VOCAL-FOLD 3D MICRO-ARCHITECTURE AND MICRO-MECHANICS: A MULTIMODAL IMAGING STUDY

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Objectives: Current understanding of the histological features of the vocal folds is still insufficient to make the link to their vibromechanical performance. In particular, the 3D microscale rearrangement of the loaded tissues is still to be explored. Thus, the aim of this work is to characterize the 3D histological specificities of human vocal folds' fibrous networks and their strain-induced microstructure evolutions under tensile loading, at the scale of the muscular, collagen and elastin microfiber bundles.

Introduction: The 3D *ex vivo* observation of vocal folds at micron scale is a challenging task using micro Magnetic Resonance Imaging (limited spatial resolution), multiphoton nonlinear scanning microscopy (limited depth of field) and X-ray microtomography in absorption mode (low contrast) [1,2,3]. Recently, the 3D hierarchical architecture of human vocal folds was revealed by means of synchrotron X-ray microtomography with phase retrieval imaging mode [4]. High-resolution (voxel size of $0.65^3 \mu m^3$) and in depth 3D images of soft tissues (subvolumes of $1.3^3\, mm^3$) were acquired with fast scanning times (1-2 min), to quantify structure descriptors of *lamina propria* and *vocalis* fibrous networks at multiple length scales. In continuation of such developments, this work presents the preliminary *in situ* tensile tests on vocal-fold tissue coupled to synchrotron X-ray imaging.

Methods: 10 tissue samples made of *lamina propria* and *vocalis* sublayers were excised from vocal folds of the same human larynx (male donor, 76 yo), to avoid inter-subject variability, and enable imaging at micro-scale resolution. The dissection yielded to 5 samples from each left/right vocal fold [4], of typical dimensions $15\times5\times3 mm^3$. Several conditions of tissue conservation were used before testing (ethanol, gel, cryopreservation). First, a multimodal imaging study was conducted to investigate the tissue fibrous architecture at rest, thanks to three complementary techniques: (1) Synchrotron 3D X-ray imaging at high-resolution ($0.65^3 \mu m^3$), using the microtomographs of the ESRF's ID19 beamline; (2) Biphotonic 3D microscopy imaging ($0.35\times0.35\times0.70 \mu m^3$) using the confocal microscope of Léon Bérard's Cancer Center (LSM 780, Laser 900nm); (3) Histological stainings (Hematoxylin-Eosin-Saffron), differentiating vocal-fold constituents on 2D micrographs. Then, several samples were subjected to uniaxial tensile tests interrupted by relaxation steps, and combined to 3D X-ray imaging using a mechanical device positioned onto the ESRF microtomographs. Holding conditions were optimized to avoid stick-slip effects and tissue damage.

Results: Micromechanisms of deformation of the vocal fold tissues under tensile loading will be described and quantified by tracking various structure descriptors as a function of the applied strain: shape and size of their layered fibrous architectures within the *lamina propria* and the *vocalis*; orientation, shape and size of muscle fibres as well as collagen and elastin fibre bundles constituting these layers. Based on the multimodal study, a specific focus will be paid to the distinction of the extracellular matrix fibres within the *lamina propria*. Finally, the impact of the contrast agent on both the tissue mechanics and the quality of the acquired images will be assessed, depending on the immersion procedure (diffusion time, concentration).

Conclusions: Based on advanced micro-imaging techniques, this study provides a quantitative database of the 3D and multiscale descriptors of vocal fold tissues, and their evolution during a mechanical loading.

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