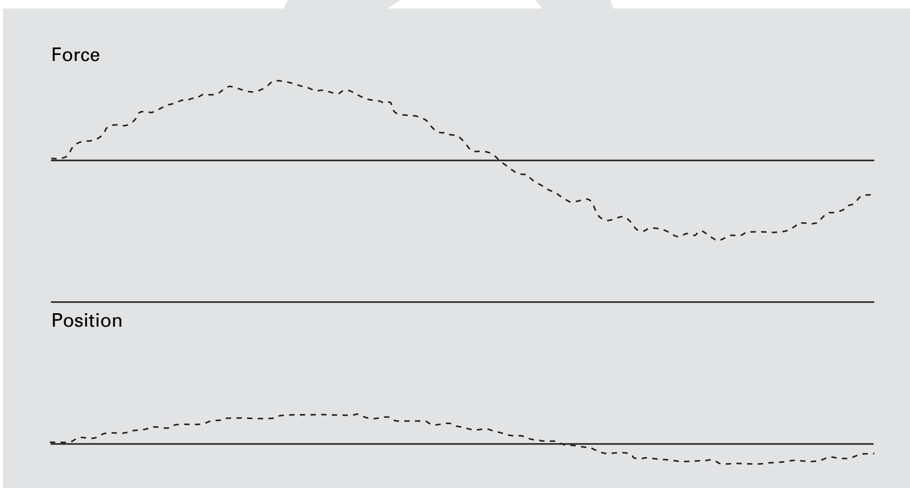

Fig. 3. A pair of real force and displacement traces.

T, the phase shift between the two signals.

The DSR is given simply by the formula F_{max}/P_{max}. Derivatives are calculated and expressed as grams per millimetre, millimetres per newton and micrometres per gram. The DSR is a non-frequency-dependent measurement of the elasticity of the material under test.

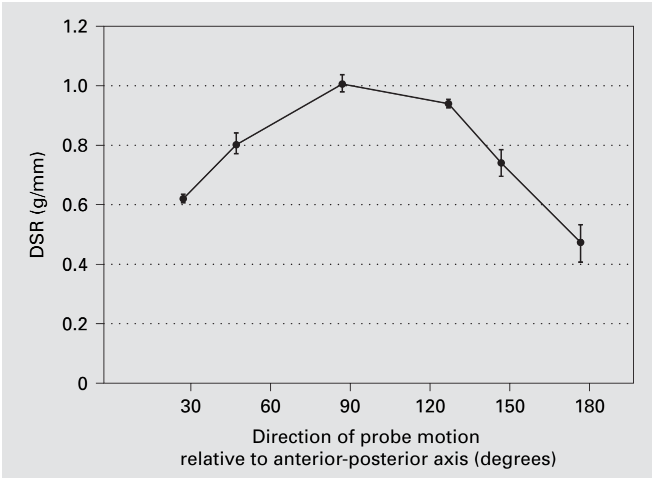

Fig. 4. The viscous component may be inferred by calculating the area of ellipse, i.e. by the angle of probe motion (study 2).

The viscous component is often inferred by calculating the area of the ellipse shown in figure 4. A more rigorous approach is to perform a regression on the original sinusoidal data in order to solve the equations:

$$F = F_{max} \sin(t) \qquad (1)$$

$$P = P_{max} \sin(t + T) \qquad (2)$$

where F = instantaneous force, F_{max} = peak force, t = time for one complete cycle in seconds, P = instantaneous displacement, P_{max} = peak displacement, T = phase shift in radians.

Having solved these equations, it is then a straightforward problem to solve the integral over one cycle that represents the area of the ellipse:

$$\int_0^{2\pi} F_{max} \sin(t) P_{max} \cos(t + T)$$

The LSR software solves the above equations for both elastic and viscous components of the data. These are subsequently displayed, directly after measurement. For this study only the non-frequency-dependent DSR readings were recorded.

Bench Tests on Laryngeal Specimens

Laryngeal specimens from animal sources were prepared by hemisection, taking care to leave the vocal fold attachment to the thyroid cartilage at the anterior commissure region intact. The specimens were pinned to a wooden base attached to a small XY/rotary machinist's table that allowed for accurate positioning and rotation. The specimens were kept moist with physiological saline, and measurements were made at room temperature (roughly 20°C). Most measurements were made using needle-tipped probes. The most effective of several designs tested was made from a

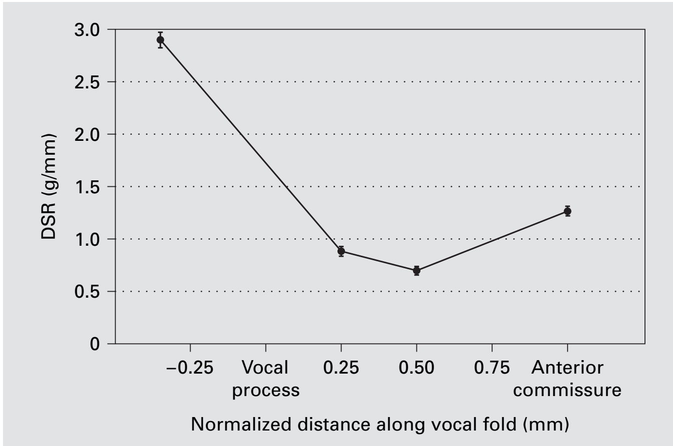

Fig. 5. Measurements of study 1: normalized distance along vocal fold.

spring steel rod 1 mm 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 the insertion depth to 1.5 mm. In some instances we used a suction-based probe made of light-weight aluminium tubing with an internal diameter of 1.4 mm. The human specimen was first tested intact, in order to determine the feasibility of obtaining perpendicular measurements from within the larynx. It was then split in a similar manner to the animal specimens, and the measurements were repeated.

Study 1 – Variation in DSR Properties along the Length of the 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 was plotted with respect to the position of the measurement from the vocal process. The complete set of measurements is given in figure 5. Stiffness was greatest near the vocal process and anterior commissure. The DSR over the vocal process was about 3-fold higher than over the membranous vocal fold.

Study 2 – 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, as shown by figure 4. The lowest DSR was found to be perpendicular to the long axis (90°) and maximal for displacements along the long axis.

Study 3 – Elasticity of the Vocal Folds within an Intact Human Larynx

A freshly excised male adult larynx was deep frozen (–80°C). After thawing, the intact larynx was mounted such that the LSR probe could be inserted into the larynx

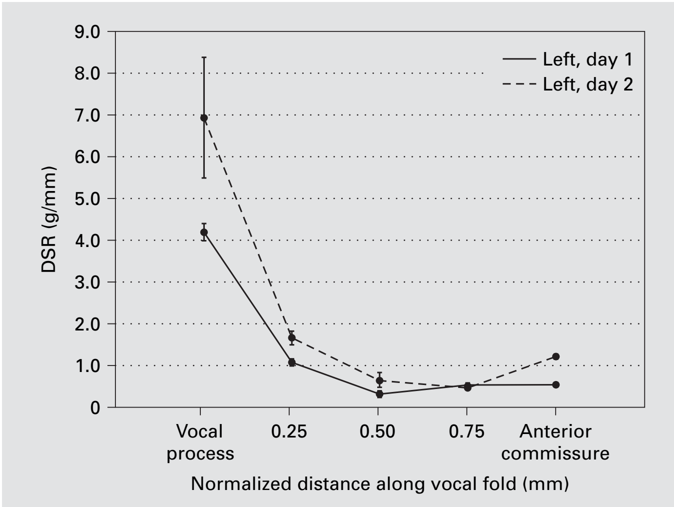

Fig. 6. Elasticity of the left vocal folds within an intact human larynx.

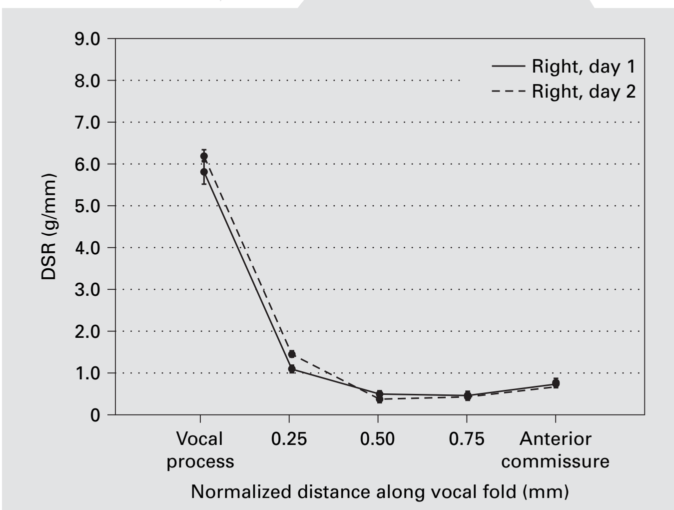

Fig. 7. Elasticity of the right vocal folds within an intact human larynx.

and attached to either vocal fold. Ten readings were taken at 5 approximately equidistant points, starting at the vocal process and finishing at the anterior commissure. The readings were retaken 1 day later (storage at room temperature keeping the larynx moistened with saline). Both days' results are shown in figures 6 and 7.

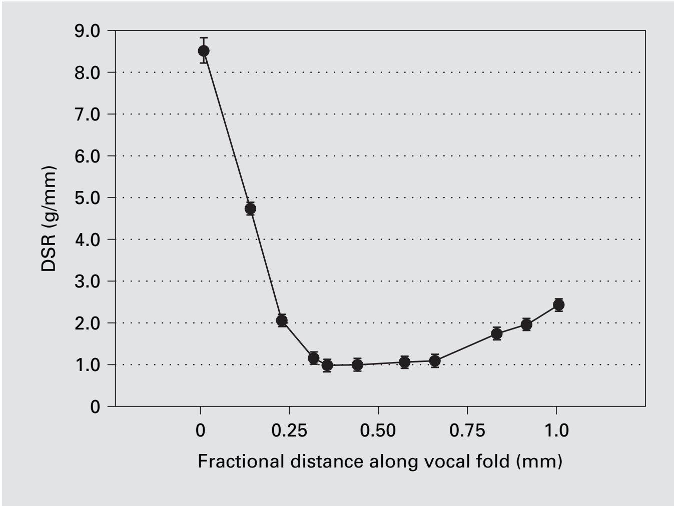

Fig. 8. Elasticity of the vocal fold of a split larynx. Fractional distance along vocal fold: 0.0 = vocal process, 1.0 = anterior commissure.

Study 4 on Day 2 – Elasticity of the Vocal Fold of a Split Larynx

On day 2, the intact larynx was split, and the left vocal fold was retested. This arrangement allowed a more precise measurement of the position from the vocal process to be determined. A millimetre rule was attached to the left vocal fold, and 5 readings were taken from different points. The results are shown in figure 8.

Conclusions

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 understand the anatomy, pathology, aging, modelling and repair of the vocal folds. For excised specimens, parallel plate rheometry has become a standard for determining the shear visco-elasticity parameters of tissue from animals, cadavers or surgical specimens, of candidate augmentation materials and of animal vocal folds previously implanted with augmentation materials [5–15]. Complementary stress/strain studies on excised vocal fold layers have provided data on longitudinal visco-elastic properties essential for understanding vibratory behaviour and contributions of the different layers to vibration as a whole [16–19].

Measurements on intact vocal folds have been technically difficult but are 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 [20, 21], lateral displacement of the vocal fold with a transoral calibrated lever [14, 22, 23] and medial aspiration of the mucosa with a calibrated suction catheter [24]. The latter two methods have been tested on human subjects under general anaesthesia.

In contrast to standard in vitro rheological methods, the LSR is not capable of determining absolute values for visco-elasticity parameters on a unit area or volume basis because the exact volume of tissue that is deformed cannot be determined. Data obtained so far are 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 oscillate 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

The most recent studies have demonstrated that the technique can be used to extract measurement data from an intact human larynx. We now have sufficient confidence in this measuring technique to justify a more extensive study using a quantity of freshly excised human larynges.

We further intend to examine the feasibility of developing a compact LSR device that will be capable of being inserted through a direct laryngoscope speculum, thereby allowing in vivo measurements to be taken from patients during general anaesthesia.

Acknowledgements

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