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On the development of new composite hydrogels mimicking vocal-fold histological and mechanical features

Daniel Ferri⁴, Hamid Yousefi-Mashouf^{2,3}, Lucie Bailly², Laurent Orgéas², [Jerome Sohier](#)¹

¹ CNRS, UMR 5305 - Laboratoire de biologie tissulaire et ingénierie thérapeutique, Lyon, FR; ² University Grenoble Alpes, CNRS, Grenoble INP, 3SR, Grenoble, FR; ³ University Grenoble Alpes, CNRS, Grenoble INP, GIPSA-lab, Grenoble, FR; ⁴ CNRS, UMR5510 MATEIS, Villeurbanne, FR

Introduction

Human vocal folds are soft laryngeal structures able to endure very large and reversible deformations (up to 50% stretching), despite the stresses they are subjected to during voice production. These outstanding properties are related to their multi-layered structure (*epithelium*, *lamina propria* and *vocalis* muscle) and fibrous microstructures¹. Vocal folds can accommodate with many traumas, but severe or cancerous lesions require microsurgical intervention that may induce dysfunctional scarring lesions. Few biomaterials have been envisaged to treat vocal folds' lesions, mainly because their unique mechanical properties are not clearly understood.

A way to better comprehend these properties and pave the way to future clinical applications would be to mimic the fibrillar and elastomeric structure of vocal folds. In this respect, a tailorable mimetic hydrogel (poly-lysine dendrimers (DGL) and polyethylene glycol (PEG-NHS)) could be of interest². Its association with polycaprolactone (PCL) nanofibers could provide composites of modular mechanical and vibratory properties, close to native tissues. Thus, this work aims at (i) characterizing the organization of collagen and elastin of the human vocal folds, and (ii) reproducing it through the design and mechanical evaluation of different nanofibres/hydrogels composites (nFHC) with tuned hydrogel elasticity, nanofibrillar orientation and mono/multi-layered organization, (iii) comparing their mechanical response with those of native human tissues.

Experimental Methods

Microstructural organization of vocal folds excised from donated bodies was assessed with biphotonic microscopy for collagen and elastin fibres (Zeiss® LSM 780). PCL nanofibres of adjustable orientations (iso- and anisotropic) were processed using a jet-spraying method³. Diameter distribution and orientation of collagen, elastin and PCL fibres (SEM FEI quanta) were analysed using Fiji®. Hydrogels of varying mechanical properties were obtained by mixing DGL (Colcom®) and PEG-NHS aqueous solutions of various concentrations, PEG molecular weights (2 k, 10 k or 40 kDa) and geometry. Different nFHC were prepared by percolation of hydrogel solutions through the nanofibers. Swelling of hydrogels and composites was measured over 48 h. Their mechanical properties were characterized in cyclic tension upon finite strains (BOSE® EF3200). Similar experiments carried out previously with Human vocal folds were used as reference data⁴.

Results and Discussion

The microstructural analysis of human vocal folds revealed specific organizations of collagen and elastin fibres in the different anatomical layers. Epithelium was devoid of either, few were present in the muscle layer while the *lamina propria* was rich in both. Within the latter, both elastin and collagen wavy fibers were arranged in an entangled network of antero-posterior orientation, showing density gradients from superficial to deep layers (Fig 1A). Collagen fibers were thicker than elastin ones.

PCL nanofibers in the processed mats were of orientations and diameters similar to collagen and elastin fibers (Fig 1B). Thanks to the very high porosity of nanofibrillar networks (more than 96%), nanofibers could be impregnated in a continuous DGL/PEG hydrogels matrix to create composites. (Fig 2A). Nanofibers incorporation reduced their swelling, revealing a coherent integration and the effect of stronger PCL fibres on composite properties (Fig 2B).