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Multi-axial mechanical properties of hydrogel-based materials upon finite strains: towards the design of tailored vocal-fold composite replicas

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Introduction

In vitro modeling of phonation requires materials able to mimic the vibro-mechanical features of vocal-fold tissues: ability to (i) endure large deformations under physiological multiaxial loadings and strain rates [1], (ii) adapt their vibro-mechanical behavior to external loadings and environmental changes. Among relevant candidates [2], hydrogels are attractive materials due to their tissue-like water content [3,4]. However, the mechanical characterization of hydrogels is often limited to single monotonic loadings, mainly in compression, or to standard DMA analyses [5]. These configurations are far from those endured by the tissue *in vivo*. Here, we manufactured hydrogels with tunable mechanical properties that we characterized under realistic, finite strain, cyclic tension, compression and shear loadings.

Methods

Materials. Two types of hydrogels were used. The first one was based on 10 %w/v porcine Gelatin (Ge) aqueous solution (300 g Bloom, Type A). After molding and freeze-drying, dried plate samples were crosslinked by immersion in Glutaraldehyde (GA) solutions of various concentrations. The second hydrogels were obtained by casting aqueous solutions of poly-lysine dendrimers (DGL) and functionalized polyethylene glycol (PEG-NHS) with various concentrations and molecular weight MW [6].

Mechanical characterization. Hydrogel samples were tested using a tensile testing machine (INSTRON® 5944) equipped with a ±10 N load cell. Three loading modes were studied, as previously done on vocal-fold tissues [7]: simple tension, compression and shear. Samples were first subjected to quasi-static cyclic paths ($10^{-3}s^{-1}$) with increasing strain amplitude up to failure. Shear DMA measurements were also carried out to study the cyclic behavior of hydrogels at various frequencies for small and finite strains. All tests were conducted within a thermo-hygro-regulated chamber at 25°C and saturated humidity (RH ≈100%).

Results and discussion

Typical tensile stress-strain curves recorded for both hydrogels at various compositions are plotted in Figure 1, together with the corridors corresponding to the tensile behavior of the vocal-fold sublayers. Ge-GA hydrogels present proper tensile behavior, of the same orders of magnitude as observed for the *lamina propria*, with weak stress-hysteresis and suitable stiffness up to 10-20% strains. However, their strains at break exhibit weak values which should be improved (up to 30-50%) to approach the mechanics of the *lamina propria*. PEG-NHS hydrogels display similar tensile behaviors, with lower stiffness and higher strains at break, and closer to the behavior of the *vocalis*. By modulating the Ge-GA (resp. PEG/DGL) ratio, the hydrogel stiffnesses can be easily tuned. In addition, increasing the PEG MW allowed the hydrogel elongation at break to be significantly improved, from 30% to 90%.

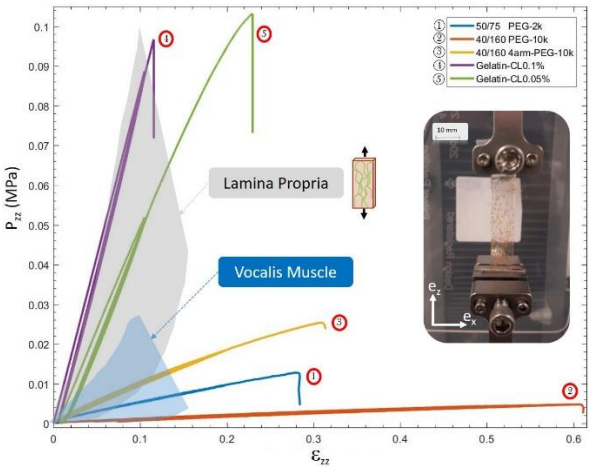

Figure 1: Tensile behavior of the Ge-GA and PEG-NHS hydrogels (Piola-Kirschoff stress P_{zz} vs. Hencky strain ε_{zz}) together with the corridors recorded for the lamina propria and the vocalis [7].

The compression and shear behaviors of the hydrogels are roughly in accordance with those observed for the *vocalis* and the *lamina propria*. Nonetheless, due to their isotropy, hydrogels are stiffer than the highly anisotropic living tissues. modifying the hydrogel nanostructures with proper processing routes or reinforcing them with orientated nanofibers are anticipated solutions to explore.

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