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Abstract

During phonation, human vocal fold tissues are subjected to combined tension, compression and shear loadings modes from small to large finite strains. Their mechanical behaviour is however still not well understood. Herein, we complete the existing mechanical database of these soft tissues, by characterising, for the first time, the cyclic and finite strains behaviour of the *lamina propria* and *vocalis* layers under these loading modes. To minimise the inter or intra-individual variability, particular attention was paid to subject each tissue sample successively to the three loadings. A non-linear mechanical behaviour is observed for all loading modes : a J-shape strain stiffening in longitudinal tension and transverse compression, albeit far less pronounced in shear, stress accommodation and stress hysteresis whatever the loading mode. In addition, recorded stress levels during longitudinal tension are much higher for the *lamina propria* than for the *vocalis*. Conversely, the responses of the *lamina propria* and the *vocalis* in transverse compression as well as transverse and longitudinal shears are of the same orders of magnitude. We also highlight the strain rate sensitivity of the tissues, as well as their anisotropic properties.

Keywords : Vocal folds, Mechanical tests, Tension, Shear, Compression

1. Introduction

Human vocal folds are anisotropic soft tissues, comprising two principal layers : the *lamina propria*, *i.e.*, a loose connective tissue made of collagen and elastin fibers, and the *vocalis*, composed of skeletal "muscle fibers" (Fig. 1, [19]). The fiber arrangement within these layers exhibits a pronounced alignment along the antero-posterior (or longitudinal) direction $\mathbf{e}_z$ of the vocal folds (Fig. 1, [19, 32]). During phonation, vocal folds are deformed due to pulmonary airflow and laryngeal motions, enduring vibrations of various amplitudes, frequencies, and degrees of collision. These multiple configurations imply complex and coupled multiaxial mechanical loadings experienced by the tissue upon finite strains and at various strain rates. These loadings include combined longitudinal tension and compression which are mainly due to laryngeal muscular contractions along $\mathbf{e}_z$ (Fig. 1), but also transverse compression due to aerodynamic forces and vocal-fold collision along $\mathbf{e}_x$, as well as longitudinal and transverse shears due to oscillatory motion along $\mathbf{e}_y$ and friction stresses between both vocal folds [31]. These observations are confirmed with finite element simulations of vocal folds oscillations during phonation [16, 17, 41, 40, 39], bringing fruitful semi-quantitative information. However, current simulations suffer from a lack of experimental data, to use more relevant constitutive mechanical models of vocal tissues.

To investigate the mechanics of vocal-fold tissues, several experimental works have been conducted during the last twenty years [15, 31, 11]. Most of them focused on the *lamina propria* response during longitudinal tension. They highlighted the non-linear behaviour of this tissue showing a J-shape stress-strain curve upon loading, and thus, an increasing tangent longitudinal modulus E_z^t from 10 kPa to 2000 kPa [22, 24, 9, 30, 21]. Viscoelastic properties of this layer were also investigated using either standard shear Dynamic Mechanical Analysis (DMA), *i.e.*, within the linear regime [7, 6, 14, 37], or more recently using Large Amplitude Oscillatory Shear (LAOS) [5]. These works allowed to characterise the shear storage G' and loss G'' moduli (DMA) of the *lamina propria*, as well as its cyclic and finite strains shear behaviour (LAOS) within the (x, y) plane. Several conclusions can be drawn therefrom. Firstly, G' and G'' (i) are of the same order of magnitude and exhibit a non-linear shear rate stiffening, (ii) vary within a wide range of values (from 1 Pa to 10 kPa), that are much lower than those recorded for E_z^t. Point (i) proves that viscous effects play a key role on the mechanics of the *lamina propria*. Point (ii) emphasises the anisotropy of the *lamina propria* behaviour, which is directly induced by its structural anisotropy [2]. Secondly, the *lamina propria* cyclic shear stress-strain curves also exhibited a J-shape, with an increase of the strain stiffening above a shear strain around 0.5 [5].

Despite this important database, there are still some issues to be tackled to understand and model the mechanics of human vocal folds. Among them is the difficulty to analyse experimental results due to the large variability of the mechanical response between subjects, and within the tissues themselves [9, 7, 37], as for other soft living materials [4]. It is thus challenging to compare data obtained with different mechanical loadings, *e.g.*, tension and shear. In addition, the mechanics of the *lamina propria* during transverse compression has never been studied so far. This constitutes a crucial lack in current knowledge, keeping in mind that the quality of contact between vocal folds is a key factor in voice quality, and that high-impact transverse compressive stresses are believed to generate common lesions in the *lamina propria* after a phonotrauma [18, 26]. Finally, the mechanics of the *vocalis* has been often discarded up to now, although being a major vocal-fold sublayer used to tune the phonation process, in its active but also passive state.

Therefore, this study aims to provide a new mechanical dataset of human vocal-fold tissues, subjected to a series of physiological loadings, *i.e.,* longitudinal tension, transverse compression as well as longitudinal and transverse shear. These testing conditions were achieved sequentially on each sample, thereby minimising inter-sample variability. We studied and compared the finite strains mechanical responses of both upper layers, including *epithelium* and *lamina propria* (Fig. 1), to those of the *vocalis* muscle for each loading mode. Finally, we quantified the strain rate sensitivity of these tissues, as well as their mechanical anisotropy.

2. Materials and Methods

2.1. Vocal folds

Experiments were carried out with 4 healthy human larynges, noted L_i, $i \in [1, 2, 3, 4]$, excised from donated bodies (Table 1) within 48 h *post-mortem*. Procedures were conducted following the French ethical and safety laws related to Body Donation. All but one larynx (*fresh* larynx L2) were preserved by freezing ($-20\,°C$). Before any manipulation, each frozen sample was slowly thawed for 30 min in tepid water ($T \approx 20\,°C$). Vocal folds were then dissected from each laryngeal specimen with portion of thyroid and arytenoid cartilages. Excised vocal folds can be approximated as parallelepiped beams owning a sandwich lamellar structure, oriented along the longitudinal (antero-posterior) direction $\mathbf{e}_z$, as schemed in Fig. 1(b), and pictured in Supplementary Figs. S1(a) and S2(a): they were made of all sublayers from their *epithelium* to the *vocalis* (Fig. 1), and noted L_i-F_j ($j = 1$ and $j = 2$ standing for left and right vocal folds of larynx L_i, respectively). Finally, samples L_3-F_1 and L_4-F_1 were cut in half along the plane ($\mathbf{e}_x$, $\mathbf{e}_z$), as shown in Supplementary Fig. S2(b). One half was dedicated to histological analyses, following the protocol detailed in Bailly *et al.*, 2018 [2]. The second half was dedicated to mechanical testing.

Larynx name	Gender	Age [y]	Height [m]	Weight [kg]
L_1	Female	78	1.40	40
L_2	Male	80	1.55	50
L_3	Male	79	1.70	65
L_4	Female	79	1.60	45

Table 1: Origin of the tested larynges.

2.2. Experimental protocol

We designed a protocol to characterise the finite strains mechanics of the *vocalis* and upper layers (*lamina propria* + *epithelium*) under tension, compression and shear, while minimising the inter and intra-individual variability.

2.2.1. *Hygro-mechanical set-up*

Mechanical tests were conducted at proper hygrometric conditions to prevent the tissues from air drying [34], using a chamber (Fig. 2(a)) in which a saturated air flow ($\approx 95 - 100$ %RH) was regulated with a humidifier (Fisher and Paykel HC150). We also used a tension-compression micro-press inserted inside the chamber and designed for soft samples (load cell 5 N, relative displacement between crossheads measured with a LVDT sensor) [27, 20, 28, 25, 2]. For simple tensile tests, specially designed knurled clamps (26 mm width, 7 mm height) were used to facilitate the sample positioning and restrain its slippage (Fig. 2(b)). For simple compression tests, compression platens were hydrated by a film of Phosphate-buffered saline solution, avoiding friction (Fig. 2(b)). For simple shear tests, plates (10 mm length and width) were coated with sand paper to restrain sample slippage (Fig. 2(b)).

2.2.2. *Testing protocol*

- Tensile tests were first carried out with vocal folds L_i-F_j (Fig. 2(b)), the gauge length ℓ_0 and cross-section S_0 of which are reported in Table 2. Tests were performed along $\mathbf{e}_z$, *i.e.,* the main fiber orientation. The cell force f signal and the LVDT displacement δ were used to estimate the first Piola-Kirchoff stress $P_{zz} = f/S_0$, as well as the Hencky tensile strain $\varepsilon_{zz} = \ln(1 + \delta/\ell_0)$. Each sample was subjected to 10 load-unload cycles at a strain rate $|\dot{\varepsilon}_{zz}| = |\dot{\delta}/\ell_0| \approx 10^{-3}s^{-1}$, up to a moderate tensile strain $\varepsilon_{zz}^{max} = 0.1$ to restrain sample damage.

- Samples were then unmounted, and their upper layers (further labelled L_i-LP_j) were separated from the *vocalis* (L_i-M_j). *Epithelium* was left intact as a remaining part of the L_i-LP_j layer. Care was taken to preserve cartilages parts on both layers. Then, each sample was again subjected to tension loading along $\mathbf{e}_z$ following the aforementioned procedure (see Table 2 for their dimensions).

- Therewith, samples L_i-LP_j and L_i-M_j ($i = 3, 4$) were released from their cartilaginous ends and resized in smaller parallelepiped samples in order to fit compression and shear plates, as described in Fig. S2(c) (see Table 2, "compression" column for their adjusted dimensions). They were then subjected to compression along $\mathbf{e}_x$ (Fig. 2(b)). During the tests, compression stress $P_{xx} = f/S_0$ and compression strain $\varepsilon_{xx} = \ln(1 + \delta/\ell_0)$ were recorded. Samples were subjected to 10 load-unload cycles up to $\varepsilon_{xx}^{min} = -0.2$. This procedure was carried out at two strain rates, $|\dot{\varepsilon}_{xx}| = |\dot{\delta}/\ell_0| \approx 10^{-3}s^{-1}$ and $10^{-2}s^{-1}$, respectively.

- Finally, we conducted two consecutive shear tests with the same samples along two different directions, "longitudinal" plane ($\mathbf{e}_z$, $\mathbf{e}_x$) and "transversal" plane ($\mathbf{e}_y$, $\mathbf{e}_x$), respectively. During the tests, shear stress $P_{zx} = f/S_0$ (resp. $P_{yx} = f/S_0$) was measured as a function of shear strain $\gamma_{zx} = \delta/\ell_0$ (resp. $\gamma_{yx} = \delta/\ell_0$), while subjecting samples to 10 load-unload cycles up to $\gamma_{zx}^{max} = 0.6$ (resp. $\gamma_{yx}^{max} = 0.6$) at a shear rate $|\dot{\gamma}| = |\dot{\delta}/\ell_0| \approx 10^{-3}s^{-1}$.

	Sample gauge dimensions					
	Tension		Compression		Shear	
Name	ℓ_0 (mm)	$S_0^\dagger$ (mm^2)	ℓ_0 (mm)	$S_0^\dagger$ (mm^2)	ℓ_0 (mm)	$S_0^\dagger$ (mm^2)
L_1–F_1	10.1	28.9±2.4	-	-	-	-
L_1–LP_1	15	8.9±4.8	-	-	-	-
L_1–M_1	7	9.8±4.2	-	-	-	-
L_1–F_2	10.3	40.8±6.1	-	-	-	-
L_1–LP_2	8.5	5.4±3.6	-	-	-	-
L_1–M_2	7.2	14.4±5.8	-	-	-	-
L_2–F_1	16.8	49.2±7.2	-	-	-	-
L_2–LP_1	18	21.3±7.6	-	-	-	-
L_2–M_1	17.8	24.3±9.8	-	-	-	-
L_2–F_2	19.8	38.6±7.2	-	-	-	-
L_2–LP_2	22.3	13.2±7.1	-	-	-	-
L_2–M_2	18.3	19.9±9.3	-	-	-	-
L_3–F_1	14.2	39.0±11.5	-	-	-	-
L_3–LP_1	10	7.4±2.9	1.5	80.0±0.7	1.0	68.5±4.7
L_3–M_1	10	8.8±4.4	1.9	91.9±0.8	1.3	99.0±9.5
L_3–F_2	17.8	82.3±20.2	-	-	-	-
L_3–LP_2	15.7	14.7±4.7	1.1	74.9±5.8	0.9	78.9±0.6
L_3–M_2	11.5	29.3±10.1	2.6	120.1±15.8	2.1	78.0±7.1
L_4–LP_1	15.2	5.8±4.5	0.9	44.8±1.6	0.6	44.6±4.1
L_4–M_1	15.7	11.9±4.0	1.5	81.7±1.2	1.0	84.4±0.3
L_4–LP_2	14	11.7±5.4	1.3	88.6±0.7	1.3	99.0±9.5
L_4–M_2	15.3	14.4±5.6	2.2	93.7±0.9	2.1	72.9±11.9

Table 2: Sample dimensions used to determine stresses and strains. ℓ_0 is the initial distance between platens or clamps (Fig. 2(b)). The mean initial cross section S_0 and its standard deviation were estimated optically from the width and the thickness profiles of the samples once put onto a flat surface.

3. Results

3.1. General trends in tension

Tensile responses of the vocal folds and their sublayers along $\mathbf{e}_z$ are reported in Fig. 3 for the first cycle, together with the evolution of the longitudinal tangent moduli $E_z^t = \mathrm{d}P_{zz}/\mathrm{d}\varepsilon_{zz}$ with ε_{zz} (for the first loading only).

Variability – Fig. 3 first emphasises a large scattering of the mechanical responses. For instance, at $\varepsilon_{zz} \approx 0.09$, the ratio of maximal and minimal stresses registered in the case of the L_i-F_j samples rises up to 5. This well-known inter-individual variability is ascribed to tissue histological singularities of each donor, which depends on age, gender, tobacco smoking profile [7, 8, 9]. Conversely, note that the intra-individual variability is much less marked : the ratio of maximal to minimal stresses at $\varepsilon_{zz} \approx 0.09$ is only 1.3 for left and right vocal-fold samples. Similar conclusions are drawn for upper layers L_i-LP_j and *vocalis* L_i-M_j.

Shape of stress-strain curves and corresponding stiffness – Whatever the sample, stress-strain curves exhibit non-linear responses with a J-shape strain-hardening (Fig. 3, *left)* and a strain hardening of tangent moduli (*right*). These trends are related to the recruitment and reorientation of wavy fibers during tension [30, 13, 12]. They are less marked for *vocalis* samples, the muscle fibers being straighter than the collagen/elastin fibers of the upper layers at rest (Fig. 1, [2]). In addition, reported stress-strain curves exhibit stress hysteresis with a non-negligible residual strain after unloading, which may be ascribed to viscoelastic effects together with structure rearrangements.

Comparison between sublayers – Fig. 3 also proves that stress levels in upper layers are much higher than those recorded for the *vocalis*. For example, at $\varepsilon_{zz} = 0.1$, nominal stresses P_{zz} vary from 14 to 50 kPa, from 8 kPa to more than 100kPa, and from 0.5 kPa to 28 kPa for vocal folds, upper layers, and *vocalis* samples, respectively. A similar conclusion is drawn for the tangent moduli E_z^t (Fig. 3).

3.2. Mechanics in tension, compression and shear for single samples

To get rid of the aforementioned inter or intra-individual variablility, focus is now made on samples L_3-LP_2, L_4-LP_2, L_3-M_2 and L_4-M_2, subjected to the protocol purposely designed. Despite this procedure, some stress scatterings remain due to sample dimensions (Table 2): in Figs. 4 and 5 presented hereafter, they have been highlighted with gray corridors surrounding nominal values of stresses. The first figure gives stress-strain curves after subjecting samples L_3-LP_2 and L_3-M_2 to 10 load-unload cycles in tension, compression and shear. The second one reports similar data for samples L_4-LP_2 and L_4-M_2.

Shape of stress-strain curves – Compared to tension along $\mathbf{e}_z$, the compression along $\mathbf{e}_x$ yields to similar J-shape stress-strain curves during the first loading. However, during unloading, compression stress-strain curves exhibit a marked hysteresis with higher residual strains. In addition, stress levels of upper layers are of the same order of magnitude than those of the *vocalis*, which is also different to what is observed in tension. By contrast, stress-strain curves obtained in shear do not exhibit a J-shape, but a practically constant strain hardening. Note that the apparent strain softening produced at the end of the load (notably for the *vocalis*) is probably due to experimental artefacts, such as sample rocking. In addition, despite a slightly stiffer response for the *vocalis* compared to the upper layers, orders of magnitude of stress levels generated within both sublayers are comparable up to 0.2 shear strain. Lastly, the shear stress hysteresis as well as the residual shear strain are limited.

Effect of cycling – Whatever the samples, repeating load-unload sequences yields to progressive (i) decrease of maximal stresses, (ii) decrease of the stress hysteresis, (iii) increase of the residual strains. These evolution are commonly observed while cycling soft tissues [36] and consistent with previous studies [42, 23, 9]. For both sublayers, these effects are limited in shear (Figs. 4 and 5), but pronounced in tension and compression.

Effect of strain rate – Fig. 6(a) shows typical stress-strain curves obtained with sample L_4-LP_2 compressed at $|\dot{\varepsilon}_{xx}| \approx 10^{-3}\ s^{-1}$ and $|\dot{\varepsilon}_{xx}| \approx 10^{-2}\ s^{-1}$. As expected, the tissue viscoelasticity yields to a moderate to strong increase of stress levels and hysteresis with the strain rate. Fig. 6(b) displays the compression stress ratio P^2_{xx}/P^1_{xx} of the 8 tested samples with the compression strain during the first loading, where P^1_{xx} (resp. P^2_{xx}) is the stress at $10^{-3}\ s^{-1}$ (resp. $10^{-2}\ s^{-1}$) : this ratio ranges within 1.1 and 3.3. For most of samples, it exhibits a slight increase during compression. Furthermore, for the same vocal fold (at fixed i and j-values), the ratios of samples L_i–LP_j and L_i–M_j follow roughly close evolutions.

Anisotropy – The stress-strain response of sample L_3-LP_1 in shear parallel to the ($\mathbf{e}_y$, $\mathbf{e}_x$) and to the ($\mathbf{e}_z$, $\mathbf{e}_x$) planes is presented in Fig. 6(c). For both shear directions, the shape of the curve is similar but stresses are about twice higher in the ($\mathbf{e}_y$, $\mathbf{e}_x$) plane, emphasising a marked anisotropy. This feature is confirmed in Fig. 6(d), displaying the anisotropic ratio G^t_{yx}/G^t_{zx} of the tangent shear moduli G^t_{yx} and G^t_{zx} with the shear strain for the 5 tested samples. Except for sample L_3-LP_1, the ratios G^t_{yx}/G^t_{zx} mainly range between 3 and 1 (mean value 1.55) and tend to decrease towards 1 ($G^t_{yx} \approx G^t_{zx}$). No clear difference was found between both sublayers.

4. Discussion and concluding remarks

This study provides original biomechanical data (20 samples) for excised human vocal folds, their upper layers and the *vocalis*, by completing the knowledge of their finite strains mechanics in tension, compression and shear.

With respect to literature data, the database conjures up the following comments. In tension, the orders of magnitude of the longitudinal tangent modulus E^t_z of the upper layers are in agreement with previous *ex vivo* data. For instance, in the linear regime, Min *et al.* [30] gave values around 20–50 kPa for the *lamina propria*. Other values range between 10 and 600 kPa for the isolated "cover" (*i.e., epithelium* + *lamina propria* superficial layer) [9, 23, 24, 22, 31], and between 10 to 110 kPa for the "vocal ligament" (*i.e., lamina propria* mid and deep layers) [9, 23, 24, 22]. For tensile strains above 0.4, the range is even wider, from 20 to 500 kPa for *lamina propria* [30], up to 1850 kPa for cover [9] and 3300 kPa for ligament. The sources of such a scattering are mainly related to intra/inter-individual variability. This was here again emphasised and conducted us to design a procedure to better compare experimental results (tension, compression and shear). In addition, to our knowledge, tensile data recorded for the human *vocalis* are original and cannot be compared with other literature data. Alternatively, close stress levels can be found on other skeletal muscles stretched along the main fiber direction such as *Longissimus dorsi* samples (10 kPa stress at a strain of 0.1), albeit taken from fresh pig tissues [38]. Furthermore, our shear data showed that the tangent shear moduli of the upper layers range from 0.36 to 2.3 kPa and from 0.25 to 2.5 kPa in the longitudinal and transverse direction, respectively. These values are similar to those obtained while shearing vocal-fold covers (without specifying the shearing plane), *i.e.,* between 1 Pa to 1 kPa [6]. Our estimates are also consistent with apparent elastic properties recorded with a linear skin rheometer (LSR) with vocal fold and sublayer samples [37]. In this study, the anisotropy of the apparent shear moduli was emphasised but the shear anisotropy ratio remained below 1 (0.5–0.75) at small apparent strains, whereas ours are such that $1 < G^t_{yx}/G^t_{zx} < 3$ within a larger strain range. Any further comparison is limited by uncertainties related to the assumptions stated both for the sample dimensions and the stress-strain state homogeneity using the LSR technique. Lastly, although transverse compression is a key mechanical loading during voice production [39, 31, 40], compression experiments on vocal folds have not been reported so far. Few other biological materials have been characterised in compression, this loading being usually preferred for very soft tissues, the shear or tensile behaviour of which are tricky to characterise [29, 33]. For instance, results can be found on adipose porcine tissue [10], lung tissue [1] or muscles [35, 3]: they emphasise non-linear properties similar to the trends highlighted here.

The present database shows that vocal-fold layers behave as many other soft living tissues subjected to finite strains, *i.e.,* with a non-linear viscoelastic and anisotropic behaviour exhibiting strain hardening and strain rate sensitivity, stress hysteresis and accommodation. These features are connected both to their inner gel-like ground substances and their orientated fibrous architectures which reorient, rearrange and deform differently with the loading mode and direction. In addition, by minimising the inter or intra-individual variability, the proposed experimental procedure allowed a quantitative comparison of results. Thus, important differences are emphasised for the aforementioned mechanical features as a function of the loading modes. The mechanical role of the sublayers on the vocal folds mechanics is also better established. For instance, our data prove that the passive mechanical behaviour of the *vocalis* in tension is minor with respect to that of the upper layers. By contrast, stress levels achieved in compression and shear are close for both the upper layers and the *vocalis*. In addition, it is interesting to note that stress levels obtained in compression and shear are much (resp. moderately) lower than those obtained in tension for the upper layers (resp. the *vocalis*). Hence, by completing the existing database, these results constitutes a quantitative information for the validation of biomechanical models of phonation. It should also be completed, *e.g.,* by further scrutinising the strain rate sensitivity at higher strain rates, the accommodation and damage mechanisms, the link between the sublayer mechanics and the evolution of their inner fibrous architecture, and the active mechanics of the *vocalis*.

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Conflict of interest statement

The authors declare no conflict of interest.

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