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The shear modulus of the human vocal fold, preliminary results from 20 larynxes

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Abstract Quantification of the elastic properties of the human vocal fold provides invaluable data for researchers deriving mathematical models of phonation, developing tissue engineering therapies, and as normative data for comparison between healthy and scarred tissue. This study measured the shear modulus of excised cadaver vocal folds from 20 subjects. Twenty freshly excised human larynxes were evaluated less than four days post-mortem. They were split along the saggital plane and mounted without tension. Shear modulus was obtained by two different methods. For method 1 cyclical shear stress was applied transversely to the mid-membranous portion of the vocal fold, and shear modulus derived by applying a simple shear model. For method 2 the apparatus was configured as an indentometer, and shear modulus obtained from the stress/strain data by applying an established analytical technique. Method 1 shear model for male larynxes yielded a range from 246 to 3,356 Pa, with a mean value of 1,008 and SD of 380. The range for female larynxes was 286–3,332 Pa, with a mean value of 1,237 and SD of 768. Method 2 indentometer model for male larynxes yielded a range from 552 to 2,741 Pa, with a mean value of 1,000 and SD of 460. The range for female larynxes was 509–1,989 Pa, with a mean value of 1,332 and SD of 428. We have successfully demonstrated two methodologies that are capable of directly measuring the shear modulus of the human vocal fold, without dissecting out the vocal fold cover tissue. The sample size of nine female and 11 male larynxes is too small to validate a general conclusion. The high degree of variability in this small cohort of subjects indicates that factors such as age, health status, and post-mortem delay may be significant; and that there is range of 'normality' for vocal fold tissue.

Keywords Elasticity · Shear modulus · Vocal fold biomechanics · Shear modulus of the vocal fold

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Introduction

Intraoperative measurements of vocal fold pliability would be very useful in the practice of phonosurgery. As a preliminary step in designing a system to make such measurements we conducted a study on fresh cadaver vocal folds. The purpose of this study was to obtain some preliminary data regarding the range of normal shear modulus values for male and female vocal folds, and to compare two methods for obtaining these data. Two different techniques were developed to measure the shear modulus of the tissue at the mid-membranous position without the need to dissect out the vocal fold out of the larynx. One method was the application of cyclical shear stress transversely to the axis between the vocal process and the anterior commisure; the resultant stress/strain characteristics are used to derive the tissue modulus using a simple shear model. The second method was the use of an indentometer to compress the tissue normal to the surface; the mathematical model developed by Hayes et al. [5] can then be applied to the resultant stress/strain data to obtain another measure of the tissue modulus.

There are very few published reports that give the shear modulus for a group of human vocal folds. Those that do exist employed a range of different techniques, such as ultrasonics, optics, and mechanics. The ultrasonic and optical methods infer shear modulus from secondary phenomena, whereas the mechanical methods directly measured the biomechanical response of the tissue. Our results compare most favourably with those obtained from human tissue using mechanical methodologies.

Materials and methods

All measurements were made with a Linear Skin Rheometer (LSR) [10, 14]. The LSR is a precision instrument originally designed to measure the viscoelastic properties of the stratum corneum. Based upon the concept of the Gas Bearing Electrodynanometer (GBE) [14] developed by Hargens in the 1960s, the LSR uses modern micro-mechanical components to achieve force feedback control in real time and precision position measurement. It is now being successfully used to measure the more delicate tissue of the vocal fold [4, 6, 7].

Method 1: simple shear model

A flat probe is attached to the vocal fold epithelium, mid-membranous between the anterior commisure and vocal process. A sinusoidal force F is applied to the material under test in a transverse direction so as to apply a shear stress to the vocal fold, and the resultant displacement P is logged. The driving force was set to be 0.5 g, which typically results in a displacement of 1 mm. The frequency of operation was set to be 0.3 Hz. The value of 1 mm was chosen as this represented a realistic displacement with respect to the geometry of the tissue structure, the measurement frequency is the one used by researchers when applying the LSR to analysis of the stratum corneum; the results can therefore only be used to measure the elasticity of the tissue and not the viscosity. When comparing our results with other published results we have whenever possible selected data obtained using comparable frequencies; and the closest methodology to ours is the study carried out by Tran et al. [15], which was effectively at DC.

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

$$P = P_{\max}\sin(t + R), \tag{2}$$

where F is the instantaneous force, $F_{\max}$ the maximum force, t the time over one cycle in radians, P the instantaneous displacement, $P_{\max}$ the maximum displacement, and R the phase shift in radians.

The Dynamic Spring Rate (DSR) of the tissue is $F_{\max}/P_{\max}$, and is expressed in units of grams force per millimetre. The DSR can then be used to determine the shear modulus using knowledge of the geometry of the test site as follows:

The shear stress σ is the applied force F per unit area A given by

$$\sigma = F/A. \tag{3}$$

The resultant shear strain ϵ is given by lateral displacement P per material thickness T.

$$\varepsilon = P/T. \tag{4}$$

Shear modulus G is defined as stress per unit strain

$$G = \sigma/\varepsilon. \tag{5}$$

$$G = (F/P)\cdot(T/A). \tag{6}$$

As DSR = F/P then

$$G = \mathrm{DSR}\cdot T/A. \tag{7}$$

Data are obtained by gluing the flat tip of a probe arm to the tissue with cyanoacrylate. This is a very fast acting adhesive that internally polymerises in the presence of a small amount of water, achieving the bond by in-filling crevices on the surface of the epithelium. The adhesive works by first forming a surface skin, with polymerisation continuing at a high rate internally. In view of the speed of action, and the manner of the bonding, we consider that it is highly improbable that the adhesive solvents would have time, or be able to penetrate into the tissue to the extent that it would perturb the results.

The area of attachment (A) is determined by direct measurement. The simple shear model does not take account of tissue that is attached to the column that is directly stressed. The Hayes formula provides a mathematically rigorous correction for indentometer data, which in addition to compressing tissue with the indentor also exerts shear stresses to surrounding tissue. No such similar rigorous solution has been found for pure shear stresses; the derivation of such a solution will form part of a new study.

The additional surface area that was subjected to the applied stress was observed to be typically 0.75 mm around the area of direct attachment; therefore, the dimensions were increased by this amount on all four sides. The thickness of the vocal fold tissue (T) is typically 1 mm.

Using these geometric values a range of shear moduli can be derived for each sample. Ten readings were taken from each test site, and middle of each range was averaged. These results are referred to as the 'shear model' in the text.

Method 2 indentometer

In the second approach the LSR was used as an indentometer. In this arrangement a 1 mm diameter flattened probe tip is pushed into the tissue, and the force-displacement data is then captured in real time. The measurement point was chosen to be the centre of the area used for the shear model method already described in order to enable a meaningful comparison. The indentor was located clear of the tissue with an air gap of 1 mm, it was then driven into the vocal fold for a distance of 2 mm at a speed of 1 mm/s.

For a homogeneous material the resultant relationship will be logarithmic, forming a classic compression cycle curve. However, many researchers have correctly stated that indentation of a soft tissue does not follow this simple rule because surrounding tissue remains in contact with the depressed section to which a shear stress is applied.

One widely accepted model is the one originally proposed by Fung, from which Hayes et al. [5] developed a rigorous mathematical solution. This mathematical device is based upon a solution for Yung's 3D partial differential equations that explain the deformation of soft tissue [16]. This solution offers a 'correction factor' to Yung's equations that takes account of the shear strain surrounding the indentation, which requires knowledge of the tissue's Poisson's ratio (v). v is the relationship between a materials' elongation and sheer strains. For an incompressible material it is 0.5.

The correction factor (κ) is a function of the indentor radius (a), the tissue thickness and Poisson's ratio (v), assumed to be 0.5. Our indentor radius (a) is 0.5 mm, and we assume the thickness of the tissue to be 1 mm. Hayes gives the following expression in his paper as the definition of κ together with a table of solutions.

$$k = (F \cdot (1 - v))/(4aGw), \tag{8}$$

which can be rearranged to give

$$G = \frac{(F/w) \cdot (1 - v) \cdot 9.80665}{(4a\kappa)}, \tag{9}$$

where κ is the Hayes correction factor obtained from the published table, F the applied force, v the Poisson's ratio, a the indentor radius, G the shear modulus, and w the depth of penetration.

The 9.80665 converts the units for shear modulus (G) into Pa.

Each sample was indented ten times. From the resultant stress/strain curves we select the initial linear section, apply a least squared fit, and obtain the best value for F/w in units of g/mm. All the other values are known. These results are referred to as the 'indentometer model' in the text.

Results

Overall results

Please refer to tables and graphs for the full set of results (Figs. 1, 2; Tables 1, 2). The graphs show the distribution of shear modulus with respect to the donor's age.

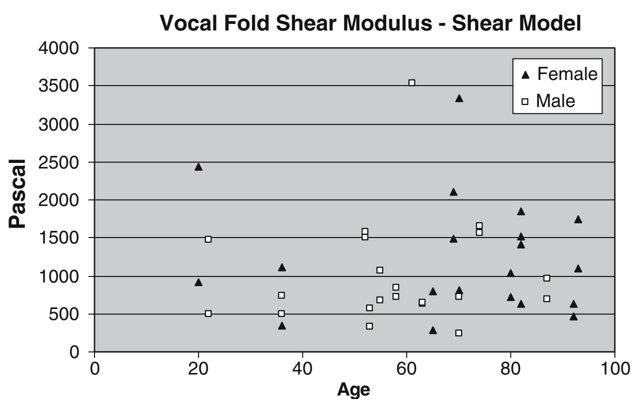

Fig. 1 Distribution of shear model modulus with age

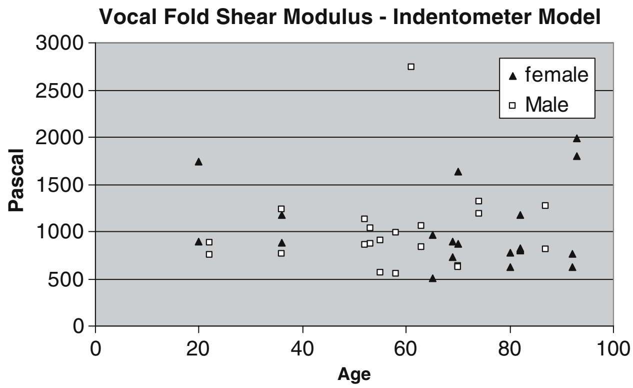

Fig. 2 Distribution of indentometer model shear modulus with age

Table 1 Shear modulus of female larynxes

Age (left then right)	Shear model (Pa)	CofV %	Indentation model (Pa)	CofV %
20	912	2.9	893	18
20	2,439	8.1	1,746	8.2
36	351	9	1,179	3.3
36	1,110	3	879	15
65	286	14	963	40
65	802	17.5	509	16
69	1,491	3.3	892	18
69	2,101	5.9	735	33
80	1,749	5.8	1,796	14
80	1,100	4.9	1,989	11.5
82	1,844	15.4	827	24
82	1,520	8.2	1,181	18.6
82	628	8.8	809	22
82	1,408	4	802	25
92	723	21.1	782	19.7
92	1,042	3.5	627	12.3
93	472	10.5	765	16
93	633	2	621	29

The results can be summarised as follows:
Male shear modulus

1. Range = 246–3,536 Pa (shear model).
2. Mean = 1,008 Pa (shear model).
3. SD = 380 (shear model).
4. Range = 552–2,741 Pa (indentometer model).
5. Mean = 1,000 Pa (indentometer model).
6. SD = 460 (indentometer model).

Female shear modulus

1. Range = 286–3,332 Pa (shear model).
2. Mean = 1,237 Pa (shear model).
3. SD = 768 (shear model).
4. Range = 509–1,989 Pa (indentometer model).
5. Mean = 1,332 Pa (indentometer model).
6. SD = 428 (indentometer model).

Table 2 Shear modulus of male larynxes

Age	Shear model (Pa)	CofV %	Indentation model (Pa)	CofV %
22	1,474	3.5	877	7.3
22	501	5.2	753	33.7
36	491	6.1	1,241	12.7
36	742	4.2	761	9.2
52	1,507	3.4	1,134	9.4
52	1,582	13	856	16
53	336	7.2	868	22
53	565	32	1,031	16
55	1,069	9.4	904	23.5
55	684	14	567	23
58	840	11.3	984	19.6
58	729	8.3	552	17.2
61	3,536	2.3	2,741	7.7
63	637	6.1	1,056	26
63	644	6	832	18.9
70	718	4.5	631	25
70	246	19	618	16.9
74	1,658	3.5	1,188	14.9
74	1,652	11.3	1,321	19.9
87	958	5.4	1,272	22
87	696	3.9	808	22

Comparison between methods 1 and 2

We can compare the data obtained by both methods from the same tissue sample as a validation procedure. The total data set consists of 39 pairs of data from the left- and the right-hand sides of the 20 larynxes (one hemi-larynx was damaged during hemisection).

A standard statistical tool that is used to compare two sets of results with each other is the correlation coefficient (CC), on a scale of 0–1; where 1 is a perfect match.

Using all 39 pairs of data we obtain a CC of 0.66. This is a fair result, but not perfect. By selectively rejecting ~20% of the data set the CC rises to a far more acceptable value of 0.8; indicating that we require a larger sample group to improve our confidence in both methods.

Comparison between left- and right-hand sides

A further validation of the methodology can be obtained by comparing the results obtained from the left- and the right-hand sides of the same larynx. In total there are 19 left/right pairs of data available for each method. The CC for all data pairs is poor, being 0.22 for the shear model and 0.47 for the indentometer model. By rejecting a few obvious outliers it is possible to obtain a CC of 0.7 or better. This result again demonstrates the need for expanding the size of the data set.

Discussion

Few researchers have reported data obtained by direct measurement of the mechanical properties of intact larynxes. Results have either been inferred from observations of acoustic or optical effects, or the vocal fold cover has been excised and tested mechanically out of anatomical context.

Kaneko et al. [9] and Tamura et al. [13] amongst other have reported the derivation of visco-elastic properties using ultrasound in vivo and with excised larynxes. However, they do not offer comparable data relating to the elastic modulus.

Hsiao et al. [8] have reported success in obtaining values for Young's Modulus using colour Doppler imaging in vivo. If we assume a Poisson's ratio of 0.5 then these results translate to shear modulus ranges of 10,000–40,000 Pa for men and 40,000–100,000 Pa for women.

McGlashan et al. [11] have reported a method to infer vocal fold properties using an in vivo optical technique that generated a series of dynamic surface maps, from which they derived the velocity of the mucosal wave. A more recent conference report gives a shear modulus of 2,500 Pa.

Chan and Titze [3] have measured shear modulus in excised tissue using a parallel plate rheometer. Their earlier work gives a value of between 10 and 1,000 Pa for shear modulus. Their later papers report a range of values for different subjects, taken under differing conditions. Values ranged from as low as 10 to 300 Pa, for low frequency cycling.

The in vivo data obtained by Tran et al. [15] offers a range of shear modulus from 2,450 to 29,400 Pa. Berke and Smith [2] describe the apparatus used in more detail, and give some results for Young's Modulus using canine data. The medial result equates to a shear modulus of 1,450 Pa. Perlmann et al. [12] have obtained canine data using excised tissue with a range of 9,460–41,200 Pa for a variety of conditions. Alipour's excised canine results [1] for Young's Modulus equate to a shear modulus of 13,960 Pa for a Poisson's ratio of 0.5.

Our results are indicative of a transverse shear modulus for the vocal fold of between 300 and 4,500 Pa. As yet there is insufficient data to enable us to draw generalised conclusions; and the difficulty of obtaining repeatable measurements from soft tissue is demonstrated by the poor coefficients of variance relating to the original raw data. Our results compare most favourably with those obtained from intact larynxes, by direct mechanical measurements (Tran and Berke), or by inference using the optical technique (McGlashan). We believe that this is because these techniques measured or derived shear modulus from intact larynxes, with the vocal fold cover still attached to the underlying structures.

Our intention is to continue this study in order to improve the repeatability of the results, to reduce the CofV of the raw data, and to expand the data sets to achieve a statistically acceptable sample size. Our measure for success will be an improved convergence of data obtained by both methods and an improved correlation between data obtained from the left- and right-hand sides.

The resultant strains that both our methods are applying are small, typically between 0.5 and 1 mm; we believe that we are mainly acting on the vocal fold cover, but this hypothesis needs to be demonstrated. In future studies we will complete the analysis by dissecting out the vocal fold tissue and measuring it in isolation enabling a more in-depth analysis of our results.

Conclusion

We have shown that it is possible to directly measure the shear modulus of the vocal fold without dissecting the tissue from its surroundings, using two mechanical methodologies. Thus, the data obtained is more representative of the elastic response during phonation than data obtained from dissected tissue measured in isolation.

The two techniques outlined offered similar results and therefore support each other. They are also similar to data obtained by other researchers using direct mechanical methodologies, and obtained from intact larynxes. Insufficient data have yet been obtained to make generalised conclusions, or to provide a statistically acceptable data set. Both methods are promising, and will be improved in future studies. For the shear modulus method the primary cause of error is the uncertainty relating to the area of attachment. This will be addressed by the use of a more repeatable probe design and the development of a mathematical model that adequately takes account of the attached tissue that is not under direct stress. The indentometer has consistently shown more consistent results, and this apparatus will be redesigned to remove observed mechanical problems.

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