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1. Introduction

Composed of collagen, elastin and muscular fibrous networks, vocal folds are soft multilayered structures of the larynx. However, the impact of their histological features on their unique vibromechanical performance is still poorly understood. This gap needs to be filled to meet the future challenge of sustainable voice restoration using suitable vibrating biomaterials.

Since the late 1950s, to overcome the limitations of *in vivo* testing conditions, various artificial larynges were developed to mimic human phonation *in vitro*. In recent decades, vocal-fold replicas have evolved from rigid-walled models to deformable replicas able to generate flow-induced self-sustained vibrations and audible sound sources (Kniesburges et al. 2011). Recently, a few studies have presented extensible, articulated vocal-fold replicas capable of adjusting fold elongation in the anterior-posterior direction, *i.e.* a key mechanism to control pitch during phonation (Shaw et al., 2012, Tur et al., 2023, Luizard et al., 2023). This aspect has long been overlooked, even though the vocal folds can stretch up to 50% *in vivo*. Despite these promising advances, further research is needed to design replicas with greater biomimetic vibro-mechanical performances, with the ability to withstand large reversible deformations and to oscillate with a fundamental frequency from 50 Hz to 1500 Hz. We assume that these native performances are highly related to the fibrous microstructure of the folds, showing specific fibre's type, density and arrangement. However, to date, most of the materials manufactured for *in vitro* oscillators are made up of homogeneous elastomers with isotropic microstructures and quasi-linear stress-strain responses, far from those experienced by the folds.

Thus, by combining the work of Luizard et al. (2023) on articulated albeit homogeneous vocal-fold replicas with that of Ferri-Angulo et al. (2023) showing how to elaborate versatile fibre-reinforced "biomimetic" vocal-fold layers, this study explores how a fibrous reinforcement incorporated into a vibrating replica with an adapted architecture can influence its phonation capabilities and its aeroacoustic correlates.

2. Methods

2.1 Vocal-fold composite replica

As shown by Ferri-Angulo et al. (2023), the fibrous collagen network of the upper vocal-fold layers can be reproduced by polymeric materials such as polycaprolactone (PCL), when formulated into fibrous mats with controlled fiber diameters, orientations and volume fractions using a jet-spraying method. Thus, several mats of aligned PCL nanofibers were elaborated to mimic the vocal-fold microstructure, as illustrated in Fig. 1a (right picture).

Then, each fibrous mat was embedded into a vocal-fold replica made of a homogeneous silicone rubber (Smooth-on, Ecoflex series^{TM} 00-30 − noted EF30), commonly used in previous *in vitro* testbeds of phonation (Kniesburges et al., 2011). To do so, a dedicated two-step molding procedure was developed, extending the

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processing route described in Luizard et al. (2023) to manufacture isotropic EF30 replicas: a first 3D-printed mold was used to cast the deepest layer of the vocal fold ("body"), covering 2/3 of the total volume. After 2h curing, the "body" was demolded and wrapped in the fibrous mat. EF30 was then poured into a second dedicated mold to process the upper part of the replica, covering the remaining third of the fold volume. The wrapped body was immersed in this uncured liquid until covered, then left to cure for 2h. Once demolded, the final composite replica was inserted into an articulated larynx testbed to adjust the prephonatory strain ε_{ap} in the anterior-posterior direction (Fig. 1b; Luizard et al., 2023).

2.2 Vibro-mechanical characterization

First, mechanical tests were performed on the composite materials selected for the vocal-fold replica, using a uniaxial tensile testing machine (Instron 5944) equipped with a ±10N load cell.

Then, the 3D laryngeal replica was connected to a pressurized airflow in order to explore and characterize the conditions under which vibrations induced by fluid/structure interactions occur (Fig. 1b). Once settled, various aeroacoustic parameters were recorded and averaged over a 4s-time window, including the subglottal air pressure, the audio frequency (f_o) and the sound pressure level (Luizard et al., 2023). Experiments were repeated for several vocal-fold replicas, with and without fibrous mats.

3. Results and discussion

The effect of fibrous reinforcement on vocal-fold vibrations and resulting sound was measured on several aeroacoustic descriptors. In particular, Fig. 1c compares the evolution of the audio frequency f_o measured as a function of the fold pre-strain ε_{ap} for two types of replicas, with and without reinforcement.

Several results are highlighted:

- For vocal-fold replicas without PCL mat, pre-stretching the folds has a decreasing impact on f_o, corroborating previous results on "biomimetic" candidates with quasi-linear, isotropic mechanical properties (Shaw et al., 2012, Luizard et al, 2023). Though minor (a few Hz from rest to $\varepsilon_{ap} \sim 0.20$), such variations are at odds with *in vivo* observations on folds during a *glissando*, where fo increases with ε_{ap}.

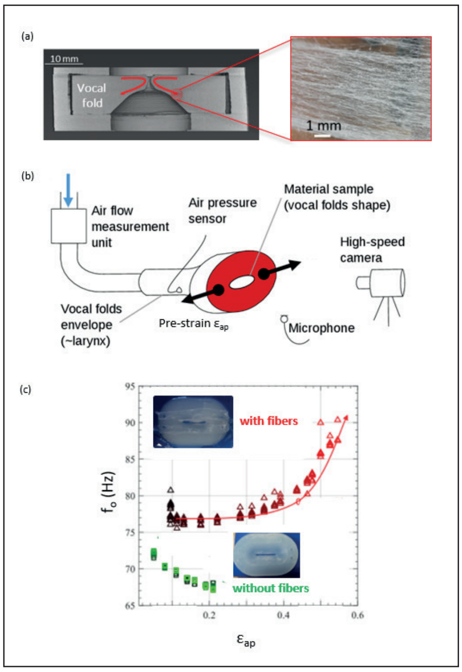

Figure 1. (a) Vocal-fold replica: mid-coronal X-ray tomographic view, scheme and zoom of an embedded PCL fibrous mat; (b) Overall in vitro testbed of phonation; (c) Fundamental frequency of oscillation against the applied anterior-posterior pre-strain.

- In comparison, the trends are reversed for anisotropic structures (EF30 with aligned PCL mats), in line the qualitative variations observed during phonation. The strain-induced fo-increase can be explained by the non-linear strain-hardening of tangent moduli measured on the composite materials during quasi-static tension, due to the progressive rearrangement and mechanical response of the fibrous network during loading (Ferri-Angulo et al., 2023).

Although improved thanks to its non-linear and anisotropic mechanical properties, the vibratory performance of our current *in vitro* idealization (with f_o between 75 and 90 Hz) is still far from that a biological larynx (with f_o rarely below 100 Hz in modal speech). This may be due to geometric and structural discrepancies that have not yet been optimised. In particular, the multi-layered mesostructure of the folds and gradients of mechanical properties in their sublayers should also be taken into account for more realistic f_o-range (Tur et al., 2023).

4. Conclusions

This work demonstrates the importance of the architectured fibrous networks making up vocal tissues in their flow-induced vibratory performances. It reinforces current studies aimed at refining composite replicas of vocal folds, to propose more realistic candidates in terms of microstructure and mechanics, and thus be able to characterise the multi-scale deformations of vocal folds during vibrations.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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